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ZOONOSIS

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Volume 2

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PREFACE

The well-being of humans and animals is pretty much interdependent. It's impossible to ensure human health, without considering animal health and vice versa.

The need to enhance the collaboration between animal health workers and medical professionals, researchers and academicians has moved the editors to develop this publication. The book takes into account the major threats of animal and human health. This book provides the core concepts of Zoonosis with a critical focus on the key challenges and their effective management. The objective is to cover epidemiological interactions of various infectious diseases and their ecological implications as an emerging threat.

It is anticipated that this book would be of great use to a variety of readers. University students, graduates, practitioners, animal healthcare providers and health professionals would definitely find this book of great importance. The language of book has been intentionally kept easier for a non-technical person to grasp the concepts on interdependence of animal and human health. The editors wish to publish a series on the subject keeping in view the urgency to highlight these areas for awareness, research and development.

Editors

Contents
Volume 2

Mini-Devils: Exploring the Zoonotic Potential of Ticks

01

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ABSTRACT

Ticks are prominent blood-sucking arthropods and are considered one of the biggest threats to the human and animal health globally because of their vector function for a wide range of bacterial, viral and protozoal zoonotic pathogens. After mosquitoes, ticks are regarded as the second most threatening vector for human health. The economic impact of diseases caused by ticks possesses notable significance and tends to rise every year. The spread of these pathogenic organisms is mostly unnoticed in the nature of the enzootic tick-vertebrate cycles. However, these may be responsible for causing remarkable morbidity and mortality in humans and animals when attacking in abundance. The anatomy and physiology of a tick helps it in exhibiting its behavior. The mouth-parts of ticks play a significant role in their penetration to their hosts. As ticks are blood-sucking parasites, so their survival, growth, development and reproduction depend on blood meals. There is secretion of certain substances when they feed on their hosts in order to anchor themselves to the host. These substances act as sedatives that cover up the pain from the bites and prevent coagulation of blood. This tenacious and efficient-feeding behavior makes them the potential vectors of zoonotic infections. Therefore, both the ticks infesting various hosts at the wildlife-domestic animal-human interface and the pathogenic organisms transmitted by these tick species occupy prime importance in one health perspective. The in depth knowledge of the host, tick disease and pathogen triangle involving various habitats and distribution of ticks, changes in the environmental conditions and global warming is of prime significance and must be considered while devising policies involving migration and trade of animals.

Keywords: Ticks, Zoonoses, Blood-sucking parasites, Mortality, Survival, Sedatives, Pathogen triangle.

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INTRODUCTION

Zoonoses are the "diseases and infections that are naturally transmitted between vertebrate animals and man," according to the definition proposed by the World Health Organization (WHO) Expert Committee on Zoonoses in 1951. Rudolph Virchow coined the term zoonosis (zoonoses, plural) at the end of the nineteenth centuryfor the diseases caused in humans by animals. It is the fusion of two Greek words (*zoon*, animals and *noson*, disease). The word 'zoonosis ' is also viewed as more straightforward and shorterthan 'anthropozoonosis' (animals to humans) and 'zooanthroponosis' (humans to animals), which stemmed from the prevailing trend of transference between man and other vertebrates. The definition of zoonoses should comprise the term 'infestations' to envelop more properly parasitic infections (Chomel 2009). Fig. 1 illustrates the epidemiological classification of zoonoses.

Zoonoses occupy a crucial place in the global health program of the World Health Organization (WHO). A considerable number of animal species are involved in the transmission of zoonotic diseases in humans, either directly or indirectly. These zoonotic diseases are about four-fifths of overall infections that occur in humans and are responsible for causing remarkable mortality and morbidity in both sexes and all age groups (Pal 2005). Therefore, the treatment and control of zoonotic diseases is of prime importance.

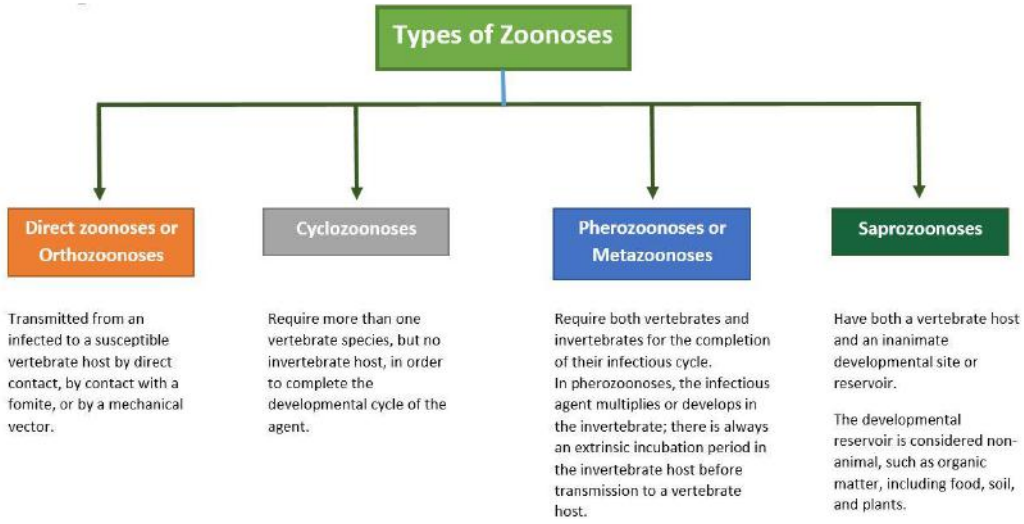

Fig. 1: Different types of zoonoses.

In the past few years, one-third of the emergence of infectious diseases is due to zoonotic vector-borne infections, which have a harmful impact on human and animal health and welfare on a global scale (de la Fuente et al. 2015). Especially, the occurrence of Lyme disease in the USA is anticipated to hike up by about 20% in the coming years due to changes in climatic conditions (Dumic et al. 2018). After mosquitoes, ticks are regarded as the second most threatening vector for human health as these are involved in the transmission of a wide range of pathogenic organisms (Toledo et al. 2009). In order to alleviate the production losses of livestock and bad effects on animal welfare and mitigate disease exposure in humans, the transmission channels of tick-borne diseases must be comprehended (Tamzali 2013). This is important as the incidence of tick-borne zoonotic diseases is elevating in the 21st century due to climate change's effects on the tick lifecycle and the transboundary locomotion of animals infested with ticks (Parola et al. 2013).

The economic impact of diseases caused by ticks possesses noteworthy significance and escalates every year. In the USA, the reported expense per patient diagnosed with Lyme disease was USD $8172 in the year 2002(Zhang et al. 2006), which equals to $11 838 in the year 2019,according to CPI inflation calculator (Rochlin and Toledo 2020). On the basis of 42 743 cases reported to the Centers for Disease Control and Prevention (Xiao et al. 2021) in 2017, the estimated cost was over USD $500 million yearly. These expenses can be even greater in patients with post-treatment Lyme disease syndrome (Adrion et al. 2015). Economic additional expenditures are associated with tourism and hospitality in endemic regions (Donohoe et al. 2015). The economic importance of Lyme disease is not limited to the USA only. The costs of millions of euros are also found to be associated with Lyme disease in Europe (Mac et al. 2019). As the cumulative impact of all tick-borne zoonotic infections remains unquantified in most situations, it is highly significant to study and comprehend tick-associated zoonoses.

2. BIOLOGY OF TICKS AND THEIR BEHAVIOUR

Ticks are one of the most prominent blood-sucking arthropods. Their body is generally divided into two major parts, i.e. a flat capitulum or head called gnathosoma and an oval-shaped body called idiosoma (Anderson 2002). Like other arthropods, nymphs and adult ticks have eight legs while in the larval stage ticks have six legs. A distinct head is not present in the ticks. The mouth-parts of ticks are present on the capitulum (John and Anderson 2008). These are made up of hypostome and chelicerae. These mouth-parts play a significant role in the penetration ticks into their hosts. As ticks are blood-sucking parasites, so their survival, growth, development and reproduction depend on blood meals. Substances are secreted by the ticks when they feed on their hosts in order to anchor themselves to the host. These substances act as sedatives that cover up the pain from the bites and avoid blood coagulation. As ticks are tenacious and efficient feeders, they are potential vectors of zoonotic infections(John and Anderson 2008).

Ticks undergo three basic stages throughout their life, i.e. larva, nymph and adult (Anderson 2002). They spend each life stage on a different host. Fig. 2 briefly describes the general life cycle of ticks.

Ticks generally don't fly, jump or drop from trees on the hosts. They seek out their hosts. This host-seeking behaviour of ticks is known as "questing"(Richardson et al. 2022). This behaviour refers to a sequence of actions or behaviour in which the ticks mount vegetation especially tall grasses, stop and extend their forelimbs and wait for a host to get attached to (Holderman 2013).

3. MAJOR TICK-BORNE PATHOGENS CAUSING ZOONOTIC INFECTIONS

Ticks are considered one of the biggest threats to the human and animal health globally because of their vector function for a wide range of bacterial, viral and protozoal zoonotic pathogens. The dissemination of these pathogenic organisms is mostly unnoticed in the nature of the enzootic tick-vertebrate cycles. However, these may be responsible for causing remarkable morbidity and mortality in humans and animals when attacking in abundance (Jahfari 2016). Fig. 3 demonstrates various viral, bacterial and protozoal tick-borne pathogens.

4. COMMONLY FOUND TICK-BORNE ZOONOTIC INFECTIONS

Different species of ticks carry different types of zoonotic infections. Table 1 indicates various types of zoonotic diseases, their causative agents, the tick vectors, the symptoms and the geographical distribution of that zoonotic infection.

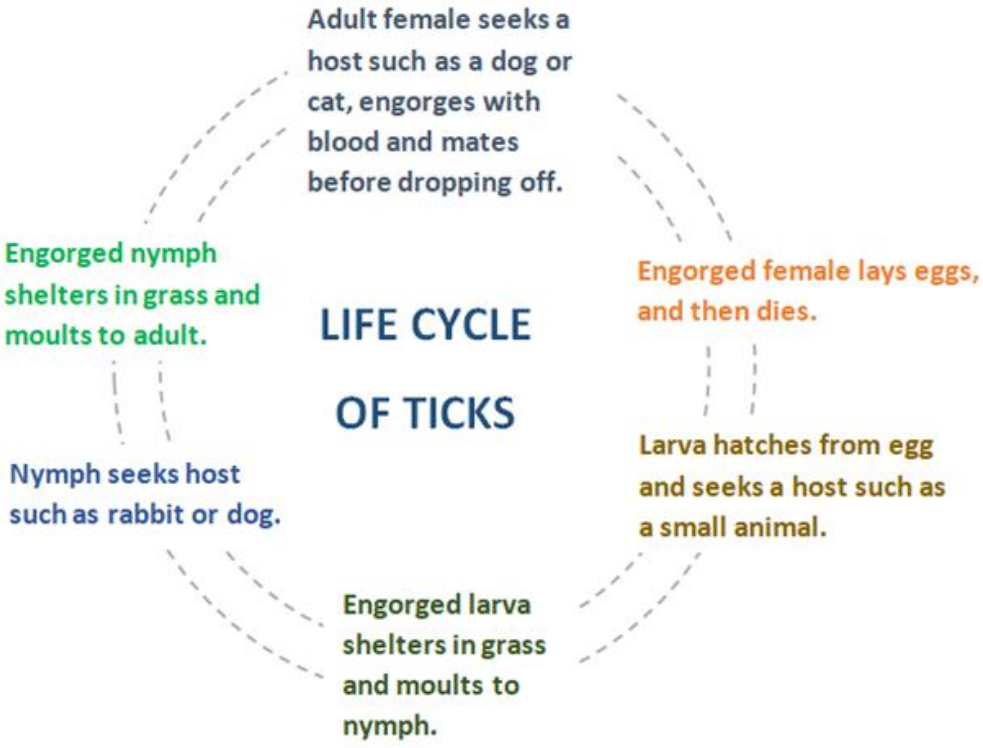

Fig. 2: General life cycle of ticks

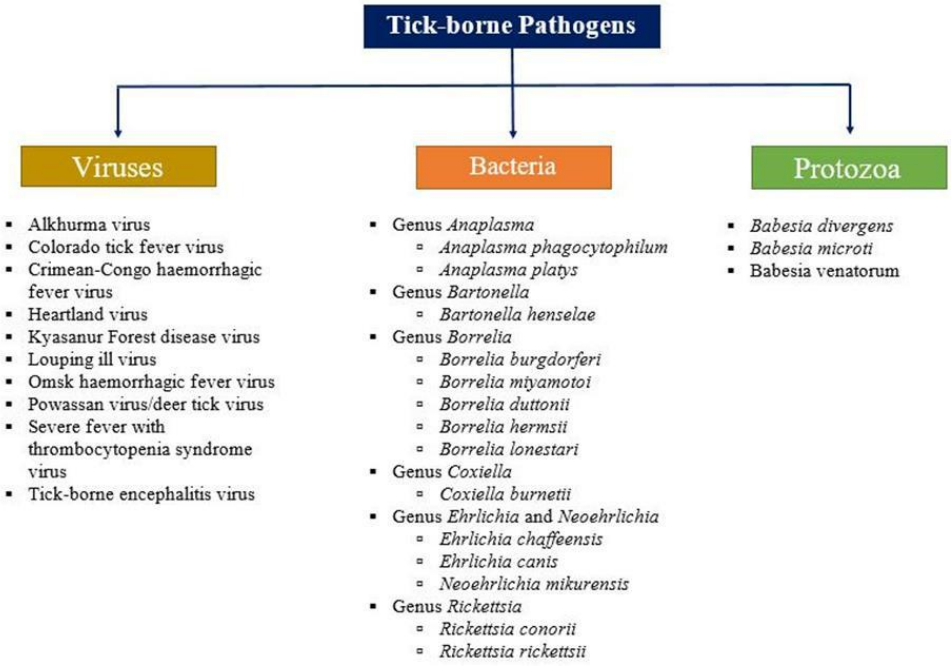

Fig. 3: Major tick-borne pathogens causing zoonotic infections

5. DIAGNOSTIC APPROACHES AND TREATMENT OF TICK-BORNE DISEASES

Earlier only microscopic examination of blood smears was considered to be the widely used method for diagnosis of tick-borne diseases. Although it is a quick and economical method, other methods are being used nowadays; these methods are faster and more sensitive.

5.1. NUCLEIC ACID AMPLIFICATION TECHNIQUES

Nucleic acid amplification techniques are generally more sensitive and faster than other methods like pathogen culture as pathogen culture is slow and difficult (Springer et al. 2021).

5.2. REAL-TIME QUANTITATIVE PCR

Real-time qPCR is quite beneficial in observing the course of infection as the DNA load of the given pathogen tells the level of infection in patient (Che et al. 2019). That is why real-time qPCR is considered to be hypersensitive and faster than conventional PCR (Springer et al. 2021).

5.3. REAL-TIME DIGITAL PCR

Real-time dPCR detects and analyzes the rarely found target sequences by segregating the sample into several parallel PCR reactions which makes it a hypersensitive test (Das et al. 2020).

5.4. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (Langford et al.2015)

LAMP is a cost-efficient DNA amplification method (Marins et al. 2013; Wang et al. 2017; Singh et al. 2019). LAMP assays require a constant temperature and do not need a thermo-cycler to work (Becherer et al. 2020).

5.5. MASS SPECTROMETRY

On the basis of comparison between protein signatures and existing databases, mass spectrometry–based techniques are generally used to detect cultured pathogens in laboratories (Neumann-Cip et al. 2020). This method is found beneficial in the diagnosis of *Babesia microti* infections (Magni et al. 2020). It may help in the detection of *Babesia canis* infections in canines (Adaszek et al. 2014).

5.6. ENZYME-LINKED IMMUNOSORBENT ASSAY (de Souza et al. 2021)

ELISA tests are likely to be performed but these tests are less specific. So, techniques like immune-blotting (Sanchez et al. 2016) or sero-neutralisation tests (Reusken et al. 2019) are usually recommended to confirm the ELISA tests.

5.7. IMMUNOFLUORESCENCE ANTIBODY TEST (Rifat et al. 2023)

IFATsare generally used to identify and analyze antibodies against *Babesia* (Sanchez et al. 2016; Solano-Gallego et al. 2016) and *Ehrlichia* (Dumler et al. 2007). This approach is time-consuming and could be subjective to some extent (Springer et al. 2021).

5.8. RAPID IMMUNOCHROMATOGRAPHIC TESTS

These tests are easy to use and available commercially but they only give binary outcomes (either positive or negative results) and do not allow analysis of antibody titers. Such tests are shown to be less sensitive (Smit et al. 2015; Liu et al. 2018; Springer et al. 2021).

Table 1: Various types of zoonotic diseases, their causative agents, the tick vectors, the clinical manifestations and regional prevalence of that zoonotic infection

Type of Disease	Causative agent(s)	Tick-vector(s)	Clinical manifestations	Regional Prevalence	References
Anaplasmosis	*Anaplasma marginate* *Anaplasma phagocytophilum*	*Ixodes ricinus* *Ixodes trianguliceps,* *Ixodes scapularis,* *Ixodes pacificus*	Fever, anorexia, dyspnea, anemia, reduced milk production in cattle	Australia, South Africa and South America	(De La Fuente et al. 2005; Bown et al. 2008; Foley et al. 2008; Aubry et al. 2011; Keesing et al. 2012; Aktas et al. 2017; Hove et al. 2018; Akram et al. 2021)
Babesiosis	*Babesia bigemina* *Babesia equi* *Babesia microti*	*Ornithodo roserraticus,* *Ixodes scapularis,* *Ixodes dammini*	Fever, headache, myalgia, chills, fatigue and sweats	Central and South America, Africa, Australia, and Europe	(Homer et al. 2000; Boozer and Macintire 2003; Bock et al. 2004; Beugnet and Moreau 2015; Vannier 2020)
Colorado Tick Fever	Coltivirus	*Dermacentor andersoni*	Headache, fever, rash, myalgia, lymph-adenopathy, photophobia, GI disturbances	Mountain regions of Canada and the US	(Naides 2012; Petney et al. 2012; David Beckham 2015)
Ehrlichiosis	*Ehrlichia canis* *Ehrlichia chaffeensis* *Ehrlichia equi* *Ehrlichia phagocytophila*	*Amblyomma americanum,* *Ixodes ricinus,* *Ixodes scapularis,* *Rhipicephalus sanguineus*	Anaemia, arthritis, fever, anorexia, fatigue, diarrhoea, vomiting, dyspnea, and weight loss	South-Central America, Europe, Asian countries including Korea, China, and Siberian Russia	(Yabsley et al. 2002; Skotarczak and Medicine 2003; Demma et al. 2005a; Dumler et al. 2007; Vieira et al. 2011; Paulino et al. 2018; Springer and Johnson 2018)
Lyme disease or Lyme borreliosis	*Borrelia burgdorferi* *Borrelia afzelii* *Borrelia garinii*	*Ixodes scapularis*	Myalgia, malaise, fever, lymph adenopathy, headache, and joints pain	Europe, North America and some regions of Asia	(Steere 2001; Wormser 2006; Mead 2015)
Powassan Virus Disease	Powassan Virus (*Flavivirus*)	*Ixodes scapularis,* *Ixodes cookei*	Nausea, vomiting, dizziness, fatigue, fever, rashes, blurry vision, hemi-plegia, paralysis	Russia and North America	(Ebel 2010; Raval et al. 2012; Piantadosi et al. 2016; Santos et al. 2016; Fatmi et al. 2017; Hermance et al. 2017; Kemenesi and Bányai 2018)
Q Fever	*Coxiella burnetii*	*Amblyomma triguttatum triguttatum* *Dermacentor andersonii*	High-grade fever, malaise, myalgia, headache, flu-like symptoms, Pericarditis	All over the world except New Zealand	(McDiarmid et al. 2000; Parker et al. 2006; Sally et al. 2007; Angelakis and Raoult 2010; Duron et al. 2015b; Pierre-Edouard Fournier 2020; Körner et al. 2021)

Rocky Mountain Spotted Fever	*Rickettsia rickettsii*	*Rhipicephalus sanguineus Dermacentor spp.*	Fever, rash, myalgia, GI disturbance, headache	South and North America	(Masters et al. 2003; Demma et al. 2005b; Dantas-Torres 2007; Luke and Chen 2008; Alvarez-Hernandez et al. 2022)
Tick-borne Encephalitis	*Tick-borne encephalitis virus* [TBEV] (*Flavivirus*)	*Ixodes spp*	Fever, fatigue, flu-like symptoms, body aches, anorexia, slightly increased body temperature, and nausea	Northern regions of China, South Korea, Japan, Eastern and Central Europe and Russia	(Gritsun et al. 2003; Lindquist and Vapalahti 2008; Bogovic and Strle 2015; Valarcher et al. 2015)
Tularemia	*Francisella tularensis*	*Ambloyomma americanum Dermacentor andersoni Dermacentor variabilis*	Fever, malaise, myalgia, chills, headache, and sometimes regional lymph adenopathy	Korea, Japan, China, mainland Europe, and North America	(Parola and Raoult 2001; Feldman 2003; Sjöstedt 2007; Tärnvik and Chu 2007)

5.9. ENZYME-LINKED IMMUNOSPOT ASSAY (ELISPOT)

ELISPOT is considered to be a highly sensitive and specific technique to evaluate T cells cytokine response on antigen stimulation (Kalyuzhny 2005).

5.10. LYMPHOCYTE TRANSFORMATION TESTS (LTTs)

LTTs detect the T cell's proliferative responses upon specific-antigen stimulation. These tests can be used for identification of human Lyme borreliosis but are not generally suggested as they are not highly specific (Dessau et al. 2014).

6. ONE HEALTH PERSPECTIVE

A number of tick species effectively transmit zoonotic pathogenic organisms, but some of these exhibit exceptional behavior due to their function as vectors for many zoonotic pathogens. Therefore, both the ticks infesting various hosts at the wildlife-domestic animal-human interface and the pathogenic organisms transmitted by these tick species occupy prime importance in one health perspective. *Ixodes scapularis*, *Ixodes ricinus* and *Ixodes persulcatus* are among the relatively significant tick vectors. These belong to the Ixodes ricinus complex which is a group that comprises 14 Ixodes species having global distribution (Xu et al. 2003). Ticks, belonging to Ixodes ricinus complex, are confirmed vectors of various bacteria (e.g. Rickettsiales and *Borrelia* spp.), three flaviviruses (Powassan virus, TBEV, and Louping ill) and protozoa (*Babesia* spp.) that are zoonotic. Moreover,*Amblyomma americanum*, *Dermacentor andersoni,* and *Dermacentor variabilis* are specifically crucial in North America considering the one health perspective (DE 2018) because of their vector function for a diverse range of zoonotic viral (e.g., Heartland and Powassan virus) and bacterial (e.g., *Ehrlichia* spp. and *Rickettsia* spp.) pathogenic organisms. Although hard ticks are involved in the transmission of most tick-borne diseases, soft ticks may also act as vectors (Akram et al.2022;Dantas-Torres et al. 2012). Several *Ornithodoros* spp. may also play a role in the transmission of relapsing fever borreliae (Talagrand-Reboul et al. 2018), and this tick genus can also be involved in the transmission of Alkhurma fever virus (Sawatsky et al. 2014) and *Coxiella burnetii*(Duron et al. 2015a).

7. TICK AND TICK-BORNE ZOONOSES CONTROL

According to the recommendations of the CDC, the best defense against tick-borne infections is personal protection (Beard et al. 2014), alongside landscaping alterations and the application of acaricides (Clark and Hu 2008; Hook et al. 2015). However, there is little observation regarding the effectiveness of any of these methods in lowering the tick exposure or incidence of disease (Connally et al. 2009; Hinckley et al. 2016). It is essential to reduce the tick population and tick prevalence in highly endemic areas in order to achieve adequate personal protection. Target deer or mice as hosts for either immature or adult life stages of ticks is the only area-wide available control choice to lower the number of tick population (Eisen et al. 2012). As the use of pesticides may induce adverse effects on animal as well as human lives, there is a need to devise alternative methods and approaches in order to control ticks and tick-borne zoonoses. Oils and organic compounds derived from plants can be used to control the population of ticks. These plant-derived products hinder blood feeding in the ticks.

8. CONCLUSION

Ticks affect animal and human health badly. This is because ticks are the vectors for various zoonotic pathogens. This makes tick-borne zoonotic infections perfect examples of the concept of One Health. Tick-borne zoonoses are an important issue worldwide. Significantly, countries with warm and humid climates provide favorable conditions for infections in animals and humans. So, control programs and the development of effective disease management strategies are needed. Moreover, animal movement due to trade or migration should be kept in mind for possessing probable infection as a risk of zoonoses. Strict quarantine measures need to be imposed in order to avoid the probability of reintroductions of ticks and tick-borne infections. In order to suppress the increased population of ticks, a better understanding of stimuli leading to distribution, accumulation, stability, and density-dependent mortality is required. The knowledge of the host, tick disease, and pathogen triangle concerning various habitats and distribution of ticks, changes in the environmental conditions, and global warming must be considered while devising policies involving migration and trade of animals. The administration of anti-tick agents and recombinant vaccines to prevent related infections can be done in order to disrupt this triangle, especially in the case of animals. The promotion of disease-resistant animal breeds instead of susceptible ones may be another tick control strategy. Overall, in-depth studies revolving around the control of tick-borne zoonoses should be considered a top priority plan.

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Vector-Borne Zoonotic Diseases

02

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ABSTRACT

The global health program of the World Health Organization recognizes zoonoses as a significant threat, encompassing around 250 diseases transmissible from animals to humans. Zoonotic diseases, often vector-borne, pose a dual threat by causing severe illnesses in humans and endangering animal health, leading to substantial economic losses in the livestock industry. Vector-borne zoonoses, transmitted by creatures like mosquitoes and ticks, have far-reaching consequences for public health and the environment. These diseases, including West Nile virus, Malaria, Lyme disease, and dengue fever, contribute significantly to global morbidity and mortality. Vectors act as bridges, facilitating the transmission of infectious agents between animals and humans, potentially sparking epidemics or pandemics. The environmental impact is profound, influenced by factors like temperature changes and ecological disruptions, altering the dynamics of these diseases. The economic toll is substantial, with higher medical expenses, lost productivity, and reduced agricultural output in affected regions. The interdependence of human, animal, and environmental health necessitates a collaborative One Health approach for effective management and prevention. Vector-borne zoonoses remain a concern due to urbanization, climate change, and globalization, posing challenges to surveillance, diagnosis, and control. Vectors, such as mosquitoes, ticks, flies, fleas, and lice, play a crucial role in transmitting infectious agents actively or passively. Mosquito-borne diseases, influenced by complex interactions between the environment and population dynamics, present a serious global health challenge. This chapter explores various aspects of vector-borne zoonoses, emphasizing their significance and the need for collaborative strategies to address emerging threats.

Keywords: Zoonotic diseases, Vector-borne diseases, One Health, Global health program, Mosquito-borne diseases

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1. INTRODUCTION

The World Health Organization's global health program includes zoonosis as a significant component. 250 zoonotic ailments can be contracted by humans either directly or indirectly from a wide range of animal species. Primary health systems prioritize the treatment and control of zoonotic diseases. Zoonosis is primarily considered an animal disease and human acts as an aberrant host (Bezerra-Santos et al. 2021). Zoonosis functions as a two-edged weapon. One by spreading deadly diseases to people, and the other by endangering the health and production of animals and generating significant financial losses to the livestock industry (Judson and Rabinowitz 2021). Throughout the world, numerous infections caused by viruses, bacteria, or parasites are recognized as zoonosis transmitted by ticks, and diseases caused by them are referred to as vector-borne diseases (VBDs). In other words, these infections are transmitted especially from animals to humans (Springer et al. 2021). The importance of vector-borne zoonosis is seen in its effects on the environment and public health.

Following are some crucial details emphasizing the significance of vector-borne zoonosis:

1-Zoonotic disorders transmitted by vectors are a serious hazard to human health on a global level. A notable portion of morbidity and mortality worldwide is brought on by these illnesses, which include West Nile virus, Malaria Lyme disease, and dengue fever.

2-Infectious agent transmission between animals and people, including the transfer of viruses, bacteria, and parasites, is greatly aided by vector-borne zoonosis. By acting as a bridge, they enable infections to get across species boundaries and may even start epidemics or pandemics.

3-Environmental Impact: Environmental factors are directly related to vector-borne zoonosis. The distribution and behavior of vectors can be impacted by changes in temperature, land use, and ecological disruptions, which can change the dynamics of zoonotic illnesses.

4- Economic Effects: Vector-borne zoonosis has significant negative economic effects. These illnesses can result in higher medical expenses, lost productivity, and lower agricultural output in the afflicted areas. In regions where these diseases are endemic, the financial burden might be very high.

5- Vector-borne zoonosis brings attention to the interdependence of human, animal, and environmental health. Public health professionals, veterinarians, ecologists, and other stakeholders must collaborate to manage and prevent these diseases using a One Health strategy.

6-Vector-borne zoonosis continues to represent a concern because of things like urbanization, climate change, and globalization. The introduction of novel illnesses via vectors into new geographical areas might provide difficulties for surveillance, diagnosis, and control (Hassall et al. 2023).

2. VECTORS AND THEIR TYPES

A vector, which is a living creature, transmits an infectious agent from an infected animal to a human or another animal. Vectors include arthropods such as mosquitoes, ticks, flies, fleas, and lice (Swei et al. 2020). Infectious diseases can be transmitted actively or passively via vectors:

Some biological vectors, such as ticks and mosquitoes, can carry illnesses that multiply within them and transfer to new hosts via biting.
Mechanical vectors, such as flies, can pick up infectious pathogens and distribute them through direct physical contact.
Some vectors can go long distances. This may have an impact on the range of zoonotic diseases spread by vectors (Combs et al. 2022).

3. MOSQUITO-BORNE DISEASES

Mosquitoes-borne diseases are one the serious health problem around the globe and their transmission rely upon perplexing interactions between the environment and the sensitivity, vulnerability, and versatile capacity of populations (Colón-González et al. 2021). When we talk about the list of these diseases there are a number of diseases and in this chapter we will discuss a few as given below.

4. WEST NILE FEVER

This disease is spread by arbovirus i.e. West Nile Virus belongs to the *"flaviviridae" family*. It is spherical, enveloped, and has approximately 40-60 nm diameter according to the electron microscope. For perpetuation in nature, this virus depends on a taxonomically diverse host viz. Mosquitoes and Birds. These hosts differ evidently in their response to infection. Some ways of transmission include hematophagy, ingestion, aerosol and direct contact (Habarugira et al. 2020). According to CDC, transmission of West Nile Virus is shown in Fig. 1.

5. SIGNS AND SYMPTOMS

Partial paralysis, head pressing, weight loss, weakness particularly in hind legs, muscle twitching, impaired vision, stumbling, grinding teeth, an inability to swallow, circling and convulsions are typical signs of WNF in horses (Cantile et al. 2000 and WOAH).

6. DIAGNOSIS

Diagnosis is performed by serological testing for detection of Ig M antibodies in the serum of infected person via ELSIA test (Clark and Schaefer 2019).

7. TREATMENT AND CONTROL

The treatment of this virus is mainly supportive care along with symptoms management. For its control in horses, vaccines are available. Moreover, key to prevent west Nile Fever is to control mosquito population and surveillance programs to educate people regarding severity of this disease (Castillo-Olivares and Wood 2004; WOAH).

8. MALARIA

Millions of people around the world are afflicted with malaria, a potentially fatal infectious disease spread by mosquitoes that is most prevalent in tropical and subtropical areas. It has been brought on by the Plasmodium parasite. Despite enormous attempts to combat it, this disease has long been a major cause for public health concern and still poses a significant threat to global health (Zambare et al. 2019).

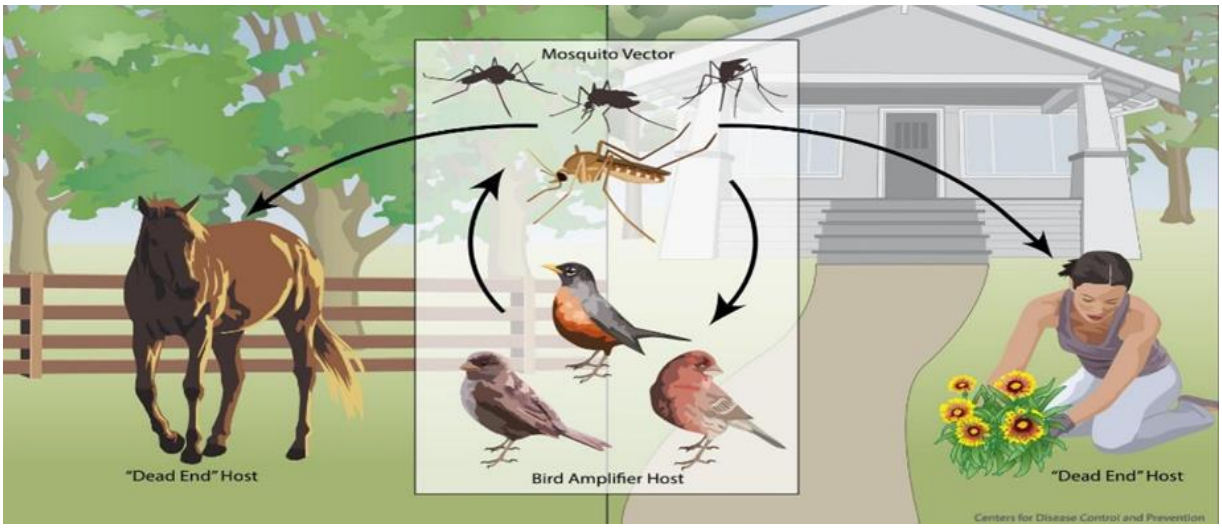

Fig. 1: Transmission of West Nile Virus (CDC).

9. CAUSATIVE AGENT OF MALARIA AND ITS LIFE CYCLE

There are five species of Plasmodium known to infect human beings and are the causal agents of malaria*: Plasmodium falciparum, Plasmodium malariae, Plasmodium Knowles and Plasmodium vivax* (Talapko et al*.* 2019). Majority of fatal cases of malaria are brought on by *Plasmodium falciparum*, which is the most threatening for all species.

10. LIFE-CYCLE

Plasmodium has a wildly complex life cycle consist of three stages shown in Fig. 2.

10.1. GAMETOCYTES STAGE 1

The male gametocytes (microgametocytes) and female gametocytes (macrogametocytes) are transmitted by an anopheles mosquito during a blood meal. Inside the mosquito, these gametocytes develop into a sporozoite. The male and female gametocytes then mate within the mosquito's gut, forming a parasite known as a sporozoite after about 15 to 18 days (Kuehn and Pradel 2010; Deshmukh 2023).

10.2. SPOROZOITES STAGE

When the infected mosquito bites a human, the sporozoites are transmitted through the mosquito's saliva into the human's bloodstream. Inside the liver cells, the sporozoites mature into schizonts. These schizonts then rupture, releasing merozoites (Deshmukh 2023).

10.3. MEROZOITES STAGE 3

Over the next one or two weeks, each schizont multiplies, giving rise to several merozoites. These merozoites exit the liver, entering the bloodstream once again, where they attack the red blood cells. As they grow and multiply, they destroy the blood cells. Some merozoites develop into gametocytes, which

are ingested by a mosquito during a blood meal, restarting the whole cycle. When the red blood cells are destroyed by the merozoites, they release a toxin that causes severe chills and fever. Extreme cold chills and high fever are classic symptoms of malaria in humans (Deshmukh 2023).

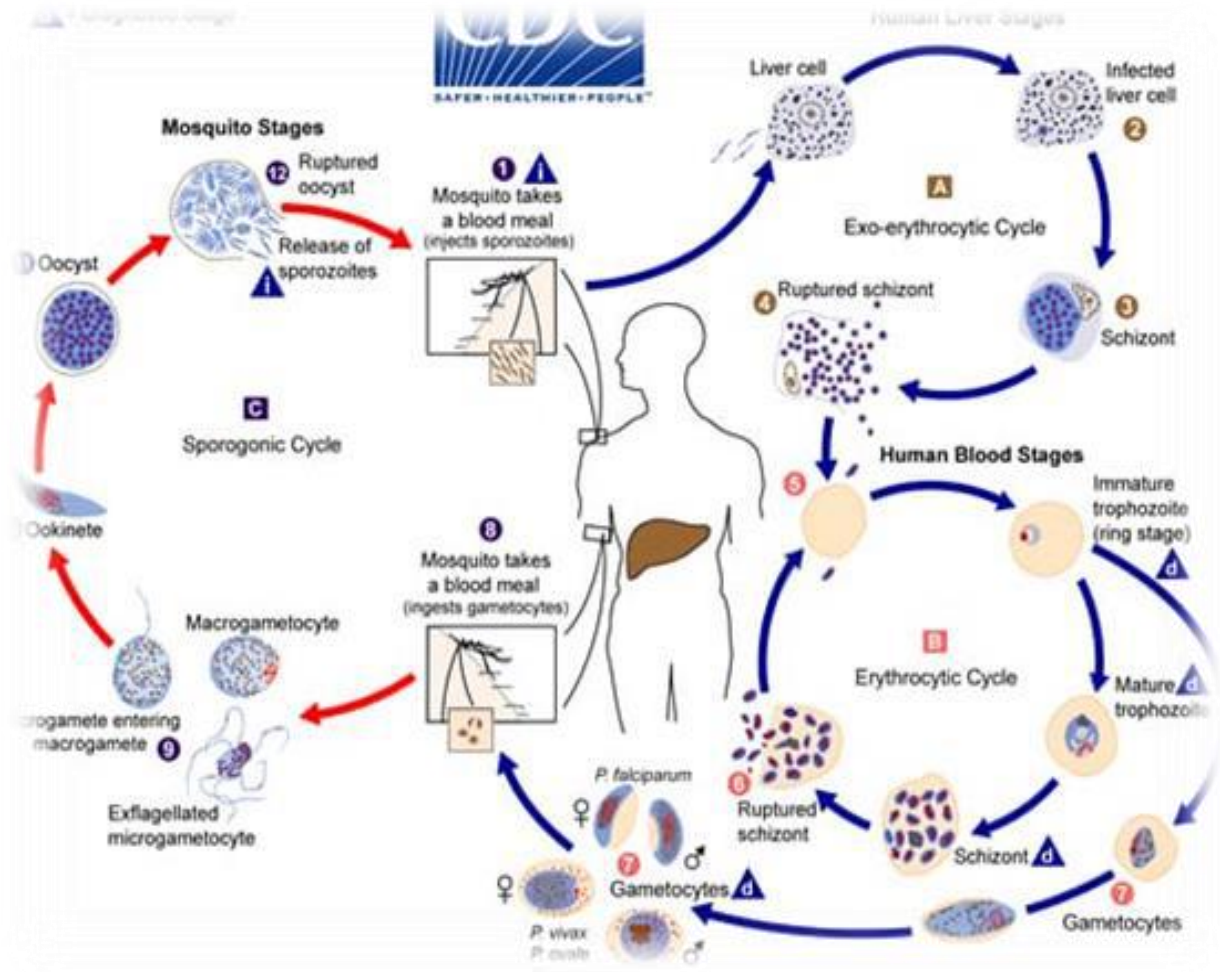

Fig. 2: Plasmodium life cycle (CDC) http://www.cdc.gov/.

11. PATHOGENICITY AND CLINICAL SIGNS OF MALARIA

The attack of malaria is called Paroxysm. Liberation of merozoites and malarial pigment and merozoites; RBC debris into the bloodstream. -*P.vivax*. Attack occurs once after every other day (48 hours). *P.falciparum* attacks 36/48hours. They show a lead of 3 stages 1. The cold stage (having chill), lasting for 30 min to 1 hour. 2. The hot stage (having fever), 1 to 4 hours. 3.Sweating stage that lasts 1 to 2 hours (Schiess et al. 2020).

12. SPLENOMEGALY AND ANEMIA

Rupture of infected RBCs and destruction of normal RBCs increase phagocytosis, causing phagocytes to multiply and function better, eventually leading to anemia and spleen enlargement.

13. RELAPSE

A unique attack that occurs months or even years after the initial strike. The bradysporozoites in the liver relax and slumber for months or even years before developing into exoerythrocytic and erythrocytes stages. At this point, the patient experiences paroxysm, displaying recurrent fever similar to the main bouts; this is known as relapse.

14. MALIGNANT MALARIA

P. falciparum malaria is more severe than malaria produced by other plasmodia. Cerebral malaria (involvement of the brain), Blackwater fever (massive hemoglobinuria), acute respiratory distress syndrome, severe gastrointestinal symptoms, shock, and renal failure may result in mortality (Batte et al. 2021).

15. LABORATORY DIAGNOSIS

Under microscopic examination, the demonstration of malarial parasites in the blood film (Thin or Thick) is the gold standard test for malaria diagnosis. (Gitta and Kilian 2020).

16. TREATMENT

Anti-erythrocytic stage drugs like Chlorquine and quinine. Anti-exoerythrocytic stage drug like Pyrimethamine. Primaquine for all stages of all species (Hanboonkunupakarn and White 2022).

17. PREVENTION AND CONTROL

Chemoprophylaxis, which includes chloroquine and pyrimethamine, is used to prevent malaria. Chemotherapy should be started one week before entering the endemic area and continued for four weeks after returning.Mosquito control. Reconstruction of the environment: eliminate mosquito breeding sites. Insecticides such as DDVP and others should be sprayed. To protect yourself from mosquito bites, use mosquito netting, screens, or repellents. (Tegegne et al. 2019).

18. DENGUE FEVER

It is vector borne diseases prone by dengue virus and transmitted by mosquitos of Aedes genus particularly *Aedes Aegypti.* It is RNA virus and a member of family Flaviviridae and have four interlinking serotypes. Approximately, 50-100 million people annually affected by this disease. Currently, this disease become a major health problem and endemic in almost 112 countries of the world (Ullah et al. 2018).

19. TRANSMISSION

It is transmitted from the bite of mosquitoes, mother to fetus and rarely by blood transfusion, organ transplantation etc.

20. LIFE CYCLE

This life cycle of Aedes Mosquitoes consists of following stages as shown in shown Fig. 3.

20.1. EGGS

Female mosquitoes lay their eggs on wet container walls. These eggs can survive for 8 months even if they dry out. Even a little water can attract them.

20.2. LARVA

After eggs are covered by water, larvae come out. They eat tiny living things in the water and change their skin three times before becoming a pupa.

20.3. PUPA

Pupa is a stage where a young mosquito develops inside the water until it becomes an adult.

20.4. ADULT

After becoming an adult, male mosquitoes drink flower nectar, while females need blood to lay eggs. Aedes aegypti mosquitoes are attracted to people and can be found near homes and buildings where there are no screens or open doors.
Eggs look like dirt, larvae and pupae are in the water, and when they grow up, female mosquitoes bite people for blood (Day 2016).

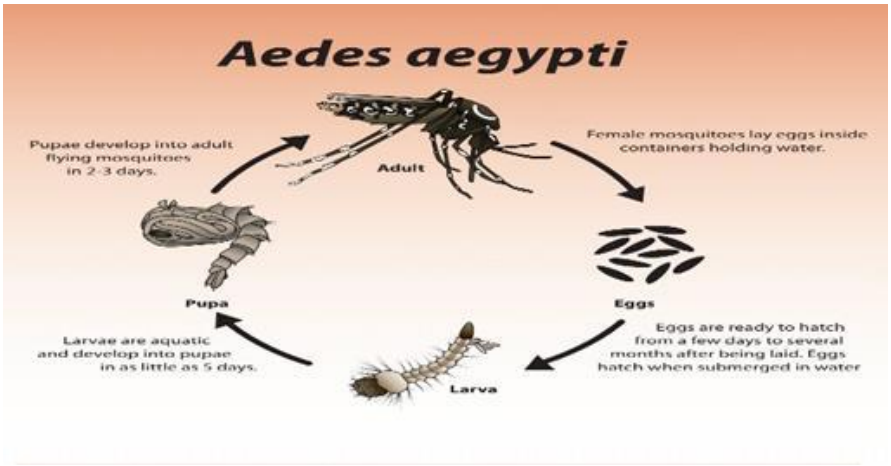

Fig. 3: Life cycle of Aedes Mosquitoes (CDC) http://www.cdc.gov/.

21. SIGNS AND SYMPTOMS

Fever, headache, retro orbital pain, rash, low white blood cell count and myalgia's are common signs. Due to severe pain in muscles, bones and joints, it is also known as "break-bone fever" (Li and Wu 2015). Repeated infection of this disease results in lots of complications including Hepatitis, Encephalopathy, ARDS, Dengue Hemorrhagic Fever and Dengue Shock syndrome (Ullah et al. 2018).

22. DIAGNOSIS

Diagnosis is performed by serological testing for detection of Ig M antibodies in the serum of infected person via ELIZA test (Raafat et al. 2019).

23. TREATMENT AND CONTROL

Supportive and symptomatic treatment are predominant therapeutic approach. Antiviral drugs are widely used for its treatment but its role is limited (Wiwanitkit, V. 2010). For its control, there is no specific vaccine available in market. However, Tetra valent vaccine has been reported against this virus. Proper sanitation conditions and control of the population play a significant role in its control (Li and Wu 2015). Moreover, educating people regarding the usage of mosquito control chemicals on their breeding sites also plays a vital role in the prevention of this virus (Wiwanitkit 2010).

24. YELLOW FEVER

It is historically dangerous infectious RNA viral disease belong to *Flaviviridae family*. It is transmitted to human through an *Aedes*, *Sabethes* and *Heamogogus* mosquitoes genera and monkey (Douam and Ploss 2018).

25. TRANSMISSION

This virus have 3 transmission cycles *viz*. Jungle (sylvatic) cycle in which none human primates (monkey) involve, virus transmitted from mosquitoes to monkey to human when human jungle; Intermediate (savannah) cycle, in which virus transmitted mosquitoes to monkey and monkey to human and human to human through mosquitoes; urban cycle In which infection transmit from urban mosquitoes to to human through mosquitoes; urban cycle in which infection transmit from urban mosquitoes to human (Monath and Vasconcelos 2015).Transmission of Yellow Fever to human through an *Aedes*, *Sabethes* and *Heamogogus* mosquitoes genera and monkey as shown in Fig. 4.

26. SIGNS AND SYMPTOMS

After contraction, virus incubates 3-6 days in body and then prone infection in one or two phase. First one is acute phase elucidates fever, muscle pain, loss of appetite, nausea, vomiting, headache and backache. This phase usually last 2-3 days and that symptom disappear. Nevertheless, 15% patients face toxic phase after 24 hours of acute phase. In this phase, patient suffers with high fever, jaundice, blood ooze out from mouth, nose, ears and stomach. Most of the organs affected, kidney functions deteriorate and death occurs usually 10-15 days. If organs arenot affected in the toxic phase the patient recovers from this phase as well (WH0-2014).

27. DIAGNOSIS

It is diagnosed by several laboratory tests. For instance, for molecular detection RT-qPCR and serological tests MAC- ELISA were performed to detect IgM antibodies (Silva et al. 2020).

28. TREATMENT AND CONTROL

Supportive and symptomatic treatment area used for this fever. Vaccines are available for its prevention which are cheap and affordable. Additionally, insecticides are used to control mosquitos' population for the control of this infection. Furthermore, prompt detection of this virus and vaccination campaign play significant role for the control of this viral fever (WH0-2014).

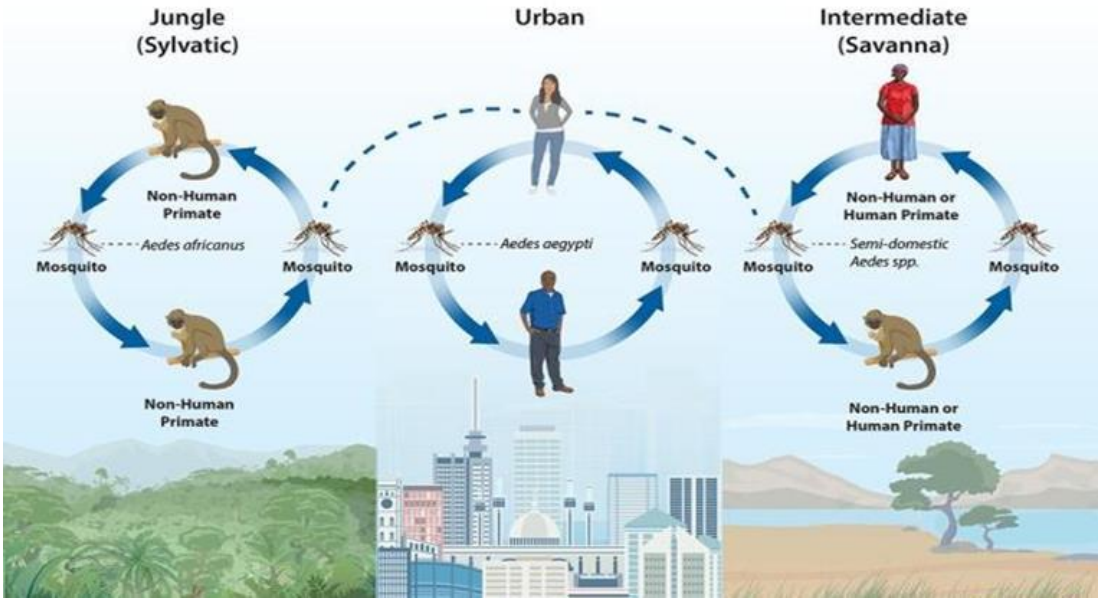

Fig. 4: Transmission of Yellow Fever (CDC) http://www.cdc.gov/.

29. ZIKA VIRUS

It is positive sense RNA virus belong to *Flaviviridae family*. This virus first isolated from Zika Forest of Uganda therefore its name is Zika Virus (Noorbakhsh et al. 2019)

30. TRANSMISSION

It is transmitted to human via bite of *Aedes Mosquitoes* particularly *Aedes Agypti*. It is also transmitted person to person while sexual intercourse, by blood transfusion, mother to fetus during pregnancy and during breast feeding (Rawal et al. 2016). Life cycle of Zika Virus according to CDC are shown in Fig. 5.

31. SIGNS AND SYMPTOMS

Low grade fever with maculopapular rash, conjunctivitis and arthralgia (involve small joints of hands and feet) are the common signs of this virus. It prone various complication include abortion, congenital microcephaly and Guillain–Barré syndrome (Rawal et al. 2016).

32. DIAGNOSIS

Zika virus is diagnosed by different serological tests against Ig M antibodies, by autopsy tissues, from flow cytometry of whole blood, by RT-PCR and from aptamer- based ELISA assay against NS1 protein (Noorbakhsh et al. 2019).

33. TREATMENT AND CONTROL

There is no antiviral therapy or vaccine available for this virus. Symptomatic and supportive treatment is used. For the control, doctor advice avoidance of sexual intercourse by using barrier methods i.e. condoms and taking environmental control measures to control mosquito breeding (Rawal et al. 2016).

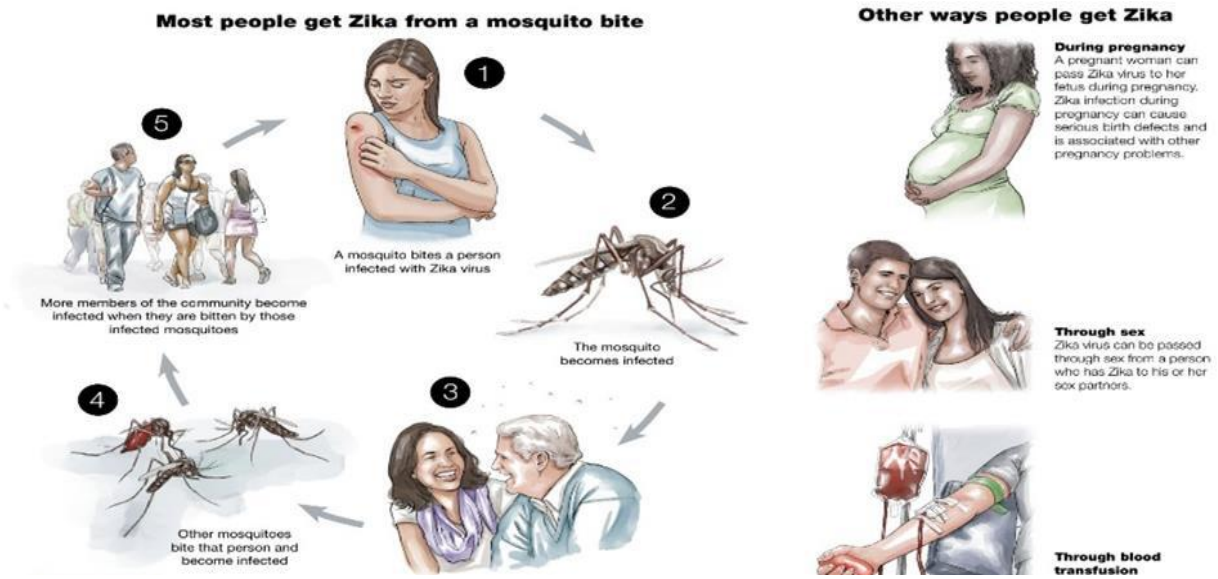

Fig. 5: Life cycle of Zika Virus (CDC) http://www.cdc.gov/.

34. JAPANESE ENCEPHALITIS VIRUS

It is mosquito-borne virus that belongs *Culex species*, and is a member of the *Flaviviridae family*. The most significant viral encephalitis in the world is called Japanese encephalitis (JE), which is brought on by infection with the Japanese encephalitis virus (JEV). With a death rate of 10,000–15,000 per year, JE affects about 35,000–50,000 persons annually (Zheng et al. 2012).

35. TRANSMISSION

Japanese encephalitis (JE) is a virus that spreads through the bite of infected mosquitoes, particularly *Culex tritaeniorhynchus* (Fig. 6). These mosquitoes pass the virus between animals like pigs and wading birds, which are natural carriers as shown in 6. Humans can get infected too, but they can't pass the virus to other mosquitoes. The virus is mostly found in rural areas with rice fields and flooding. In some parts of Asia, this can also happen near cities. In cooler parts of Asia, JE occurs more in summer and fall, while in warmer areas, it can happen all year, especially during the rainy season (Mackenzie-Impoinvil 2014).

36. SIGNS AND SYMPTOMS

The typical febrile disease of JE appears as anorexia, headache, backache, myalgia, and a sudden onset of fever. These symptoms last for a week. Following this are changes in mental state, speech, gait, and other motor function problems. In children, it may cause gastrointestinal symptoms like nausea and stomach pain (Unni et al. 2011).

37. DIAGNOSIS

Virus isolation, molecular tests, plaque reduction neutralization test, the haem- agglutination test, the complement fixation test, the immunofluorescence assay, and the enzyme-linked immunosorbent assay are some of the traditional diagnostic methods used to identify JEV. Biosensors, a new diagnostic instrument designed specifically for viruses, will be applied (Roberts et al. 2022).

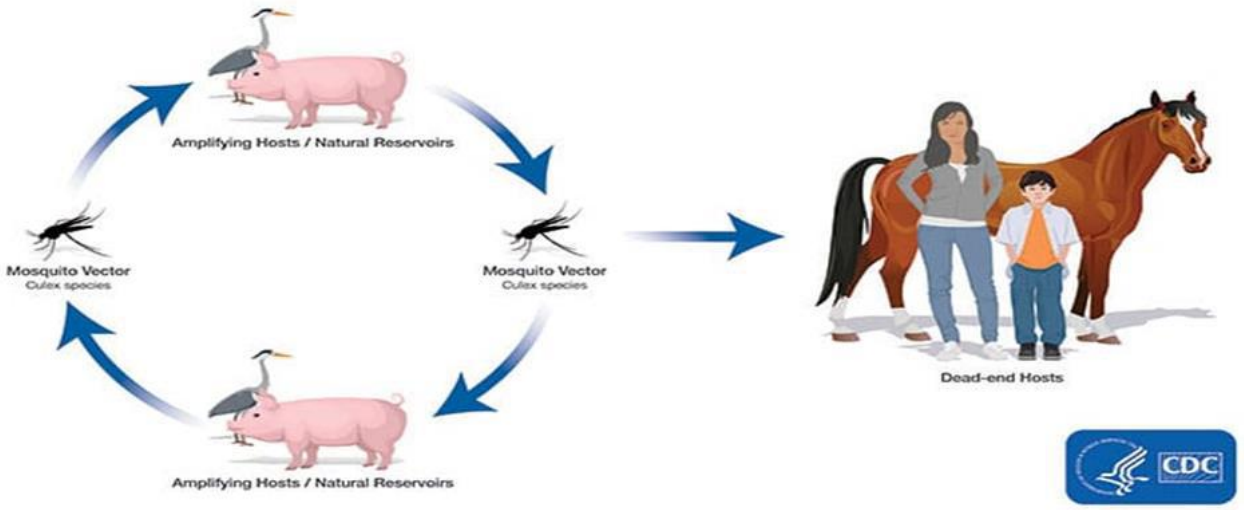

Fig. 6: Transmission of Japanese Encephalitis Virus (CDC) http://www.cdc.gov/.

38. TREATMENT AND CONTROL

JEV vaccinations were created as early as 1930. There are various vaccinations on the market, and more are being developed. Currently, there are three different vaccination forms (cell-culture-derived live-attenuated, inactivated derived from mouse brain and SA 14-14-2 JE vaccination is used in Asian countries with some degree of efficacy. Alarmingly, flaviviral infections are spreading to new regions, demanding management measures. In general, the flavivirus control schemes involve human vaccination, pig immunization, and mosquito control by spraying pesticides and using impregnated mosquito nets (Unni et al. 2011).

39. CHIKUNGUNYA VIRUS

The chikungunya virus (CHIKV) is an arthropod-borne virus that is primarily responsible for acute and chronic articular symptoms and is spread by Aedes mosquitoes. An alphavirus called the chikungunya virus (CHIKV) is spread by mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus.*

40. TRANSMISSION

Chikungunya virus is mostly spread by mosquitoes that bite infected people as shown in Fig. 7. However, it can also be spread through blood, like when healthcare workers handle infected blood or through a pregnant woman to her baby. But it's not commonly found in breast milk, so breastfeeding is still recommended even if the mom has the virus. The chances of passing the virus to a mosquito or through blood are highest during the first week of being sick (Diallo et al.1999).

41. SIGNS AND SYMPTOMS

High fever, back pain, arthralgia's, and headache are the most common signs. Antipyretics have a poor response to fever, which is usually high. In adults, Intense fatigue, myalgias, anorexia, nausea, and vomiting are common, in adults, while older patients may also experience transient confusion, and

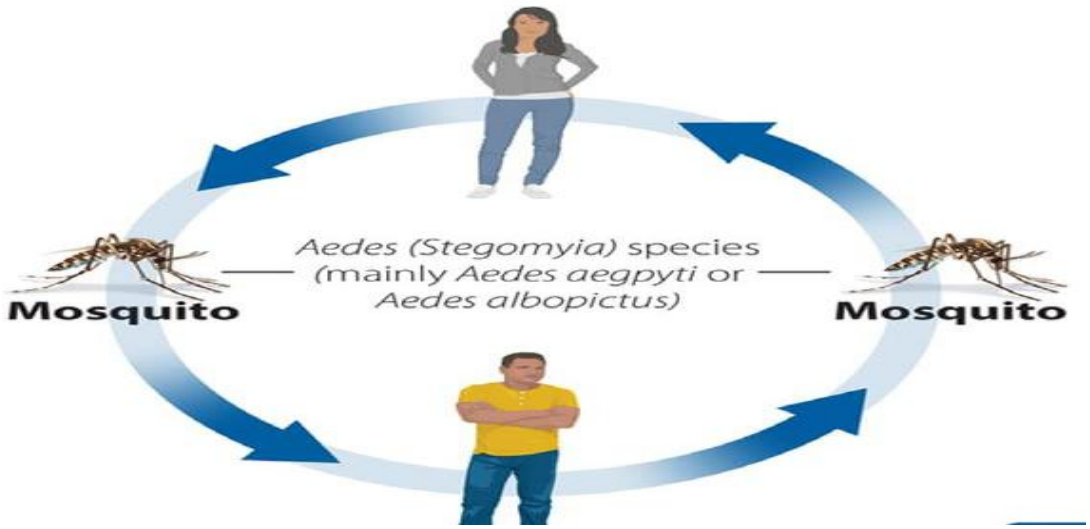

Fig. 7: Transmission cycle of Chikungunya virus (CDC) http://www.cdc.gov/.

symptoms on the GIT, skin, and mucosa are frequent. After the short-lived recovery following the acute stage, the life of the patient recently infected with CHIKV can be adversely affected by early exacerbation, long-lasting rheumatism, inflammatory relapses, and a significant loss in the quality of life (Simon 2011).

42. DIAGNOSIS

CHIKV can be diagnosed by virus isolation, nucleic acid amplification, or serology, depending on the timing of the patient's blood specimen collected in relation to the onset of symptoms. The most specific test and gold standard test is the viral culture in Vero. The sample should be taken within the first three days of illness for the best chance of effective isolation. Cell culture also offers the possibility of virus isolation, making it helpful for obtaining novel or unexpected agents. Since reverse transcription-PCRl 5 is a potent method that can identify nucleic acid from non-viable viruses, blood samples collected more than three days ago may be used (Sam 2006).

43. TREATMENT AND CONTROL

Treatment for CHIKV is mostly supportive, with rehydration and analgesics as necessary. Clinically, no antivirals have been used. Chloroquine was found to improve signs of chronic arthritis patients following CHIKV infection in an open trial. The United States Army developed a live CHIKV vaccine that was found to be immunogenic and safe in Phase II studies, but not further tested. Similar to dengue, control and prevention of outbreaks has been targeted on community education and vector control techniques like spraying of insecticides and eradication of breeding sites'. Surveillance is also crucial for early detection of outbreaks (Sam 2006).

44. FLY-BORNE DISEASES

44.1. LEISHMANIASIS

Leishmaniasis is caused due to sand flies and is a parasitic disease. It is present in Found in 88 different countries (Mann et al. 2021). There are three types of Leishmaniasis. Cutaneous refers to skin damage

caused by the bite of sand-fly. While viscera mostly affects the liver, the spleen, and specially bone marrow. Lastly Mucocutaneous affecting the nose and mouth mucous membranes as a result of dissemination from a neighboring cutaneous lesion (extremely rare). The most prevalent species in the Middle East are these two (L. major and L. tropical). Skin infection is due to L. major. While L. tropical leads to skin and visceral infection, as well as mucocutaneous infection in rare cases. Incidence rate of cutaneous Leishmaniasis is 1.5 million per year while 500 000 /year for visceral Leishmaniasis worldwide. In Desert Storm soldiers, 20 instances for cutaneous Leishmaniasis (L. major/L tropical) and twelve instances for visceral infection (L. tropical) were documented. (Abadías-Granado et al. 2021).

45. CUTANEOUS LEISHMANIASIS

The most common type is distinguished by a number of papules, nodules, or skin lesions. Sores are sometimes described as mimicking a volcano with an elevated perimeter and core crater because they can alter in size and shape over time. Even though sores are typically not painful, they might become so if they develop into secondary infections. Near the lesions, there may be swollen lymph nodes. The majority of sores develop within a few weeks of a sand-fly bite, yet they can take months to manifest.

46. VISCERAL LEISHMANIASIS

The most intense form of this illness can be fatal if remain untreated. Weight loss, pyrexia and an enlarged liver or spleen are common symptoms. Anemia manifesting (low red blood cells), leukopenia manifesting (low white blood cells), and thrombocytopenia manifesting (low platelets) are all prevalent. Lymphadenopathy is possible. Visceral Leishmaniasis in the Middle East is typically milder and has less particular findings (Martins et al. 2021).

47. LIFE CYCLE

Visceral Leishmaniasis completes its life cycle in following steps as shown in Fig. 8. Leishmania, a tiny germ, spreads through the bite of infected sand flies. When a person is bitten, the germs enter the body and change into a different form. They multiply inside the body and can be passed on to more sand flies when they bite. Inside the sand fly, the germs transform back to their original form and can infect another person when the fly bites again (Sachdeva 2016).

48. CLINICAL SIGNS AND SYMPTOMS

Typically accompanied by a pyrexia, weight loss, and enlargement of the spleen and liver. If Thrombocytopenia (low platelets), Anemia (low RBC), and leukopenia (low WBC) are all prevalent conditions. Lymphadenopathy is a possibility. If a sore appears on the face, it may leave behind painful scars and be disfiguring ((Chakravarty et al. 2019).

49. DIAGNOSIS

The diagnosis requires a biopsy. If local medical staffs are qualified and there is a Leishmania diagnostic capability, a biopsy can be performed. Special laboratories will perform microscopy, culture, and PCR. Sores that still not heal must be sent for further identification, even if they are not "typical" of Leishmaniasis. People experiencing high temperatures, weight loss, GIT problems, and unusual liver tests needs to be evaluated (Castelli et al. 2021).

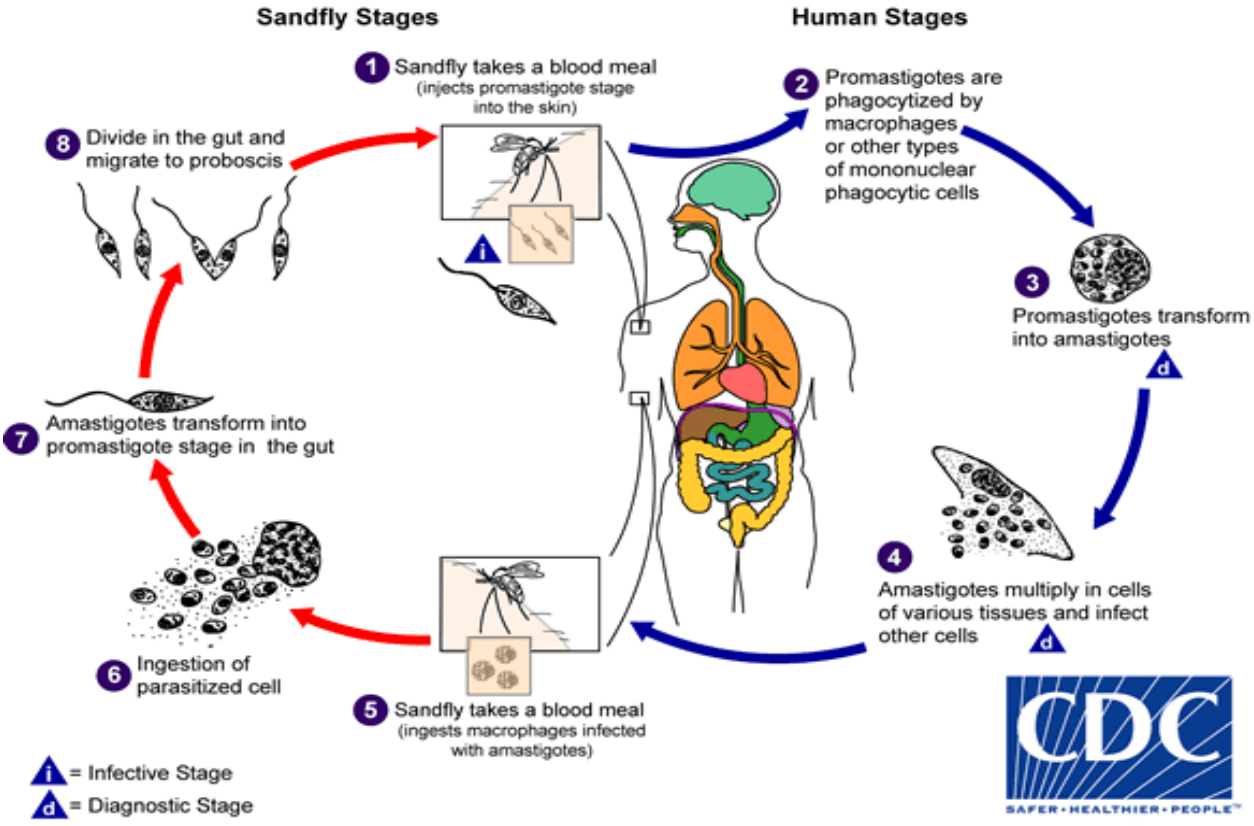

Fig. 8: Life Cycle of Visceral Leishmaniasis (CDC) http://www.cdc.gov/.

50. PREVENTION AND CONTROL

The reservoir is suppressed using dogs, gerbils rats, and several other small animals and rodents. Remove the vector: Sand-fly, essential for disease prevention in stagnant troop populations. Prevent sand-fly bites by doing the following: Personal defense at night, use sleeved-down, repellents for insects including DEET, permethrin-treated uniforms, and permethrin-treated sleeping nets (Ibiapina et al. 2023).

51. TREATMENT

51.1. CUTANEOUS LEISHMANIASIS

The drug of choice is antimony (Pentostam®, Sodium stibogluconate), which is administered intravenously for 20 days. Fluconazole may shorten the healing time after an L. major infection. To determine species, it requires a biopsy and culture, while Six weeks of therapy are required (Azim et al. 2021).

51.2. VISCERAL LEISHMANIASIS

The drug of choice is liposomal amphotericin-B (AmBisome®), with a dosage rate of 3 mg/kg per day on days 1-5, 14, and 21. Pentostam® is a complementary therapy. A total of 28 days of therapy are necessary (Mazire et al. 2022).

52. TICK BORNE DISEASES

52.1. ANAPLASMOSIS

A contagious and transmittable ailment called anaplasmosis is characterized by increasing anemia as well as other recognizable signs. Cattle are affected by this disease, which is spread by ticks (Rajan and George 2021). Although it can infect humans, it primarily affects animals, particularly domesticated livestock and pets. Many places of the world are affected by the disease, especially those where tick populations are enormous (Villar et al. 2023).

53. ETIOLOGY

Humans are susceptible to anaplasmosis due to the bacterium *Anaplasma phagocytophilum.* Other species of Anaplasma are accountable for the disease's occurrence in animals like cattle and dogs. Ticks, especially those of the *Ixodes genus* (often called deer ticks or black-legged ticks), are the main vectors for spreading the bacteria to hosts.

54. LIFE-CYCLE

Anaplasmosis spreads through tick bites as shown in Fig. 9. When an infected tick bites a cow or a deer, it passes the Anaplasma bacterium to them. The bacteria then multiply in the animal's blood, causing weakness and anemia. Other ticks can get infected by biting these sick animals, continuing the cycle (Dantas-Torres and Otranto 2017).

55. SIGNS AND SYMPTOMS

Anaplasmosis symptoms that are frequently observed are listed. It is crucial to remember that not everyone will experience all of the symptoms, and that each person will experience a unique set of symptoms (Shaukat et al. 2019). Anaplasmosis symptoms among humans typically start to show up 1 to 2 weeks after a tick bite Sigs in Fever, chills, rigors, Severe headache, Malaise, Myalgia, Gastrointestinal symptoms (nausea, vomiting, diarrhea, anorexia), Rash (<10%).

56. GENERAL LABORATORY FINDINGS

Typically seen within the first week of clinical illness onset, mild anemia, thrombocytopenia, and leukopenia are all symptoms of leukopenia. Hepatic transaminase increases range from mild to high.

57. DIAGNOSIS

Blood smear testing is insensitive and shouldn't ever be used to rule out anaplasmosis completely. However, the presence of morulae in the cytoplasm of granulocytes during blood smear analysis is extremely suggestive of a diagnosis. DNA detection in whole blood using PCR. This method's sensitivity is highest in the first week of an infection; after taking tetracycline-class antibiotics, sensitivity may decline (Mahmoud et al. 2023). Using the indirect immunofluorescence antibody (IFA) assay on paired serum samples, it was shown that the IgG-specific antibody titer had increased by four times. First sample should be collected within the first two weeks of illness, and second sample should be collected between two and four weeks afterwards. Organisms from skin, tissue, or bone marrow biopsies are stained using immunohistochemistry (Jamil et al. 2023).

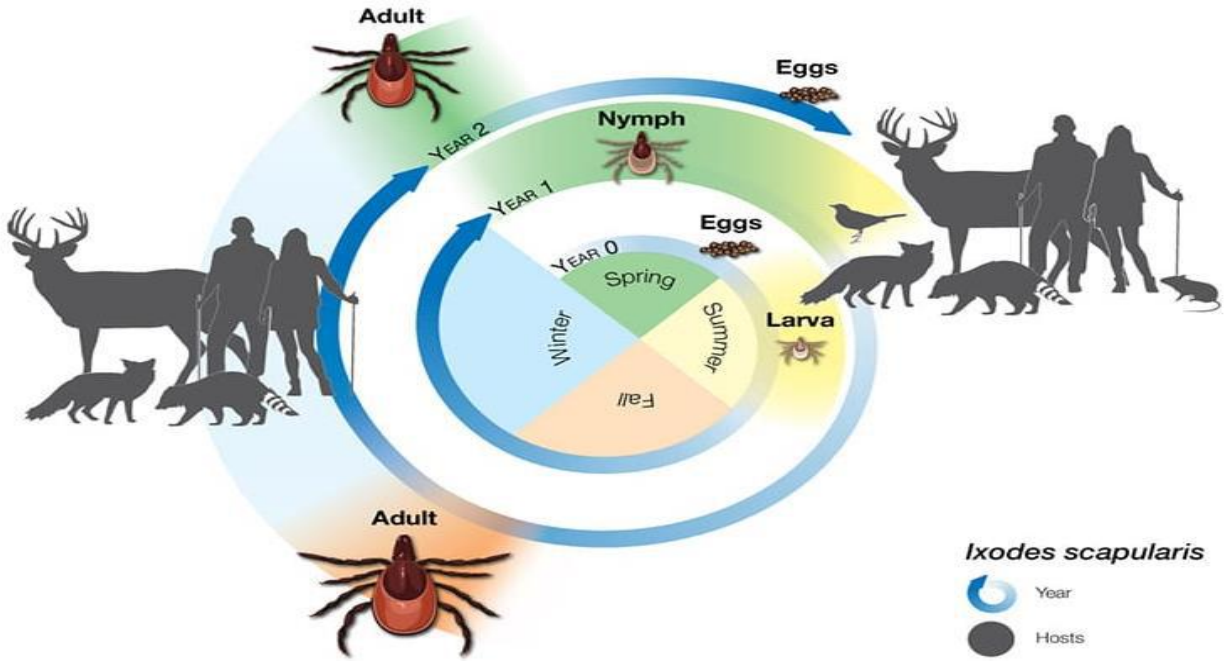

Fig. 9: Life Cycle of Anaplasmosis (CDC) http://www.cdc.gov/**.**

58. TRANSMISSION OF DISEASE

The Northeastern and Upper Midwest regions of the United States, which coincide with the known geographic distribution of Lyme disease and other Ixodes scapularis-transmitted infections, are where anaplasmosis cases are most frequently reported from. Co-infection with A. phagocytophilum and B. burgdorferi, Babesia microti, or Powassan virus is possible as a result of the shared vector. A phagocytophilum is primarily spread through the bite of an infected tick, although it can also be contracted through organ or blood transplants (Jiménez et al*.* 2019).

59. PREVENTION AND CONTROL

The risk of anaplasmosis must be reduced by avoiding tick bites. The risk of infection can be reduced by taking precautions including wearing protective clothes, using insect repellents, and properly checking for ticks after outdoor activity (Reppert 2019).

60. TREATMENT

If anaplasmosis is detected, it is critical to seek medical care right away because, if untreated, the condition can result in serious complications. Use of antibiotics like doxycycline, which is typically successful in battling the infection, is part of the routine treatment (Sarli et al*.* 2021).

61. TICK- BORNE ENCEPHALITIS

Three genera—*flavivirus, hepaciviruses*, and *pestiviruses*—represent the family of viral diseases known as *Flaviviridae*. Numerous human diseases are caused by *flavivirus*. Common *Flavivirus* infections are caused

by the *Japanese encephalitis virus* (JEV), *Dengue virus* (DENV), *West Nile virus* (WNV), *Yellow fever virus* (YFV), and *tick-borne encephalitis virus* (TBEV). Since ticks and mosquitoes are the primary vectors for the majority of flaviviruses to infect humans, and is also referred to as Arboviral infections (Unni, et al. 2011). The tick-borne encephalitis virus, which is mostly transmitted to people by tick bites, causes an infection of the central nervous system (Bogovic and Strlex 2015).

62. LIFE CYCLE

When a tick feeds on an infected animal, it can catch the infection. This can happen at any stage of the tick's life. The virus can also spread between young ticks feeding on the same host. TBEV in the host's blood infects the tick through its belly, then moves to its spit glands and can be passed to the next animal it bites (Fig. 10). In young ticks, the virus spreads as the tick grows, so it stays infectious for its whole life. Adult ticks that are infected can produce infected eggs, passing the virus to the next generation (Pulkkinen et al. 2018).

63. SIGNS AND SYMPTOMS

Tick-borne encephalitis is more prevalent in adults than in children. Clinically disease extends from mild meningitis to the severe meningoencephalitis specifically with or without paralysis (Bogovicand Strle 2015).

64. DIAGNOSIS

The diagnosis of TBEV is simple: typically, when CNS symptoms appear in the second phase of the disease, TBEV-immunoglobulin M (IgM) and typically TBEV-IgG antibodies are found in the initial serum samples collected. Additionally, multiplex PCR and haemagglutination inhibition and ELISA are widely used (Lindquist and Vapalahti 2008).

65. CONTROL AND TREATMENT

Tick-borne encephalitis cannot be treated with a specific antiviral medication. People who live in or travel to places where tick-borne encephalitis is endemic should consider getting the vaccine since it can successfully prevent the illness. Non-specific preventive strategies include pasteurization of milk, personal protective procedures, and reduction of the tick population (Bogovic and Strle 2015).

66. ROCKY MOUNTAIN SPOTTED FEVER

Rocky Mountain spotted fever (RMSF) is a major life-threatening disease that is caused by *Rickettsia rickettsii*, an obligatory intracellular bacterium and is spread to human beings by infected ticks.

67. TRANSMISSION

Rocky Mountain spotted fever spreads through tick bites (such as those from the lone-star tick, American dog tick, or lone-star tick) or contact with tick faeces or blood on the skin. There is no human-to-human transfer.

68. SIGNS

Patients with RMSF shows a wide range of systemic, cardiac, cutaneous, pulmonary, renal, ocular, neurological, gastrointestinal and skeletal muscle manifestations.

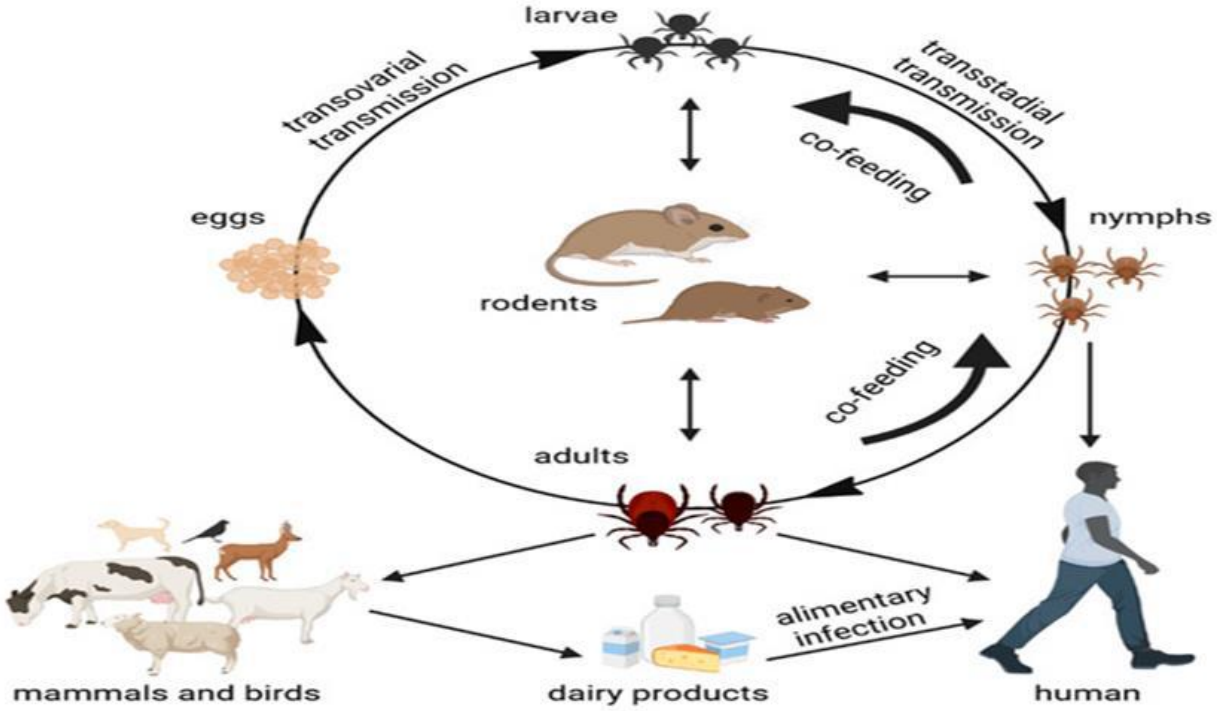

Fig. 10: Life Cycle of Tick- Borne Encephalitis (CDC) http://www.cdc.gov/**.**

69. DIAGNOSIS

The diagnosis of RMSF is done by physical examination of the patient and recent epidemiological data. Quantitative PCR assay, (new PCR-based method) have developed for the quantification and detection and of R rickettsia.

70. CONTROL AND TREATMENT

The only effective drugs for the treatment of RMSF are Tetracyclines and chloramphenicol. Because of the development of safe and effective antibiotics, the development of vaccines remains a low priority against rickettsial diseases.
Thus, it is best way to emphasize on control of tick-infested habitats like heavily wooded areas to prevent RMSF (Dantas-Torres 2007).

71. LYME BORELIOSIS

Borrelia burgdorferi sensu lato, often known as Lyme Borrelia, is a family of related spirochetes that causes Lyme disease or Lyme boreliosis and is transmitted by certain *Ixodes spp*. Ticks (Stanek 2012).

72. LIFE CYCLE

Blacklegged ticks have a life cycle of 2 to 3 years and go through four stages as shown in Fig. 11. These stages are egg, larva, nymph, and adult stage. They need blood meals to advance to the next stage and for egg production. Lyme disease bacteria can infect ticks when they feed on infected animals, and they

can transmit the bacteria to humans during their next blood meal. Deer are essential for the survival and movement of ticks but do not carry Lyme disease bacteria. Ticks usually need to be attached for 36 to 48 hours before they can transmit the disease, so prompt removal can reduce the risk of infection. In the eastern US, Lyme disease risk is high from spring to fall. Nymphs, being tiny and abundant, are hard to detect and pose a significant risk. Adults are more noticeable but can also transmit the bacteria. Many Lyme disease cases occur without the person realizing they were bitten by a tick (Burn et al. 2023).

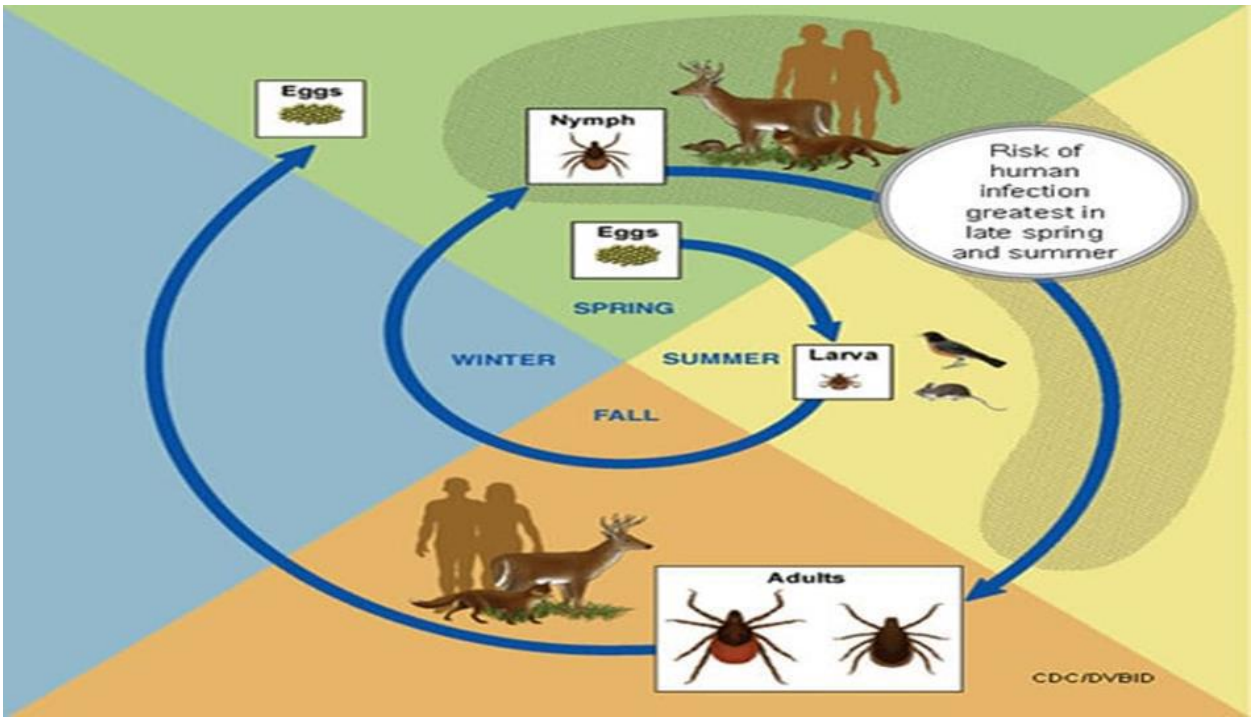

Fig. 11: Life Cycle of Lyme boreliosis (CDC) http://www.cdc.gov/.

73. SIGNS AND SYMPTOMS

Skin lesion, musculoskeletal, cardiac, nervous system, ocular manifestations are major signs in this disease (Stanek 2011).

74. DIAGNOSIS

Skin biopsy samples from erythema migrans lesions, as well as PCR-based and ELISA testing for Borrelia burgdorferi, are routinely used procedures. However, PCR is not a reliable test for current infection. Serological testing is the only generally available and practicable tool for confirming Lyme boreliosis diagnosis (Steere 2016).

75. CONTROL AND TREATMENT

Personal preventive measures are the main focus of interposition for the prevention of Lyme boreliosis. Avoiding tick-infested regions, wearing protective clothes, using acaricides and insect repellents, checking for ticks, and altering the landscape around residential areas to make them less tick-friendly are all

examples of preventative strategies. However, according to research carried out in the United States, most people can avoid developing Lyme boreliosis by taking one tablet of doxycycline if it is taken within 72 hours of a tick bite. NSAIDs are effective with antibiotic treatment. Neither North America nor Europe currently manufactures a human Lyme boreliosis vaccine (Steere 2016).

76. CRIMEAN-CONGO HEMORRHAGIC FEVER CCHF

The most common disease transmitted by ticks is Crimean-Congo hemorrhagic fever (CCHFV), which is brought on by the CCHFV. (Nasirian 2020).

77. LIFE CYCLE

The CCHFV GN and GC proteins play a role in the early stages of the CCHFV replication cycle (Fig. 12). GC helps the virus attach to cellular receptors and merge with the cell in endosomes. CCHFV relies on the cell's structure for internalization, assembly, and release. Although the specific receptors for CCHFV haven't been found, there seems to be a connection between CCHFV GC and cell surface nucleolin. After CCHFV attaches to the cell's outer layer, it gets pulled inside through a process called clathrin-mediated endocytosis. Cholesterol and low pH levels seem to be important for this. The virus then moves to early endosomes and later to multivesicular bodies where the CCHFV membrane fuses with the endosome's membrane. This releases the genetic material of the virus into the cell, which then starts the replication process. The L protein and N are essential for this replication. As CCHFV is a type of virus that carries its genetic information in a negative strand of RNA, this genetic material is used to produce capped mRNA. This mRNA is then used for making proteins and new viruses. The process continues with the production of more viral proteins and the final maturation of the virus. The assembly and release of CCHFV likely happen similarly to other bunyaviruses (Watts et al. 2019).

78. SIGNS AND SYMPTOMS

The majority of CCHF cases are unprovoked or moderate. Mild cases might show a variety of clinical signs or nonspecific symptoms, including headache, joint pain, fever, myalgia, nausea, and vomiting. However, a small percentage of cases possessed sudden onset, rapid bruising, and severe hemorrhage (Temur et al. (2021).

79. DIAGNOSIS

Diagnosis can be done by examining epidemiologic factors, clinical manifestations, and the abnormal laboratory tests. The laboratories involved with CCHFV diagnoses must conduct quality control assays. There is a need for high sensitivity and specificity testing as well as standardized assays. In this case, PCR is more sensitive than ELIZA. The use of early diagnosis in therapeutic and preventative strategies are helpful (Nasirian 2020).

80. CONTROL AND TREATMENT

The main way to stop tick bites and control CCHF is to use tick repellents. Acaricides are a good option for tick management in well-run animal production operations. Light clothing that covers the arms and legs should be worn to stop tick exposure and attachment, and skin and clothes should be checked often for ticks to avoid coming into touch with infected cells or blood. Permethrin can be applied to the sleeves and legs of clothing, and tick repellent must be applied directly to the skin. Regular CCHF awareness programs

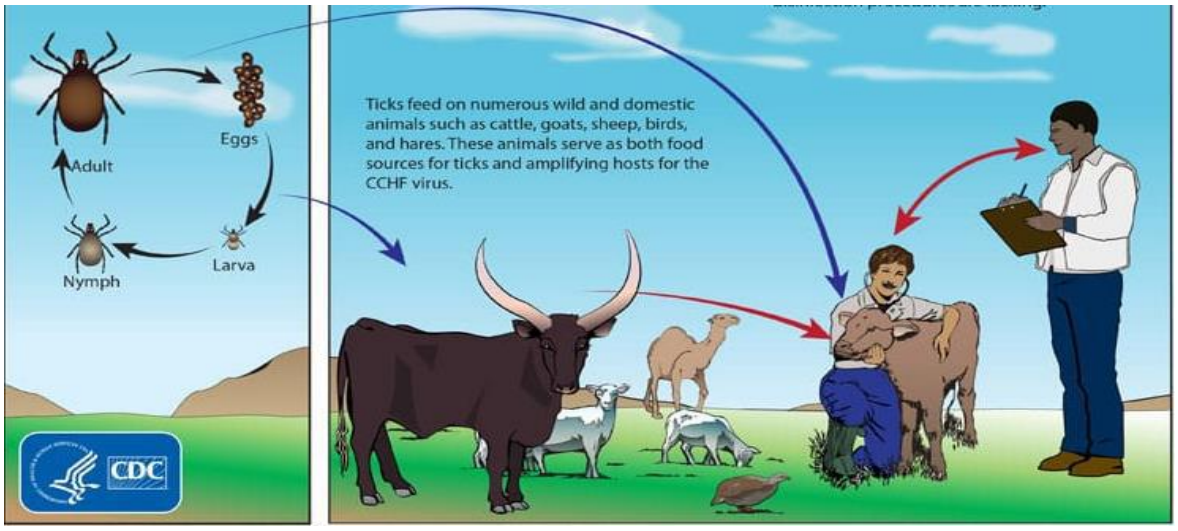

Fig. 12: Life Cycle of Crimean-Congo hemorrhagic fever (CDC) http://www.cdc.gov/.

are required at the country level by various communication channels for health workers, animal handlers, working housewives, and other people at risk since the cases rise during Eid-al-Adha. In CCHF treatment, majorly intravenous ribavirin has been suggested, whereas oral ribavirin has been suggested for post-exposure prophylaxis. Symptomatic and supportive treatments are usually done as there is no approved vaccine for CCHF is available yet (Nasirian 2020).

81. FLEA- BORNE DISEASES

81.1. PLAGUE

Yersinia Pestis, the pathogen that causes plague, is not frequently seen in hospitals, despite the fact that there are many natural plague foci all over the world. It has been determined that *Y. pestis* is a category A bioterrorism agent. A missed diagnosis will have serious consequences.

82. TRANSMISSION

Plague bacteria mostly spread through flea bites. When many rodents die from plague, the fleas that fed on them look for other blood sources. If people or animals go to these areas, they can get infected from flea bites (Fig. 13). Dogs and cats can also bring infected fleas home. Flea bites can cause bubonic or septicemic plague (Barbieri 2021).

83. SIGNS AND SYMPTOMS

The early signs of plague are similar to those of flu, an elevated temperature (up to 39 to 40°C), malaise, chills, and headache. Contact history with wild animals in natural plague foci or with other plague patients is an important clue for suspecting the plague. In an area where plague is endemic, if a patient develops an instant high fever after close contact with dead animals like (rodents or other wild animals) may indicate serious concerns. These symptoms include bubonic plague (regional lymph node swelling), and septicemic plague (sudden high fever and chills) or pneumonic plague (severe coughing and pneumonic signs by X-ray) (Yang 2018).

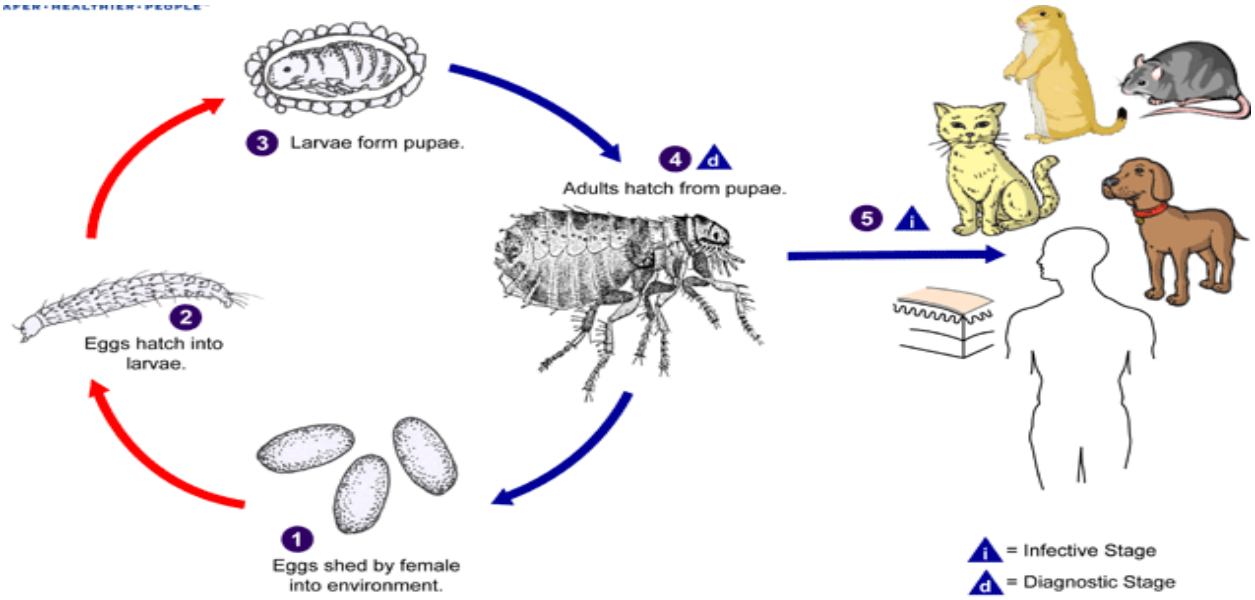

Fig. 13: Transmission cycle of PLAGUE (CDC) http://www.cdc.gov/.

84. DIAGNOSIS

According to the WHO guidelines, plague should be interpret as a suspected, presumptive, or confirmed case (World Health Organization 2006). The gold standard test in case of plague diagnosis is the isolation and identification of the plague pathogen from clinical samples in the laboratory.

85. PREVENTION AND CONTROL

Aside from physical prevention, antibiotic prophylaxis using streptomycin, tetracycline and chloramphenicol are recommended by the WHO Expert Committee on Plague (1970). In bioterrorism attack setting or a large-scale plague outbreak, to treat the plague for both adult and child patients, oral doxycycline and ciprofloxacin are recommended (Yang 2018).

86. FLEA BORNE TYPHUS

The rickettsial zoonosis known as flea-borne typhus, also called endemic or murine typhus, is primarily found in warm, coastal regions of the world, including some portions of the United States (Azad 1990). Flea-borne typhus, caused by *Rickettsia typhi* and *R. felis*, is an infection that can cause serious illness or death and manifest as fever, headache, rash, and multiple organ complaints. Humans can contract flea-borne typhus by being bitten by a flea, getting an abrasion on their skin, scratching their mucous membranes, or being bitten by a flea (Anstead 2020).

87. TRANSMISSION

Flea-borne typhus comes from bacteria in flea droppings. Scratching the droppings into a bite or wound, or getting them in your mouth or eyes, can cause the infection as shown in Fig. 14. Pets like cats, dogs, and wildlife can carry the infection without looking sick. Fleas need animals to survive and are always

around in Orange County. Giving pets flea medicine all year helps stop the disease from spreading. Flea-borne typhus can come from fleas on lots of animals like cats, dogs, opossums, rats, and more. Any animal with fleas can spread the disease (Eisen and Gage 2012).

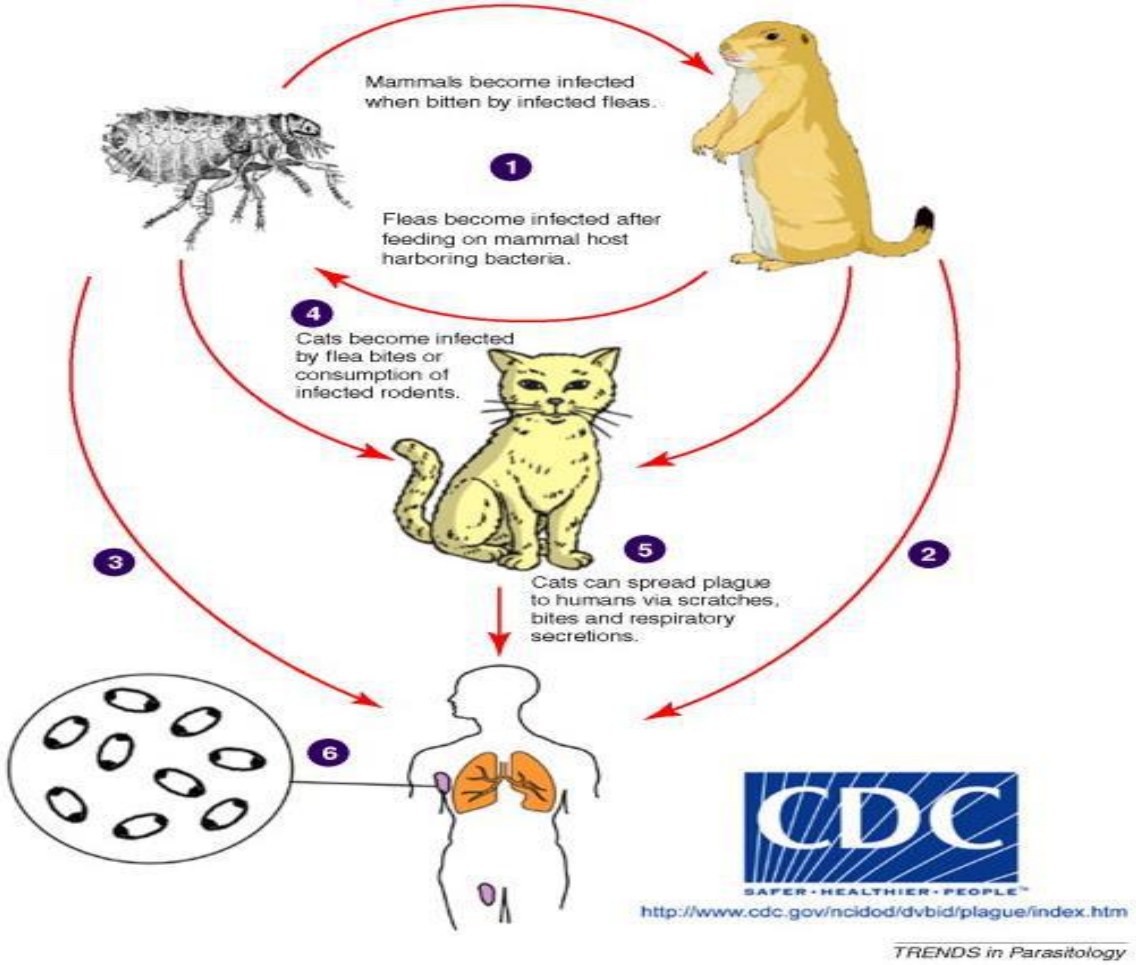

Fig. 14: Transmission cycle of Flea borne typhus (CDC) http://www.cdc.gov/.

88. SIGNS AND SYMPTOMS

The advanced level of disease that patients reported at initial hospital presentation may have been a confounding factor in the frequency of respiratory and neurologic signs identified in this group. The risk of ailments is thought to increase with age, according to previous research. Therefore, patients above the age of 50 may be more likely to experience negative results. (Pieracci et al 2017).

89. DIAGNOSIS

Tetracyclines, which are effective antibiotics for FBT, first entered clinical use in the late 1940s. The Weil-Felix test, the original diagnostic method for FBT, was reported to show inadequate specificity and sensitivity and was then replaced by complement fixation and the indirect fluorescent antibody test. R. felis, a second organism that causes FBT, was identified in 1990 (Anstead 2020).

90. PREVENTION AND CONTROL

"FBT is treated in the same way as other acute infectious condition. About all that can be advised is complete bed rest, attentive nursing, adequate nourishment, plenty of fluids, and treatment of symptoms as they arise. For nervous symptoms and headaches, opiates are usually needed. While sulfa medications first appeared in the American Pharmacopoeia in the 1930s, this class of medications had negative effects on the treatment of rickettsial infection and it was later advised to avoid them for FBT.

To prevent the spread of this disease, manage crowded, unhygienic environments and vectors in those places (Anstead 2020).

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An Overview of Filariasis

03

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ABSTRACT

Filariasis is endemic in the tropics and subtropics, caused by nematode parasites, "filariae," which belong to the family “Filarioidea” It consists of many species of slender, long worms that inhabit the tissues of various vertebrate hosts. The adult worms have a width of 0.25-0.30 mm and a length of 80-100 mm. This infection is circulated via arthropod vectors. More than 882 million individuals across 44 countries globally are still at risk of lymphatic filariasis and need preventive treatment to halt the spread of this parasitic disease. In addition, filariae are frequent worms in both animals and humans since parasites belong to the invertebrate class. However, it is difficult to prove that a filarial infection is a zoonosis; this illness may spread spontaneously from animals to people. To generalise and categorise whether the different filariasis are zoonoses. However, generalisations and classifications have been made using mosquito host preferences and the relative vulnerability of humans and other animals to infection. Given the correct circumstances, virtually every animal that is a host for filaria parasites has the potential to infect and spread to humans.

Key words: Filariasis, Zoonosis, Public Health.

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1. INTRODUCTION

Filariasis is endemic in the tropics and subtropics, caused by nematode parasites, "filariae," which belong to the family "Filarioidea" It consists of many species of slender, long worms that inhabit the tissues of various vertebrate hosts. The adult worms have a width of 0.25-0.30 mm and a length of 80-100 mm (Rebollo et al. 2017; Shukla et al. 2019). This infection is circulated via arthropod vectors. More than 882 million individuals across 44 countries globally are still at risk of lymphatic filariasis and need preventive treatment to halt the spread of this parasitic disease. In addition, filariae are frequent worms in both animals and humans since parasites belong to the invertebrate class (Maldjian et al., 2014). However, it is difficult to prove that a filarial infection is a zoonosis; this illness may spread spontaneously from animals to people. To generalize and categorise whether or not the different filariasis are zoonoses. However, generalisations and classifications have been made using mosquito host preferences and the relative vulnerability of humans and other animals to infection. Given the correct circumstances, virtually every animal that is a host for filaria parasites can potentially infect and spread to humans (Kalyanasundaram et al., 2020).

Filariasis is a disease that is endemic in the tropics and subtropics, and this disease is circulated via arthropod vectors. A group of infectious diseases that can affect both animals and humans. The nematode parasites, or "filariae," consist of several hundred species of slender, long worms residing in various vertebrate hosts' tissues. Filariasis, a parasitic infection, arises due to roundworms belonging to the Filarioide family. All worms are transmitted by blood-feeding insects such as flies and mosquitoes (CDC et al.2010). These diseases are known as helminthiases. The parasitic worms living in human tissue and blood cause filariasis (CDC et al. 2010; Paniker 2007). The adult worms have a width of 0.25-0.30 mm and a length of 80-100 mm. The male worms are smaller than the females, but the females are viviparous and produce microfilariae that might be identified in the cutaneous tissues or fringe blood, based on the species (Paniker 2007).

Several mosquito genera facilitate filariasis transmission, including Culex, Ochlerotatus, Mansonia, Aedes, and Anopheles. Female mosquitoes are sullied after taking a blood feast containing microfilaria from people; the starting stage of hatchlings needs around 12-15 days to frame into the grown-up phase of hatchlings in mosquitoes (Burkot et al.2002). One of the most crippling tropical excused diseases, filariasis has a high prevalence of despair, a slow pace of mortality, and a variety of clinical symptoms. According to the World Health Organisation (WHO), 1.34 billion people are predicted to reside in places where filariasis is prevalent and are at risk of contracting the disease. The disease affects 120 million individuals from 81 different countries (Rebollo et al. 2017). Parasitic infections are particularly dreadful and deadly all around the world. One of the most crippling and undertreated tropical illnesses is filariasis. The illness filariasis, which is native to the jungles and subtropics and is transmitted by arthropod vectors, is the cause of friendly shame. A class of contagious illnesses that can affect both people and animals. The "*filariae,"* often known as nematode parasites, are hundreds of long, slender worms living inside the tissues of other vertebrate hosts. The bulk of this parasite, known to infect people, belongs to the genera *Dipetalonema, Mansonella, Loa, Wuchereria, Brugia,* and *Onchocerca* (Burkot et al. 2002). They can be found in the lymphatic system, connective tissues, muscles, and body cavities of vertebrate hosts, among other locations.

The adult worm may be split into three major groups based on where it lives: the lymphatic group, the cutaneous group, and the body depression group. Table 1 includes a few filarial species infecting people and the illnesses they transmit to their middle hosts as a result of their habitat as adult worms. The infection is carried by intermediate hosts of the order Diptera, which are always blood-sucking arthropods. According to Taylor et al. (2010), two genera, Wuchereria and Brugia, are principally to blame for human lymphatic filariasis. *Setariadigitata* and *S. cervi* in cattle, *Dirofilariaimmitis* and *D. uniforms* in

dogs, *Litomosoidescarinii* and *Dipetalonem*a vitae in gerbils, *Brugiapahangi* in cats, and *Acanthocheilonemaviteae* in birds are the most prevalent animal parasites.

Table 1: Shows Species with their Habitat, Intermediate Host, and Disease

Filarial worm	Habitat	Intermediate host	Disease
Wuchereriabancrofti	Lymphatics	Mosquito sp.	Elephantiasis
Brugiamalayi	Lymphatics	Mosquito sp.	Malayan filariasis
B. timori	Lymphatics	Mosquito sp.	Timor fever
Loa loa	Connective tissue	*Chrysopsis* sp. (*C. dimidiata*) horse flies	Loaiasis
Mansonellaozzardi	Serous membranes	*Culicoides* sp. (*C. furens*) biting midges	Ozzard's filarial
Onchocerca volvulus	Skin	*Simulium* sp. (*S. damnosum*) black flies	Onchocerciasis

2. SIGNS AND SYMPTOMS

Early signs include scrotal lymphedema and a high temperature (Fateh et al. 2019). but also the oedema of the testis, the thickness of the spermatic cord, and enlargement of the lymph nodes that are clinical indicators of filariasis in the testicle (Knott et al. 1939), symptoms of *lymphatic filariasis edema* with thickening of the skin and underlying tissues (WHO, 2013). Additionally, Lymphatic system function is crucial for the lymphatic system's regular operation, which includes maintaining bodily fluid balance and physiological interstitial fluid transfer. Filarial parasites typically target the lymphatics and impede the lymph flow. Lymphedema, a primary condition associated with filarial infection and brought on by the lymphatics' inability to contract, is one of these disorders (Chakraborty et al., 2013).

3. ETIOLOGY

Filariasis is a parasitic illness caused by *Filarioidea* roundworm infection (Centres for Illness Control and Prevention). *Wuchereriabancrofti, Brugiamalayi, Brugiatimori,* and *Setariacervi* are the primary species responsible for filariasis, and it is transmitted by mosquito genera such as *Aedes*, *Anopheles, Culex*, and *Mansonia*. Several studies have found that Dirofilaria species include the intracellular symbiont*Wolbachiabacteriae*, which is critical in the embryogenesis and proliferation of microfilariae as well as the disease's inflammatory pathophysiology (Simón et al. 2017; Dreyer et al. 2000).

4. THE GENERAL LIFE CYCLE OF FILARIAL WORM

The adult filarial worm dwells in the lymphatic system of man, the final host of filarial worms. Adult females discharge live embryos termed microfilariae (290u). *Microfilariae* circulate in the peripheral circulatory system without metamorphosis and can survive for a long time until they are taken up by the intermediate host, culicine mosquitoes, during a blood meal (Nutman et al. 2011). *Microfilariae* are picked up from the peripheral circulation by the vector while feeding on the host, and then Micofilarie reaches the gut where escheatment occurs; it again penetrates the thorax and settles down parallel to the thorax muscle; inside the thorax, microfilariae shorten to become sausage stage (1st stage) moult twice to develop into 2nd and 3rd stage larvae as seen in Fig. 1 L3 moults twice and transform into adult worms in the definitive host, host cycle is mentioned above.

5. ZOONOTIC ASPECT OF FILARIASIS

Since the invertebrate stages of parasites in humans and animals are comparable, it is challenging to confirm that filarial infections are zoonotic diseases that spread naturally from animals to humans. To

generalize and categorise whether or not the different filariasis are zoonoses, however, generalizations and classifications have been made using mosquito host preferences and the relative vulnerability of humans and other animals to infection. The midnight periodic shape of *B. malayi* does not meet the criteria for a zoonotic illness (Laing et al. 1961); the variant of anopheline and aedine carriers are predominantly found in rural areas, where the quantity and diversity of animals are constrained. For the limited instances of infections in domestic animals, including cows, goats, cats, and dogs, it is believed that they most likely originated from humans. At the same time, the subperiodic strain of *B. malayi* is considered to represent zoonosis. Mansonia mosquitoes, which generally feed on people and hosts of wild animals like forest monkeys, are the vectors of this strain (Wharton 1963; WiJers et al. 1977). Rarely do *Onchocerca* infections fit the definition of a zoonosis, even though gorillas and spider monkeys (Caballero and Caballero 1985). These animals are not believed to represent a substantial human onchocerciasis reservoir since they have been naturally infected, and the chimpanzee is a perfect laboratorical host for *O. volvulus* (Duke 1962).

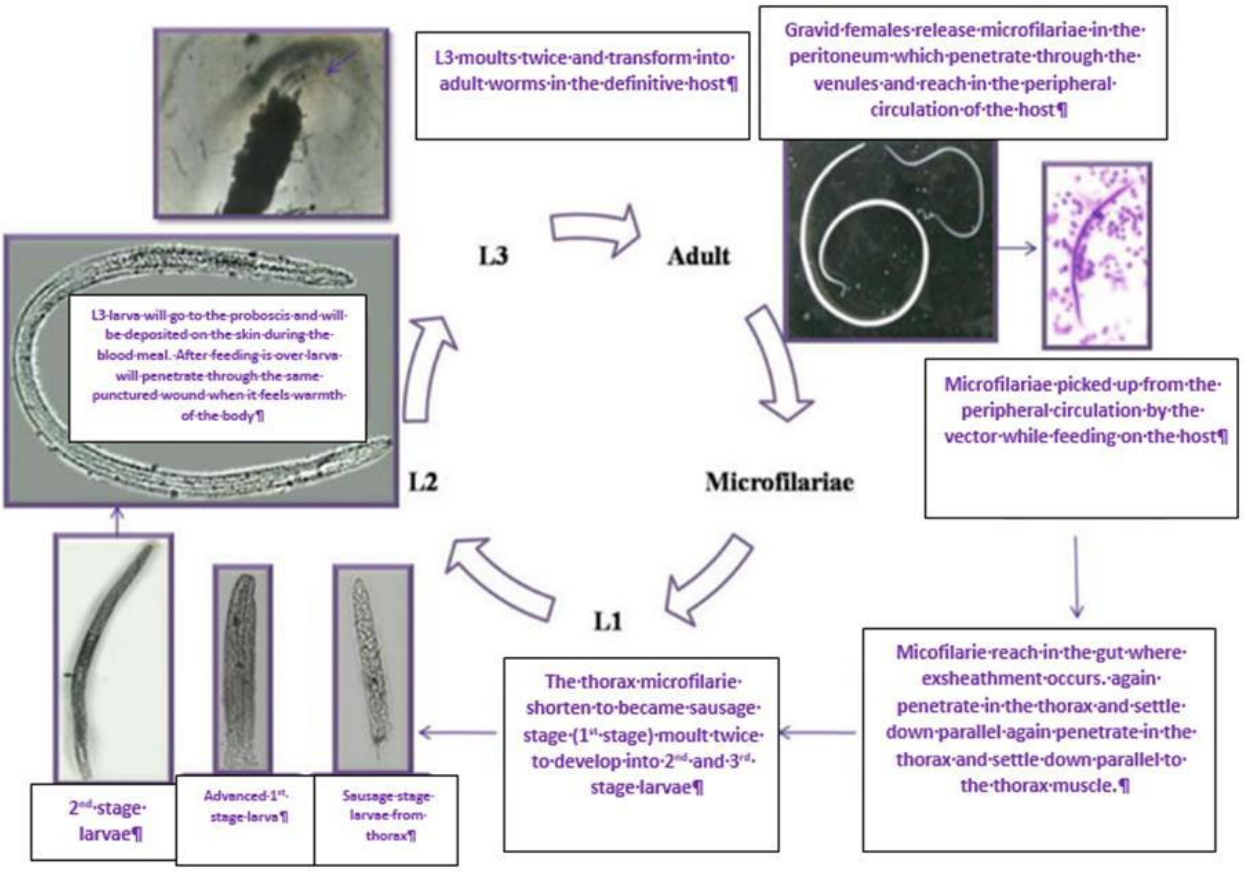

Fig. 1: The life cycle of the filarial worm *Setaria cervi* was given by Prof. Wajihullah and Dr. Sharba Kausar.

Research recorded in West Africa suggested that the transmission of *S. damnosum* may have resulted in the forms of *Onchocerca* associated with 0. Volvulus (Disney and Boreham 1969; Duke 1967). Like *Brugia* species, which cannot be identified from cattle and wild antelope, microfilaria onchocerca and adult worms isolated from humans cannot either (Garms 1983). According to Cameron et al. (1928), these animals may participate in the transmission process. It's probable that in some regions of Africa, the

same simulid species is dispersing both human and bovine *Onchocerca* species. To effectively address these critical problems, deoxyribonucleic acid (DNA) probes designed for parasites of humans or other animals may one day be developed (McReynolds et al. 1986). The circumstances surrounding *L. loa* are similar to those around *O. volvulus*. Despite the possibility of human strains infecting nonhuman primates like the drill, it is not believed that these or any other animals serve as substantial reservoirs of infection for human populations (Ottesen et al. 1984). Fig. 2 shows the dirofilariasis epi-system and essential connections between the implicated species, the climate, and the elements influenced by human behavior.

6. IMPACT ON PUBLIC HEALTH

The parasitic disease filariasis, which is caused by filarial worms, has a significant effect on general health. According to Zeldenryk et al.(2011), it can result in physical impairment, particularly lymphatic filariasis, producing aberrant body part enlargements that cause discomfort, severe impairment, and social shame. According to Wynd et al. (2007), the persistently disabling symptoms of this disorder,

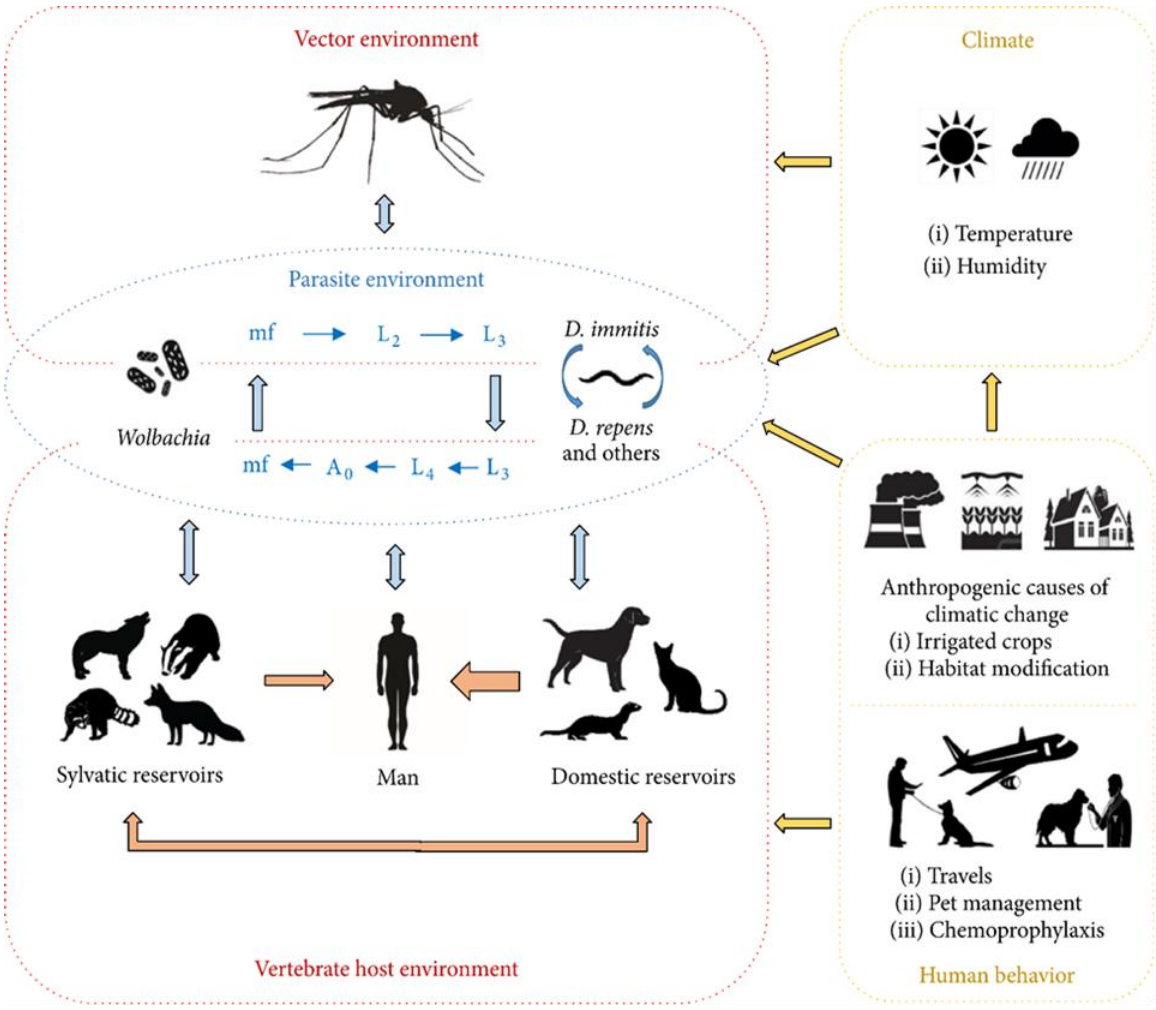

Fig. 2: The system of dirofilariasis, which includes connections between the affected species, climate, and human-influenced factors (Simón et al. 2017).

Which includes inflammation of both limbs, Chest/udder, and external genital organs, and has a significant adverse effect on the general well-being of the person who is affected. It imposes a substantial economic burden on individuals, families, and communities. According to Mathew et al. (2020), the yearly economic impact of lymphatic filariasis is predicted to be around USD 5.8 billion. The disease can lead to loss of productivity, increased healthcare costs, and decreased quality of life (Turner et al. 2016; Sawers and Stillwaggon 2020). Filariasis can lead to social stigma and isolation, particularly in cases of visible physical disability (Abdulmalik et al., 2018; Zeldenryk et al., 2011). According to Eneanya et al. (2019), The social and financial effects of lymphedema and hydrocele can lead to anxiety, difficulty concentrating, sleep issues, and social isolation because of stigma. Over 120 million people in 72 nations in the tropics and subtropics of Asia, Africa, the Western Pacific, and portions of the Caribbean and South America are affected by filariasis, which is common in many tropical and subtropical regions of the world. In nations where the illness is prevalent, it has a significant negative social and economic impact (Newman and Juergens, 2023). Filariasis may spread from animals to people through mosquito bites carrying the disease. Rural locations with less access to healthcare have a higher prevalence of the condition (Otsuji 2011).

7. PREVENTION AND CONTROL

The Worldwide Programme to Eliminate Lymphatic Filariasis was established in 2000 with the eradication of lymphatic filariasis as Public Health issued in 2020 (WHO 2013; CDC 2020). The program has provided approximately 763 million individuals with 5.6 billion treatments, significantly lowering transmission in many areas (WHO 2013). Some techniques to prevent and control filariasis include preventive chemotherapy, vector control measures, and health education programs (CDC 2020). Filariasis has a significant impact on public health, causing physical disability, economic burden, and social stigma and affecting many people in endemic regions. Preventive chemotherapy, vector control measures, and health education programs are strategies to prevent and control filariasis.

8. RESEARCH AND FUTURE DIRECTIONS

Current research into filariasis aims to provide novel diagnostic techniques, therapeutic choices, and possible vaccinations. Preventive vaccination against lymphatic filariasis is currently being researched. Several subunit-candidate vaccination antigens have been tried in lab animals with varying degrees of success (Kalyanasundaram et al., 2020; Samykutty et al., 2010). In a mouse model, a combination vaccination combining BmALT-2 and BmHSP showed significant effectiveness in protecting against a complicated B. malayi infection (Samykutty et al. 2010). The fusion protein vaccine has also been used to optimise the intermediate development in place of a vaccine against human lymphatic filariasis (Melendez et al. 2020). According to Malina et al. (2019), the current anti-filarial are only partially effective against the long-lived microfilariae and need recurrent, protracted therapy over the years. Corallopyronin A is a potential antibiotic for treating filariasis (Katiyar and Singh 2011). Researchers are investigating novel pharmacological targets and prototypes for antifilarial chemotherapy. The Global Programme to Eliminate Lymphatic Filariasis was established in 2000 to eradicate lymphatic filariasis as a public health issue by stopping transmission through the Mass Drug Administration (MDA).

Additionally, providing the best security to people suffering from lymphoedema brought on by the infection is crucial. Despite the remarkable progress, not everyone will be able to meet the initial deadline of 2020. The updated deadline for ending lymphatic filariasis, set by the World Health Organisation, is 2030. Alternative diagnostic techniques for lymphatic filariasis are being researched,

including anti-filarial IgG1 serologic enzyme immunoassay testing. These assays offer an alternative to the microscopic examination of blood smears to find microfilariae. Managing molecular reagents, standard operating procedures (SOPs), and filarial parasites is the responsibility of the Filariasis Research Reagent Resource Centre (FR3). The Parasite Resources Division offers molecular strategies and diagnostic assays to recognize and distinguish all filarial parasites in blood and mosquitoes.

9. CONCLUSION

Filariasis, a mosquito-borne parasitic infection, encompasses a group of diseases causing severe morbidity. Its impact extends beyond physical health, contributing to socioeconomic challenges in affected communities and warranting comprehensive efforts for prevention and control. Continuing filariasis research aims to provide novel diagnostic techniques, therapeutic choices, and possible vaccines. By 2030, The overall Global Programme to Eliminate Lymphatic Filariasiscondition as a public health issue. The Filariasis Research Reagent Resource Centre also offers molecular resources for researchers researching filariasis.

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Recent Advances in Diagnosis of Filariasis

04

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ABSTRACT

Filariasis is a mosquito-borne parasitic disease caused by filarial nematode worms. Various mosquito genera are responsible for the transmission of this disease condition. Filarial worms have endemic presence in tropical and sub-tropical regions of the world. Filariae exhibit distinctive properties and are regarded as substantially significant in human as well as veterinary medicine as a wide range of filarial worms infest wild and domestic animals around the globe. Precise parasite identification not just helps in clinical settings but also provide significant assistance in research. This chapter highlights the various advancements made regarding the diagnosis of filariasis in the recent years. The traditional methods used for the diagnosis include blood examination, skin biopsy, urine and sputum analysis. However, there are certain barriers that hinder the usage of these traditional methods for the diagnosis of Filariasis. These days, different diagnostic approaches are being used including molecular, serological and imaging techniques. Recently, continuous advancements are being observed regarding the development of better molecular and diagnostic techniques. The significance of genetic and genome-based information is growing on a substantial rate for the detection and characterization of zoonotic parasites. The accurate diagnosis of Filariasis is significant because it will aid the treatment and epidemiological monitoring of disease burdens. Although many recent advances have been made regarding the diagnosis of filariasis still there is room for development of better and more reliable techniques. Development of economical, specific and more reliable techniques can lead to the timely diagnosis of filariasis and thus can be treated more effectively.

Key words: Filariasis, Zoonoses, Diagnosis, mosquito-borne disease, recent advances

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1. INTRODUCTION

Vector-borne diseases (VBDs) are transferred by the bites of hematophagous arthropods like ticks, mosquitoes, blackflies, and sandflies. These VBDs can be bacterial, viral or parasitic (Obradovic et al. 2022). The disease conditions caused by the mosquitoes are among the most crucial public health issues faced globally, causing mortalities in humans, livestock, and wildlife and considerable financial losses (Tolle 2009). Filariasis, one of the mosquito-borne parasitic diseases, is an important disease caused by filarial nematode worms. Different mosquito genera, such as, Anopheles, Culex, Mansonia, Aedes, Ochlerotatus, and Armigeres transmit this disease condition(Foster and Walker 2019). Filarial worms are vector-borne parasitic nematodes, having endemic presence in tropical and sub-tropical regions of the world. In terms of form and structure, the filarial worms are narrow and long having no pharynx and buccal capsule. Esophagus consists of two parts i.e. anteriorly muscular part and posteriorly glandular part. Males possess asymmetrical spicules and are generally smaller in size as compared to the females. Vulva is positioned anteriorly where fully formed larvae are born. These larvae are termed as microfilariae(Simón 2001). Filarial worms have been observed in the central nervous system, subcutaneous tissues, the eye, the heart and lungs, and the lymphatic system (Orihel and Eberhard 1998).Filariae exhibit unique attributes and are regarded as substantially significant in human as well as veterinary medicine (Evans et al. 2022). A wide range of filarial worms infest wild and domestic animals around the globe (Satjawongvanit et al. 2019; Kaikuntod et al. 2020). Moreover, various filarial genera, like Meningonema, Wuchereria, Brugia, Loaina, Dirofilaria, Dipetalonema, and Onchocerca have also been identified in humans. *Wuchereria bancrofti* and *Brugia* spp. cause lymphatic filariasis (elephantiasis)(WHO 2022),*Onchocerca volvulus* causes onchocerciasis (river blindness) (World Health Organization 2019), *Loa loa* causes loiasis (African eye worm) (Tatuene et al. 2014), and *Mansonella* spp. causes mansonellosis (Akue et al. 2011). Many of these neglected tropical diseases (NTDs) are severe public health issues in the endemic regions as these may induce stigmatizing pathologies, exerting socio-economic burden in the people who are affected (Karunakaran et al. 2023). The estimated population that fell a prey to onchocerciasis is estimated to be around 21 million, where 14.6 million is suffering from skin diseases and about 1.15 million with vision dysfunction(WHO 2022).Lymphatic filariasis is the most common type of filariasis and is highly prevalent in Indonesia, Malaysia, India, Pakistan, Nigeria, Philippines, and Bangladesh. Beside these developing countries, it can also be found in developed countries like China, Western Pacific and some areas of America (Abbas et al. 2022). Onchoreasis is common in Africa but it can also be found in Yemen, South and Central America, and Saudi Arabia (Abbas et al. 2022). Fig. 1 shows major types of filariasis and basic difference among them.

As filariasis a mosquito-borne disease so its cycle starts when an infected mosquito bites the skin of the vertebrate host and injects the third stage larvae or L3 larvae into the body of the host. These L3 larvae move towards the lymphatic system where they mature. Maturation of L3 larvae is a slow and gradual process which takes almost 6 to 12 months (Chandy et al. 2011). This maturation leads to the formation

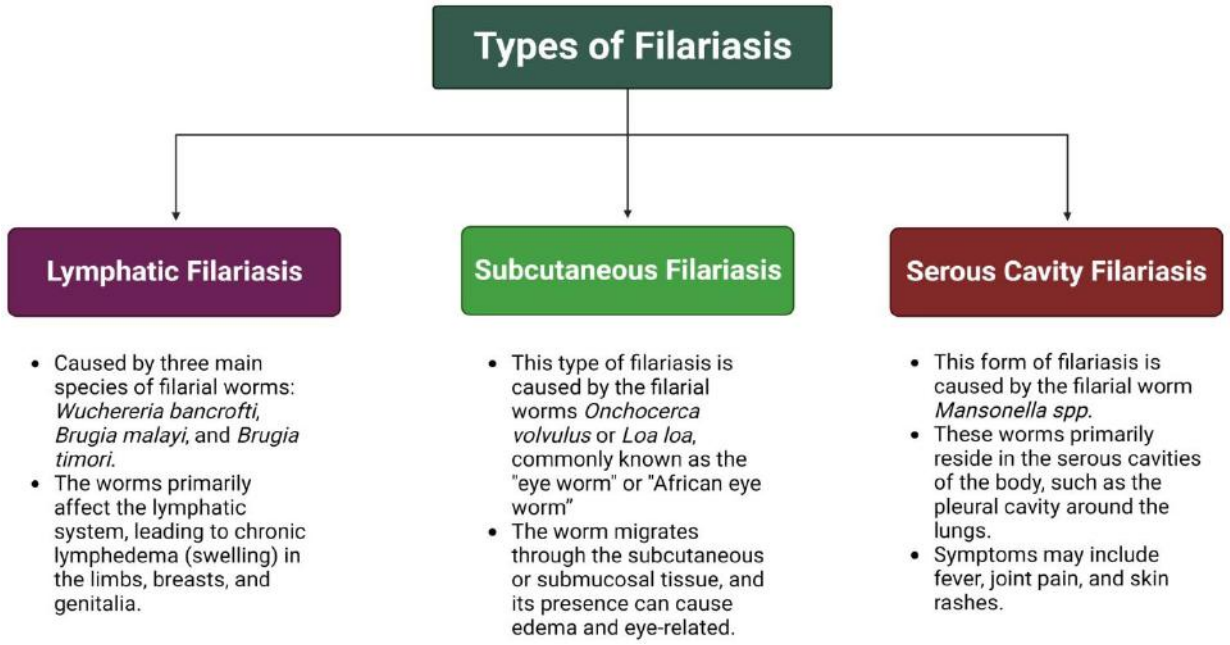

Fig. 1: Common types of filariasis.

of fourth stage larvae or L4 larvae which develop into adult larvae. These adult larvae give birth to the first stage larvae or L1 larvae commonly called microfilarae. These microfilarae flow in the blood stream. The mosquito's uptake the microfilarae where microfilarae develop into infective L3 larvae. L3 larvae move towards their proboscis and penetrate the host's skin when the mosquito bites the host. This continues the cycle. The penetration of larvae triggers the immune responses and symptoms like lymphoedema and hydrocele(Chandy et al. 2011) (Fig. 2).

These parasitic nematodes are generally long-living and hard to identify, often inducing chronic disease conditions spanning over years. Due to these reasons, proficient diagnostic tests are important for their control. Filariae at adult stage are likely to occupy far-off anatomical sites in the hosts. However, microfilariae disseminate profusely in the skin or blood which facilitates transmission and uptake by the blood-feeding arthropods in order to successfully complete their life cycle. The identification of this microscopic life form indicates integral form of test performed for diagnosis. DNA-based methods and immunological diagnostic techniques have since been devised for various filarial worms along with the techniques for the visualization of adult worms in situ (Gruntmeir et al. 2023). All of these approaches have their own pros and cons; therefore, effective diagnosis is mostly the integration of these methods. Precise diagnosis is essential for the detection of lethal infections like canine heartworm and is important for burgeoning zoonoses, such as *Onchocerca lupi*, and potential animal reservoirs, as in the case of *Brugia malayi.* Precise parasite identification not just aids in clinical settings but also provide significant assistance in research (Evans et al. 2022). In this chapter we will delve into the various advancements made regarding the diagnosis of filariasis in the recent years.

2. CONVENTIONAL METHODS USED FOR THE DIAGNOSIS OF FILARIASIS

2.1. DETECTION OF MICROFILARIAE IN BLOOD

Procedures like Knott's technique and membrane filtration technique are generally used in order to detect the presence of microfilariae in the body fluids (e.g. blood etc.) (Garcia and Procop 2016).

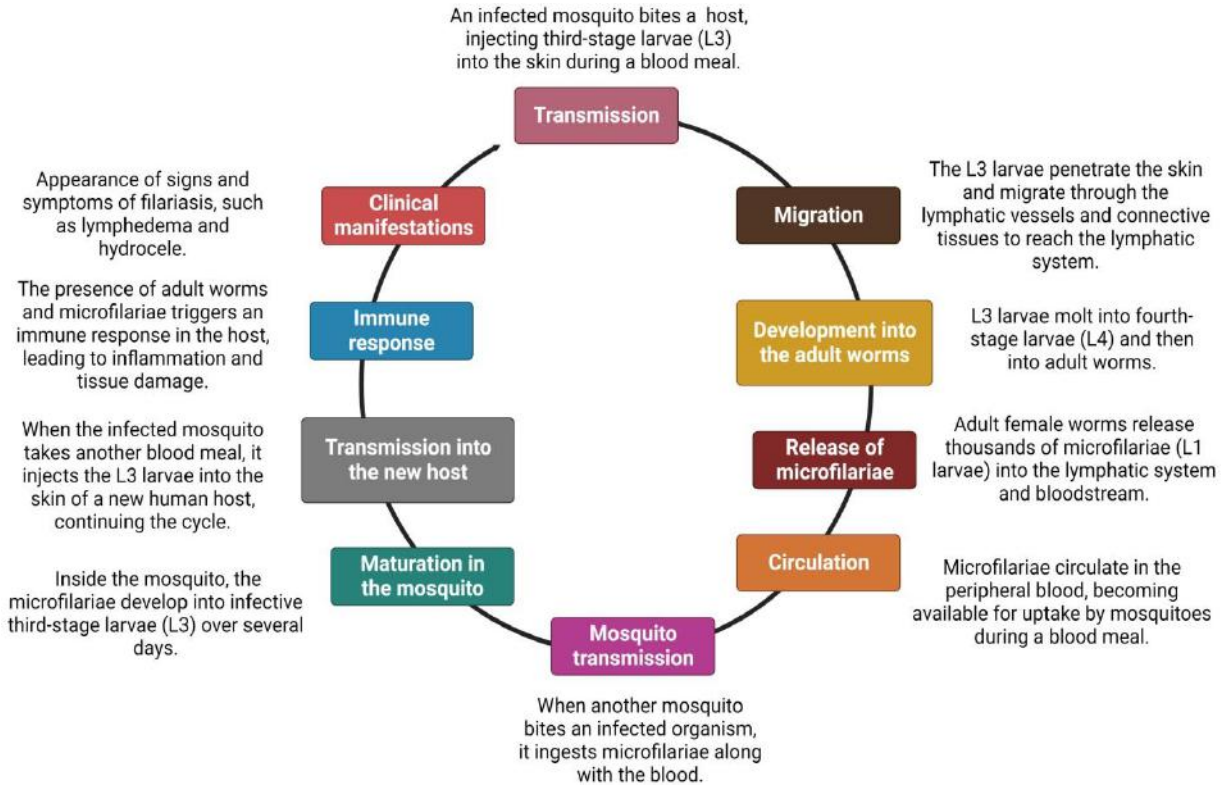

Fig. 2: Pathogenesis and transmission of filarial infection.

2.1.1. KNOTT'S TECHNIQUE

This technique is more commonly used. For this procedure, around 1 milliliter of blood is collected in EDTA (Ethylenediaminetetraacetic acid) or citrate through venepuncture. Alongwith 10 ml of 10% formalin, it is kept in a centrifuge tube. It is shaken in order to facilitate disintegration of red blood cells. This is centrifuged at 300 times *g* for around 120 seconds. The supernatant is removed. A small amount of sediment is observed (World Health Organization 2000; Mathison et al. 2019).

2.1.2. MEMBRANE FILTRATION TECHNIQUE

Fresh blood is collected in EDTA (Ethylenediaminetetraacetic acid) or sodiumcitrate. 10 mL of 10% Teepol saline sol. is combined with around 1 mL of blood. A filter paper is moistened and placed firmly in a filter holder. The Teepol-Blood mixture is poured in a 20 mL syringe and passed though the filter smoothly. Then water is slowly run through filter 2 to 3 times. Around 3 mL of methanol is flushed gently via filter to set microfilariae. Filter is removed and put on a slide. Let the filter to be dried completely. Then the slide is observed when it is entirely dry (World Health Organization 2000; Ash and Orihel 2007).

2.2. SKIN BIOPSY

A skin biopsy is usually carried out for the infections caused by the nematodes inhabiting the tissues. For biopsy, a sterilized needle is used for slightly lifting skin and the lifted skin is shaved off with the aid of

sterile blade. The skin sample is placed in a small tube with normal saline for 3 hours. Then the sample is examined to observe the mobile microfilariae (World Health Organization 2000).

2.3. INVESTIGATION OF SPUTUM AND URINE SAMPLES

Microfilariae are generally observed in blood but they can be detected in other body fluids. *Wuchereria bancrofti*have been witnessed in the urine of Chyluria infected organisms (Verma and Vij 2011). Hydrocele fluid, sputum and urine samples are centrifuged and microfilariae can be observed in the sediment (World Health Organization 2000; Garcia and Procop 2016).

2.4. BARRIERS TO THE USE OF TRADITIONAL METHODS

These traditional methods have low sensitivity so their use is not generalized (Eick et al. 2019). Limitations to the investigation of blood specimens are because ofinsufficient saturation of filter-paper, varying results with various filter-papers, and threat to the breaking down of specimens due to raised temperature or humidity(Arkell et al. 2022).

3. MOLECULAR TECHNIQUES FOR THE DIAGNOSIS OF FILARIASIS

Molecular investigations are not generally used. These techniques can be performed in research centers and specialized labs. Polymerase chain reaction (Mendoza et al. 2009) and loop-mediated isothermal amplification are two commonly known molecular techniques (Mathison et al. 2019).

3.1. REAL-TIME PCR

RT– PCRis favourable for labs in the countries that are developed as those countries have proper and developed labs (Mathison et al. 2019). Real-Time PCR is a cost effective, highly sensitive and specific method. It discovers the DNA fragments of the microfilariae in the infected persons as well as animals (McCarthy 2000; Chandy et al. 2011).

3.2. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Loop-mediated isothermal amplification or LAMP is a better choice for the countries being developed because the price of reagents is lower in comparison with that of RT – PCR. It can identify the microfilariae like Wuchereria *bancrofti*, *Brugia* spp., *Onchocerca volvulus*, and *Loa loa* (Drame et al. 2014; Poole et al. 2017; Mathison et al. 2019).

3.3. DNA SEQUENCING

This diagnostic method does not allow the analysis of whole genome. Some microfilariae species can be detected by DNA sequencing approach (Mathison et al. 2019).

4. SEROLOGY

4.1. IMMUNOCHROMATOGRAPHIC TESTS (ICTS)

Immunochromatographic tests are done for the identification of microfilariaeantigens. These tests are rapid, highly specific and sensitive. *Wuchereria bancrofti* can be detected by ICTs (Chandy et al. 2011).

4.2. ANTIBODIES IDENTIFICATION

Antibodies tests are very specific. These tests are usually done against *Brugia malayi* and *Wuchereria bancrofti* (Mathison et al. 2019). For antibodies test, blood specimen is collected in a simple vial and serum is separated. Then the test is done with the help of specialized test kit. *WbSXP-1*, a recombinant antigen, identifies the specialized antifilarial antibodies for *Brugia malayi* and *Wuchereria bancrofti*.

4.3. FILARIASIS TEST STRIP

The Filariasis Test Strip is a *Wuchereria bancrofti* specific RDT (Rapid Diagnostic Test). A Rapid Diagnostic Test or RDT is the one that is fast, simple and accurate. Filariasis Test Strip (FTS) identifies the CFA (Circulating Filarial Antigens) that are usually found in the blood or blood tissues of an individual. It was first used by the company Alere in the year of 2013 (Weil et al. 2013; Chesnais et al. 2016). For the test, the test strips are placed in the trays where we have to work prior to the addition of sample. Then the blood is collected in a micropipette. Blood is gradually added to the strip by pressing the bulb of the micropipette. Results can be read approximately after ten minutes. There are two lines in the test strip (Chesnais et al. 2016). One is test line and the other is control line. If both lines appear pink, then the result is said to be positive. On the other hand, if only control line shows pink colour then the result is said to be negative (Weil et al. 2013). If only test line appears pink or none of the lines show pink colour then the test result in invalid (Fig. 3). Filariasis Test Strip (FTS) is more economical, sensitive and has prolonged product life-span (Weil et al. 2013; Yahathugoda et al. 2015).
*T = Test line
**C = Control line

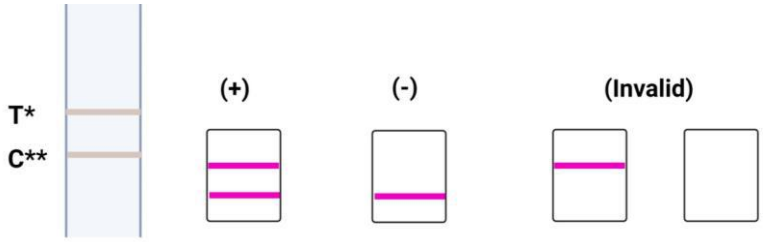

Fig. 3: Possible results for Filariasis Test Strip

5. IMAGING TECHNIQUES

5.1. ULTRASONOGRAPHY

Ultrasonography can help in detection of moving filarial nematodes or the adult filarial nematodes. On examination, movement of filarial nematodes can be seen. This is termed as “Filarial Dance Sign”. This sign can be observed in breast, cords, scrotum, axillary lymphatic, and limbs (Medeiros et al. 2021).

5.2. LYMPHOSCINTIGRAPHY

It is a diagnostic approach for the detection of any abnormality in lymphatic system (Hsueh et al. 2001; Pai 2023). Even in the asymptomatic condition of filariasis, the microfilariae infected organisms may present the abnormalities in the lymphatic system (Chandy et al. 2011).
Table 1 manifests the specific diagnostic approaches used for the diagnosis of certain species of filarial worms.

***Periodicity =**the time of day when the microfilariae circulate substantially in the blood of the vector or host (Aoki et al. 2011).

6. ONE HEALTH APPROACH AND FILARIASIS

Zoonotic filariasis is the filariasis in humans caused due to animal worms and has worldwide occurrence. It was first reported more than a 100 years ago. Since then, the number of reported cases and the parasites involved have gradually increased. Animal filaria require biological vectors like hematophagus insects to infect humans, which fed previously on a diseased animal in a suitable time frame. On the global scale, most people are at some risk but those who are more likely to interact with the vectors can be at higher risk. But there is also a possibility of unrecognized risk factors, as the animal filaria present worldwide distribution (Otranto and Eberhard 2011).There are three forms of filariasis depending upon the worms' predilection sites i.e. serous cavity filariasis, subcutaneous filariasis, and lymphatic filariasis. Lymphatic filariasis is also referred as elephantiasis. It is because, this condition is associated with the blockage of lymphatic system, distention, enlargement of testes, breasts, and limbs (Fassari et al. 2021). Onchocerciasis, also termed as river blindness, is another important disease caused by filarial worms. Black fly of genus Simuliumtransmits the causative agent of this disease i.e., *O. volvulus*. This condition is called river blindness because black flies breed near streams and rivers. It is also one the leading causes of blindness worldwide (Vinkeles Melchers et al. 2021). Dirofilariasis, induced by the filarial worms belonging to genus Dirofilaria, is another zoonotic infection which occurs mostly in canids and cause heart disease in them. *D. repens* and *D. immitis* are the well-known zoonotic species of *Dirofilaria* (Dantas-Torres and Otranto 2020). Out of nine species of Genus Mansonella, *M. streptocerca*, *M. perstans*, and *M. ozzardi* are widely-known zoonotic species. These parasitic worms are transmitted by female *Culicoide* and inhabit the cutaneous membrane of their host (Klion 2013).

Recently, continuous advancements are being observed regarding the development of better molecular and diagnostic techniques. The significance of genetic and genome-based information is growing substantially for the detection and characterization of zoonotic parasites. A proliferation of cross-host species relationships has lately been found out, which may have significant implications from evolutionary and epidemiological point of view (King et al. 2015; Lamberton et al. 2015). Due to enhancements in the molecular diagnostic techniques and genome sequencing of parasitic organisms, evidence revolving around the intermixing of genetic material is also being gathered (Webster et al. 2016). Precise diagnosis of parasitic infections is significant for the treatment and epidemiological monitoring of disease burdens. Diagnosis comprises of the utilization of clinical history, geography, travel history, and laboratory methods (Medeiros et al. 2021).

7. FUTURE PERSPECTIVES

Filariasis eradicating programs are advancing towards the goals set for the elimination of the filariasis as it is a problem all over world especially the developing countries. Proper diagnostic tools are important for supporting the goals of the programs. Limitations of the current tools used for the diagnosis of filariasis make it tough and challenging to ensure that the objectives of the program are attained. Some diagnostic tools are specific for specific species such as CFA (Circulating Filarial Antigens) tests are usually limited to the detection of*Wuchereria bancrofti*in blood smears. Their results cannot be trusted in case of *Loa loa*. There is an urgent need of discovery of techniques that can diagnose all forms of filariasis at once as it requires more cost and instruments to detect other forms or species regarding filariasis. Although many advances have been made regarding the diagnosis of filariasis still there is room for development of better and more

Table 1: Specie specific diagnosis of filarial nematodes.

Species	Regional prevalence	Shape and size	Periodicity*	Diagnostic approaches	References
Brugia malayi.	It is more prevalent in Southeast Asian countries including Malaysia, South Korea, India, Vietnam, Indonesia and Philippines.	They have nucleate tails and large cephalic space. The length of microfilariae of *B. malayi*is 0.177 to 0.230 mm and the width is 0.005 to 0.006 mm. They give pink colour with Giemsa stain.	Nocturnal	ELISA (Enzyme-Linked Immunosorbent Assay) PCR (Polymerase Chain Reaction) assays	(Lizotte et al. 1994; Fischer et al. 2003; Rao et al. 2006; Ash and Orihel 2007; Hotez 2009; Fox 2018; Mathison et al. 2019; Mulyaningsih et al. 2019)
Brugia timori.	Prevalent in Indonesia especially Lesser Sunda Islands	Microfilariae of B. timori have large cephalic space and nucleated tails. They have an average length of 0.31 mm and the width ranges from 0.006 to 0.007 mm. They do not give pink colourwith Giemsa stain.	Nocturnal	Antigen testing PCR (Polymerase Chain Reaction) assays	(Fischer et al. 2002; Supali et al. 2002; Fischer et al. 2004; Fischer et al. 2005; Ash and Orihel 2007; Mathison et al. 2019)
Loa loa.	Generally affects the organisms of Western and Central Africa	The microfilariae of *L. loa*possess less cephalic space and nuclear tail. They have length of 0.231 to 0.250 mm. They give no colour with Giemsa stain.	Diurnal	Loop-Mediated Isothermal Amplification (LAMP) Immuno-chromatographic card test (ICT)	(Ash and Orihel 2007; Fink et al. 2011; Wanji et al. 2015; Mathison et al. 2019; Campillo et al. 2022)
Mansonella ozzardi	It is commonly found in South and Central America and the Caribbean.	The microfilariae of *M. ozzardi* do not have a sheath but have a slender, nuclear tail. The length of *M. ozzardi*ranges from 0.163 to 0.203 mm.	Aperiodic	RT-PCR (Real-Time Polymerase Chain Reaction)	(Orihel et al. 1982; Ash and Orihel 2007; Tang et al. 2010; Medeiros et al. 2015; Lima et al. 2016; Medeiros et al. 2018; Raccurt 2018; Mathison et al. 2019; Ferreira et al. 2021)
Mansonella perstans	It is more prevalent in Sub-Saharan Africa and some parts of South and Central America.	Microfilariae of *M. perstans*do not have a sheath but have round, nuclear tail. Their length ranges from 0.19 to 0.2 mm.	Aperiodic	Serological assays Loop-Mediated Isothermal Amplification (LAMP)	(Meyers et al. 2000; Ash and Orihel 2007; Downes and Jacobsen 2010; Simonsen et al. 2011; Bassene et al. 2015; da Silva et al. 2017; Mathison et al. 2019; Bobkov et al. 2021)
Mansonella streptocerca	It is more prevalent in Sub-saharaincluding , East Africa, West Africa,	The microfilariae of *M.streptocerca*do not have a sheath but have a hook-shaped nucleated tail. The length of *M. streptocerca*generally	Aperiodic	Skin biopsy PCR (Polymerase Chain Reaction) assays	(Fischer et al. 1998; Ash and Orihel 2007; Downes and Jacobsen 2010; Fox 2018; Mathison et al. 2019)

	Southern Africa and Central Africa.	ranges from 0.18 to 0.24 mm.			
Onchocerca volvulus	It is usually found in the Sub-Saharan Africa, South and Central America and Yemen.	Microfilariae of *O. volvulus*has crooked, anuclear tails. They have length of 0.304 to 0.315 mm.	Aperiodic	Antibody tests Skin Biopsy PCR (Polymerase Chain Reaction) Sequencing Luciferase Immunoprecipitation Systems (LIPS)	(Orihel and Ash 1995; Meyers et al. 2000; Lipner et al. 2006; Ash and Orihel 2007; Osei-Atweneboana et al. 2007; Burbelo et al. 2009; BS 2018; Crowe et al. 2018; Mathison et al. 2019; Nyagang et al. 2020; Schmidt et al. 2022)
Wuchereria bancrofti.	Usually found in the Tropic of Cancer in Africa, Asia, the Caribbean, subtropics of South Pacific and South America.	Microfilariae have less cephalic space and an anuclear tail, with the length of 0.244 to 0.296 mm and the width of 0.0075 to 0.01 mm. They are generally colourless with Giemsa stain.	Nocturnal	ELISA (Enzyme-Linked Immunosorbent Assay) RT-PCR (Real-Time Polymerase Chain Reaction)	(Ramzy et al. 1997; Chansiri and Phantana 2002; Fischer et al. 2003; Lammie et al. 2004; Rao et al. 2006; Ash and Orihel 2007; Hotez 2009; Abdel-Shafi et al. 2017; Mathison et al. 2019)

reliable techniques. Loop-Mediated Isothermal Amplification (LAMP) assays are the novel techniques and undoubtedly have many benefits still there are drawbacks in the usage of LAMP assays. The primary drawback is that when there is Primer to Primer interaction or contamination, these LAMP assays give false positive results. So, there is dire need of the development of upgraded and more efficient diagnostic techniques and procedures to surveil and examine different forms of filariasis in humans as well as animals.

8. CONCLUSION

Filariasis is one of the neglected tropical diseases that are affecting the populations all over the world especially the developing countries. Its proper diagnosis and treatment in time is necessary as it can be fatal if not treated. With the development in the medical field, there are also advances in the diagnostic approaches used for the detection of filariasis. DNA sequencing, RT-PCR, LAMP, RDTs (Rapid Diagnostic Tests), FTS (Filarial Test Strip) and lymphoscintigraphy are some of the recent diagnostic methods that are being used for the identification of filariasis in organisms in the present day. Though these methods are better than the traditional methods that were being used in the past still there is need of development of more advanced and trustable diagnostic techniques in order to cope with this NTD (Neglected Tropical Disease). Development of inexpensive, appropriate and more reliable techniques can lead to the timely diagnosis of filariasis and thus can be treated more effectively.

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Diagnosis and Control of Lymphatic Filariasis with Special Emphasis on Gene Editing Method

05

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ABSTRACT

Lymphatic filariasis stands with a significance global public health concern. In order to proceed for the control and treatment of the disease, the analysis of its diagnosis deserves crucial attention. Likewise other diseases, the diagnostics of lymphatic filariasis includes several physical examinations, molecular assays as well as serological methods of diagnostics. The efforts have been made of achieving milestone in developing drug with specific efficacy. Drug therapy through anti filarial drugs has been a cornerstone in order to control and eliminate LF globally. With pharmaceutical intervention, health professionals attempts to increase effectiveness of these drugs through designing drug combinations. However, it is important to note that prevention is favored to control LF. Disease control through certain drugs is employed by MDA program. It is majorly aiming to target agents for transmission (worms and microfilariae). Complementary strategies to lessen the prevalence of filarial vectors include vector control. Systematic tracking, surveillance, community engagement and strengthening healthcare infrastructure in endemic areas are some collaborative efforts to control the disease.

The limitations of the control strategies such like drug resistance lead to emergence of promising technique i.e gene therapy. The Cas9 CRISPR technology has provided revolutionary treatment in all recent times. The genomes of filarial parasites are being edited and modifications are being made. This will create hindrance in transmission cycle, reproduction cycle of parasites and worms. In the result, parasite become inactive and is destructed. The gene therapy delivered by viral vectors, nano particles or any other molecular vehicles. However, the Cas9 system is known for its precision, versatility and transformative effects. Still there is intervention of regulatory, ethical considerations and not this technique is able to be delivered to all targeted population of world. The Genome editing has the potential to transform the strategies for disease management. It can revolutionize the lymphatic filariasis control and prevention agenda. The comprehensive identity of genome editing if used in union of existing techniques, can execute sustained disease elimination.

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1. INTRODUCTION

Elephantiasis, also known as lymphatic filariasis, is a parasitic condition brought on by Wuchereria bancrofti, Brugia malayi, and Brugia timori, three species of thread-like worms (nematodes) known as filarial worms. It is mainly spread to people via mosquito bites that are contaminated. The illness is a serious public health hazard and is common in tropical and subtropical areas of the world (Chandrasena et al. 2018; Lourens and Ferrell 2019). This disease can be deadly but early diagnosis can be the smart strategy towards the management of the disease. This includes the detection of microfilariae in the blood of humans which is transmitted through mosquito bites. The diagnostics cover many techniques like night blood smears, PCR, detection of antigens, etc.

The screening of lymphatic filariasis helps in identifying the target disease in the population at risk and also in the implementation of prevention strategies (Pastor et al. 2019). It can be done by conducting surveys, vector control measures, etc. But it's also important to consider effective drug development, knowing the health technologies through gadgets, and innovative ways combined with traditional ones. In recent times the most hyped and capable tool CRISPR-Cas9 as genome editing to generate new possibilities in the site of treating lymphatic filariasis (Kwarteng et al. 2021). This technology destroys the life cycle of filarial worms. Through identifying the target genes of both culprit and victims i.e. mosquito and human respectively. The approach is to create resistance for parasitic worms to become mature, reproduce, and host in humans. However, there is no such record that an approved vaccine is developed for this disease. But the trials are being made to introduce a vaccine specifically for Wuchereria bancrofti. The complicated life cycle of filarial parasites hinders to inducement of any immune response to fight against disease. However, the trials made particular targets to identify the antigens that as a result be used for developing vaccines (Das, Chakraborty et al. 2023).

2. DIAGNOSIS AND SCREENING

Lymphatic filariasis is a parasitic disease caused by roundworms that is transmitted to humans through the bite of an infected mosquito. The following methods are commonly used to diagnose and study this disease.

2.1. PHYSICAL EXAMINATION

Doctors may examine patients for physical symptoms, such as swollen limbs (lymphedema) or swollen scrotum (edema), which are characteristic signs of lymphatic filariasis (Mahalingashetti, Subramanian et al. 2014).

2.2. LABORATORY TESTS

Microscopic examination of blood smears: A blood sample is examined under a microscope to check for the presence of microfilariae, the larval stages of heartworms, in the blood. Serological tests: These tests detect antibodies to roundworms in the blood, indicating infection. These are particularly useful for detecting early or asymptomatic cases (Organization 2021).

2.3. MOLECULAR TECHNOLOGY (PCR)

The polymerase chain reaction (PCR) test can detect the presence of filarial DNA in blood samples, providing a highly sensitive and specific diagnostic method (Rao, Atkinson et al. 2006).

2.4. SCREENING PROGRAMS AND STRATEGIES

Screening programs are in place in endemic areas to combat lymphatic filariasis. This may involve high-dose antifilarial drugs (MDA) across at-risk populations, even in asymptomatic cases. This helps reduce the number of parasites in the community and stop transmission (Bockarie, Taylor et al. 2009).

2.5. TREATMENT AND MANAGEMENT

In addition, other preventive measures include vector control (mosquito control) and health education to raise awareness about the disease, its transmission and how to prevent it.
Early detection and prompt treatment are critical to prevent serious complications and reduce the prevalence of lymphatic filariasis. A regular inspection program is essential to control and ultimately eradicate the disease in affected areas.
Lymphatic filariasis is a parasitic infection transmitted by mosquito bites. Let's look at treatment and management options.

2.6. DRUG THERAPY

2.6.1. ANTIFILARIAL DRUGS

Diethylcarbamazine, ivermectin, and albendazole are commonly used to treat infections. Diethylcarbamazine helps kill adult worms, while ivermectin and albendazole target larvae. These drugs are usually given in combination (Shenoy, Suma et al. 2009).

2.7. DRUG COMBINATIONS AND TREATMENT PLANS

The specific drug combination and treatment regimen will depend on the severity of the infection and the recommendations of your health care professional. A combination of antifilarial drugs is usually prescribed to maximize effectiveness (Olsen 2007).

2.8. SURGICAL INTERVENTION FOR ADVANCED DISEASE

Advanced lymphatic filariasis may require surgical intervention to reduce complications. Surgical intervention is aimed at improving lymph flow and reducing swelling. These procedures are usually considered for severe lymphedema or elephantiasis (2019).

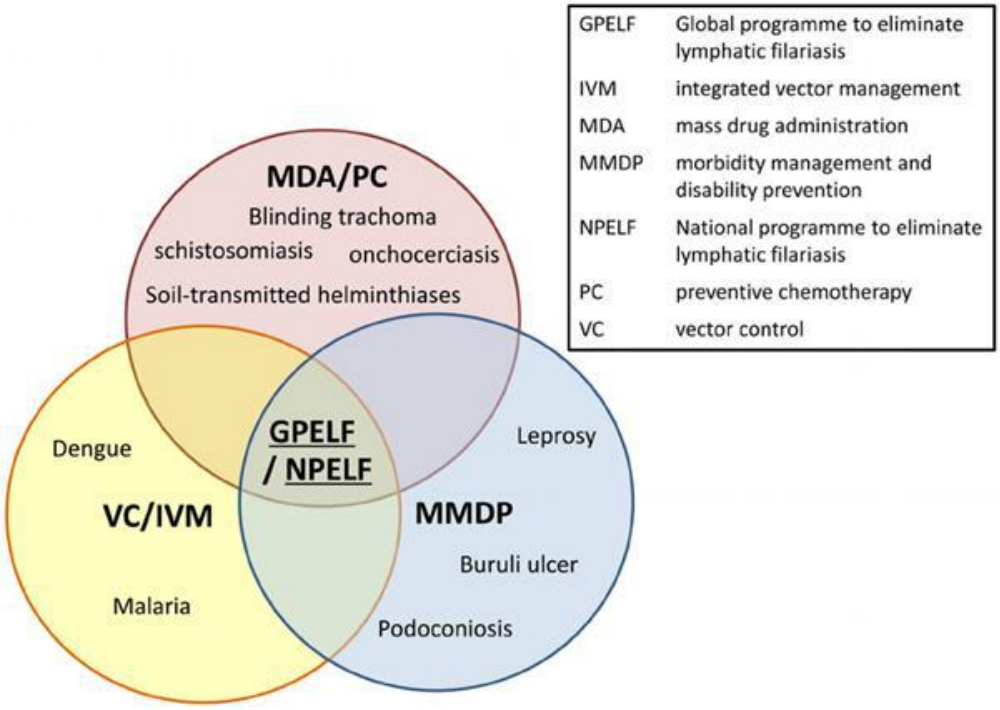

Fig. 1: Prevention and control of Lymphatic Filariasis

2.9. TREATMENT OF ASSOCIATED COMPLICATIONS (SUCH AS SECONDARY BACTERIAL INFECTIONS)

Secondary bacterial infections can occur in skin areas affected by lymphedema. Proper wound care and antibiotic treatment are essential to manage these complications and prevent further health problems. It is important to note that prevention is essential to control lymphatic filariasis. These include mosquito control measures and mass drug administration to vulnerable populations in endemic areas. If you suspect that you or someone you know has lymphocytic filariasis, it is essential to see a doctor for proper diagnosis and treatment (Boccardo, Campisi et al. 2012).

3. PREVENTION AND CONTROL

Prevention and control of lymphatic filariasis are essential to reduce its transmission, prevent disability and suffering, and eventually eliminate the disease. Several strategies are used to achieve these goals as shown in Fig. 1:

3.1. MASS DRUG ADMINISTRATION (MDA)

The most effective approach to control lymphatic filariasis is through the distribution of preventive medications. The World Health Organization (WHO) recommends using a combination of two drugs, usually ivermectin and albendazole or diethylcarbamazine (DEC) and albendazole, to kill the microfilariae (larval stage) of the filarial worms. MDA involves administering these drugs once or twice a year to all eligible individuals in endemic areas, even if they show no symptoms. Treating the entire at-risk population helps reduce the number of microfilariae circulating in the community and interrupts the transmission cycle (Talbot, Viall et al. 2008).

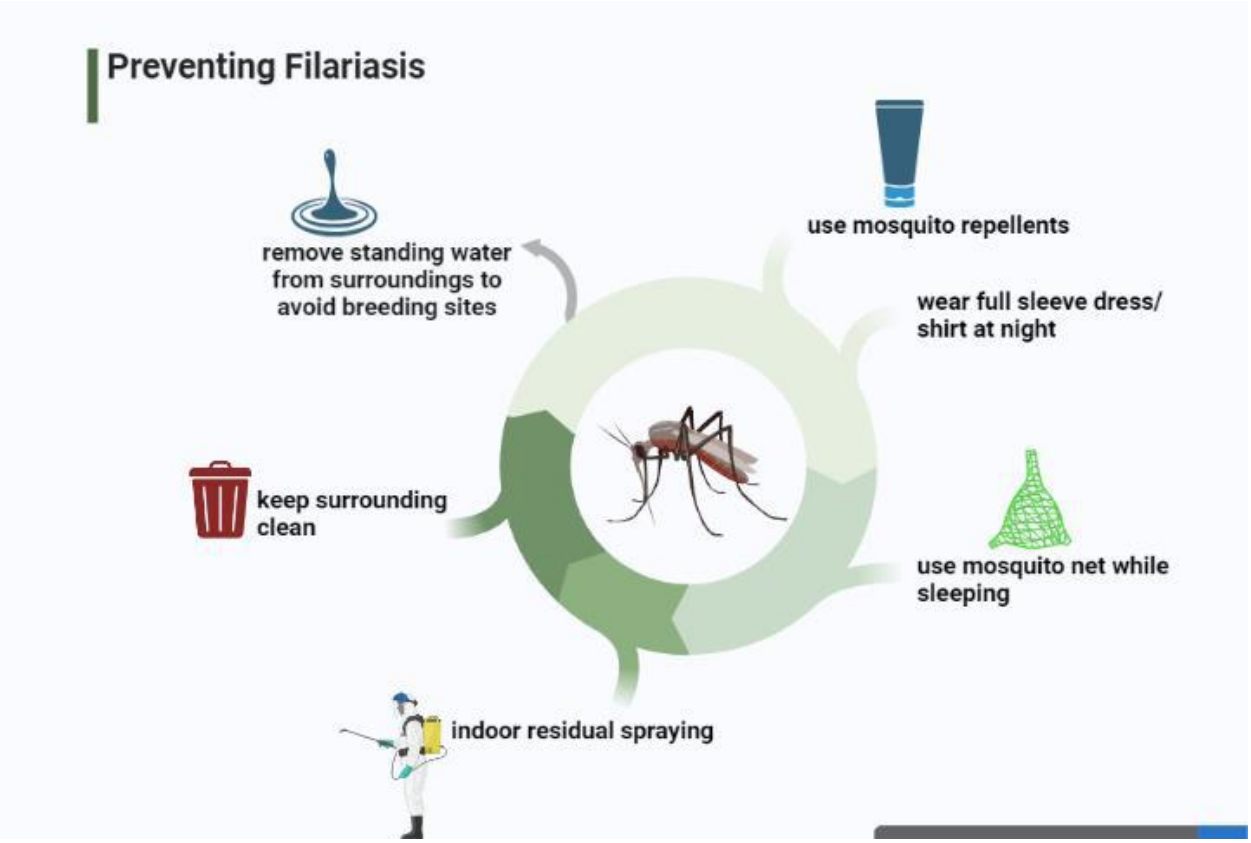

Fig. 2: Prevention and Control of Vector.

3.2. VECTOR CONTROL

Since lymphatic filariasis is transmitted by infected mosquitoes, controlling the mosquito population is crucial. This can be accomplished by taking steps including employing bed nets sprayed with pesticide, indoor residual spraying, and managing the environment to eliminate mosquito breeding grounds as show in Fig. 2 (Bockarie, Taylor et al. 2009).

3.3. PREVENTIVE HEALTH STRATEGIES AND COMMUNITY ENGAGEMENT

To promote community involvement in control efforts, it is essential to raise awareness about the disease, its transmission, and prevention methods. Education campaigns help communities understand the importance of MDA and vector control, as well as the benefits of personal protective measures like using bed nets and wearing long-sleeved clothing (Agrawal and Sashindran 2006).

3.4. MORBIDITY MANAGEMENT AND DISABILITY PREVENTION (MMDP)

For individuals already affected by lymphatic filariasis and suffering from chronic symptoms like elephantiasis or hydrocele (swelling of the scrotum), it is essential to provide appropriate care and support. This includes managing acute attacks, preventing secondary infections, promoting hygiene, and providing physical therapy and rehabilitation services to improve the quality of life (Chandrasena, Premaratna et al. 2018).

3.5. SURVEILLANCE AND MONITORING

Regular monitoring and surveillance systems are necessary to assess the effectiveness of control programs, track progress, and identify areas that still require intervention.

3.6. STRENGTHENING HEALTH SYSTEMS

Building and strengthening healthcare infrastructure in endemic areas helps ensure the efficient delivery of prevention and control measures. It involves training healthcare workers, improving diagnostic capabilities, and ensuring the availability of essential drugs and supplies (Zulu, Maritim et al. 2022).

3.7. COLLABORATION AND PARTNERSHIPS

A multi-sectorial strategy involving governments, international organizations, non-governmental organizations (NGOs), and affected communities is necessary to combat lymphatic filariasis. Collaborative efforts enhance the effectiveness of control programs and resource mobilization (ASSEMBLY and SANTÉ 1997).
It Is important to note that the success of prevention and control efforts depends on sustained commitment and funding from governments and international partners. Combined efforts can result in the eradication of lymphatic filariasis as a public health issue and decrease the suffering of the millions of people infested by this crippling illness (Organization 2010).

4. LYMPHATIC FILARIASIS: SOCIOECONOMIC AND PSYCHOSOCIAL IMPACTS

The parasitic worms that cause lymphatic filariasis are spread through mosquito bites from affected individuals. It is a neglected tropical disease. As a result of the parasites' primary impact on the lymphatic system, body parts like the limbs and genitalia expand. This chronic and debilitating condition has significant socioeconomic and psychosocial impacts on affected individuals and communities (Wynd, Melrose et al. 2007).

4.1. ECONOMIC BURDEN ON INDIVIDUALS AND COMMUNITIES

4.1.1. MEDICAL COSTS

Lymphatic filariasis requires long-term management, including medication, regular check-ups, and possible surgeries. These medical expenses can impose a substantial financial burden on both individuals and healthcare systems (Gyapong, Gyapong et al. 1996).

4.2. REDUCED WORK PRODUCTIVITY

The physical impairments caused by the disease can hinder an individual's ability to work. Swollen limbs or genitalia may prevent people from engaging in certain occupations, leading to reduced productivity and income.

4.3. STIGMA AND DISCRIMINATION

The visible disfigurement and disabilities resulting from lymphatic filariasis can lead to stigmatization and discrimination in employment and social settings. This can further hinder affected individuals from finding or maintaining jobs, exacerbating their economic struggles (Ramaiah, Das et al. 2000).

4.4. OPPORTUNITY COST

When individuals are unable to work or participate fully in daily activities, they miss out on potential economic opportunities, further perpetuating poverty cycles.

4.5. REDUCED COMMUNITY PRODUCTIVITY

If the disease affects a significant portion of a community, the overall productivity of that community may decline due to the cumulative effects of lost workdays and diminished labor capacity.

4.6. SOCIAL STIGMA AND DISCRIMINATION

4.6.1. APPEARANCE-BASED STIGMA

Swollen limbs, genitalia, or other visible manifestations of the disease can lead to social isolation and discrimination. Affected individuals may be subjected to ridicule, negative attitudes, and avoidance by others (Abdulmalik, Nwefoh et al. 2018).

4.7. MARITAL AND SOCIAL EXCLUSION

In some cultures, individuals with lymphatic filariasis may face difficulties in finding a partner for marriage due to societal perceptions about the disease. This exclusion from social and family life can lead to feelings of loneliness and depression (Person, Bartholomew et al. 2009).

4.8. EDUCATIONAL DISRUPTION

Children affected by lymphatic filariasis might experience discrimination and teasing by their peers, which can lead to reduced school attendance and hinder their educational development.

4.9. COMMUNITY REJECTION

Entire families may face discrimination within their communities, affecting their social integration and overall well-being.

4.10. PSYCHOLOGICAL IMPACT ON AFFECTED INDIVIDUALS

4.10.1. MENTAL DISTRESS

Living with a chronic and stigmatized condition can lead to anxiety, depression, and feelings of hopelessness in affected individuals.(Ton, Mackenzie et al. 2015).

4.11. BODY IMAGE ISSUES

The physical disfigurements caused by the disease can negatively impact body image, leading to low self-esteem and self-confidence.

4.12. SOCIAL ANXIETY

Fear of rejection and social isolation can lead to increased social anxiety in individuals with lymphatic filariasis (Krishna Kumari, Harichandrakumar et al. 2005).

4.13. EMOTIONAL BURDEN

Coping with the long-term effects of the disease, including pain and physical limitations, can place an emotional burden on affected individuals and their families.
Overall, the socioeconomic and psychosocial impacts of lymphatic filariasis highlight the importance of comprehensive approaches to disease management. To improve the general wellbeing of impacted people and communities, these strategies should not only focus on medical therapies but also address social stigma, offer mental health assistance, and encourage economic development. The impact of this neglected tropical disease can be significantly reduced by public health initiatives that emphasize prevention, early detection, and treatment.

4.14. SUCCESS STORIES AND CHALLENGES IN DIFFERENT REGIONS

Positive progress has been done for the elimination and transmission control of Lymphatic filariasis and many different strategies like MDA, MMDP and vector control have played a big role for the disease control.

4.15. (INTIMATE'S STORY)

A local inhabitant of Orissa, India, Indurate (mother of seven and 58-year-old widow) thought that can never be out of this disease like all the other Lymphatic Filariasis patients and that will be forever self-isolated. She has been suffering by lymphedema (swelling) of the leg because of LF for nearly 25 years. Indurate was even reluctant to visit her girls, for dread they and their grandchildren would be terrified in response to her sickness.
LF is one of the world's most health draining diseases, with 120 million people already affected by it. Symptoms are painful which includes swelling of arms, legs, and breasts. Another effect of LF is that many individuals who suffer from LF are rejected by their communities.
Many NGOs along with Disease Control and Prevention (CDC) and India's Church's Auxiliary for Social Action (CASA) are working in collaboration to help the patients of Lymphatic Filariasis. CDC ran different campaigns which involve yearly distribution of medicine to groups at risk, to halt the spread of infection.
CASA workers have started programs in Intimate's village and are raising awareness on LF. Management of Lymphatic Filariasis includes exercise of the affected limbs and cleaning the affected regions with water and soap. These minimal measures help to stop severe attacks of LF.
Since workers helped Indurate learn new techniques, she is now much better without the severe Lymphatic Filariasis attacks and now living a normal life. "Because of [CASA] now I am able to lead a better life. Now my grandchildren come to me without any hesitation," Indurate reported ecstatically (Babu, Acharya et al. 2001).

5. GENOME EDITING AS CONTROL INSTRUMENT FOR LF

5.1. MECHANISM OF TREATMENT FOR LYMPHATIC FILARSIS BY GENOME EDITING

As of the last update in September 2021, there were no widely accepted genome editing-based cures for lymphatic filariasis. However, I can provide you with some insights into the concept of using genome editing for potential cures.

Genome editing techniques like CRISPR-Cas9 have revolutionized the field of genetics and offer potential opportunities for developing therapies against various diseases, including infectious diseases caused by parasites like *Wuchereria bancrofti*. crispr-cas9 works as follows as shown in Fig. 3.

5.2. IDENTIFYING TARGET GENES

Scientists first identify specific genes that are important for the survival or reproduction of the *Ucherella bancroft* parasite.

5.3. DESIGN OF THE CRISPR-CAS9 SYSTEM

Once the target gene is identified, the guide RNA (gRNA) is designed to be complementary to the specific gene sequence. This gRNA is a molecular guide directing the Cas9 enzyme to the exact spot in the parasite genome where the target gene resides.

5.4. DELIVERY OF CRISPR-CAS9 TO THE PARASITE

Next, the CRISPR-Cas9 system is delivered to the parasite, which can be a complex and difficult step. Various delivery methods can be considered, including the use of viral vectors, nanoparticles, or other delivery vehicles that can effectively deliver the CRISPR components to the parasite (Kwarteng, Sylverken et al. 2021).

5.5. EDITING THE PARASITE GENOME

Once inside the parasite, the CRISPR-Cas9 system recognizes target genes and induces cleavage of their DNA. The parasite's repair machinery may attempt to repair this damage, but often makes mistakes that cause genetic mutations that disrupt target gene function.

5.6. INACTIVATION OR DESTRUCTION OF THE PARASITE

With essential genes disrupted or modified, the parasite may become unable to survive or reproduce, effectively neutralizing its ability to cause lymphatic filariasis in the human host.

5.7. MONITORING AND VALIDATION

Extensive monitoring needed for CRISPR-Cas9 treatment efficacy and safety without side effects (Torres, Prince et al. 2022).
It is guided by small RNA sequence provides site for endonucleases. The CRISPR/Cas9 system is a simple endonuclease system consisting of Cas9, two RNA molecules, and the Cas9 protein. The system is guided to the target genomic locus by a short RNA sequence called crRNA. The second RNA, Trans activating crRNA or tracrRNA, pairs with the crRNA to create a lops-based RNA structure, directing Cas9 to the target locus with a PAM sequence. NHEJ or homology-directed repair are options for fixing the DSB.
CRISPR-Cas9 genome editing techniques for treating lymphatic filariasis remain in theoretical and experimental research, with challenges like efficient delivery, avoiding off-target effects, and ethical considerations requiring further development.
As of September 2021, information is accurate; updates may occur. Consult the latest scientific literature or a qualified medical professional for the most up-to-date information.

5.8. REVOLUTIONIZING LYMPHATIC FILARIASIS CONTROL: THE TRANSFORMATIVE APPLICATIONS OF GENOME EDITING

Elephantiasis, or lymphatic filariasis, is a parasitic illness brought on by nematode worms that are spread by mosquito bites. It causes swelling in legs and genital areas, potentially causing permanent disability.

The application of genome editing in relevance to lymphatic filariasis can be explored in several ways:

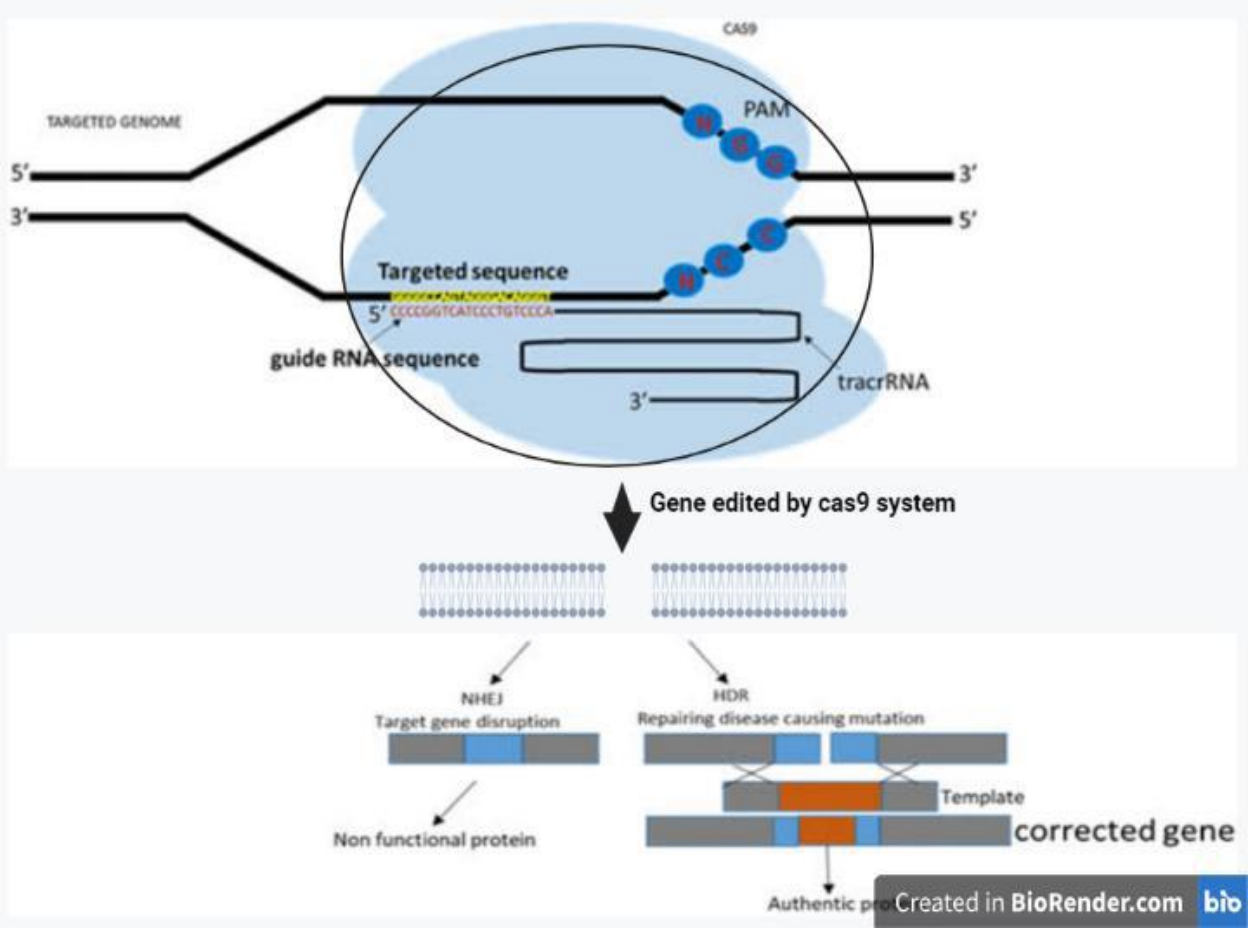

Fig. 3: Gene edited by CRISPR Cas9 system.

5.9. GENE EDITING OF THE PARASITE

Gene editing of the filarial parasite involves targeting essential genes, preventing its survival or propagation within the human host. Researchers can use CRISPR-Cas9 or other tools to disrupt infection or reduce virulence.

5.10. GENE EDITING OF THE MOSQUITO VECTOR

Genome editing can modify mosquito genes responsible for transmitting filarial worms, potentially reducing or eliminating their ability to carry and transmit the disease to humans (Severson and Behura 2012).

5.11. HOST GENETIC MODIFICATION

Genome editing can modify genes in the human host to confer resistance to lymphatic filariasis, enhancing immune response and reducing disease impact.
Genome editing holds potential, but challenges and ethical considerations must be addressed for safe, responsible deployment in real-world scenarios (Nutman 2013).

5.12. OFF-TARGET EFFECTS

Genome editing technologies must be specific to prevent unintended changes and potential consequences (Krotneva, Coffeng et al. 2015).

5.13. DELIVERY

Effective delivery of the gene-editing tools to the target cells or organisms, whether parasites, mosquitoes, or human hosts, remains a challenge.

5.14. REGULATORY AND ETHICAL CONSIDERATIONS

Genome editing in disease control raises ethical concerns, including ecological consequences and informed consent for human interventions (Kouassi, Barry et al. 2018).

5.15. SAFETY AND EFFICACY

Preclinical research and clinical trials are crucial for genome editing safety and efficacy.
Genome editing research advances rapidly, so consult recent sources for latest developments in combating lymphatic filariasis and other diseases.

5.16. ADVANCING BIOINFORMATICS AND BIOTECHNOLOGY IN THE BATTLE AGAINST LYMPHATIC FILARIASIS: A PIONEERING APPROACH

An infection with nematode worms known as lymphatic filariasis, often called elephantiasis, is spread by mosquito bites. It is a major public health issue in tropical and subtropical regions. Bioinformatics and biotechnology are essential for understanding, developing diagnostic tools, and developing control and treatment strategies.

5.17. GENOME SEQUENCING

Bioinformatics analyzes genomic data using computational tools. Sequencing filarial parasite genomes, like *Wuchereria bancrofti* and *Brugia malayi*, offers insights into their biology, aiding researchers in drug targets identification and understanding resistance mechanisms (Williams, Lizotte-Waniewski et al. 2000).

5.18. IDENTIFICATION OF DRUG TARGETS

Analyzing filarial parasite genomic and proteomic data enables bioinformaticians to identify essential genes and proteins for worm survival and reproduction. These targets can be used for drug development or repurposing, effectively treating the disease.

5.19. VACCINE DEVELOPMENT

Bioinformatics is crucial in identifying and designing potential vaccines against lymphatic filariasis. By analyzing parasite genomes and their interactions with the host immune system, researchers can identify antigens that may elicit a protective immune response. This information aids in rational vaccine design and preclinical and clinical trials (Kalyanasundaram, Khatri et al. 2020).

5.20. TRANSCRIPTOMICS AND PROTEOMICS

Transcriptomics and proteomics aid researchers in understanding filarial parasite gene expression and protein profiles, identifying differentially expressed genes and revealing mechanisms underlying parasite development and transmission.

5.21. DIAGNOSTICS

Bioinformatics can enhance diagnostic tools for lymphatic filariasis by analyzing filarial worm genetic material, enabling sensitive PCR-based assays to detect parasite presence in human samples. These molecular diagnostic methods are more accurate than traditional methods, aiding early disease detection and monitoring (Misra-Bhattacharya and Shahab 2018).

5.22. POPULATION GENETICS

Bioinformatics tools analyze filarial parasite population genetic data, providing insights into spread and distribution, tracking transmission patterns, and identifying intervention areas.

5.23. DRUG RESISTANCE MONITORING

Biotechnological tools monitor drug resistance in filarial parasites by analyzing genomic data, revealing genetic mechanisms, and adjusting treatment strategies accordingly (Sharma, Vadlamudi et al. 2013).
Integrating bioinformatics and biotechnology is crucial for understanding lymphatic filariasis, developing treatments, and eradicating the disease. This facilitates data-driven research and provides powerful tools for effective global health challenges.

5.24. RESULTS AND DISCUSSION LYMPHATIC FILARIASIS

Lymphatic filariasis, also known as elephantiasis, is a parasitic disease caused by filarial worms, Wuchereria bancrofti and Brugia malayi and timori. These worms primarily affect the lymphatic system and are found in Africa, Southeast Asia, the Indian subcontinent, the Pacific Islands, and some parts of the Americas.

5.25. TRANSMISSION

Lymphatic filariasis is transmitted through infected mosquito bites, where microfilariae enter the bloodstream, migrate to lymphatic vessels, and grow into adult worms, which can live for years in the human host.

5.26. LIFECYCLE

Filarial worms have a life cycle consisting of two stages: the human host and the mosquito vector. In the human host, worms' mate, releasing microfilariae into the bloodstream. These microfilariae can be picked up by mosquitoes during a blood meal. Once inside, they mature into infective larvae, which can be transmitted to another human.

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Basic Insights into Lymphatic Filariasis

06

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ABSTRACT

Lymphatic filariais, a debilitating disease which is caused by parasitic worms and transmits by bite of a mosquito. The epidemiology of the disease refers to the certain environment conditions which promote the breeding of mosquitos' carrying the LF disease. There is High risk population and socioeconomic impacts that have increased the prevalence of the disease to certain regions. It is also associated with the spread of causative agents which shows persistence in topical and subtropical regions. Moreover, the burden of disease due to lack of treatment opportunities is much dense in regions like India and Nigeria. The disease is neglected yet complex due to reciprocity between humans and mosquitos. The morphology circulates around the injection of the microfilariae by mosquito from blood meal, developing infection causing larvae and again transmits to human but a new one this time. The understanding of transmission dynamics of LF is a key step to address disease precisely. However, in mosquito microfilariae undergoes several life stages and after the L3 stage it penetrates to human lymphatic system. The pathogenicity of LF can be assessed by host parasite interaction and certain immune responses. The recognition of parasite as an antigen stimulates innate immune system, T cell activation linked with APC's. but if the LF infection replicates it causes severe stages of LF. Lymphedema caused by chronic infection in worst cases can lead to disability. Hydrocele, lymphangitis and elephantitis are the hallmark of clinical manifestation predilection debility of lymphatic system.

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1. INTRODUCTION

1.1. *LYMPHATIC FILARIASIS*; INSIGHT TOWARDS ITS ETIOLOGY

Lymphatic filariasis, also known as elephantiasis, is a parasitic disease caused by thread-like filarial worms that belong to the nematode family. Being a vector-borne disease, Lymphatic filariasis refers to an infection causing which is mostly neglected all over the world. Lymphatic filariasis is transmitted through the bite of infected female mosquitoes, from the genera Culex, Anopheles, and Aedes. The nematodes which are the real cause of the disease are passed on through mosquitos. Humans become hosts for the vectors, but the adult worms still do not multiply in humans. That is why tourists and travelers exposed to the infection are shortly affected, and it persists for a short time. However, children are more prone to get the transmission, and pervasiveness gets more with age.

These parasites are transmitted to humans by mosquito biting and the human gets infected. The worm at this point is only 1mm in length. The larvae then travel to the lymphatic vessels of the human and transform within 9 months with 7-10cm in length. for the areas in which the mosquitos have maximum activity at night, the larvae have many possibilities to be detected in the bloodstream during the night and vice versa for areas with maximum activity in the daytime. The microfilariae in blood are ingested by mosquitoes while the mosquito takes human blood as a meal. Microfilariae penetrates the midgut of the mosquito and develops itself into L1 larvae. The L1 larvae develop into L3 larvae which are again transmitted to humans through mosquito biting. By the source of this whole life cycle, the repeated bits of mosquitoes spread lymphatic filariasis to humans. Filariasis is much influenced and dependent on the time of exposure to mosquito bites. And also depends on the presence of antigens of adult worms in vessels.

However, the detection of the microfilaria is done blood microscopy. It can also be done by detecting filarial antigen in blood using molecular technique like ELISA or by detecting the filarial DNA using the techniques like PCR. The most preferable technique is detecting the antigen in the blood through ELISA because it doesn't specify the timings of taking blood sample and it's easy to run. The adult worms can also be detected using ultrasonography. As the adult worms are distinguishable because of their sizes or quick or so-called dancing movements with is different from other blood movement.

2. EPIDEMIOLOGY OF LYMPHATIC FILARIASIS

Geographical Distribution:

Region	Frequency level	Endemicity
East Asia	Low	Occasional
Oceania	Moderate	Regional
Southeast Asia	Moderate	Regional
South Asia	High	Common
Pacific Islands	High	Common
Central America	Low	Occasional
Europe	Negligible	Eradicated

In Asia, *Lymphatic filariasis* was widely spread in some areas of Southeast Asia, and the Western Pacific region. India and Bangladesh are marked with a very high frequency of Lymphatic filariasis. While China is less endemic, in America, Lymphatic filariasis was widely spread in some areas of the Caribbean and South America (Michael et al. 1996).
Not very common in regions of Europe. Rural areas suffer more from Lymphatic filariasis because of poor health and un-hygienic condition in those areas.

2.1. HIGH-RISK POPULATIONS AND SOCIOECONOMIC IMPACT

Lymphatic filariasis has mostly affected the tropical and subtropical regions. As such tropical and subtropical areas provide more favorable climatic circumstances for pests to spread the disease, and Lymphatic filariasis is likely to occur there more frequently (Ottesen 2006) Areas with more density of mosquitos are at more risk of Lymphatic filariasis transmission as the mosquitos transfer their contaminated maggots from one carrier to another person. Another reason could be thatun-hygienic sites and bad conditions of places can initiate the rookery for mosquitos that could be very favorable for the transmission of disease. Another big reason is when infected persons move from one place to another, they transmit disease to the healthy person another reason could be poverty. (Ramaiah et al. 2000).
LF can have different indirect effects on individuals' health and socioeconomic factors. For example, *Lymphatic filariasis* can affect different body parts like the limbs which cause them to be swollen and this eventually reduces the working abilities and activities of a person which effects overall community. As children with disease cannot attend schools because of their health and also because of the risk of transmitting the disease, so it largely affects their education and their future career (Shenoy et al. 2009). When the infected bread earner couldn't go outside for earning, the house income will be very low and this could lead towards poverty. Infected people will not be able to attain enough education which causes less social climbing and ultimately no growth of the society. LF will cause major effect on the industries. The people who are infected and have any disability or abnormal body part have a high possibility of being victimized and discriminated, they will separate themselves from other people shrinking their social circle and life (Mak 2007).

2.2. BURDEN OF DISEASE

The number of cases of Lymphatic filariasis vary from time to time because of the amount and type of treatments, betterment of monitoring of the disease and different other factors. It is an estimate that around one-twenty million population was infected due to this disease *Lymphatic filariasis* and about a billion people were endangered of being infected with the disease in 80 countries.(Edeson et al. 1960) It is thought that India was in the no.1 in the list of infected countries, followed by Nigeria which was on the no.2 with most infected LF patients. Other regions included, South America, Africa and Western Pacific sides. Mass Drug Administration is being applied in many regions of Ghana. (Gyapong et al. 1996).
Wuchereria bancrofti, Brugia malayi and *Brugia timori* are the three filariasis responsible for the disease Lymphatic filariasis. *Wuchereria bancrofti* caused about 90% of the LF cases and the second two filariasis caused around 10% of cases (de Souza et al. 2014).

2.3. DISABILITY-ADJUSTED LIFE YEARS (DALYs)

Is a measure used to estimate the life lost in deaths, any type of disability or in bad health conditions. As Lymphatic filariasis can lead to many health abnormalities like disabilities of body parts or specifically Elephantiasis, which reduces the life expectancy of individuals, losing their

DALY's. Different health policies and measures are changed as WHO monitors the risks of Lymphatic filariasis and evaluate their DALY's (Addiss et al. 2000).

3. PARASITE BIOLOGY AND LIFE CYCLE

3.1. TYPES OF FILARIAL PARASITES INVOLVED

The filarial parasites mentioned in lymphatic filariasisare mentioned below in Fig. 1. (Paily et al. 2009).

3.1.1. *WUCHERERIA BANCROFTI*

This is the most common and widespread filarial parasite responsible for causing lymphatic filariasis in humans. It is primarily found in tropical and subtropical regions of Africa, Asia, the Pacific Islands, and parts of the Americas.

3.1.2. *Brugia malayi*

Another important filarial parasite that causes lymphatic filariasis, primarily found in Southeast Asia and parts of the Western Pacific.

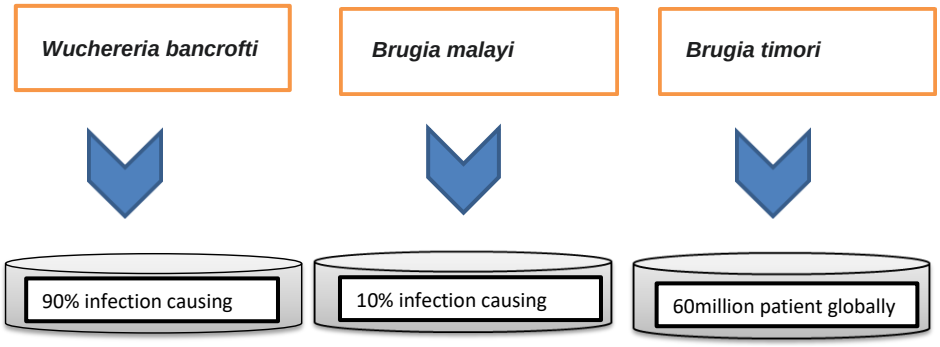

Fig. 1: Parasites responsible for Lymphatic Filariasis with their infection level

3.1.3. *Brugia timori*

Similar to *Brugia malayi*, this filarial parasite also causes lymphatic filariasis, primarily found in certain regions of Southeast Asia (Babu and Nutman 2014).

3.2. MORPHOLOGY AND LIFE STAGES OF THE PARASITES

The filarial parasites have a complex life cycle that involves multiple stages, both in the human host and the mosquito vector as mentioned in Fig. 2. (Cheng 1973). The key life stages of filarial parasites are as follows:

3.2.1. MICROFILARIAE

These are the first-stage larvae of the filarial parasites. *Microfilaria*e are tiny, elongated, and thread-like, measuring about 200 to 300 micrometers in length. They circulate in the bloodstream and lymphatic system of the infected human host (Famakinde 2018).

3.2.2. MOSQUITO STAGE

When an infected mosquito of the appropriate species feeds on a human, it ingests the microfilariae along with the blood.

3.2.3. L1 STAGE

Inside the mosquito, the microfilariae shed their sheaths and penetrate the mosquito's gut, becoming first-stage larvae or L1.

3.2.4. L2 STAGE

Over the course of about one to two weeks, the L1 larvae molt into the second-stage larvae or L2.

3.2.5. L3 STAGE

After further development (approximately one to two weeks), the L2 larvae molt into the infective third-stage larvae or L3. These L3 larvae migrate to the mosquito's proboscis (mouthparts), ready to be transmitted to the next human host.(Lawrence and Devaney 2001).

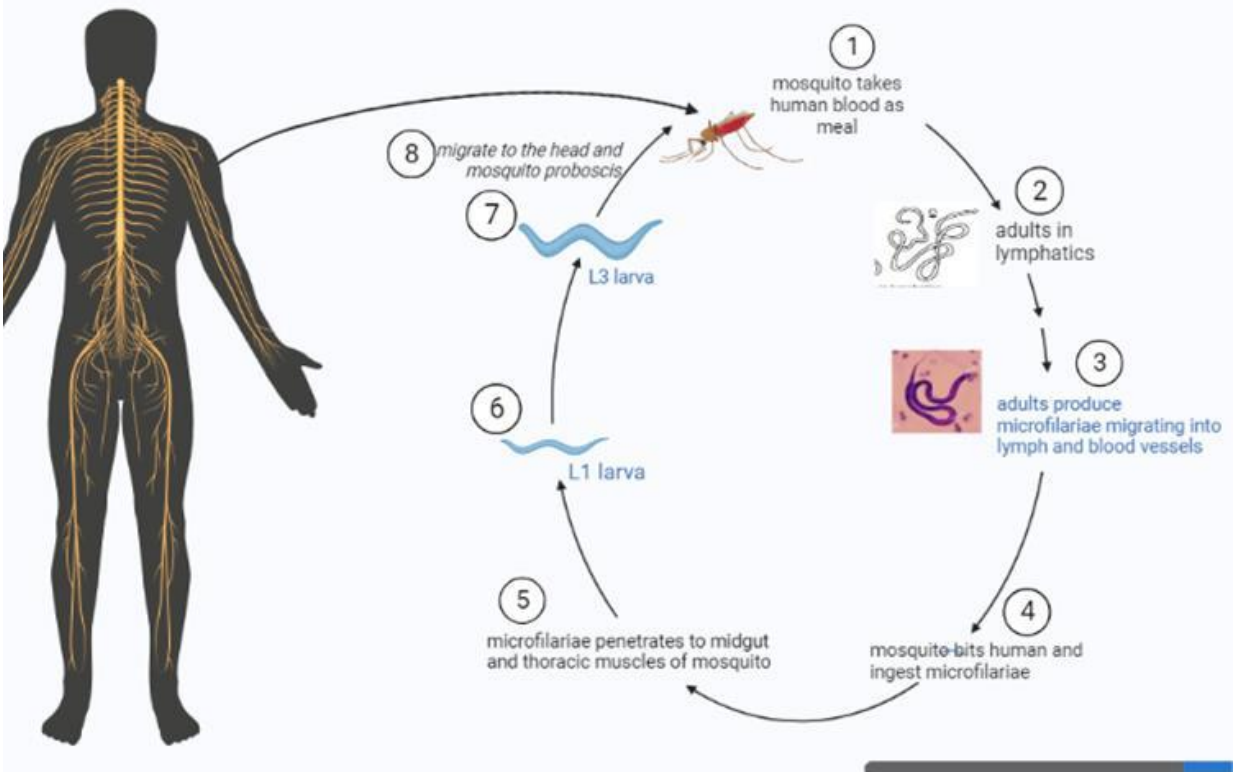

Fig. 2: Life stages of Filarial Worm CDC)

3.2.6. HUMAN STAGE

When an infected mosquito takes a blood meal from a human, it deposits the infective L3 larvae onto the skin.

3.2.7. L3 TO L4 MOLT

The L3 larvae penetrate the bite wound and enter the human host, where they continue to migrate through the subcutaneous tissues. Over several days, the L3 larvae molt into the fourth-stage larvae or L4.

3.2.8. L4 TO ADULT MOLT

The L4 larvae continue to migrate, typically reaching the lymphatic vessels and lymph nodes. There, they molt into immature adults and then further into sexually mature adult filarial worms (Roberts et al. 2009).

3.2.9. ADULT STAGE

The adult filarial worms reside in the lymphatic vessels and lymph nodes of the human host, where they mate and produce microfilariae, completing the life cycle (Wilke and Marrelli 2015).

3.3. TRANSMISSION MECHANISMS (MOSQUITO VECTORS) OF LYMPHATIC FILARIASIS

➢ Lymphatic filariasis is transmitted through the bites of infected mosquitoes, which act as vectors for the filarial parasites. The main mosquito species involved in the transmission of *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* are usually from the genera Culex, Anopheles, and Aedes.

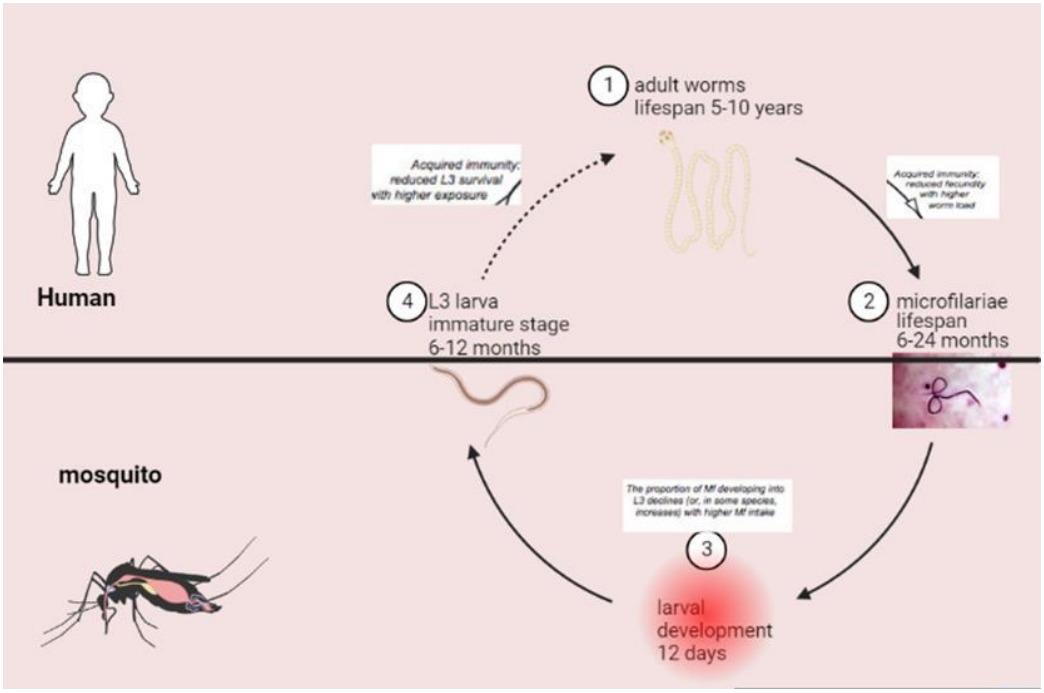

Fig. 3: Cycle of transmission for LF. It shows W. bancrofti life cycle. Microfilariae live in lymphatic system for 5-10 years. Female worms reproduce many MF in blood living for 6-24 months. L3 develops inside mosquitoes in 12 days and can cause infection in humans. While mosquito take other bite L3 is transmitted to the human body migrating to blood and lymphatic system. This immature period takes 6-12 months. And grows to mature adult worms.

- The transmission cycle begins when an infected mosquito takes a blood meal from a human already infected with adult filarial worms. During the blood meal, the mosquito ingests the microfilariae that circulate in the bloodstream and lymphatic system of the human host (Cheng 1973; Amuzu et al. 2010).
- Inside the mosquito, the microfilariae go through several larval stages (L1 to L3) and become infective third-stage larvae (L3) in the mosquito's proboscis. When the mosquito feeds again, it deposits these infective L3 larvae onto the skin of another human, usually during its nighttime feeding activities (Witt and Ottesen 2001).
- The L3 larvae penetrate the skin through the bite wound created by the mosquito and then migrate through the subcutaneous tissues. Eventually, they reach the lymphatic vessels and lymph nodes, where they mature into adult worms, continuing the cycle of infection (Day 1991).
- It is important to note that not all mosquito species are capable of transmitting filarial parasites as mentioned in Fig. 3, and the transmission dynamics can vary depending on the geographical region and specific mosquito vectors involved. Controlling the mosquito population and preventing mosquito bites are essential strategies for controlling and preventing lymphatic filariasis. Mass drug administration of antifilarial drugs to affected communities is also used to reduce the number of microfilariae in the human population, thereby decreasing the transmission potential (Stolk et al. 2015).

3.4. SIGNALING PATHWAY OF LYMPHATIC FILARIASIS

Lymphatic filariasis, commonly known as elephantiasis, is a parasitic disease caused by thread-like worms called filarial nematodes, mainly *Wuchereria bancrofti, Brugia malayi*, and B*rugia timori*. These worms are transmitted to humans through the bites of infected mosquitoes.
The signaling pathways involved in lymphatic filariasis primarily revolve around the host's immune response to the presence of the filarial worms and their antigens. When the mosquito bites and deposits the infective larvae into the human host, they migrate through the lymphatic vessels and develop into adult worms that live within the lymphatic system.
Here is an overview of the signaling pathway and immune response in lymphatic filariasis:

3.4.1. RECOGNITION OF PARASITE ANTIGENS

The immune response begins with the recognition of filarial antigens by the host's innate immune system. These antigens can be derived from the parasites themselves or released from their dead or dying cells.

3.4.2. ACTIVATION OF INNATE IMMUNE CELLS

Dendritic cells, macrophages, and other antigen-presenting cells (APCs) are crucial in detecting and capturing the filarial antigens. Once they engulf the antigens, they process and present them on their cell surfaces using major histocompatibility complex (MHC) molecules (Sreenivas et al. 2017).

3.4.3. ANTIGEN PRESENTATION AND T CELL ACTIVATION

The APCs then migrate to lymph nodes where they present the filarial antigens to T cells. This presentation activates CD4+ T helper cells, which play a central role in orchestrating the immune response.

3.4.4. TH1 AND TH2 RESPONSE

Lymphatic filariasis triggers a complex balance between two types of T helper cells: Th1 and Th2. Th1 cells produce pro-inflammatory cytokines like interferon-gamma (IFN-gamma) that activate

macrophages to kill parasites. Th2 cells produce anti-inflammatory cytokines like interleukin-4 (IL-4) and interleukin-13 (IL-13) that stimulate B cells to produce antibodies (Babu et al. 2005).

3.4.5. ANTIBODY PRODUCTION

The activation of Th2 cells leads to the production of specific antibodies, particularly IgG4 and IgE, against filarial antigens. These antibodies may not directly kill the parasites but are believed to facilitate their elimination by other immune cells.

3.4.6. GRANULOMA FORMATION

Chronic infection with filarial worms can lead to the formation of granulomas, which are aggregates of immune cells around the parasites. Granulomas are an attempt by the immune system to contain the infection and limit its spread (Chakraborty et al. 2013).

3.4.7. REGULATORY T CELLS (TREGS) AND IMMUNE SUPPRESSION

The filarial parasites have developed strategies to evade the host's immune response. They induce the production of regulatory T cells (Tregs) that help dampen the immune response and create an immunosuppressive environment, allowing the parasites to persist (Babu et al. 2006).

3.4.8. LYMPHATIC DAMAGE AND ELEPHANTIASIS

Long-term infection and chronic inflammation can cause damage to the lymphatic vessels. This damage, along with the accumulation of fluid and immune cells, can lead to the development of lymphedema, swelling of limbs, and in severe cases, elephantiasis.
It's important to note that the immune response in lymphatic filariasis is complex and varies from person to person. Some individuals may be more susceptible to severe disease, while others may have a milder course of infection. Additionally, treatments for lymphatic filariasis often focus on antiparasitic medications and managing the associated symptoms such as lymphedema (Edeson et al. 1960).

4. CLINICAL MANIFESTATIONS

Elephantiasis, or Lymphatic filariasis, is a parasitic condition brought on by an infection with filarial parasites, which are worm-like parasites. The primary culprits behind the disease are three species of worms: *Brugia timori*, *Wuchereria bancrofti*, and *Malaysian brugia*. Humans contract the illness from mosquito bites that have been contaminated (Dreyer et al. 2000).
Once the parasites enter the human body through mosquito bites, they migrate to the lymphatic system, which is in charge of preserving fluid balance and warding off infections. In the lymphatic system, the adult worms produce microfilariae, which are tiny, immature forms of the parasites. The microfilariae then circulate in the bloodstream and are taken up by mosquitoes when they bite an infected person, completing the life cycle (Rajan and Gundlapalli 1997).
Lymphatic filariasis can manifest in different ways, depending on the stage of the infection. In the early stages, most individuals remain asymptomatic, but they still contribute to the transmission of the disease (WHO 2013).

4.1. ASYMPTOMATIC PHASE

Many infected individuals do not show any immediate symptoms. This phase can last for years, during which microfilariae can be found in the blood when tested (Mak 2012).

4.2. ACUTE PHASE

In some cases, people may experience acute attacks of lymphangitis, which is inflammation of the lymphatic vessels. This can cause symptoms such as fever, pain, and swelling in the affected limbs or body parts.

4.3. CHRONIC PHASE

Over time, chronic inflammation of the lymphatic vessels and lymph nodes can occur. This leads to the characteristic symptoms of lymphatic filariasis, (Partono 2007) such as:

4.4. LYMPHEDEMA

Swelling of limbs (usually the legs or, less commonly, the arms) due to the accumulation of fluid and blocked lymphatic vessels.

4.5. ELEPHANTIASIS

A severe form of lymphedema where the affected limbs or body parts become extremely enlarged and thickened, resembling an elephant's skin.

4.6. HYDROCELE

Fluid accumulation in the scrotum (in males) or labia major (in females) due to lymphatic obstruction.

4.7. PREVENTION AND TREATMENT

Lymphatic filariasis prevention involves controlling mosquito populations, using insecticide-treated bed nets and indoor residual spraying and using mass drug administration programs to provide antifilarial medications to at-risk populations.
Lymphatic filariasis treatment focuses on managing symptoms and preventing complications. Anti-inflammatory medications are used for acute attacks, while physical therapy, compression bandaging, and hygiene manage swelling and prevent secondary infections.
Early diagnosis and intervention are crucial for preventing lymphatic filariasis progression to chronic stages. Elimination requires preventative measures and targeted treatment programs in endemic regions.

4.8. CLINICAL MANIFESTATIONS

4.8.1. ASYMPTOMATIC STAGE

In many cases, individuals infected with lymphatic filariasis may remain asymptomatic for years or even decades. During this stage, the parasites reside in the lymphatic vessels and nodes without causing noticeable symptoms. However, even in the absence of symptoms, the parasites can still cause damage to the lymphatic system, leading to chronic inflammation and scarring (Paily et al. 2009).

4.8.2. ACUTE STAGE

Some people may experience acute episodes of inflammation known as acute adenolymphangitis (ADL). These episodes are characterized by sudden and painful swelling of the affected limb(s) (Fig. 4). ADL can be triggered by various factors, including secondary bacterial infections. The frequency of ADL episodes tends to increase over time (Dixit et al. 2007).

Lymphadenitis, Lymphangitis, and Lymphatic Filariasis are related conditions involving the lymphatic system. Let's explore each of them in detail:

4.9. LYMPHADENITIS

Lymphadenitis refers to the inflammation of the lymphatic system is filled with tiny, bean-shaped structures called lymph nodes. A vital role for lymph nodes in filtering lymph fluid, trapping harmful microorganisms and mounting an immune response to infections as shown in Fig. 5. (Mohapatra and Janmeja 2009).

4.9.1. CAUSES

Lymphadenitis is often caused by a bacterial or viral infection. Common pathogens include Staphylococcus aureus and Streptococcus pyogenes. The infection can occur when bacteria or viruses enter the body through wounds, bites, or other openings.(Colovic et al. 2008).

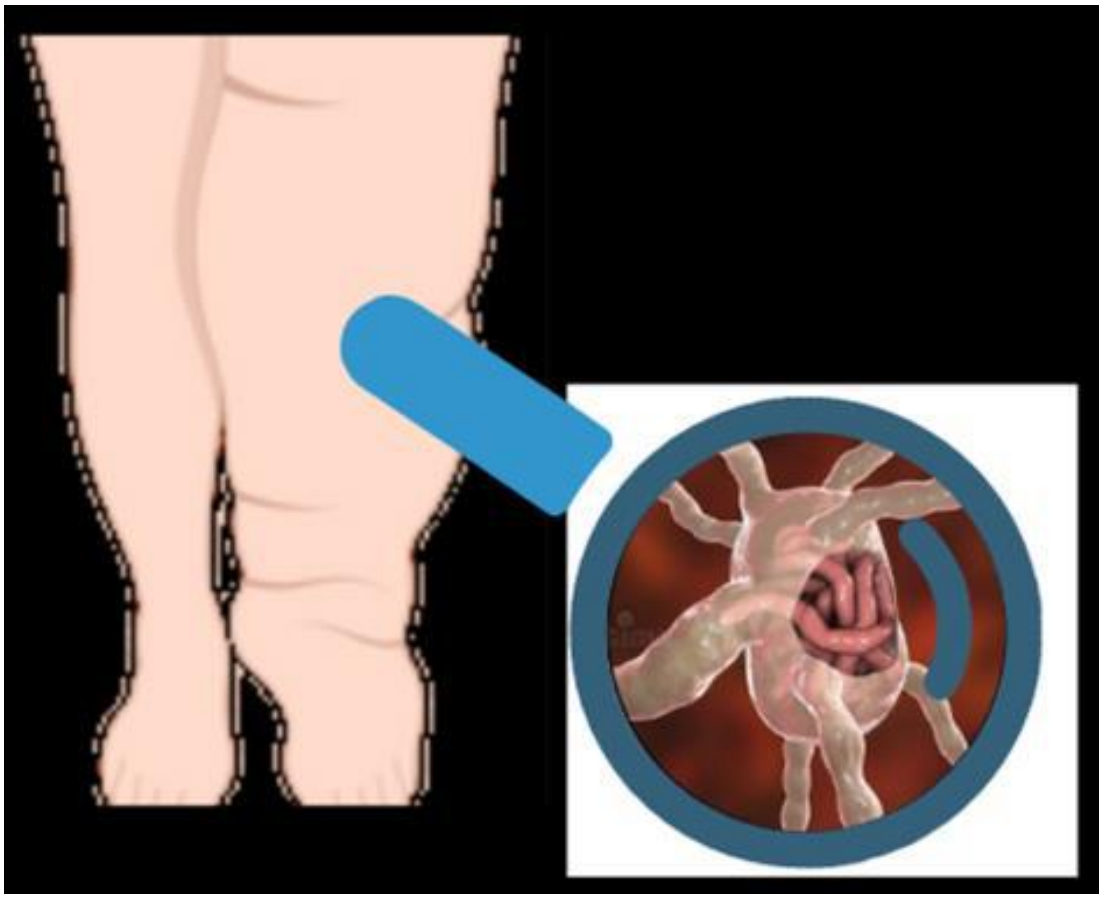

Fig. 4: Microscopic view of a person's leg with elephantiasis.3D view blockage of lymph node with filariasis worms.

4.9.2. SYMPTOMS

The affected lymph nodes become swollen, tender, and may feel warm to the touch. In some cases, the overlying skin might appear red or inflamed. Depending on the severity of the infection, one may experience fever, chills, and general malaise.

4.9.3. TREATMENT

Treatment typically involves antibiotics to address the underlying infection. Rest, pain relief medication, and warm compresses may also be recommended to alleviate symptoms (Weiler et al. 2000).

4.10. LYMPHANGITIS

Lymphangitis refers to the inflammation of the lymphatic vessels that transport lymph fluid from tissues to lymph nodes. It often develops as a complication of a skin infection, most commonly caused by Streptococcus or Staphylococcus bacteria.(Bruce et al. 1996).

4.10.1. CAUSES

The condition usually arises when bacteria enter the body through a wound or skin infection, spreading through the lymphatic vessels to the nearby lymph nodes.

4.10.2. SYMPTOMS

Lymphangitis is characterized by red streaks extending from the site of the wound or infection towards the nearest lymph nodes. The affected area may be swollen, warm, and painful. Other symptoms such as fever and chills may also be present (Klimek 2019).

4.10.3. TREATMENT

Antibiotics are the primary treatment for lymphangitis. Elevating the affected limb, rest, and warm compresses can help alleviate symptoms and promote recovery (Olszewski 2019).

4.10.4. CHRONIC STAGE

Over time, repeated episodes of ADL and ongoing inflammation lead to chronic lymphatic obstruction. This condition is the hallmark of lymphatic filariasis and is responsible for the most visible and disabling symptoms associated with the disease.

4.10.5. LYMPHEDEMA

Chronic lymphatic obstruction causes a progressive buildup of fluid (lymph) in the affected limbs, leading to lymphedema. Lymphedema primarily affects the legs but can also occur in the arms, breasts, and genitals (Warren et al. 2007). The affected limb becomes swollen, heavy, and may exhibit skin changes, such as thickening and hardening (fibrosis). This chronic swelling can lead to permanent disability and disfigurement, resulting in the classic "elephantiasis" appearance as mentioned in Fig. 4 and 6. (Rockson 2001).

4.10.6. ELEPHANTIASIS

In severe cases of chronic lymphedema, the affected body parts can become grossly enlarged and thickened, giving rise to the term “elephantiasis.” This condition most commonly affects the legs and genitalia and can result in significant physical and psychological distress for the affected individuals (Sisto and Khachemoune 2008).

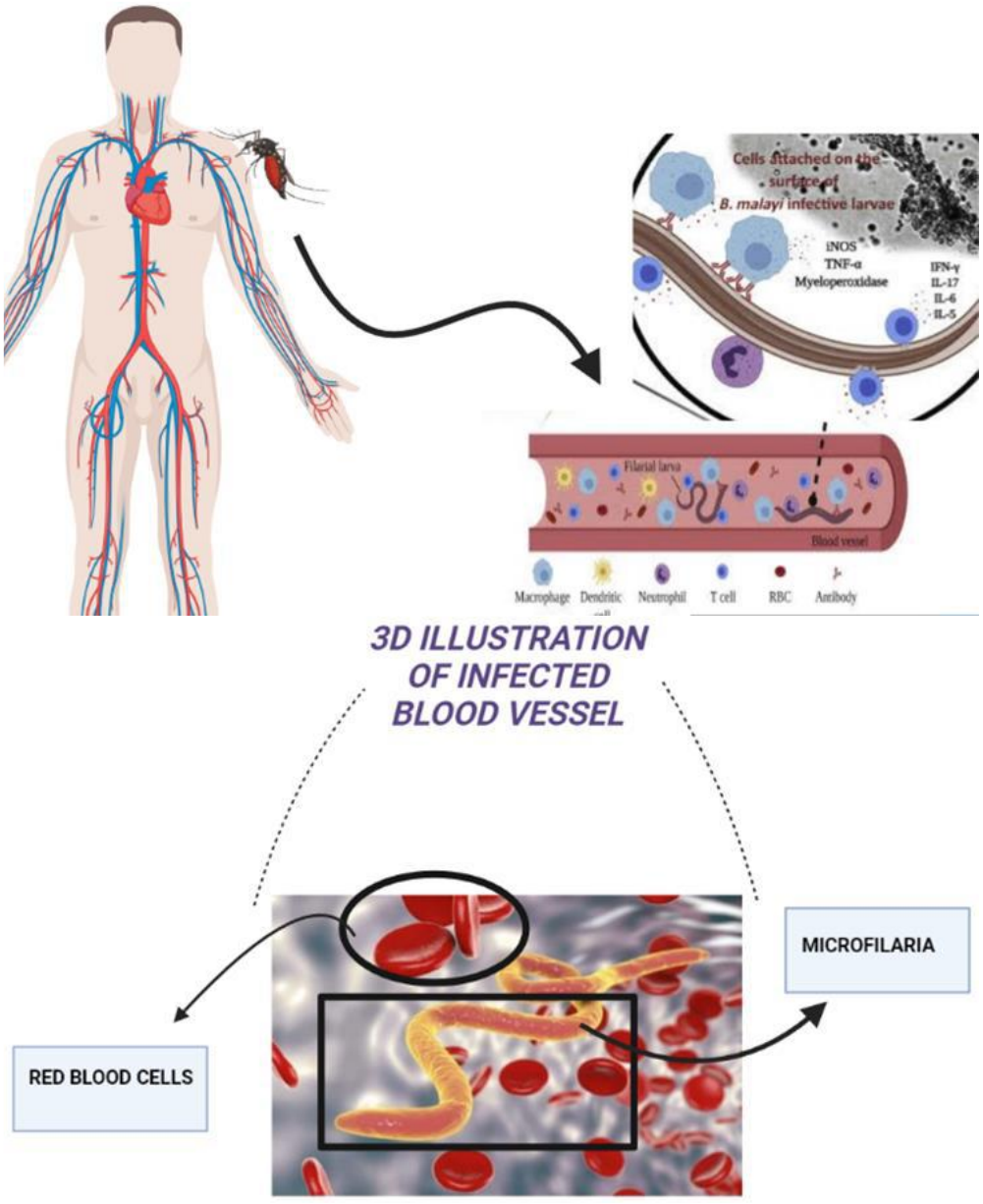

Fig. 5: Illustration of lymphadenitis. Microfilariae, nematodes parasites in blood vessel causing Lymphatic filariasis.

4.10.7. HYDROCELE

In males, lymphatic filariasis can lead to the accumulation of fluid around the testicles, causing swelling known as hydrocele. The hydrocele can become quite large and cause discomfort (Dandapat et al. 1990).

4.10.8. UNDERSTANDING TROPICAL PULMONARY EOSINOPHILIA: CAUSES, SYMPTOMS, AND TREATMENT

Tropical pulmonary eosinophilia (TPE) is a condition caused by the immune response to microfilariae as shown in Fig. 7 , which are tiny worm larvae transmitted through the bites of infected mosquitoes in areas with endemic lymphatic filariasis (Mullerpattan et al. 2013). When

these microfilariae migrate to the lungs, they trigger an exaggerated immune reaction, leading to the accumulation of eosinophils, a type of white blood cell, in the lung tissues. Immune response triggers cough, wheezing, shortness of breath, and night fevers (Ottesen and Nutman 1992). TPE is a distinct clinical manifestation of lymphatic filariasis, characterized by elevated blood eosinophil levels and specific antibodies. Timely diagnosis and treatment with antifilarial drugs, such as diethylcarbamazine, can effectively manage TPE, preventing further complications and helping to control the spread of lymphatic filariasis in affected regions (Vijayan 2007).

4.10.9. LYMPHATIC FILARIASIS OF THE BREAST

In some cases, the lymphatic system of the breast may be affected, leading to swelling and enlargement of the breast.
It's essential to note that the severity of lymphatic filariasis can vary widely between individuals. Some may experience mild symptoms or remain asymptomatic, while others may develop severe and debilitating manifestations (Thongpiya et al. 2021).

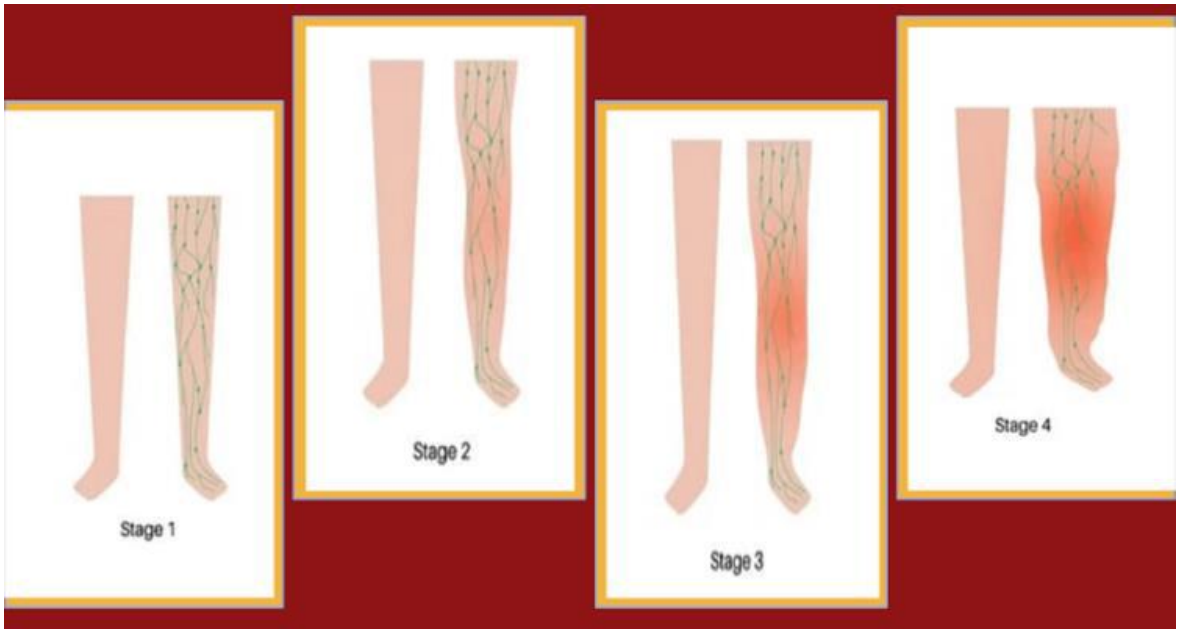

Fig. 6: Stages of Lymphedema Disease (Created by Biorender)

4.11. PREVENTION AND TREATMENT

Preventive measures primarily involve mosquito control, such as using insecticide-treated bed nets and mosquito repellents, and taking measures to reduce mosquito breeding sites (Dayal and Selvaraju 2010).
Mass drug administration with antifilarial drugs (such as diethylcarbamazine, ivermectin, and albendazole) is used to treat infected individuals and prevent the spread of the disease in endemic areas. For those already affected by the disease, managing and alleviating symptoms through good hygiene, elevation of affected limbs, compression bandaging, and exercises may help improve their quality of life. In severe cases, surgical intervention may be considered to reduce limb swelling and manage complications. Early diagnosis and timely intervention are crucial in preventing long-term disabilities associated with lymphatic filariasis (Chandy et al. 2011).

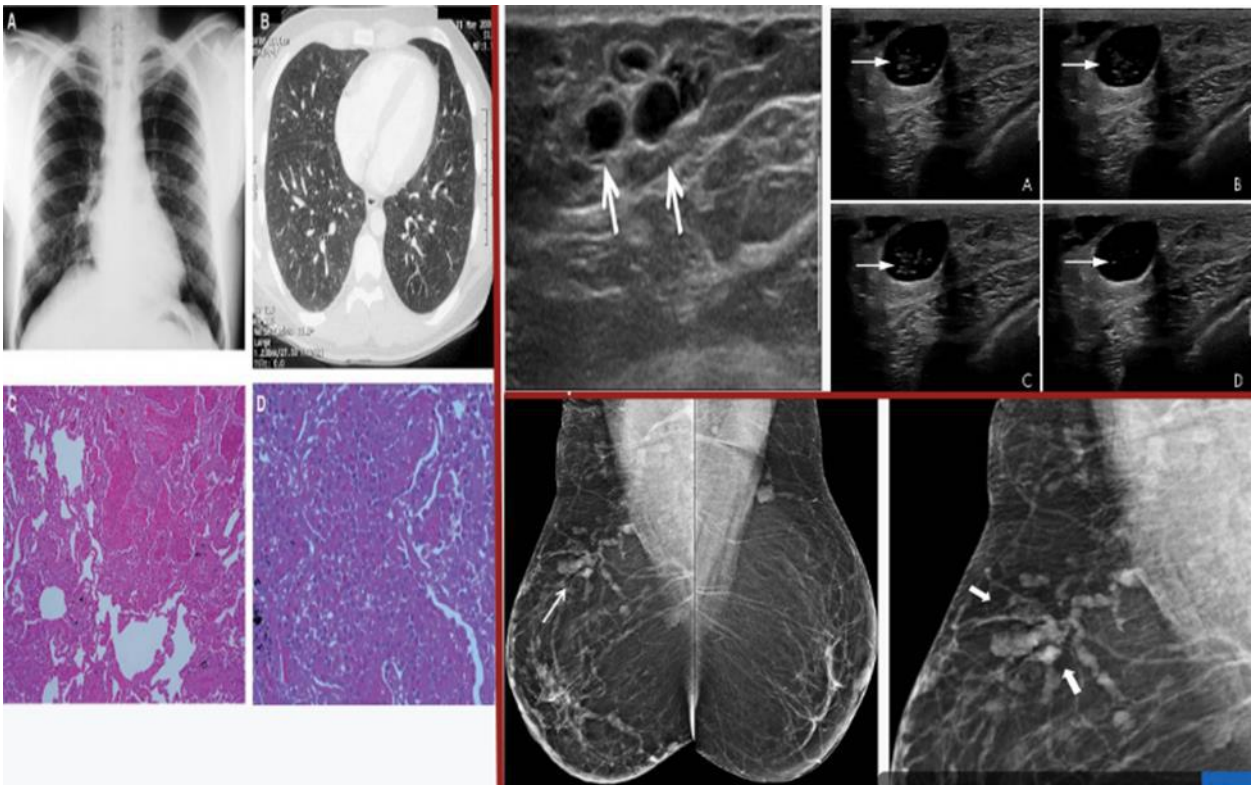

Fig. 7: Tropical pulmonary eosinophilia. Chest X-ray shows nodules, pattern of bilateral micronodule. Lung specimen with eosinophilic infiltration of alveolar sacs.

5. CONCLUSION

Parasite infections often persist despite attempts to completely eliminate them, as doing so might require harmful immune reactions from the host. Consequently, the manifestation of disease in many parasitic infections is often linked with immune-related damage. The most effective host response involves maintaining a balance in controlling the parasite within tolerable levels, thereby preserving immune equilibrium without causing permanent harm to tissues. Filarial infections exemplify the delicate interplay between hosts and parasites, where a harmonious immune-parasite equilibrium is occasionally disrupted, leading to severe consequences due to overwhelming host immune reactions.

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Overview of Rift Valley Fever

07

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ABSTRACT

Emerging viral diseases pose significant threats to global health and economies, exemplified by recent epidemics of Ebola and Zika viruses. Rift Valley Fever (RVF), caused by the Rift Valley fever virus (RVFV), is a zoonotic disease primarily affecting domestic ruminants and camels. Originating in Kenya in 1931, RVF has a broad geographic distribution, with endemicity in sub-Saharan Africa and occasional outbreaks in the Arabian Peninsula and beyond. The virus is transmitted through mosquitoes, with zoonotic transmission occurring through direct contact with infected animals or their products. Mosquito control, hygiene practices, and personal protective measures are crucial for mitigating zoonotic transmission risks. RVF outbreaks coincide with heavy rainfall and flooding, impacting livestock, trade, and human health. Risk factors include mosquito exposure, direct contact with infected animals, handling infected tissues, and consuming contaminated animal products. The pathogenesis involves entry, replication, systemic spread, immune response, hepatic involvement, hemorrhagic manifestations, neurological complications, and fetal complications in pregnant women. Clinical manifestations range from mild flu-like symptoms to severe hemorrhagic fever and neurological complications. Diagnosis involves serological and molecular tests, with vaccination, vector control, and surveillance essential for prevention and control. Future research priorities include vaccine development, enhanced surveillance, innovations in vector control, climate change impact studies, and antiviral therapies. Collaborative efforts, incorporating the One Health approach, are critical to addressing RVF challenges comprehensively. Continued investment in research and development is essential for advancing knowledge, improving prevention strategies, and minimizing the socioeconomic consequences of RVF outbreaks, safeguarding public health and animal populations.

Keywords: Rift Valley Fever (RVF), Zoonotic Transmission, Epidemiology, Prevention, Research Priorities

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1. INTRODUCTION

Rift Valley Fever (RVF) is a viral disease primarily affecting domestic ruminants and camels, commonly seen in sub-Saharan Africa (Søren et al. 2020). The disease is caused by the Rift Valley fever virus (RVFV), transmitted by mosquitoes and blood-feeding flies. While the virus primarily affects animals, it also can infect humans (Małgorzata et al. 2021). Human infections can result from contact with the blood or organs of infected animals or from the bite of infected mosquitoes. The disease in humans can range from a mild flu-like illness to severe hemorrhagic fever, which can be lethal. Most people with RVF have either no symptoms or a mild disease, but a small percentage can develop more severe symptoms. Human-to-human transmission of RVF has not been documented (Daniel et al. 2019).

The first discovery of RVF was in sheep in the Rift Valley province of Kenya in 1931. Since then, outbreaks have been reported in sub-Saharan Africa and North Africa. The disease has also been reported outside Africa, with the first cases reported in Saudi Arabia and Yemen in 2000 (Mohamed et al. 2019).

The outbreaks of RVF have demonstrated the potential for these diseases to impact both animal health and the global economy negatively. For example, following small sporadic outbreaks of RVF, a widespread epidemic occurred in South Africa in 2010 and 2011, leading to significant economic losses due to high mortality rates in young animals and waves of abortions in pregnant females (Daniel et al. 2019). The outbreaks of other emerging viral diseases, such as the Ebola virus in 2014-15, 2018, and 2020, and the Zika virus in 2015-16, have also demonstrated the potential for these diseases to negatively impact both human health and the global economy in unpredictable ways (Safder et al. 2021).

2. ZOONOTIC NATURE OF RIFT VALLEY FEVER

The primary means by which the RVF virus is transmitted to ruminants is through mosquito bites, particularly those from infected mosquitoes of the Aedes and Culex genera (Alex et al., 2022). During outbreaks, most cases result from mosquitoes becoming infected after feeding animals the virus and

transmitting it to others during subsequent blood meals. This mosquito-borne transmission is the primary driver of RVF infections (Yusuf et al., 2020). However, other pathways of zoonotic transmission have also been observed. Direct contact with infected animal tissues, such as handling infected carcasses, blood, or other fluids, can transmit the infection to humans (Fabian et al., 2022). Certain occupations, including livestock farming, veterinary care, and meat processing, increase the risk of zoonotic RVF transmission due to regular exposure to infected animals or their products (Elysse et al., 2019).

Numerous studies have highlighted the zoonotic potential of RVFV (Keli et al. 2022). Serological surveys conducted in regions where the disease is endemic have identified RVFV-specific antibodies in individuals involved in animal husbandry, indicating past exposure to the virus (Caroline et al. 2020). Case reports and epidemiological investigations have also linked human infections to direct contact with infected animals or their products (Jonathan et al. 2022).

To mitigate the risk of zoonotic transmission, it is crucial to implement preventive measures and raise awareness about RVF. Mosquito control strategies, such as applying insecticides and eliminating mosquito breeding sites, are essential for reducing the mosquito population. Promoting good hygiene practices, including safe handling and proper disposal of animal carcasses, and implementing personal protective measures for individuals at high occupational risk can help minimize the chances of zoonotic RVF transmission (Mehmood et al. 2021).

3. EPIDEMIOLOGY

3.1. GEOGRAPHIC DISTRIBUTION OF RIFT VALLEY FEVER

Rift Valley Fever (RVF) primarily occurs in sub-Saharan Africa as an endemic disease, with occasional outbreaks documented in other parts of the world (Keli NG et al. 2022). The geographical distribution of RVF can be summarised as follows:

3.2. SUB-SAHARAN AFRICA

RVF is endemic in several countries across sub-Saharan Africa, including Kenya, Tanzania, Uganda, Sudan, South Sudan, Somalia, Ethiopia, Madagascar, and Senegal. These countries have experienced recurring outbreaks of varying magnitudes. The prevalence of RVF in this region is closely associated with ecological factors, such as rainfall patterns and competent mosquito vectors (Earl et al. 2022) (Getahulivestock et al. 2022).

3.3. ARABIAN PENINSULA

RVF outbreaks have been reported in the Arabian Peninsula, specifically in Saudi Arabia and Yemen. The first documented outbreak occurred in Saudi Arabia in 2000, followed by animal and human outbreaks. The climate and landscape of the Arabian Peninsula provide conducive conditions for RVFV transmission by mosquito vectors (Mutaz 2019).

3.4. OUTBREAKS BEYOND AFRICA AND THE ARABIAN PENINSULA

Although RVF is primarily concentrated in Africa and the Arabian Peninsula, isolated cases and outbreaks have been reported outside these regions. These occurrences are often linked to travel or the movement of infected animals or animal products. For instance, RVF outbreaks have occurred in Egypt, Mauritania,

and Niger, where infected livestock or animal products were imported from endemic areas (Yahya et al. 2022).

4. INCIDENCE AND PREVALENCE

4.1. INCIDENCE OF RIFT VALLEY FEVER

The occurrence of RVF cases is primarily associated with the circulation of the virus among animal reservoirs. Mosquitoes, particularly those of the Aedes and Culex species, are crucial in transmitting the virus between animals and humans. RVF outbreaks often coincide with periods of heavy rainfall and flooding, which facilitate mosquito breeding and the subsequent spread of the disease (Wanjama et al. 2022).

4.2. PREVALENCE OF RIFT VALLEY FEVER

RVF prevalence varies based on geographical location, environmental factors, and the presence of suitable vectors and animal reservoirs. The disease primarily affects domestic livestock, particularly cattle, sheep, and goats, leading to high mortality rates among newborn animals and causing abortions in pregnant animals. RVF outbreaks have severe economic consequences, resulting in livestock losses, reduced milk production, and disruptions in livestock product trade (Cynthia et al. 2021).
Human prevalence of RVF is closely linked to occupational exposure to infected animals or their products. Individuals at high risk include veterinarians, farmers, slaughterhouse workers, and laboratory personnel (Keli et al. 2023).

5. RISK FACTORS

The key factors that contribute to the transmission of the virus.

5.1. EXPOSURE TO MOSQUITOS

Mosquitoes, particularly species like Aedes and Culex, play a crucial role in transmitting Rift Valley Fever. Individuals living or working in areas with high mosquito populations face an elevated risk of contracting the virus. This is particularly true for those involved in agriculture, livestock farming, or regions with inadequate mosquito control measures (Andrea et al. 2020).

5.2. DIRECT CONTACT WITH INFECTED ANIMALS]

Direct contact with infected animals, especially during birthing or slaughtering, poses a significant risk of RVFV transmission (Abdala et al. 2020).

5.3. HANDLING INFECTED ANIMAL'S TISSUES AND BLOOD

Handling infected animal tissues, blood, or other biological fluids can expose individuals to the Rift Valley Fever virus. This risk is especially relevant in slaughterhouses, where workers may encounter contaminated materials. Infection can occur through cuts, abrasions, or mucous membranes (Elysse et al. 2019).

5.4. CONSUMPTION OF CONTAMINATED ANIMAL PRODUCTS

Consuming raw or undercooked meat, milk, or other animal products from infected animals increases the risk of RVFV transmission. Thorough cooking of meat and pasteurization of milk is essential to eliminate the virus and reduce the risk of infection. Lack of awareness about the disease and its transmission routes contributes to consuming contaminated animal products (Mohamed et al. 2019).

5.5. OCCUPATIONAL EXPOSURE

Certain occupations, such as veterinarians, healthcare workers, laboratory personnel, and researchers studying the virus, face an elevated risk of acquiring Rift Valley Fever. These individuals are more likely to contact infected animals or handle virus specimens. Adherence to biosafety protocols and using personal protective equipment (PPE) is critical in mitigating this risk (Veerle et al. 2019).

5.6. CLIMATE AND ENVIRONMENTAL FACTORS

Environmental conditions and climate significantly influence the prevalence and transmission of Rift Valley Fever. Heavy rainfall followed by periods of drought creates favorable breeding sites for mosquitoes, leading to increased transmission. Floods can also displace animals, contributing to the spread of the virus. Climate change and environmental disruptions can potentially expand the geographic range of RVFV, thereby increasing the risk of transmission to new areas (Dan et al. 2023).

6. PATHOGENESIS

The pathogenesis of RVF involves a series of events following infection with the RVFV. Here is an explanation of the pathogenesis of RVF:

6.1. ENTRY AND INITIAL REPLICATION

RVFV enters the body through mosquito bite or contact with infected animals or their tissues. The virus primarily targets and infects cells of the liver, spleen, and lymph nodes. The virus replicates in these cells upon entry, producing new viral particles (Lieza et al. 2021).

6.2. SYSTEMIC SPREAD

Once the virus replicates, it can disseminate throughout the body via the bloodstream. RVFV can infect various organs and tissues, including the liver, spleen, kidneys, and central nervous system (CNS). This systemic spread contributes to the diverse clinical manifestations observed in RVF (Lukas et al. 2022).

6.3. IMMUNE RESPONSE

The immune response plays a critical role in the pathogenesis of RVF. The virus triggers the activation of immune cells, including macrophages, dendritic cells, and lymphocytes, producing pro-inflammatory cytokines and chemokines. The immune response aims to control viral replication, but excessive or

dysregulated immune activation can contribute to tissue damage and disease severity (Ashgan et al. 2020).

6.4. HEPATIC INVOLVEMENT

The liver is a significant target of RVFV infection, and liver damage is a characteristic feature of RVF. Hepatocytes, the primary cells of the liver, become infected and undergo cell death, leading to inflammation and disruption of liver function. This can manifest as elevated liver enzymes, jaundice, and hepatomegaly (Muqadas AS et al. 2023).

6.5. HEMORRHAGIC MANIFESTATIONS

RVFV infection can cause hemorrhagic manifestations in some cases. The virus can damage blood vessels, leading to bleeding tendencies. This can result in hemorrhagic diathesis, petechiae (small red or purple spots on the skin), and more severe bleeding complications (Trevor et al. 2019).

6.6. NEUROLOGICAL INVOLVEMENT

In severe cases, RVFV can invade the central nervous system (CNS), leading to neurological complications. Meningitis, encephalitis, and meningoencephalitis can occur, causing symptoms such as headache, confusion, seizures, and coma (Tasneem et al. 2021).

6.7. PREGNANT WOMEN AND FETAL COMPLICATIONS

Pregnant women are particularly vulnerable to RVFV infection. The virus can cross the placental barrier and cause severe fetal complications, including fetal malformations, stillbirths, and neonatal deaths (Cynthia et al. 2021).

7. CLINICAL MANIFESTATIONS OF VALLEY FEVER

The clinical manifestations commonly associated with Rift Valley fever in humans:

7.1. MILD SYMPTOMS

Many cases of RVF present as a mild illness resembling the flu. Symptoms can include fever, headache, muscle and joint pain, weakness, fatigue, and loss of appetite (Daniel et al. 2023).

7.2. HEMORRHAGIC FEVER

Severe cases can progress to hemorrhagic fever, characterized by bleeding tendencies. This may include bleeding from the gums, nose, or other mucous membranes, as well as the presence of small red or purple spots (petechiae) or more extensive bruises (ecchymoses) on the skin (Compton et al. 2020).

7.3. OCULAR MANIFESTATIONS

Rift Valley fever can occasionally affect the eyes, leading to symptoms such as redness, inflammation, and impaired vision (Madeline et al. 2022).

7.4. HEPATIC INVOLVEMENT

The virus can cause inflammation of the liver (hepatitis), resulting in elevated liver enzymes, jaundice (yellowing of the skin and eyes), and abdominal pain (Leanne et al. 2022).

7.5. NEUROLOGICAL COMPLICATIONS

Rarely, RVF can lead to neurological manifestations, including inflammation of the brain (encephalitis) and the membranes surrounding the brain and spinal cord (meningitis). These complications can cause confusion, seizures, coma, and potential long-term neurological issues (Dominique et al. 2020).

8. DIAGNOSIS AND MANAGEMENT

8.1. DIAGNOSTIC TESTS FOR RIFT VALLEY FEVER

The RVF diagnosis involves clinical evaluation, laboratory tests, and consideration of epidemiological information (Christelle et al. 2019). Several diagnostic tests are commonly used for Rift Valley fever.

8.2. SEROLOGICAL TESTS

These tests detect antibodies the immune system produces in response to (RVFV) infection. Examples include enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA). Serological tests can indicate whether a person has been exposed to the virus, but they may not be effective during the early stages of infection (Baratang et al. 2019).

8.3. MOLECULAR TESTS

Polymerase chain reaction (PCR) tests are used to detect the genetic material (RNA) of RVFV. PCR tests can identify the virus in blood, tissues, or other samples, providing a definitive diagnosis. These tests are susceptible and specific, enabling early virus detection before symptoms appear (Changwoo et al. 2023).

8.4. VIRAL ISOLATION

Rift Valley fever virus can be isolated from blood or other body fluids in specialized laboratories. This involves growing the virus in cell cultures or using specific laboratory animals. Viral isolation is a time-consuming process that requires advanced biosafety facilities (Elisa et al. 2020).

- LFIA chromatographic tests have been explored as a potential tool for rapid and point-of-care diagnosis of diseases. These tests typically detect specific antigens or antibodies related to the disease in patient samples, such as blood or serum. The LFIA format is well-suited for use in resource-limited settings and field conditions where quick results are essential (Sören et al. 2020).
- Enzyme-linked immunosorbent assays (ELISAs) and immunohistochemistry (IHC) tests can detect viral antigens in clinical samples. Although less commonly used than serological and molecular methods, they can be valuable in certain situations (Lieza et al. 2020).

8.5. EPIDEMIOLOGICAL INFORMATION

In regions where RVF is prevalent, typical clinical symptoms, combined with a history of exposure to infected animals or mosquito bites, can raise suspicion of the disease. Epidemiological data, such as local outbreaks or reports of RVF in animals, can support the diagnosis (Franziska et al. 2022).

9. PREVENTION AND CONTROL

9.1. MANAGEMENT OF RIFT VALLEY FEVER

Managing RVF involves several critical supportive care, prevention, and control components. Here are the main aspects of RVF management.

9.2. SUPPORTIVE CARE

Patients with RVF receive supportive care to relieve symptoms and promote recovery. This includes rest, proper hydration, and over-the-counter medications like acetaminophen (paracetamol) to manage fever and pain. In severe cases, hospitalization and close monitoring may be necessary (Elizabeth et al. 2022).

9.3. MANAGEMENT OF COMPLICATIONS

Specific interventions may be required if RVF progresses to severe manifestations such as hemorrhagic fever or encephalitis. These can include blood transfusions for managing bleeding, respiratory support for severe breathing difficulties, and anticonvulsant medications for seizures associated with neurological complications (Leanne et al. 2022).

9.4. PREVENTION OF SECONDARY INFECTIONS

RVF can weaken the immune system, making patients more vulnerable to secondary bacterial or fungal infections. Appropriate antibiotics or antifungal therapy may be administered if such infections occur (Mathilde et al. 2023).

9.5. VECTOR CONTROL

To prevent the transmission of RVF to humans, controlling the mosquito population is crucial. This involves insecticide spraying, using bed nets, and eliminating mosquito breeding sites like stagnant water sources (Lotty et al. 2019).

9.6. ANIMAL VACCINATION AND CONTROL

Vaccination of susceptible animals such as sheep, goats, and cattle can be implemented to reduce the risk of transmission. During outbreaks, animal movement restrictions and quarantines may also be enforced (Edna et al. 2019).

9.7. PERSONAL PROTECTIVE MEASURES (PPM)

Individuals residing in or visiting RVF-endemic areas should take precautions to avoid exposure to infected animals and mosquito bites. This includes wearing protective clothing (e.g., long sleeves, pants), using insect repellents, and sleeping under bed nets (Keli et al. 2023).

9.8. SURVEILLANCE AND REPORTING

Early identification and reporting of RVF cases are crucial for effective outbreak response. Healthcare providers and public health authorities should promptly report suspected cases to facilitate surveillance and the implementation of control measures (Abdala et al. 2020).

10. FUTURE RESEARCH ON RIFT VALLEY FEVER

Future work on Rift Valley fever (RVF) involves various research and development areas to advance our knowledge of the disease, improve prevention and control strategies, and create new tools and interventions (William et al. 2022). Here are some potential directions for future work on RVF:

10.1. VACCINE DEVELOPMENT

Priority is given to the development of safe and effective RVF vaccines. Future research can focus on advancing vaccine candidates based on recombinant proteins, viral vectors, or nucleic acids. The goal is to enhance the availability, affordability, and accessibility of RVF vaccines for both animals and humans to prevent outbreaks (Tetsuro 2019).

10.2. ENHANCED SURVEILLANCE

Improving RVF surveillance systems is essential for early detection and timely response. This involves developing more sensitive and specific diagnostic tests, including rapid and point-of-care tools. Integrating surveillance data with advanced modeling techniques can improve prediction and forecasting capabilities (Aurélie et al. 2021).

10.3. INNOVATIONS IN VECTOR CONTROL

Innovative approaches to vector control can help reduce mosquito populations and limit RVF transmission. Future research can explore novel insecticides, improved delivery systems, alternative vector control strategies (e.g., biological controls), and the study of ecological factors that influence mosquito populations (Hassani et al. 2020).

10.4. CLIMATE CHANGE AND RVF

Investigating the potential impact of climate change on RVF transmission is critical. Understanding the relationship between climate variability, mosquito ecology, and RVF outbreaks can help develop adaptation strategies and early warning systems for at-risk regions (Rania et al. 2019).

10.5. ANTIVIRAL THERAPIES

Research can explore antiviral therapies specific to RVF. This involves studying the viral replication cycle, identifying potential drug targets, and developing antiviral agents with efficacy against RVFV (Nicholas et al. 2022).

Continued research, innovation, and collaboration are necessary to address the challenges posed by RVF. By focusing on these areas, we can advance our understanding of the disease, develop effective prevention and control strategies, and ultimately reduce the burden on human and animal health.

11. CONCLUSION

In conclusion, RVF presents a significant risk to human and animal well-being. The RVFV causes it and is primarily transmitted through mosquitoes. RVF outbreaks can lead to substantial socioeconomic consequences, including livestock losses and human illness or death. Efforts to prevent and control RVF necessitate a comprehensive approach involving collaboration among public health authorities, veterinary services, and other relevant stakeholders. Key strategies encompass vector control, animal vaccination, restrictions on animal movement, personal protective measures, safe handling of animal products, awareness campaigns, surveillance, and early detection.

However, there remains a need for ongoing research and development in the field of RVF. This includes the development of effective and safe vaccines, enhancement of surveillance systems and diagnostic tools, innovative approaches to vector control, strengthening the One Health approach, deepening our understanding of transmission dynamics and the impact of climate change, effective risk communication, and exploration of antiviral therapies specifically targeting RVFV. By continuing to invest in these areas, we can advance our knowledge of RVF, improve prevention and control strategies, and minimize the consequences of RVF outbreaks. This comprehensive approach is essential for protecting public health, preserving animal populations, and reducing the overall burden associated with Rift Valley fever.

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Hydatid Cyst and One Health Approach: Endangering Human and Animal Health

08

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ABSTRACT

Cystic Echinococcosis (CE) poses a formidable challenge to healthcare systems in endemic regions, straining resources with an influx of patients requiring costly surgical interventions. This zoonotic disease, primarily affecting rural populations, brings additional burdens of travel to distant tertiary hospitals, disrupting livelihoods and family dynamics. With economic ramifications extending to livestock losses, particularly in regions where dogs coexist with livestock, CE's prevalence varies globally, impacting areas like Australia, Latin America, Eastern Europe, the Middle East, and Africa. This paper delves into the taxonomic framework, emphasizing E. granulosus genotype G1 as a major contributor to human hydatidosis cases, especially in Central India. The disease's distribution is influenced by diverse factors, including agricultural practices, economic conditions, and cultural habits. The intricate life cycle involves dogs as definitive hosts, intermediate hosts like herbivores, rodents, and humans, and environmental conditions influencing transmission. Highlighting the "One Health" approach, the paper showcases collaborative efforts reducing CE prevalence and associated costs, aligning with global initiatives led by WHO, OIE, and FAO. The life cycle intricacies unfold with E. granulosus's journey from canine intestines to human organs, emphasizing the role of environmental conditions. The paper explores host-parasite interactions, showcasing the parasite's immune evasion mechanisms. Organs affected by CE include the liver and lungs predominantly, but cases extend to the spleen, heart, brain, kidney, peritoneum, and bone. The transmission, primarily through canine feces contaminating the environment, leads to significant health and economic impacts. The paper underlines the importance of diagnostics, treatment options, and public health considerations, with human cystic echinococcosis ranging from asymptomatic to potentially fatal, impacting millions globally. Financial significance becomes apparent as CE incurs direct costs in healthcare and livestock losses, affecting productivity and economic stability. The control and prevention section stress the need for comprehensive strategies, aligning with the One Health approach, to mitigate the impact of CE on both human and animal populations. This paper provides a comprehensive overview, shedding light on the multifaceted challenges posed by Cystic Echinococcosis and advocating for collaborative, interdisciplinary efforts to combat its impact.

Keywords: cystic echinococcisis, E. granulosus, one health, pathogenesis, diagnosis, hydatid cyst

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1. INTRODUCTION

Cystic Echinococcosis (CE) is an infectious ailment that poses significant challenges to healthcare systems in endemic regions. The influx of patients, often requiring costly surgical interventions, and the necessity for sustained control programs place a substantial burden on these systems. Rural populations, primarily affected by CE, face the additional hardship of being referred to distant tertiary hospitals, resulting in upheaval, loss of workdays, and strain on family dynamics. Furthermore, CE's impact extends beyond human health, encompassing economic losses tied to affected livestock, mainly sheep and goats (Larrieu et al. 2019). This disease is prevalent in regions where livestock coexist with dogs. Its incidence varies globally, with high prevalence observed in areas like Australia, Latin America, Eastern Europe, the Middle East, and Africa. The primary culprits, *E. granulosus* and *E. multilocularis*, have different transmission patterns involving dogs, livestock, and small mammals. The former leads to human cystic echinococcosis, while the latter, alveolar echinococcosis. Both pose significant public health concerns in numerous regions, even in developed countries within endemic areas (Craig et al. 2007).

Within the taxonomic framework, *E. granulosus sensu lato* encompasses several species, including *E. granulosus sensu stricto* and others like *E. equinus*, *E. ortleppi, E. canadensis*, and *E. felidis*. *E. granulosus*, particularly genotype G1, accounts for approximately 80% of human hydatidosis cases worldwide (Cucher et al. 2016; Carmena et al. 2013). Central India is an endemic region for this disease (Mahajan et al. 2004). The distribution of *E. granulosus* is influenced by various factors, including agricultural practices, economic conditions, educational levels, and cultural habits. Prevalence rates exhibit considerable variability (Andersen 1997). For instance, the prevalence rate in northern Turkana, Kenya, was high in 1982 (200/100,000 inhabitants/year), while in the central Peruvian Andes, it was 9.1/100,000 inhabitants/year. Surgical incidence rates 1992 were also notable in Tunisia (15.1/100,000 inhabitants/year) (Dziri 2001). Worldwide, at least ten E. granulosus species complex genotypes have been identified, circulating within various host populations (Thompson et al. 2006). Risk factors in regions endemic to CE include free-roaming dogs, dog ownership, and unregulated or home-based livestock slaughtering (Possenti et al. 2016). Additionally, the CE life cycle is highly contingent on environmental and climatic conditions, which impact egg survival and living conditions for humans and livestock. E. granulosus eggs can persist for varying durations under different temperatures and environmental settings, emphasizing the significance of these factors in disease transmission (Nur et al. 2017).

2. ONE HEALTH

Comprehensive care for cystic echinococcosis (CE) is one of the best illustrations of the strategies devised within the "One Health" approach. This integrated strategy aims to prevent endemic and epizootic diseases while maintaining ecosystem health for the advantage of domesticated animals, humans, and biodiversity. Its central elements are zoonoses, vectors, food security, antibiotic resistance, the effects of climate change, and the introduction and reappearance of diseases (Marcos 2013; FAO 2018).

The "One Health" model, through collaborative efforts across institutions and disciplines, coupled with active community involvement, has yielded significant outcomes. Notably, there has been a marked decline in CE prevalence in humans, associated with decreased lethality (0.5% from 1997 to 2020, with no recorded deaths in the past two years). Moreover, the health system has witnessed a noteworthy cost

reduction attributed to diminished hospitalizations and surgical interventions. The World Health Organization (WHO), the World Organization for Animal Health (OIE), and the Food and Agriculture Organization (FAO) are collaborating to promote the "One Health" initiative. It emphasizes the importance of shifting away from disease-specific therapies and toward comprehensive interdisciplinary strategies while coordinating efforts with related sectors (Laing et al. 2021; FAO 2018).

3. LIFE CYCLE

The intricate relationship between host and parasite unveils a fascinating pattern. *E. granulosus*, a diminutive tapeworm measuring a mere 7 mm in length, predominantly takes residence in the mucosa of the small intestine of definitive hosts, primarily canines. Within 4 to 5 weeks, it matures into the adult stage, attaining sexual maturity. Subsequently, gravid proglottids, each containing several hundred eggs, release and deposit the eggs into the feces of definitive hosts. Through lytic discharges, the oncosphere traverses the digestive mucosa. It enters the host's circulatory system, eventually reaching vital organs like the liver, lungs, and other sites where cystic development commences upon ingestion by intermediate hosts (including herbivores, rodents, and humans) (Paredes et al. 2011).

This process culminates in the metacestode stage, which involves a metamorphosis from the oncospheric stage. It takes four to seven days in vitro for hatchlings to develop a characteristic "bladder" with a germinal layer. Notably, the host species influences the duration of this development significantly. Typical annual growth for hydatid cysts ranges from less than 1 cm to 5 cm. Due to the unpredictability of these cysts' outcome, it may be months or even years after the initial infection before the affected areas are identified.

Cysts of *E. granulosus* undergo several phases of growth as they usually develop. The first infection, which is frequently asymptomatic, results in the development of microscopic (less than 5 cm) benign epithelial growths at organ sites. Approximately 66% of the time, cysts affect the liver, 20% of the lungs, and less than 5% of the kidneys, pancreas, heart, and bone. Twenty to forty percent of patients are affected by multiple lesions or multiple organs. There may be a symptomatic presentation if cysts exert pressure on contiguous tissues, resulting in various pathological events over months or years. Additional factors, such as cyst rupture, calcification, or content discharge into the bile duct or bronchial tree, can instantly affect cyst progression. Notably, human hosts frequently tolerate slow-growing hydatid cysts, with clinical manifestation occurring predominantly when a large cyst disrupts bodily functions or when symptoms such as eosinophilia and allergic reactions appear. Cystic masses may also be discovered incidentally during medical examinations, surgical procedures, or other clinical complications. (Eckert and Deplazes 2004).

A cell-covered membrane of varying thickness supports the inner germinal layer comprising cells constituting the mature hydatid cyst. Tegumental cells within the germinal layer form a continuous syncytium, giving rise to numerous miniature villi projecting into the laminar layer towards the host tissues. Small secondary cysts, termed "brood capsules," bud from the germinative layer and, through asexual reproduction, generate multiple protoscolices. These can either develop into adult worms in the definitive hosts' digestive tracts or secondary hydatid cysts following the rupture of a cyst in intermediate hosts. Protoscolices only develop in fertile cysts within intermediate hosts. In E. granulosus-infected cattle, immunoglobulins (IgGs) traverse the plasma membrane and tegument located between the germinal and laminar layers of the cyst. These IgGs target specific antigens involved in cell proliferation and separation processes crucial for protoscolex development in the final form. The interaction between an antigen and an antibody may result in cyst infertility by impeding cell proliferation and differentiation (Paredes et al. 2011).

The hydatid fluid obtained from the cyst's inner wall is notably clear and uncontaminated. It encompasses all components of the "inner wall," known as hydatid sand, along with the parasites' and hosts' secretions. It mirrors the components (Na, K, Cl, CO2) found in the host's serum, possesses an alkaline pH ranging from 1.008 to 1.015, and contains specific proteins conferring antigenic properties (Siracusano et al. 2012). As a blister develops slowly, several transformations may occur: the cyst's wall may fracture due to membrane detachment or microtrauma, scoleces may vesiculate or transform into vesicles, and the parasite may ultimately perish due to germinal membrane dysfunction (detachment or aging). These new vesicles, termed successors or "daughter" vesicles, inhabit the hydatid fluid and share a similar composition to the mother cyst.

Consequently, protoscolices may develop into adult parasites or give rise to another cyst in this manner. While the hydatid fluid primarily dictates antigenic propensity, the germinal layer of the cyst acts as a barrier against the host's immune cells. Therefore, cracks or crevices in the germinal layer are essential to provoke an antigenic response. Immunologic responses continue to rise indefinitely following this antigenic stimulation (Siracusano et al. 2012). This surge also occurs after measures to control cyst growth, such as surgery or incision. The parasite has likely evolved additional immune evasion mechanisms in addition to this physical barrier, allowing for its prolonged survival (Siracusano et al. 2012).

4. ORGANS AFFECTED

The distribution of cysts in the body is quite distinct, with a significant proportion found in the liver (around 70%) and lungs (approximately 20%), primarily attributed to capillary filtration (Nakamura et al. 2011). However, they can also affect other organs, including the spleen, heart, brain, kidney, peritoneum, and bone (Pakala et al. 2016). Involvement of the heart and pericardium is a rare occurrence, accounting for only 0.02-2% of cases (Engin et al. 2000). While the left ventricular wall is the most commonly affected site, any part of the heart can potentially be impacted (Kervancioglu et al. 2000). Pancreatic hydatid cysts (HC) are more frequently located in the pancreatic head, a location that can sometimes be mistaken for a pseudo pancreatic cyst, tumor, or another congenital pancreatic cyst (Lemmer et al. 1995).

Cystic echinococcosis (CE) can present clinically in a variety of ways, from quiet cysts that may not cause any symptoms to severe instances that result in anaphylactic shock from cyst rupture and the discharge of its contents (Manfredi et al. 2011; Gholami et al. 2018). Most cysts typically grow as part of their normal development, which frequently causes complications. This may result in acute or chronic pancreatitis, which is caused by symptoms including nausea, vomiting, and weight loss. Among other complications, jaundice may develop due to the cyst's pressure on the common bile duct (Hewes et al. 2000).

5. TRANSMISSION

The nematode *E. granulosus* is responsible for hydatid cyst, a parasitic disease. This particular tapeworm strain resides in the intestines of carnivores, such as dogs, which are referred to as the "definitive host." These hosts release tapeworm eggs in their feces, which are then ingested by herbivores such as sheep, referred to as the "intermediate host" (Dziri et al. 2001). After hatching in the stomach, the embryos from these eggs are discharged into the small intestine of the final host. The embryos then pass through the intestinal villi, enter the circulatory system, and grow in various organs before assuming their cystic form. This infestation primarily affects the liver and lungs (Craig et al. 2007). The mature tapeworm, typically white and measuring between 3 and 7 mm in length, is armed with a dual crown of hooks designed for affixing itself to the small intestine of dogs or foxes. Its body is segmented, housing several reproductive units or proglottids, usually around four in number. Remarkably, a mature proglottid can contain an

average of 587 fertile eggs. It's estimated that gravid proglottids are generated and discharged with faeces approximately every 15 days, leading to soil, crops, and water contamination (average of 0.071 proglottids/tapeworm/day) (Guarnera 2013). It's worth noting that dogs can harbor numerous *E. granulosus* without exhibiting any discernible disease symptoms.

The rate of cystic growth in humans is contingent upon the affected organs, with the lungs and peritoneum displaying lesser resistance compared to the liver, often correlating with the onset of symptoms (Eckert and Deplazes 2004; Craig et al. 2007). Humans are regarded as accidental hosts, where cysts may form, but the development of fertile protoscolices is not guaranteed (Rausch 2003). Hydatid cysts primarily develop in the liver and lungs, occasionally appearing in other organs, which are uncommon sites for hydatid cyst localization. Eggs, oval and ranging from 30 to 40 cm in diameter, encapsulate hexacanth embryos (also known as oncospheres or the first larval stage). These embryos are ensconced within multiple membranes and an outer keratinized, remarkably resilient thick wall. Morphologically, they cannot be distinguished from the eggs of other tapeworms like *Taenia ovis* or *Taenia hydatígena*, among others (Thevenet et al. 2005). Between the first and second year of a sheep's existence, CE cysts become fertile and persist until the animal is slaughtered. This dynamic assures the survival of *E. granulosus* in the environment for the seven to nine years that a sheep lives. Despite the absence of morbidity or mortality, the animals' productivity may be affected (Uchiumi et al. 2021).

6. PATHOGENESIS

The definitive host, including dogs and other carnivores, releases the embryonated eggs through feces. In the case of intermediate hosts such as cattle, horses, camels, and sheep, oncospheres are released upon ingestion of these eggs (Zhang et al. 2012; Moro and Schantz 2009). Oncospheres, having entered the intestinal wall, are transported via the vascular system to organs like the liver and lungs. Within these organs, protoscolices and daughter cysts gradually fill the interior of the oncospheres as they mature into cysts (Pakala et al. 2016). The final host becomes infected upon consumption of organs containing these cysts. Subsequently, the protoscolices attach themselves to the mucosa of the intestinal tract after ingestion. Following evagination, the protoscolices undergo a further transformation, eventually maturing into adult tapeworms within 32 to 80 days. Notably, there are distinctions in the life cycles *of E. multilocularis* and *E.chinococcus granulosus.*

In the case of *E. multilocularis*, its primary hosts are primarily foxes, with dogs, cats, coyotes, and wolves playing a lesser role. Small rodents serve as the intermediate hosts, and within the liver, the larval development continues indefinitely in a proliferative stage, leading to tissue infiltration. E. vogeli relies solely on dogs and bush dogs throughout its life cycle as definitive hosts (Rasheed et al. 2013). In the larval stage within the liver, the lung undergoes both external and internal growth, forming multiple vesicles. Rodents, again, serve as the intermediate hosts for this cycle. For *E. oligarchs*, rodents serve as the intermediate hosts, while wild felids function as definitive hosts throughout their life cycle. Egg ingestion by individuals leads to the formation of cysts in various organs and releasing oncospheres into the digestive tract (CDC 2018).

7. HOST-PARASITE RELATION

When the parasite infiltrates the human host, a complex interplay of innate and adaptive defense mechanisms comes into play. However, it's worth noting that not all of these responses are necessarily protective. In fact, following a parasitic infection, the emergence of defensive immunity is more of an exception than a rule. The successful survival of parasites has led them to evolve various strategies to evade or manipulate the host's defensive systems, ensuring their propagation. This can involve altering

host responses to favor parasite survival, employing simple mechanisms like finding refuge within host cells or modifying their antigenic structure to evade immune detection (Maizels et al. 2009).
In Cystic Echinococcosis (CE), the host-parasite relationship is particularly intriguing as it involves chronic infection alongside observable humoral and cellular responses directed toward the parasite. This is due to the presentation of various antigens at different stages of development, prompting independent host responses to antigenic stimuli from the invading oncosphere, the metamorphosing metacestode, and ultimately, the fully developed metacestode or hatchling (Siracusano et al. 2012).
E. granulosus uses two fundamental mechanisms to subvert the host immune response: latent encystment, in which the parasite transforms into a hydatid cyst to avoid the harmful effects of an immune response, and immune modulation, in which the parasite actively interacts with the host immune system to reduce the impact of the host's response. For a comprehensive comprehension of host-parasite interactions and the development of novel therapeutics for chronic encephalitis (CE), it is essential to conduct both animal and human clinical research (Zhang et al. 2003; Zhang and McManus 2006).

8. DIAGNOSIS

Current diagnostics for extrahepatic echinococcal disease have significantly improved with advanced imaging techniques. Treatment options now encompass a range of approaches, including surgical intervention, percutaneous drainage, and chemotherapy employing drugs like albendazole and mebendazole. However, the involvement of wild animals in sylvatic cycles can potentially intersect with the domestic sheep-dog cycle, thereby complicating control efforts (Mandal et al. 2012).

8.1. DIAGNOSTIC PROCEDURES IN INTERMEDIATE HOSTS

The most reliable method for diagnosing the parasite in intermediate hosts is the identification of cysts during postmortem meat investigation. Consequently, the presence of hydatid cysts in internal organs is a crucial diagnostic indicator (WHO 2002).

8.2. DIAGNOSTIC TECHNIQUES FOR DEFINITIVE HOSTS

Identifying grown-up tapeworm contamination in canines represents a test because of the minor and irregular shedding of sections. Minuscule recognizable proof of eggs in waste examples can't be utilized to analyze *E. granulosus* disease since these eggs are morphologically indistinguishable from those of *Taenia* species (Regassa 2019). An egg might be situated in waste, for example, utilizing the standard buoyancy procedure or on the perineal skin by applying clear sticky tape, moving it to a tiny slide, and looking at it. A legitimate morphological conclusion might be conceivable in situations where proglottids of E. granulosus are suddenly shed by canines and dominatingly found on the outer layer of waste examples (Urquhart et al. 1996).

9. DIAGNOSIS IN HUMANS

For people, the finding is affirmed through imaging methods like PC tomography (CT) checks, X-beams, and distinguishing proof of the trademark or concerning development structure. Developments are recognized utilizing imaging advances like CT sweeps, ultrasonography, and attractive reverberation imaging (X-ray). Moreover, when a growth is found, serological testing might be used to confirm the finding in people (McManus et al. 2002).

10. TREATMENT

Echinococcus tapeworms represent a more noteworthy test to destroy contrasted with other Taenia species, regardless of the accessibility of different exceptionally compelling meds, prominently praziquantel. Following treatment, canines must be confined for 48 hours to consider the assortment and removal of polluted dung. In people, careful evacuation of hydatid growths is conceivable. However, mebendazole, albendazole, and praziquantel treatments have proven viable (Taylor et al. 2015). The way to treat cystic echinococcosis relies upon the area and size of the body's hydatid cyst(s). Medical procedure stays the most dependable strategy for treating hydatid pimples in people, and chemotherapy, especially albendazole, is shown when a medical system isn't a choice. A blend of medical practice and benzimidazole, like mebendazole, forestalls protoscoleces from forming into hydatid pimples and keeps the blister dry. Layer breakdown might happen, assuming the patient controls the medication before the medical procedure (Sinan et al. 2002).

11. PUBLIC HEALTH

Human cystic echinococcosis is a disease induced by the larval stage of the Echinococcus species metacestodes. The condition can progress from asymptomatic to potentially fatal. In some regions, it is a major public health concern; in others, it may be emerging or reappearing. It is estimated that between one and two million cases occur in humans worldwide. Cystic echinococcosis is the most prevalent form in humans and domesticated animals, induced by *E. granulosus sensu lato* (CFSPH, 2011; Torgerson and Macpherson 2011).

Following essential contamination, *Echinococcus granulosus* may influence a few physical regions. Most hydatid growths are viewed in the liver (70%) or lungs (20%). Be that as it may, they can likewise be tracked down in different organs (under 2% in the mind, 2% in the spleen, and 2% in the kidney) (Pakala et al. 2016). Hydatid sores lead to extreme disease and passing in people and monetary misfortunes because of treatment costs lost wages, and yearly animal creation misfortunes (Fikire et al. 2012).

Mechanical breakdown of organs brought about by sores and extreme touchiness because of growth cracks and arrival of liquid are substantial side effects in people. The advancement of sores containing various small protoscoleces, which most regularly happen in the instinctive organs, focal sensory system, and skeletal framework, as well as thyroid organs, subcutaneous tissues, body cavities, and muscle structure, describes hydatidosis (Fromsa and Jobre 2012).

The hatching time frame for all Echinococcus species goes from months to years or even many years. It is still up in the air by the body's size and pace of blister development (Ochi et al. 2016). In people, hydatid pimples are frequently rich, and different investigations recommend that a large number of cases could be connected to expanded infectivity or pathogenicity of *E. granulosus* sensu-lato. The asymptomatic disease often wins except if other mechanical entanglements like burst, the pressure of basic designs, or draining happen. This could be ascribed to the parasite avoiding host insusceptibility (WHO 2011).

Albeit cystic echinococcosis is a possibly dangerous condition, growths are regularly very much endured except if they harm or break contiguous tissues. Numerous developments stay asymptomatic throughout an individual's life and might be found unexpectedly during a medical procedure or postmortem examination. Usually, this type of echinococcosis can be dealt with; notwithstanding, a few diseases can be deadly if the pimple cracks and triggers anaphylactic shock or harms fundamental organs. The anticipation of suggestive blisters in the mind, kidney, heart, or other significant organs is troubling (Macpherson et al. 2003).

The healing of a hydatid ulcer in the liver, lung, or other organs can cause hepatomegaly, cholestasis, jaundice, alternative biliary cirrhosis, biliary colic-like side effects, liver ulcers, calcified lesions in the liver, portal hypertension, and apoplexy. (Fato 2017). Lung growth is associated with cellular disintegration, chest pain, a chronic cough, dyspnea, hemoptysis, pneumothorax, pleuritis, and lung soreness. Heart conditions include suffering, growth, cardiovascular disease, and embolism. The adverse effects of bone and muscle disease are pain, bone extension, bone fragility, and muscle acne. Spinal and brain lesions are responsible for back pain and neurological side effects. Pain, ptosis, and visual abnormalities in the eyes, as well as biliary colic, cholestatic jaundice, cholangitis, fever, pancreatitis, and sensitivity, are all caused by the rupture of the tubercle connecting the liver and biliary tree. A blister rupture in the bronchial tree results in fever and asthma-like symptoms such as coughing, dyspnea, and hemoptysis (Moro and Schantz 2009). Table 1 shows the summary of *Echinococcus spp.* features.

Table 1: Summary of Echinococcus species' features (Gessese 2020)

Character	*Echinococcus granulosus*	*Echinococcus multilocularis*	*Echinococcus vogeli*	*Echinococcus oligarchs*
Geographic distribution	Cosmopolitan	Central and North Eurasia, Northern North America	Central and South America	Central and South America
Definitive hosts	Primarily dogs and other canids	Mainly foxes, as other canids and cats	Wild felids	Bush dog
Intermediate and aberrant hosts	Primarily ungulates also marsupials and primates, humans	Mainly Arvicola rodents, as other small mammals, humans	Rodents, agoutis, paca, spiny rats, humans	Primarily agoutis, also other rodents, humans
Nature of cyst	Unilocular, endogenous proliferation, no filtration or metastasis	Multivesicular, endogenous accumulation, metastasis	Polycystic, exogenous, and endogenous proliferation, no metastasis	Polycystic, endogenous, and exogenous proliferation, no infiltration or metastasis
Location of cyst	Visceral, primarily liver, and lungs	Visceral, primarily liver	Peripheral, primarily muscle	Visceral, primarily liver

12. FINANCIAL SIGNIFICANCE

Cystic echinococcosis is costly for humans and animals (Torgerson and Macpherson 2011). According to Torgerson and Macpherson (2011), hydatid pimple diseases in animals cause immediate financial losses such as the evaluation of cadavers and vital organs like the liver, lungs, spleen, and heart, as well as stunted development, decreased efficiency, lower milk, and meat yields, diminished nature of fleece, diminished value of stowaways and skins, and reduced birth rates.

Echinococcosis has spread to regions such as Europe, Asia, Africa, South America, Canada, and Australia (Budke et al. 2006) and has caused significant financial losses for several countries. In several locations, cystic and alveolar echinococcosis cases have been documented in detail. In contrast, cystic echinococcosis is more prevalent and has been reported in every Middle Eastern and North African Arab nation (Nejad et al. 2010).

Due to individuals, direct expenses associated with searching, hospitalization, careful support, or percutaneous drugs are related to financial hardship. This includes prescription medications, follow-up care, patient and family travel expenses, and other unforeseen expenditures such as pain and lethargy. In addition to the financial and cultural effects of incapacity caused by cases that are not promptly diagnosed and, as a result, go untreated, it is essential to consider the loss of business days or "efficiency," as well as

the abandonment of farming or other agricultural activities by affected or at-risk individuals. In addition, the majority of studies indicate that 1% to 2% of patients with cystic echinococcosis die (Torgerson and Macpherson 2011).

Despite underreporting, human cystic echinococcosis has a significant global impact in terms of disability-adjusted life years (DALYs) and monetary costs. For instance, Budke et al. (2006) estimated that the annual cost of health care for humans and animal fatalities in North African countries was approximately US$ 60 million.

13. CONTROL AND PREVENTION

Cystic echinococcosis presents a critical financial weight for the two people and creatures (Torgerson and Macpherson 2011). The judgment of cadavers and fundamental organs like the liver, lungs, spleen, and heart, combined with hindered development, diminished efficiency, lower milk and meat yields, lessened nature of fleece, diminished worth of stows away and skins, and decreased rates of birth are immediate monetary misfortunes credited to hydatid pimple diseases in creatures (Torgerson and Macpherson 2011). Numerous nations face significant economic misfortunes and general well-being challenges because of echinococcosis, crossing locales like Europe, Asia, Africa, South America, Canada, and Australia (Budke et al. 2006). Various regions have detailed instances of both cystic and alveolar echinococcosis. Notwithstanding, cystic echinococcosis is more pervasive and has been reported in all nations in the Center East and Arabic North Africa (Nejad et al. 2010).

On account of people, monetary misfortune is related to direct expenses from finding, hospitalization, careful intercession, or percutaneous medicines. This includes medications, follow-up care, travel costs for patients and their families, as well as extra backhanded costs like agony and languishing. Besides the financial and cultural effects of inability connected with undetected and like these untreated cases, one must likewise think about the deficiency of business days or "efficiency" and the deserting of cultivating or farming exercises by impacted or in danger people. Moreover, as most reports indicate, around 1% to 2% of cystic echinococcosis cases demonstrate lethal (Torgerson and Macpherson 2011). Human cystic echinococcosis has a critical worldwide effect as far as handicap-changed life years (DALYs) and monetary expenses, even after representing the underreporting of the sickness. For example, the consolidated cost of human wellbeing treatment and creature misfortunes in North African nations was assessed to be around US$ 60 million every year (Budke et al. 2006).

14. CONCLUSION

In conclusion, cystic echinococcosis substantially affects both human and animal populations globally. The economic ramifications are far-reaching, encompassing not only the loss of condemned animal carcasses and vital organs but also the direct medical expenses and indirect costs borne by affected individuals. Moreover, the disease exerts a notable influence on disability-adjusted life years (DALYs) and places a strain on financial resources.

To confront this challenge, a multifaceted approach is imperative. Proactive measures, such as rigorous control of hydatid tapeworms in dogs and the widespread vaccination of sheep, present promising avenues for curbing the impact of this parasitic affliction. Tailoring comprehensive control strategies to the unique characteristics of specific regions holds immense potential in diminishing the prevalence of echinococcosis over time.

However, it is paramount to recognize that time is of the essence. Swift and sustained efforts are indispensable in addressing the economic and public health challenges of this insidious infection. By

marshaling our resources and collective resolve, we can hope to significantly alleviate the burden imposed by cystic echinococcosis, ultimately safeguarding the well-being of both human and animal populations worldwide.

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Chagas Disease: An Overview of Current Understanding and Future Perspectives

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ABSTRACT

Chagas disease (CD), caused by the protozoan parasite Trypanosoma cruzi, is a life-threatening infection transmitted primarily by triatomine bugs. Originating in rural Latin America, CD has spread globally through various transmission routes, including organ transplantation, blood transfusion, contaminated food and drink, and hereditary transfer. Millions in vulnerable stages face poverty and inadequate medical care due to unawareness and resource constraints. The acute phase presents symptoms like fever, anorexia, fatigue, and tachycardia, progressing to the chronic phase with severe health issues, including congenital heart diseases and neurological damage. It remains a neglected tropical disease recognized by the World Health Organization. Diagnosis involves serological, molecular, and parasitological tests, with challenges such as underdiagnosis. Antiparasitic medications, including Benznidazole and Nifurtimox, offer limited effectiveness, and drug resistance is emerging. Ongoing research explores new drugs, immunotherapies, gene therapy, and combination therapies. Diagnostic advances include nucleic acid amplification tests and serological tests with recombinant antigens. Vector control is crucial, focusing on insecticide use, housing improvements, and community education. Blood screening, organ transplantation screening, and prenatal interventions contribute to prevention. The disease's global burden affects millions, with challenges in endemic and non-endemic regions. Future research must address disease mechanisms, enhance diagnostic methods, explore new treatment options, and develop effective prevention strategies. Genomics, drug discovery, immunotherapy, vector control, and health system strengthening offer opportunities. Eradication potential lies in comprehensive public health strategies, targeting vectors, blood screening, and prenatal care, underscoring the need for sustained efforts and research innovations in the fight against Chagas disease.

Key words: Chagas Disease, Trypanosoma cruzi, Vector-borne Infection, Antiparasitic Medications, Global Health Challenges

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1. INTRODUCTION

Chagas disease (CD) is an immedicable infection caused by an American vector-borne protozoan parasite, *Trypanosoma cruzi*. It is a life-threatening human disease when the infected parasite's feces enter a mammalian host's mucous membrane. It is mainly transmitted by the bite of an infected triatomine bug, commonly known as a kissing bug. Other transmission means are organ transplantation, blood transfusion, contaminated food and drink usage, and hereditary transfer (Bern et al. 2019). The original roots of CD belong to rural Latin America, and it emerged across the borders from infected immigrants from their countries of origin (Perez and Israel 2018). Millions of people who get infected with this infection at the most yielding stage are susceptible to poverty and cannot get proper medical treatment due to unawareness and lack of resources (Olivera et al. 2019). Symptoms like fever, anorexia, fatigue, body aches, tachycardia, *etc*., come under the umbrella of the acute phase. Most of the infected individuals remain asymptomatic as long as the chronic stage of the disease evolves. The chronic phase is characterized by dangerous health issues, including congenital heart diseases, heart failure, gastrointestinal disorders, and neurological damage (Lidani et al. 2019).

The World Health Organization (WHO) recognizes CD as a Neglected Tropical Disease (NTD) around the globe. A Brazilian physician, Carlos Chagas, discovered this disease while working in Brazil's Ministry of Public Health and Hygiene. He was also the first who apply intra-household vectors against malaria. As he succeeded in his work, he was honored with many awards from institutions in different countries. He also became a member of the National Academy of Medicine of Brazil. His unusual discovery was not accepted and understood by the researchers of that time. Chagas had already organized the essential characteristics of this new disease and published his discovery in a journal. Later, he consulted a patient who was the first CD case. Before 1909, CD was not mentioned in history, making his work a remarkable gain in medicine and parasitology. He narrated the principal features of a new tropical disease (Lidani et al. 2019). The CD gives rise to heart failure and even leads to death in some cases.

Identification and cure of the infection are challenging. Disease control demands the removal of the causative agents, the reduviid bugs. Control has proved successful in South American Countries but falls behind in Northern (Central) American countries (Mills 2020). Despite vector transmission, it also spreads through vertical transmission, especially in non-endemic areas with a high ratio of pregnant women infected with the parasite, most of whom were immigrants. These women can be considered latent transmitters of Congenital CD (CCD). CCD has gained attention due to its high childhood morbidity and mortality risk. Early diagnosis and mediation may improve the quality of life and low morbidity and mortality (Santana et al. 2020). Cure rates in children are considerable, and in adults, they are unsure. The test used for the reversal of CD is a negative serological test. The results are inversely proportional to the duration of the infection (Irish et al. 2022).

2. MODE OF TRANSMISSION

The CD is transmitted in many ways, such as through direct contact with kissing bugs (carriers of *T. cruzi*). Like in some areas of the world, they are freely present in their homes as family members. Feeding food

contaminated with the feces/urine of bugs is another transmission route. Wastes of the bugs carry most of the infectious form of the parasite (Eduardo et al. 2021). Vector transmission also occurs by touching the animals. These vectors enter through the mouth, open wound, or mucous membrane. The male parasite bites only at night, but the female parasite needs the host's blood to nourish eggs. Vertical transmission occurs when the baby is in direct contact with the mother's fluid. Other passages are through the transfusion of blood, the transplant of infectious organs, or unsterilized lab equipment (Han et al. 2020; Klotz and Schmidt 2020; Nguyen and Waseem 2021).

3. LIFE CYCLE OF PARASITE

The two forms of the *T. cruzi* parasite (amastigotes and epimastigotes) are divided by binary fission and are observed as clonal organisms. By the time they evolve, they undergo many changes. First, the vector enters a mammalian host through a vector bite wound and mucosal membrane. It is found in the bloodstream plasma and then binds to different cell receptors. After that, it moves into a parasitophorous vacuole (Escolano et al. 2022). After entry, it differentiates into a protist cell with unclear flagella or cilia (amastigotes) and escapes from the vacuole into the cytoplasm, where it multiplies, and here the transformation ends as it becomes flagellated. The trypomastigotes in the blood traveling through the heart, arteries, veins, and capillaries have both slender and broad forms. After completing this cycle, the parasite again undergoes the cell cycle and creates many copies until the cell becomes filled with them (Ngyen and Waseem 2021).

4. IMMUNE RESPONSE AND DISEASE PROGRESSION

CD directly targets the thymus gland, the site of maturation of T and B cells, leading to the depletion of T cells, which then damages a person's immunity. Thymus atrophy, weight loss of the thymus, is caused in the acute phase. Abnormal thymocyte is also observed in the thymus. The CD is a silent killer with three phases: acute, indeterminate, and chronic. The acute phase is symptomatic and leads to fever, diarrhea, swollen lymph nodes, headache, muscular pain, and many severe diseases like myocarditis, pericarditis, hepatosplenomegaly, lymphadenopathy, meningoencephalitis, etc. These parasites throughout the body affect many other parts of the body like glands, skeletal, nervous, lymphoid, etc. Most patients during the acute phase are asymptomatic, while the chronic symptomatic phase appears years later, with around 30% progressing toward detectable organ damage affecting mainly the cardiovascular and digestive systems. Chagas cardiomyopathy is the leading cause of nonischemic cardiomyopathy (NICM) in Latin America and affects around 30% of infected patients (Echavarria et al. 2021).

5. CLINICAL MANIFESTATIONS

The CD has an asymptomatic acute phase with higher parasitemia levels; it then enters an indeterminate chronic phase without clinical symptoms of visceral involvement (Maite et al. 2020).
Up to 30% of chronically infected people develop cardiac alterations, and up to 10% develop digestive, neurological, or mixed alterations, which may require specific treatment. In chronic patients, antiparasitic treatment can potentially prevent or curb disease progression. Recent studies have shown that treating infected women before pregnancy eliminates vertical transmission of *T. cruzi*, while treatment efficacy is also incredibly high in congenitally infected newborns, with a cure rate of 100%. In the chronic and symptomatic phase of the disease, the heart and digestive system suffer significant clinical impairment. In the digestive system, esophagopathy causes the most frequent clinical manifestations due to the destruction of the myenteric plexus of the esophagus. It triggers functional motility changes, such as hypo-

contractility, loss of peristalsis, achalasia of the lower esophageal sphincter, and megaesophagus. Esophageal motility disorder causes dysphagia, possible regurgitation of the swallowed material, heartburn, and weight loss. Dysphagia, which means swallowing difficulty, is the most frequent digestive symptom, occurring during ingesting solid and liquid foods – though most often intense with solid foods (Barja et al. 2021).

6. CLINICAL MANAGEMENT

In most cases, CD is asymptomatic and cannot be detected earlier. For this purpose, there must be a test for the early assessment of CD. When the disease remains undiagnosed, it remains untreated and thus destroys the lives of thousands of patients. Similarly, that kind of test should also be present to critically analyze the response to the pharmaceutical treatment of CCD. TPP tests (Target Product Profile) are used in different aspects: to diagnose the patients suffering in the acute phase or those who are in the chronic phase (Symptomatic or Asymptomatic) and to evaluate the feedback of antiparasitic treatment of the disease (Padilla et al. 2020). Introducing vaccines against CD will also prove beneficial in setting up a robust immune system during the infection. BNZ and NF are used in America to treat CD but show unfortunate side effects within a few weeks. So, there is a high need to research, revamp pharmaceutical compounds, generate alternative drugs, and develop innovative strategies to treat patients bearing cardiac and esophageal diseases (Ribeiro et al. 2020).

7. DIAGNOSIS

7.1. SEROLOGICAL TESTS

- Enzyme-linked immunosorbent Assay (ELISA) identifies specific antibodies against *Trypanosoma cruzi* antigens in the blood.
- Immunoblot or Western Blot identifies specific antibodies against multiple *T. cruzi* antigens (Jeffrey et al. 2019).

7.2. MOLECULAR TESTS

- Polymerase Chain Reaction (PCR) amplifies and detects *T. cruzi* DNA in blood clot samples, which is especially useful during the acute phase (Kell et al. 2021).
- Real-time PCR provides quantitative results and helps monitor treatment effectiveness (Schijman et al. 2022).

7.3. PARASITOLOGICAL TESTS

- Direct Microscopy: Examines blood smears or other fluids under a microscope for the presence of *T. cruzi* parasites, particularly during the acute phase.
- Xenodiagnosis: Involves allowing laboratory-reared triatomine bugs (vectors of CD) to feed on the patient and then examining the bugs for *T. cruzi* (Guillermo et al. 2019).

8. CHALLENGES FOR DIAGNOSIS

Limited awareness among healthcare providers, particularly outside endemic regions, can lead to underdiagnosis or misdiagnosis of CD (Amanda et al. 2020).

The disease can manifest in a range of clinical symptoms, which are generally absent or nonspecific, similar to a viral illness, specifically to CD (Amanda et al. 2018).

9. NEW DIAGNOSTIC APPROACHES

9.1. NUCLEIC ACID AMPLIFICATION TESTS (NAATS)

PCR and other NAATs have demonstrated improved sensitivity and specificity in detecting *Trypanosoma cruzi* DNA. These tests can identify the parasite during the acute and chronic phases of infection (Caryn et al. 2019; Dhésmon et al. 2021).

9.2. SEROLOGICAL TESTS WITH RECOMBINANT ANTIGENS

Newer serological tests use recombinant antigens specific to *Trypanosoma cruzi*, reducing cross-reactivity with other parasites (Carine et al. 2021). This enhances the accuracy of results and improves sensitivity and specificity compared to tests using whole parasite extracts (Gabriel et al. 2022; Pilar et al. 2023).

9.3. POINT-OF-CARE (POCS) RAPID DIAGNOSTIC TESTS

POCs for CD have been developed to enable quick and accessible diagnosis in resource-limited settings. Based on lateral flow immunoassay technology, these tests detect specific antibodies against *Trypanosoma cruzi* and provide rapid results within minutes without requiring specialized equipment (Daniel et al. 2019).

10. TREATMENT

Available therapies are the following:

10.1. ANTIPARASITIC MEDICATIONS

Standard treatment involves the use of drugs like Benznidazole and Nifurtimox. These medications attenuated or degenerated the parasite and reduced the infection's severity. They are most effective during the acute phase of the disease but can also be used in the early chronic stage (Julio et al. 2020).

10.2. SYMPTOMATIC TREATMENT

CD can cause different symptoms and complications. Managing these symptoms is essential to improving the patient's quality of life (Falk et al. 2022). Symptomatic treatment may involve medications to control cardiac symptoms, such as beta-blockers, angiotensin-converting enzyme inhibitors, and antiarrhythmics for heart rhythm abnormalities. Medications to manage digestive problems, like prokinetic agents and antacids, may also be prescribed (Jose et al. 2021).

10.3. SUPPORTIVE CARE

CD can affect multiple organs, including the heart, digestive, and nervous systems. Supportive care addresses specific symptoms and complications associated with organ involvement (García-Huertas et al.

2021). This may include interventions like pacemakers or implantable cardioverter-defibrillators (ICDs) for cardiac issues or medications to control gastrointestinal symptoms (Caldas et al. 2019).

10.4. REGULAR MEDICAL MONITORING

Patients with CD require regular medical monitoring to assess disease progression, evaluate organ function, and detect potential complications. This involves periodic blood tests, electrocardiograms (ECGs), echocardiograms, and other diagnostic procedures to assess cardiac and gastrointestinal involvement (Sulleiro et al. 2020).

10.5. VECTOR CONTROL

The CD is primarily transmitted through infected triatomine bugs. Implementing vector control measures, such as insecticide spraying, improving housing conditions, and using bed nets, is crucial for preventing new infections (Julio et al. 2019).

11. DRUG RESISTANCE

11.1. RESISTANCE TO BENZNIDAZOLE AND NIFURTIMOX

Benznidazole and Nifurtimox are the primary drugs used to treat CD. However, the parasite's resistance to these medications has been reported. The emergence of drug-resistant strains of *T. cruzi* is a concern and hampers successful treatment (Correia 2022; Juliana et al. 2022; Duschak et al. 2023).
The specific mechanisms underlying drug resistance in CD are not yet fully understood. It is believed that various factors, including genetic variations in the parasite and drug changes (Santi et al. 2022).
Drug resistance can have a significant impact on the effectiveness of treatment. Patients with drug-resistant strains may not respond well to standard therapy, leading to persistent infection and disease progression. This can result in prolonged or recurring symptoms and an increased risk of complications (Julio et al. 2019).
The emergence of drug resistance underscores the need for alternative treatment approaches. Currently, there are limited alternative drugs available to combat drug-resistant strains. Research focuses on developing new drugs or combination therapies to overcome drug resistance and improve treatment outcomes (Dumonteil et al. 2019).

12. EMERGING THERAPIES

12.1. DEVELOPMENT OF NEW ANTIPARASITIC DRUGS

Scientists are searching for novel antiparasitic drugs that effectively target and eliminate the *Trypanosoma cruzi* parasite. This involves exploring new drug compounds, repurposing existing drugs for CD, and investigating drug combinations to enhance efficacy and overcome drug resistance (de Araujo-Jorge et al. 2022).

12.2. IMMUNOTHERAPIES

Immunotherapies aim to enhance the immune response against the parasite. Researchers are developing vaccines to prevent infection or reduce disease severity. Various vaccine candidates, such

as DNA, protein-based, and vector, are being investigated to stimulate immune protection against *T. cruzi* (Subhadip et al. 2021).

12.3. HOST-DIRECTED THERAPIES

Host-directed therapies focus on modulating the host immune response to control the infection and minimize tissue damage caused by CD. These therapies seek to boost the immune system's ability to eliminate the parasite and prevent disease progression (Timothy et al. 2021).

12.4. COMBINATION THERAPIES

Combination therapies involving the use of multiple drugs with distinct mechanisms of action are being studied as a strategy to enhance treatment effectiveness and overcome drug resistance. By simultaneously targeting the parasite through numerous pathways, combination therapies can improve treatment efficacy and reduce the risk of treatment failure (Santo et al. 2020).

12.5. GENE THERAPY

Gene therapy involves modifying the genetic material of host cells to enhance their resistance to the parasite or improve the immune response against *T. cruzi*. Preclinical studies are underway to investigate the feasibility and effectiveness of gene therapy approaches in CD (Ana Lia et al. 2020).

12.6. DRUG DELIVERY SYSTEMS

Developing targeted drug delivery systems can enhance the effectiveness of antiparasitic drugs while minimizing side effects. Scientists are exploring using nanoparticles, liposomes, and other drug delivery systems to improve drug stability, increase drug accumulation in infected tissues, and optimize treatment outcomes (Nuria et al. 2022).

12.7. BIOMARKERS AND DIAGNOSTIC TOOLS

Identifying reliable biomarkers and developing improved diagnostic tools are essential for early detection, accurate diagnosis, and monitoring of CD (Carlier et al. 2019).

13. PREVENTION AND CONTROL

13.1. PUBLIC HEALTH STRATEGIES

- Vector control should be a key focus in public health strategies for CD. This involves identifying and eliminating breeding sites of triatomine bugs, improving housing conditions, and using insecticides to kill or repel the bugs.
- Screening blood and organ donors is crucial to prevent parasite transmission through transfusions and organ transplantation (Bern et al. 2019).
- Prevention of maternal and congenital transmission is essential. Pregnant women with CD should receive appropriate treatment, and newborns should be screened for early detection and intervention.
- Educating communities, healthcare providers, and the public about CD is essential. This includes raising awareness about transmission routes, prevention measures, and the importance of early diagnosis and treatment.

- Improving access to diagnostic tests and effective treatment is necessary for controlling CD (Calderón et al. 2020).

14. BLOOD SCREENING AND ORGAN TRANSPLANTATION

- Blood screening plays a critical role in preventing the transmission of CD through blood transfusions. Rigorous screening of blood donors is essential, typically involving tests to detect CD antibodies. If a donor tests positive, their blood should not be used for transfusion.
- Organ transplantation, particularly involving solid organs like the heart or liver, requires careful screening to prevent CD transmission. Donor screening includes serological tests to determine their CD status. Transplantation may be contraindicated if the donor tests are positive unless specific measures are taken, such as pre-transplantation or post-transplantation follow-up and treatment (Suárez et al. 2022).
- International guidelines provided by organizations like the World Health Organization (WHO) and the Pan American Health Organization (PAHO) offer recommendations for blood screening and organ transplantation concerning CD (Iglesias et al. 2023).

15. VECTOR CONTROL

- Effective vector control is essential in the fight against CD. It involves implementing measures to prevent the transmission of the parasite by triatomine bugs, also known as kissing bugs.
- Identifying and eliminating the breeding sites of triatomine bugs is a crucial step (Ribeiro-Jr et al. 2021). These bugs tend to inhabit poorly constructed houses, cracks in walls, animal burrows, and nests.
- The use of insecticides is a critical component of vector control. Proper application of insecticides can kill or repel triatomine bugs, preventing their bites and interrupting the transmission cycle. The choice of insecticides should be based on their effectiveness, safety, and suitability for the local context (Gürtler et al. 2021).
- Community involvement and awareness are vital for successful vector control. They are educating affected communities about and promoting the risks of triatomine bugs.
- Integrated approaches to vector management are recommended (Castro et al. 2020). This involves combining multiple strategies, such as insecticide spraying, housing improvements, and education.
- Continued triatomine bug biology, behavior, and insecticide resistance research is necessary to develop improved vector control strategies. Understanding the specific characteristics of local bug populations can inform targeted interventions.
- Collaboration among health authorities, policymakers, and communities is essential for successfully implementing vector control programs (Jennifer et al. 2019).

16. GLOBAL BURDEN AND FUTURE DIRECTION

Current Status Of Cd World Wild

16.1. ENDEMIC REGIONS

CD remains a significant public health concern in 21 Latin American countries, predominantly in Central and South America. These countries, including Argentina, Bolivia, Brazil, Colombia, Ecuador, Paraguay, Peru, and Venezuela, have a high disease prevalence and a large population at risk of infection.

16.2. GLOBAL DISTRIBUTION

Due to migration and travel, the CD has also been reported in non-endemic regions. Cases have been identified in North America, Europe, and certain parts of Asia. However, the prevalence of CD in these regions is generally lower compared to endemic areas (Amanda et al. 2020).

16.3. DISEASE BURDEN

It is estimated that CD affects 6 to 7 million people worldwide. However, the actual number of cases is believed to be much higher due to underdiagnosis and the often asymptomatic nature of the disease (Lidani et al. 2019).

17. CHALLENGES AND OPPORTUNITIES FOR FUTURE RESEARCH

17.1. DISEASE MECHANISMS

Future research should aim to deepen our understanding of the underlying mechanisms of CD, including parasite-host interactions, immune responses, and disease progression. This knowledge is crucial for developing targeted interventions (Jadel et al. 2022).

17.2. DIAGNOSTIC METHODS

There is a need to improve diagnostic tools for CD. Future research should focus on developing more accurate, affordable, and accessible diagnostic methods, including point-of-care tests and serological markers (Francisco et al. 2020).

17.3. TREATMENT OPTIONS

Current treatment options for CD have limitations. Future research should explore new therapeutic approaches, such as novel drugs or combination therapies, to improve treatment outcomes, reduce side effects, and address the chronic phase of the disease (Passos et al. 2020).

17.4. PREVENTION STRATEGIES

Developing effective prevention strategies is essential, especially in endemic areas. Future research should investigate innovative vector control methods, evaluate the impact of housing improvements, and explore interventions like vaccines or vector-targeted approaches to interrupt transmission (Jadel et al. 2022).

18. OPPORTUNITIES FOR FUTURE RESEARCH

18.1. GENOMICS AND PROTEOMICS

Advancements in genomics and proteomics offer opportunities to gain insights into the biology of the parasite, host immune responses, and disease progression. These technologies can aid in identifying drug targets, vaccine candidates, and diagnostic biomarkers (Jadelet al. 2022).

18.2. DRUG DISCOVERY AND REPURPOSING

Future research should explore new compounds and repurpose existing drugs for CD treatment. High-throughput screening and computational approaches can help identify potential candidates for further development.

18.3. IMMUNOTHERAPY AND VACCINES

Investigating immunotherapeutic approaches and developing an effective vaccine against CD are essential research opportunities. Enhancing the host immune response and preventing infection can significantly impact disease control (Kathryn et al. 2022).

18.4. VECTOR CONTROL STRATEGIES

Research should evaluate the effectiveness of existing vector control methods, explore novel insecticides and formulations, and understand the ecological factors influencing vector populations. Based on these findings, evidence-based vector control strategies can be developed (Johan et al. 2020).

18.5. HEALTH SYSTEM STRENGTHENING

Research should assess barriers and facilitators in the implementation of CD control measures. This includes evaluating cost-effectiveness, health-seeking behavior, and strategies for integrating CD into existing health programs (Batista et al. 2019).

19. POTENTIAL FOR ELIMINATION AND ERADICATION

Vector control is a primary approach, aiming to reduce the population of triatomine bugs through insecticide spraying, improved housing conditions, and community education. Blood screening programs help prevent transmission through transfusions and organ transplants (Koh, Carolina Cattoni, et al. 2023). Preventive measures, such as prenatal screening and treatment, aim to minimize vertical transmission from mother to child. Early diagnosis and treatment are crucial, and antiparasitic medications are available (Rita de Cássia Moreira de et al. 2022).

20. CONCLUSION

In conclusion, CD is a serious and potentially life-threatening infection caused by the *Trypanosoma cruzi* parasite. Infected triatomine bugs primarily transmit it, but can also be transmitted through other means, such as blood transfusions and congenital transmission. The disease has two phases, acute and chronic, with the latter leading to severe cardiac and digestive complications if left untreated. Diagnosis involves laboratory tests; treatment mainly relies on antiparasitic medications during the acute phase. Prevention and control efforts focus on insecticide spraying, improved housing conditions, bed nets, hygiene practices, and screening of blood and organ donors. While there are challenges in understanding and managing CD, future perspectives involve ongoing research to develop more effective treatments and prevention strategies to combat this global health issue.

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Recent Advances in the Diagnosis of Schistosomiasis

10

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ABSTRACT

Schistosomiasis is considered a neglected tropical disease instigated by flatworms of the genus Schistosoma, remnants a major problem in several parts of the world. A timely and accurate diagnosis is indispensable for disease management and control. Significant advances in the identification of schistosomiasis have been made in current years, improving our ability to detect and characterize infections. This chapter focuses on recent advances in schistosomiasis diagnosis, with an emphasis on new methods and approaches. Molecular diagnostic techniques, such as polymerase chain reaction (PCR), and loop-mediated isothermal amplification (LAMP), have exhibited the ability to improve schistosomiasis findings, sensitivity, and specificity. These techniques identify Schistosoma DNA in biological specimens such as blood, urine, and stool, allowing for early detection and monitoring of infections. Furthermore, the development of multiplex PCR assays allows for the finding of multiple Schistosoma species at the same time, which aids in species-specific diagnosis and epidemiological studies. In addition to molecular approaches, serological test-based antibody detection has advanced significantly. When compared to traditional methods, novel serological assays utilizing recombinant antigens and antigen-detection methods have demonstrated improved diagnostic accuracy, offering increased sensitivity and specificity. These tests not only detect current infections but also provide information about previous exposure to Schistosoma parasites. These non-invasive methods enable assisting in treatment decisions and assessing treatment efficacy. Finally, recent advances in schistosomiasis diagnosis have greatly expanded our diagnostic capabilities, allowing for more accurate and efficient infection detection. Molecular diagnostics, serological assays, and imaging modalities have all contributed to a better understanding of the disease and better patient care. Continuous research and innovation in diagnostic methods are required to improve global schistosomiasis control and elimination efforts.

Key words: Schistosomiasis, Molecular Techniques, Serological Assays, Neglected Tropical Disease.

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1. INTRODUCTION

Infectious diseases, especially parasitic infestations, are major public health concerns in both animals and humans (Alvi et al. 2023a; Alvi et al. 2023b). Schistosomiasis is considered a neglected tropical disease instigated by flatworms of the genus *Schistosoma*, remnants a major problem in several parts of the world. A timely and accurate diagnosis is indispensable for disease management and control. Significant advances in the identification of schistosomiasis have been made in recent years, improving our ability to detect and characterize infections. This chapter focuses on recent advances in schistosomiasis diagnosis, with an emphasis on new methods and approaches. Molecular diagnostic techniques, such as polymerase chain reaction (PCR), and loop-mediated isothermal amplification (LAMP), have exhibited the ability to improve schistosomiasis findings, sensitivity, and specificity.

These techniques identify *Schistosoma* DNA in biological specimens such as blood, urine, and stool, allowing for early detection and monitoring of infections. Furthermore, the development of multiplex PCR assays allows for the finding of multiple *Schistosoma* species at the same time, which aids in species-specific diagnosis and epidemiological studies. In addition to molecular approaches, serological test-based antibody detection has advanced significantly. When compared to traditional methods, novel serological assays utilizing recombinant antigens and antigen-detection methods have demonstrated improved diagnostic accuracy, offering increased sensitivity and specificity. These tests not only detect current infections but also provide information about previous exposure to *Schistosoma* parasites. These non-invasive methods enable assisting in treatment decisions and assessing treatment efficacy. Finally, recent advances in schistosomiasis diagnosis have greatly expanded our diagnostic capabilities, allowing for more accurate and efficient infection detection. Molecular diagnostics, serological assays, and imaging modalities have all contributed to a better understanding of the disease and better patient care. Continuous research and innovation in diagnostic methods are required to improve global schistosomiasis control and elimination efforts.

Schistosomiasis, also known as bilharzia, is a serious parasitic disorder initiated by blood flukes. It is still a persistent issue in various developing countries located in tropical regions. Schistosomiasis is ranked 2nd in terms of morbidity and mortality after malaria (Leblanc et al. 2023). Schistosomiasis is mainly caused by parasites *S. japonicum*, *S. mansoni*, *S. haematobium* (Mu et al. 2023a; Ullah et al. 2021), *Schistosoma mekongi*, *Schistosoma guineensis*, and *Schistosoma intercalatum* (Ajibola et al. 2018), in humans, and causes complicated conditions such as urinary bladder, colorectal, and liver malignancies (Qadeer et al. 2022). *Schistosoma* parasites can affect about 240 million people globally and have a serious health

problem prevalent in about 78 countries, approximately 0.7 billion individuals are in danger of contracting schistosomiasis (Ullah et al. 2022; Ullah et al. 2020a), and the estimated mortality due to this parasite is about 0.3 million yearly (Qadeer et al. 2021).

At present, there is no available vaccine to combat schistosomiasis, and the primary treatment approach involves the administration of praziquantel via chemotherapy. However, the widespread use of praziquantel has resulted in the emergence of drug resistance in the schistosomes that cause disease (Qadeer et al. 2022). Schistosomiasis has been detected using a variety of diagnostic methods. In conventional diagnostic methods, the *Schistosoma* eggs can be detected by traditional microscopic methods (such as Kato Katz, FECT, direct smear) in fecal or urine samples (Mu et al. 2023a). Several immunologic tests, including the indirect hemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), and rapid diagnostic tests (RDTs), have proven to be cost-effective and have been extensively employed for infection control and transmission control purposes (Lv et al. 2022). However, they may face challenges related to lower specificity when utilizing crude extracted antigens and the difficulty of distinguishing between past and present infections (Mu et al. 2023b).

Furthermore, the use of nucleic acid-based diagnostic methods has shown higher performance in detecting various schistosome species, especially in low-level infections. When compared to traditional methods such as the Kato-Katz method and miracidium hatching (Espírito-Santo et al. 2014), these DNA-based assays have significantly higher rates of positive detection. These assays, which rely on the detection of deoxyribonucleic acid (DNA), effectively reduce the occurrence of false negative results, and provide a reliable means of monitoring potential schistosomiasis exposure (He et al. 2016).

The disease can manifest in two ways: acute and chronic presentations. The acute form also referred to as Katayama syndrome, is caused by immediate or delayed hypersensitivity reactions to the immature worm migrating in the liver and other blood vessels (Coltart and Whitty 2015), and temporary lung infiltrates are typical symptoms that appear 14 to 84 days after infection (Cimini et al. 2021). Symptoms are fever, malaise, nausea, headache, diarrhea (Oliveira et al. 2020), wheezing, and eosinophilia (Coltart and Whitty 2015). While some people may have no or only mild symptoms (Oliveira et al. 2020). The chronic form, symptom may appear several months to years after the initial infection. The release of lytic enzymes, the activation of a significant T-cell mediates immune reaction, and the occurrence of delayed typed hypersensitivity reaction all contribute to the formation of granulomas and, eventually, tissue fibrosis (Zheng et al. 2020).

2. SCHISTOSOMA LIFE CYCLE

Schistosomiasis is an infectious disease resulting from parasitic trematodes belonging to the genus *Schistosoma*, which are commonly found in endemic regions of freshwater. The parasitic life cycle includes both an intermediate and a final host. The infective stage, known as cercaria, is larvae that can actively penetrate the human skin (Cimini et al. 2021). The cercaria transforms into schistosomula once inside the host and migrates through the bloodstream, eventually reaching the heart and lungs. From where it travels to the liver's portal and venous flow, wherever they develop into fully-grown worms of different sexes (sex determination occurs in fertilized eggs). To avoid the host immune response, mature worms have developed strategies such as the production of molecules that mimic self-antigens (Anisuzzaman and Tsuji 2020). Adult male-female worm pairs migrate through the host's circulation and settle in various organs depending on the Schistosoma species. The female worm starts to deposit a significant quantity of eggs, ranging from several hundred to several thousand per day, and this egg production can last for many years, even up to 20 years. In *S. japonicum*, the worms move towards the inferior mesenteric and superior hemorrhoidal veins. On the other hand, *S. mansoni* worm migrates to the superior mesenteric vein, while adult worms of S*. haematobium* travel to the vesical plexus and veins that drain the ureters, bladder, and

other pelvic organs (Cimini et al. 2021). Female worm eggs are highly immunogenic, causing a significant inflammatory response that causes damage to surrounding tissues. Furthermore, the eggs release lytic enzymes, which contribute to tissue destruction. As a result, the eggs exit the blood vessels and are excreted in urine and feces through the bowel or bladder. When the eggs hatch in the water, they discharge miracidia, the first larval stage. These miracidia enter an intermediate host, usually an aquatic snail, and reproduce asexually for several cycles within the snail. The parasite's cercaria stage eventually forms within the snail. These cercaria are then discharged from the snail into the water, where they can infect the ultimate host once more, thus perpetuating the cycle (Fig. 1) (Lindner et al. 2020).

3. OVERVIEW OF SCHISTOSOMIASIS DIAGNOSIS

To effectively control schistosomiasis in endemic areas, accurate, affordable, and user-friendly diagnostic methods are required. Microscopy of feces and urine samples (such as Kato Katz and direct microscopy) to detect parasites, as well as testing for serum antibodies, antigen detection, and DNA detection, are currently available diagnostic techniques for schistosomiasis. Nevertheless, there is a need for enhanced diagnostic methods that are affordable and user-friendly to effectively support national control programs in regions impacted by schistosomiasis. An overview of various diagnostic methods is enlisted in Fig. 2.

3.1. CONVENTIONAL PARASITOLOGICAL DIAGNOSIS

The conventional techniques were old method which is still practiced in some country due to limited resources. The conventional method was cheap, and quick but was not convenient for early diagnosis.

3.1.1. MICROSCOPIC METHODS

The first schistosomiasis diagnostic techniques relied on parasitological methods, such as identifying eggs in stool samples for intestinal schistosomiasis or urine samples for urinary schistosomiasis. These methods, however, have limitations in terms of early disease detection because they rely on detecting eggs, which are only produced by the parasites several weeks or months after infection. Consequently, these direct egg detection tests are not efficient in identifying the disease during its initial phase before the parasites become fully established and symptoms worsen (Weerakoon et al. 2015).

3.1.1.1. DIRECT FECAL SMEAR

In this method, approximately 2 mg of fresh stool or urine is taken and put on a drop of saline, properly mixed, and examined under a microscope (Utzinger et al. 2015). The direct fecal smear is a conventional method used for finding ova in fecal smears for intestinal schistosomiasis, or urine for urinogenital schistosomiasis and miracidial hatching (Chala 2023). It is widely recognized that microscopic examination of stool and urine samples remains the preferred and most reliable Schistosomiasis diagnostic test, serving as the gold standard. These methods, however, have some limitations (Ross et al. 2013). It is considered labor-intensive, time-consuming, and unsuitable for widespread disease surveillance. The effectiveness of parasitological diagnostic techniques is dependent on the rate of egg excretion, this lowers their sensitivity in regions with low disease occurrence, leading to a significant number of false negative outcomes (Chala 2023). In the case of *Schistosoma haematobium*, for example, the eggs are excreted in the urine and are typically detected through microscopic examination of concentrated urine sample obtained through sedimentation, centrifugation, or filtration, followed by passage through a paper or nitrocellulose filter (Chala 2023).

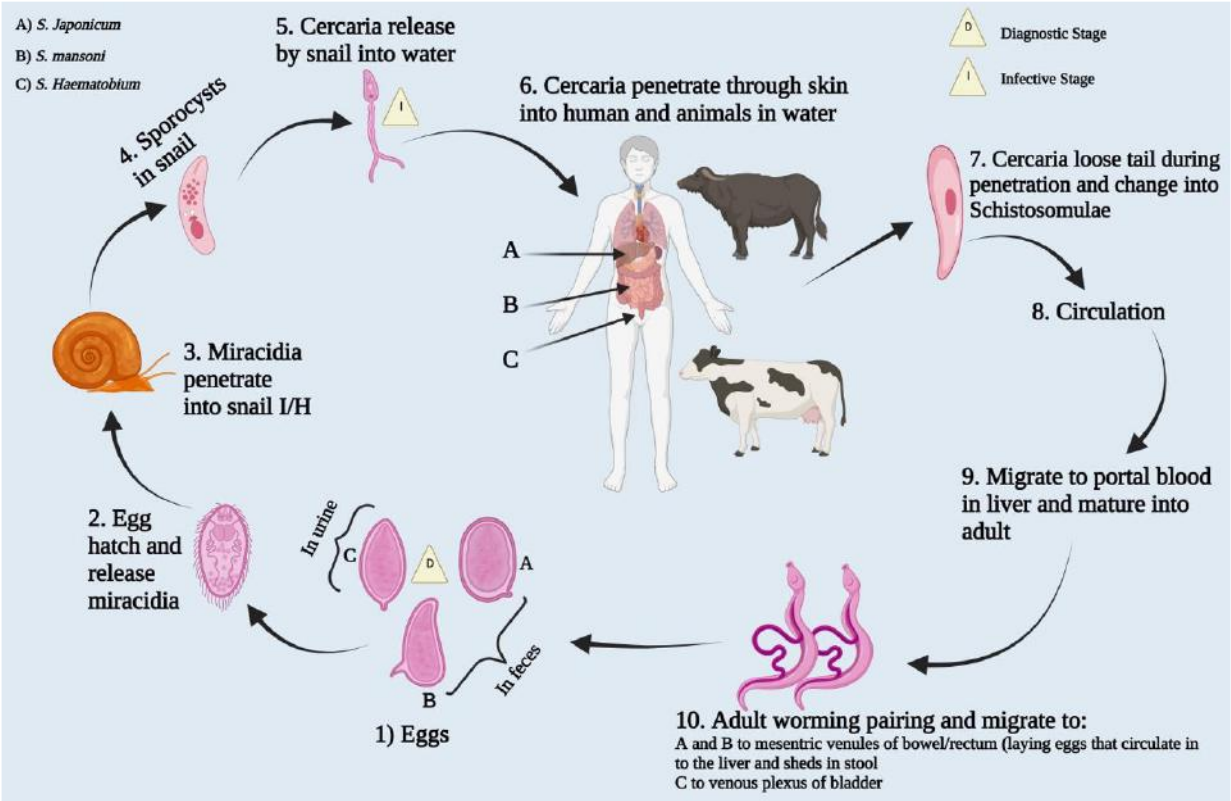

Fig. 1: Schematic life cycle of Schistosomiasis; *Schistosoma* parasites, which are trematode parasites, go through various stages of their life cycle in both bodies and water (steps 2-5) and the veins (steps 6-10) of their ultimate human/animal's hosts. The parasites rely on aquatic snails as intermediate hosts, which can be found in bodies of water. Humans/animals become infected when they encounter snail-infested waters while performing occupational or recreational activities. They then contribute to parasite transmission by contaminating local freshwater lakes and streams with urine or feces; The intermediate host snail is different for each species like *Oncomenania* species for *S. japonicum*, *Biomphalaria* species for *S. mansoni*, *Bulinus* species for *S. haemtobium* & *S. intercalatum*, *Neotricula* species for *S. mekongi*. This figure is generated by using Biorender.com.

3.1.1.2. FORMALIN ETHER CONCENTRATION TECHNIQUE (FECT)

The FECT is a laboratory technique used in high-income countries, particularly in a hospital laboratory, in conjunction with direct fecal smear analysis. Its goal is to improve the detection of parasites in stool samples (Utzinger et al. 2015).

The FECT consists of four major steps: 1) the homogenization of small amounts of stool (1-1.5gm) mixed with formalin, 2) filtration of stool through 400 µm sieve or surgical gauze to remove the debris, 3) the addition of ether to formalin-stool suspension, 4) centrifugation and examination of sediment material through a microscope. FECT generally exhibits greater sensitivity compared to direct fecal smear (Utzinger et al. 2010).

3.1.1.3. KATO-KATZ

In endemic countries, the Kato-Katz method is widely used as the standard way for detecting the occurrence and intensity of *S. japonicum*, *S. mansoni*, and other soil-transmitted helminths. There are several steps involved in the Kato-Katz procedure (Yap et al. 2012). 1) fresh stool samples are passed through a fine mesh (60 - 105 - µm) to remove large debris and to achieve a uniform consistency. 2)

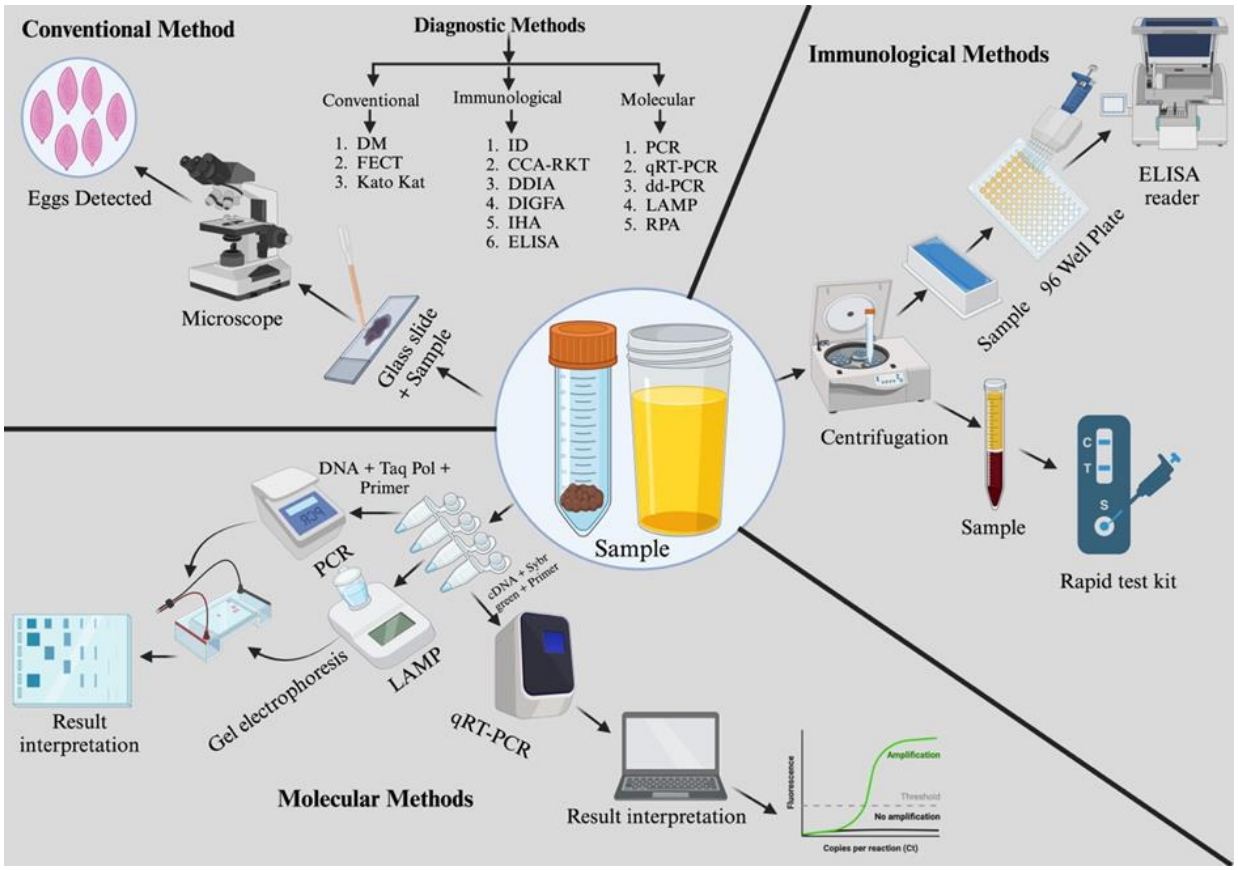

Fig. 2: General Pictorial representation of various diagnostic methods; The diagnosis of schistosomiasis can be primarily accomplished through three distinct approaches, as illustrated in the provided diagram. Each of these methods encompasses various subtypes, as depicted in the figure, with an emphasis on the commonly employed techniques.

The stool is transferred on the slide spread properly on the slide and covered with glycerol-methylene blue-soaked cellophane. The slide is then allowed to clear for at least 30 minutes, preferably 24 hours. 3) The slide is examined under a microscope after it has been cleared. The eggs seen under the microscope are counted and expressed as egg per gram of stool (EPG) by multiplying the apt factor (subject to the quantity of stool taken) (Utzinger et al. 2015). EPG values are used to determine the severity of an infection. The Kato-Katz method is a simple, inexpensive, and effective technique for detecting moderate to severe infection (Knopp et al. 2013). However, it has limitations in detecting light infections due to its comparatively low recognition threshold (around 25-50 EPG), depending on the template used. This can result in an underestimation of true prevalence, especially in low-prevalence areas, and can also complicate the confirmation of cure after treatment (Adriko et al. 2014).

3.2. NEW DIAGNOSTIC METHODS IN SCHISTOSOMIASIS

Recent advancements in proteomics and transcriptomics have led to significant breakthroughs in the study of schistosomes. These cutting-edge techniques have enabled the identification and characterization of a diverse array of molecules, including protein and other components, that are released during various stages of the schistosome life cycle. These newly discovered molecules hold great promise as a probable entrant for the development of diagnostic tools for schistosomiasis.

3.2.1. IMMUNOLOGICAL TEST:

With time various methods have been developed to assess the host's immune response by using crude or purified antigens from schistosome eggs and adult worms to detect antibodies. Immunological tests are used to detect anti-schistosomal immunoglobulins or to identify schistosomal antigens in body fluids such as plasma, serum, and urine. The following methods are commonly used for immunological detection.

3.2.1.1. INTRADERMAL TEST (ID)

It is a diagnostic test for *Schistosoma* based on immune reactions introduced by (Gan 1936), involving the use of antigens to detect IgE. These antigens are extracted from various stages of *Schistosoma*, such as fresh adult worms, frozen adult worms, eggs, and miracidia (Zhang et al. 2016). The intradermal test proved to be easily applicable, cost-effective, and highly sensitive (90 %), which led to its use in the 1950s in schistosomiasis control programmed to assess the prevalence and distribution of *S. japonica* (Maegraith 1958; Mao and Shao 1982). However, due to its low specificity, the intradermal test was eventually replaced by other diagnostic methods (Zhang et al. 2016).

3.2.1.2. CIRCULATING CATHODIC ANTIGEN (CCA) RAPID KIT TEST

This test is mostly used in humans to detect *Schistosoma* using urine samples. The CCA test, which is specifically designed for detecting *S. mansoni* is used to diagnose schistosomiasis. However, this test is less effective for the diagnosis of *S. haematobium* (Coulibaly et al. 2013). The CAA test works by binding Circulating cathodic antigen from the urine to a labeled monoclonal antibody immobilized on the sample membrane. When the solution is passed over the strip, a pink color appears if the antigen-antibody complex binds to another monoclonal antibody immobilized at the test line. The intensity of color reflects the severity of the infection (Sousa et al. 2020).

3.2.1.3. DIPSTICK DYE IMMUNOASSAY (DDIA)

It is a rapid testing kit designed to identify antibodies against *S. japonicum*. China developed the DDIA, which uses soluble egg antigen (SEA) that has been dyed colloidally. This test is particularly helpful for field screening (Zhu et al. 2005). This kit works on the principle that *S. japonicum's* soluble egg antigen (SEA) conjugates with a blue colloidal dye. This is cast-off to find antibodies in *Schistosoma*-infected patients' serum. Immunochromatography is used to immobilize the antigen-antibody complex onto a nitrocellulose membrane dipstick, employing anti-human IgG as the capturing agent (Zhu et al. 2002). In the case of S*. haematobium* infection dipstick reagent is available to detect hematuria, which is a common indicator of infection, the presence of blood can fluctuate over time (Kosinski et al. 2011).

3.2.1.4. DOT IMMUNOGOLD FILTRATION ASSAY (DIGFA)

DIGFA is a fast method used for the diagnosis of *S. japonicum*, this will detect anti-*Schistosoma japonicum* antibodies (Wen et al. 2005). The DIGFA was developed for the detection of schistosomiasis using rabbit anti-human IgG that has been tagged with colloidal gold as a probe and SEA as an antigen to make a diagnosis (Ding 1998). Tang et al. (2008) used a sheep antihuman IgM immunogold conjugate as a probe in their study to improve the precision of their assay (Tang et al. 2008). This modification produced remarkable results, with the test detecting acute schistosomiasis with 100% sensitivity and chronic schistosomiasis with 96% sensitivity (Tang et al. 2008). One of the primary benefits of this method is its

simplicity, as it does not require specialized equipment and can be performed quickly. Furthermore, when stored at 4°C, the reagent used in the test remained stable for at least 6 months (Ding 1998).

3.2.1.5. INDIRECT HEMAGGLUTINATION ASSAY (IHA)

IHA is used for the detection of *S. japonicum*. It agglutinates sheep erythrocytes with soluble antigens (Mao and Shao 1982), a process that occurs when antibodies against these antigens in patients' blood samples interact with the antigens on the cell surfaces (Zhang et al. 2016). The sensitivity of the immune-hemagglutination assay (IHA) has improved to 93-100% because of this approach, with a reduced false positive rate of 2-3% in healthy individuals from non-endemic areas (Nian-Gao et al. 2011; Yang et al. 2009). The majority of previous schistosomiasis patients have a negative test result after receiving effective, and consistent therapy for at least three years. Despite its long history of use (over 50 years), IHA is still the second most used general immunoassay in China, trailing only the COPT test (Zhou et al. 2009).

3.2.1.6. ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

ELISA is regarded as the foremost serological assay, demonstrating exceptional sensitivity and specificity in the diagnosis of schistosomiasis (Chala 2023). The reactivity between antibodies found in a patient's serum and antigens extracted from various life cycle stages of schistosomes can be assessed using the enzyme-linked immunosorbent assay (ELISA) (Weerakoon et al. 2015). The procedure involves the binding of soluble proteins, such as antigens or antibodies, to the surfaces of multi-well plates. It provides the flexibility to detect various classes of antibodies using a wide array of antigens. By virtue of the affinity between antigens and antibodies, ELISA generates qualitative or quantitative results (Othman 2013). Initially, the detection of *Schistosoma* antigens by ELISA relied on the utilization of crude soluble egg antigens (SEA) and soluble adult worm proteins (SWAP). This was subsequently followed by the introduction of purified antigens, often referred to as excretory/secretory antigens. Detecting SEA and SWAP in serum and excreta has shown significant diagnostic potential, as the levels of these antigens correlate closely with the parasitic load, enabling early treatment initiation (Chala 2023). Furthermore, assays designed for the detection of circulating schistosome adult worm antigens offer an alternative method for diagnosing schistosomiasis. The primary advantages of these circulating antigens include their high specificity, positive correlation with the worm burden, and the ability to estimate the intensity of infection. Additionally, circulating *Schistosoma* antigens rapidly disappear following treatment, making them valuable for assessing the effectiveness of a cure (Chala 2023).

3.2.2. NUCLEIC ACID-BASED TECHNIQUES

Nucleic acid tests have emerged as a prominent diagnostic tool for parasitic infections such as schistosomiasis. (Chala 2023). Because of their precision and sensitivity, these methods offer significant advantages in terms of accurate and timely parasitic detection. In this section, we will deliver a summary of current advancements in each method before delving into their utility in diagnosing Schistosome infections (Ullah et al. 2020b). The diagnosis of schistosomiasis relies on microscopic techniques, but it has poor sensitivity when the parasitic burden is low, time-consuming and a trained operator is required for it (Ross et al. 2017). Due to these problems, people are shifting towards molecular techniques for the diagnosis of schistosomiasis, it`s not only improves diagnosis but also uplifts effective research in current times (Cavalcanti et al. 2019; He et al. 2016). Following are different nucleic acid techniques used as an advanced diagnostic method in schistosomiasis diagnosis.

3.2.2.1. POLYMERASE CHAIN REACTION (PCR)

The use of PCR to detect Schistosoma DNA in urine and stool samples is a highly sensitive and specific method that offers significant improvement in diagnosing schistosomiasis in non-endemic areas with low parasitic burden (Obeng et al. 2008; Pontes et al. 2003). When used to detect Schistosomiasis in various sample types, PCR consistently shows exceptional sensitivity and specificity (Gomes et al. 2010; Ten-Hove et al. 2008). PCR specificity is 99.9% and sensitivity 94.4% when using genus-specific PCR, and specificity is 98.9% and sensitivity 100 % when using species-specific (S. mansoni) PCR (Sandoval et al. 2006). Different types of PCR are used like ddPCR, RT-PCR, qPCR, and conventional PCR for the recognition of schistosomiasis from several specimens; stool, saliva, serum, and urine (Rahman et al. 2021).

3.2.2.2. REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (QRT-PCR)

Real-time quantitative polymerase chain reaction is a technique used to quantify the quantity of PCR products, typically by incorporating fluorophores into the reaction. During the qPCR process, the fluorescence signal produced is measured in each amplification cycle. Unlike traditional PCR, qPCR eliminates the need for gel electrophoresis to visualize the DNA bands, making it less labor-intensive. Moreover, qPCR offers advantages over conventional PCR as it enables the detection of lower concentrations of target DNA (Chala 2023).

3.2.2.3. DROPLET DIGITAL PCR (DD PCR)

Recent advances in PCR technology have resulted in ddPCR (digital droplet PCR) emerging as a more sensitive and precise alternative to qPCR (Yang et al. 2014). ddPCR is highly effective in a variety of applications, including the detection of cell-free DNA (cfDNA) and the diagnosis of infection and clinical conditions such as cancer (Olmedillas-López et al. 2017). Furthermore, its efficacy in diagnosing *S. japonicum* infections in animal models and various human clinical samples has been demonstrated, allowing for quantification of infection intensity via direct target gene copy number (Weerakoon et al. 2017).

ddPCR capability to test multiple targets renders it a valuable diagnostic tool for identifying numerous parasites in an infected individual (Jongthawin et al. 2016). This characteristic enables the simultaneous detection and quantification of multiple parasite species, providing a more comprehensive thorough approach to diagnosis. Overall, the improved sensitivity, precision, and multiplexing capabilities of ddPCR make it a promising technology for research, clinical diagnosis, and infectious disease surveillance (Weerakoon et al. 2018).

3.2.2.4. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

When compared to PCR-based methods, the LAMP technique provides a cost-effective and rapid means of detecting DNA, making it particularly useful for field use. Its ease of use stems from not requiring specialized equipment such as a thermocycler or electrophoresis apparatus, making it suitable for resource-limited settings once properly optimized (Weerakoon et al. 2018). The assay is highly specific to the target sequence and remarkably sensitive recognition to the use of specific inner and outer premier sets (Xu et al. 2010). However, multiple premiers are used, and the initial optimization process can be time-consuming. Furthermore, there is a risk of carryover contamination, which could result in false positive results when reamplifying previous LAMP products (Ma et al. 2017).

Table 1: Summary of strengths and shortcomings of Schistosomiasis diagnostic techniques

Assay Type	Test Name	Strengths of tests	Shortcoming of tests	Reference
Conventional methods	Direct Microscopy	Simple, cost-effective	Ineffective in early-stage diagnosis, time-consuming,	(Chala 2023)
	FECT	Simple, time efficient	Limited sensitivity, operator dependency	
	Kato-Katz	Cost-effective, gold standard	Low detection limit	
Immunological Methods	Intradermal Test	Low cost & highly sensitive	Low specificity	(Chala 2023)
	CCA Rapid Kit test	More sensitive than conventional method	Limited accuracy and costly	
	DDIA	Rapid, easy to use, no special equipment needed, high specificity	Limited sensitivity, potential cross-reactivity	
	DIGFA	Rapid, simple, highly specific, and sensitive	Limited availability, technical requirement	
	IHA	Sensitive, wide range application, simple	Cross-reactive, technical skill requirement	(Van Lieshout et al. 2000)
	ELISA	Sensitive, high throughput, highly specific	Time-consuming, complex, cross-reactivity	
Nucleic Acid Methods	PCR	High specificity and sensitivity, can detect a small amount of template	Expensive, time-consuming,	(Ullah et al. 2020b)
	qRT-PCR	Highly sensitive and specific	More expensive than other PCR methods	(He et al. 2018)
	dd-PCR	Highly sensitive and specific	Expensive equipment required	(Weerakoon et al. 2017)
	LAMP	Rapid, highly efficient, less equipment and reagent needed	Prone to carryover contamination, chances of false positive results	(Gandasegui et al. 2018)
	RPA	Cost-effective, highly sensitive, and specific	Risk of a false positive outcome	(Poulton and Webster 2018)

LAMP assays are effective at detecting *S. mansoni* and *S. haematobium* infections, especially in areas where both parasites co-exist. This suggests that they have the potential to be used as point-of-care diagnostics for rapid and accurate detection (Lodh et al. 2017). Early detection of pre-patent schistosome infections has been made possible by the successful application of the LAMP technique in animal models (Fernández-Soto et al. 2014). Field surveys have also shown that LAMP assays an effective in detecting *S. mansoni* infection in low transmission areas, using samples from snails, humans, and animals' feces. This demonstrates the molecular approach's ability to identify transmission foci and create risk maps, which can be used to support control programs (Gandasegui et al. 2018).

Current work has investigated the feasibility of developing multiplex LAMP assays to detect multiplex parasitic species, including schistosomes, in infected people. Multiplex LAMP enables various endpoint readout options for differentiating amplified products, such as melting curve analysis or distinct gel electrophoretic banding patterns (Liu et al. 2017), facilitating species detection. Multiplex LAMP, like qPCR, is a promising method for effectively diagnosing soil-transmitted helminths (STH) co-infection or the co-infection of intestinal protozoa and schistosomes in resource-limited endemic communities (Llewellyn et al. 2016).

3.2.2.5. RECOMBINANT POLYMERASE AMPLIFICATION (RPA)

RPA is a type of isothermal amplification that works at lower temperatures, typically around 40 °C. To amplify DNA sequences, it uses DNA polymerase, DNA binding proteins, recombinase protein, and

oligonucleotides nucleoprotein complexes (Poulton and Webster, 2018). The RPA technique, like LAMP, is simple to use and can be implemented in resource-constrained settings without the need for specialized equipment such as a thermocycler, electrophoresis apparatus, or gel documentation units. This novel technique has recently been combined with chips and lateral flow devices, transforming it into a convenient and portable tool for point-of-care diagnosis (Zanoli and Spoto 2013).

RPA has demonstrated success in the diagnosis of both intestinal and urinary schistosomiasis and has been thoroughly tested field. RPA has significant advantages over microscopy and serology in terms of convenience, shorter detection time, and improved diagnostic sensitivity (Poulton and Webster 2018). However, the RPA technique has some practical limitations. The requirement to transfer the amplified products to the detection device introduces the risk of nucleic acid contamination and can result in false positive results (Weerakoon et al. 2018).

A summary of the strength and shortcomings of Schistosomiasis diagnostic methods are enlisted in Table 1.

4. FUTURE PROSPECTIVE

The ongoing research to improve existing diagnostic methods, the discovery of novel biomarkers via proteomic and transcriptomic, and the progress of multiplex assays for the simultaneous finding of multiple schistosome species or infection stages are all possibilities for the future. Furthermore, the incorporation of diagnostics and new imaging technologies into disease control programs could improve disease surveillance and targeted interventions. Collaboration among researchers, healthcare professionals, policymakers, and stakeholders is critical for fully realizing the benefits of these advances. With continued efforts and a global approach, recent advances in schistosomiasis diagnosis have the potential to have a substantial influence on disease control and improve the lives of those affected by this neglected tropical disease.

5. CONCLUSION

Significant recent developments in the identification of schistosomiasis have been made, to address the limitations of traditional methods and improve diagnostic accuracy, sensitivity, and accessibility. The emergence of rapid point-of-care tests, molecular diagnostics using PCR and LAMP, serological assay, and antigen detection tests are notable developments. These developments have shown promise in detecting Schistosome molecules at various stages of life, providing valuable diagnostic candidates. Despite these accomplishments, achieving optimal diagnosis remains a challenge. Some test's sensitivity and specificity may still need to be improved, and cost and accessibility barriers may prevent widespread implementation, particularly in resource-limited settings. Accurate differentiation of different Schistosome species remains critical for appropriate treatment, and early detection remains an important aspect of disease management.

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Zoonotic Threat of Anaplasmosis

11

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ABSTRACT

Anaplasmosis, a vector-borne zoonotic disease caused by various Anaplasma spp., poses significant threats to both human and animal health globally. A. phagocytophilum and A. marginale are notable pathogens, causing human granulocytic anaplasmosis and affecting livestock, particularly cattle. Transmitted through tick bites, the diseases exhibit a broad geographical distribution, with recent concerns arising in regions like North Africa and the Middle East. Despite advancements in understanding Anaplasma life cycles and their impact, challenges persist, necessitating further research for improved disease control and diagnostic methods. The complex life cycle involves ticks as vectors and mammalian hosts, contributing to the bacteria's wide dissemination. Clinical manifestations vary, with human cases showing acute symptoms resembling other febrile illnesses. Diagnostic methods include PCR and serological assays targeting specific antigens. Tetracyclines, particularly doxycycline, are the primary treatment, but challenges include antibiotic resistance. Control measures encompass vector management, biosecurity, and vaccination trials, notably targeting conserved antigens like A. phagocytophilum MSP4. Public health implications and zoonotic potential underscore the need for a One Health approach. Challenges in treatment, vector control, and economic considerations demand collaborative efforts for effective disease management. The interconnectedness of human, animal, and environmental health is emphasized, necessitating vigilance in surveillance, clinical awareness, and collaborative strategies to minimize public health risks associated with Anaplasma infections.

Keywords: Anaplasmosis, Zoonotic disease, Tick-borne pathogens, Diagnosis, One Health

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1. INTRODUCTION

Anaplasmosis is a vector-borne zoonotic disease caused by the Anaplasma spp. This group of bacteria includes several species that are notable for their impact on human and animal health, such as Anaplasma (A.) phagocytophilum and Anaplasma marginale (Parvizi 2021). A. phagocytophilum is known to cause human granulocytic anaplasmosis, a condition that results from the bacteria infecting white blood cells (Glatz et al. 2014). On the other hand, A. marginale is a well-known pathogen in livestock, particularly cattle. This species is prevalent in various regions around the globe, with particularly high prevalence rates noted in areas like Ghana (Heylen et al. 2023). The diseases caused by Anaplasma are transmitted to humans and animals through the bites of ticks. The role of ticks as vectors for these diseases highlights the importance of controlling tick populations to prevent the spread of Anaplasma (Madesh 2021). Furthermore, the importance of understanding and addressing these diseases is underscored by their broad geographical distribution and the wide range of animals that can serve as hosts. While a lot of attention has been focused on anaplasmosis in North America and Europe, a recent study have noted the presence of this disease in regions like North Africa and the Middle East (Parvizi 2021). While much progress has been made in understanding the life cycle of Anaplasma species and their effects on human and animal health, there remain many opportunities for further research. These include developing more effective strategies for disease control and prevention, and improving diagnostic methods for detecting infections (Suarez and Noh 2011).

Anaplasmosis, caused by several species of Anaplasma bacteria, has significant implications as a zoonotic disease, impacting both veterinary medicine and public health worldwide. These bacteria are recognized as important tick-borne pathogens (Wei et al. 2020). Species like A. phagocytophilum and A. marginale are of particular significance due to their broad distribution and the potential harm they cause to livestock and humans (Atif 2015). A. platys, another species within this genus, was initially detected in a dog from Florida and is now recognized as a causative agent of canine infectious cyclic thrombocytopenia and granulocytic anaplasmosis, both are zoonotic diseases (Atif et al. 2021). Apart from dogs, Anaplasma species have been reported in a variety of hosts, including cats, goats, and small ruminants, increasing the zoonotic potential of these diseases (Yang et al. 2017; Schäfer and Kohn 2020; Wei et al. 2020).

2. HISTORY

In Uruguay, the history of research and control efforts for anaplasmosis (alongside babesiosis) has been associated with the tick species *Rhipicephalus microplus* (Miraballes and Riet-Correa 2018). Similarly, the epizootiology and control strategies for anaplasmosis have also been studied extensively in South Africa (Potgieter 1979). In Iran, detection and diagnosis of *A. marginale* in cattle have been crucial, with several herds having a history of acute anaplasmosis (Noaman et al. 2009; Noaman 2013). Meanwhile, in Northeastern Brazil, there's a recorded clinical history of babesiosis and anaplasmosis in dairy farms, indicating enzootic stability for anaplasmosis (Souza et al. 2013). A decade-long seroepidemiological study in Belgium also highlighted the relevance of anaplasmosis in humans, especially in those with a history of tick bites and febrile illnesses (Cochez et al. 2011).

In terms of geographical distribution, anaplasmosis has been identified in a wide range of regions. For example, a significant prevalence of bovine anaplasmosis was confirmed in Egypt, with implications for public health, veterinary practice, and the livestock industry (Parvizi et al. 2020). Studies also point to the relevance of this disease in North American countries such as Canada, where human granulocytic anaplasmosis (HGA) is considered as a major emerging zoonotic disease (Kulkarni et al. 2015). In Africa, the significance of *Anaplasma* species is underscored by their detection in cattle populations in Nigeria and Ethiopia. Furthermore, anaplasmosis is prevalent in small ruminants in China, where the identification

of a novel *Anaplasma* species has raised concerns about potential public health implications (Yang et al. 2017).

3. LIFE CYCLE

The life cycle of *Anaplasma* is complex and involves both an arthropod vector, usually a tick, and a mammalian host. This life cycle is initiated when a tick that carries *Anaplasma* bacteria feeds on a mammalian host, injecting the bacteria into the host's bloodstream along with the tick's saliva (Atif 2015). This can happen when ticks, in the quest for blood meals, attach themselves to various mammalian hosts including humans, livestock, and pets. Upon entering the mammalian host, *Anaplasma* bacteria show a marked affinity for certain types of white blood cells, particularly the granulocytes. The bacteria utilize an array of specialized proteins, including Asp14, to facilitate attachment and subsequent invasion into the host cells (Kahlon et al. 2013). This invasion process is crucial for the bacteria to establish infection as it provides a protective environment against the host's immune responses and allows the bacteria to replicate unhindered. Inside the host cells, the bacteria manipulate host cellular processes to survive, grow, and multiply, creating unique cellular structures known as morulae. The infected cells eventually rupture, releasing the new bacteria which can then infect other cells, thus perpetuating the infection within the host (Rikihisa 2010). Simultaneously, ticks feeding on these infected hosts can ingest the bacteria during their blood meal, and consequently become carriers, ready to transmit the bacteria to other hosts in their future blood meals. This ensures the continuation of the bacteria's life cycle and its propagation across a wide range of hosts and geographical locations. Additionally, the movement of mammalian hosts, particularly birds and migratory mammals, contributes significantly to the spread of *Anaplasma* bacteria. These hosts can carry the bacteria over long distances, increasing its range and the possibility of encountering new potential host species (Stuen et al. 2013). The entire events involved in the life cycle are shown in Fig. 1.

4. CLINICAL SIGNS AND SYMPTOMS

Clinical manifestations of *Anaplasma* infections exhibit variability based on the particular species involved and the mammalian host affected. Notably, in the case of human granulocytotropic anaplasmosis (HGA), which is attributed to *A. phagocytophilum*, the disease typically manifests with a set of acute and nonspecific symptoms. Individuals afflicted with HGA often experience fever, accompanied by chills, headache, muscle aches, and a general sense of malaise. This clinical presentation frequently mirrors the symptomatology of other febrile illnesses, underscoring the challenge of distinguishing HGA solely based on clinical grounds (Bakken and Dumler 2006). Pro-inflammatory immune responses have been associated with clinical signs and symptoms of human anaplasmosis, indicating the involvement of the host's immune system in the disease presentation. The clinical signs of anaplasmosis in cattle and other mammalian hosts can also vary widely, presenting a complex diagnostic challenge. An outbreak of clinical anaplasmosis in dairy cattle was characterized by primary signs such as fever, anemia, jaundice, and lethargy, demonstrating the impact of *A. marginale* infections on livestock. These symptoms can lead to significant production losses, affecting both milk yield and reproductive efficiency (Schotthoefer et al. 2017).
In Turkey, laboratory-confirmed clinical cases of bovine anaplasmosis caused by *A. phagocytophilum* were identified, further highlighting the potential severity of anaplasmosis in livestock. These instances revealed that the disease could be both acute and chronic in nature, depending on factors such as the host's immune system, age, and the specific strain of *Anaplasma* involved. The geographical spread of anaplasmosis is also of significant concern. Environmental factors, vector distribution, and livestock management practices all contribute to the epidemiology of the disease, making it a considerable threat to both commercial farming and smallholder systems. Efforts to control anaplasmosis must include regular

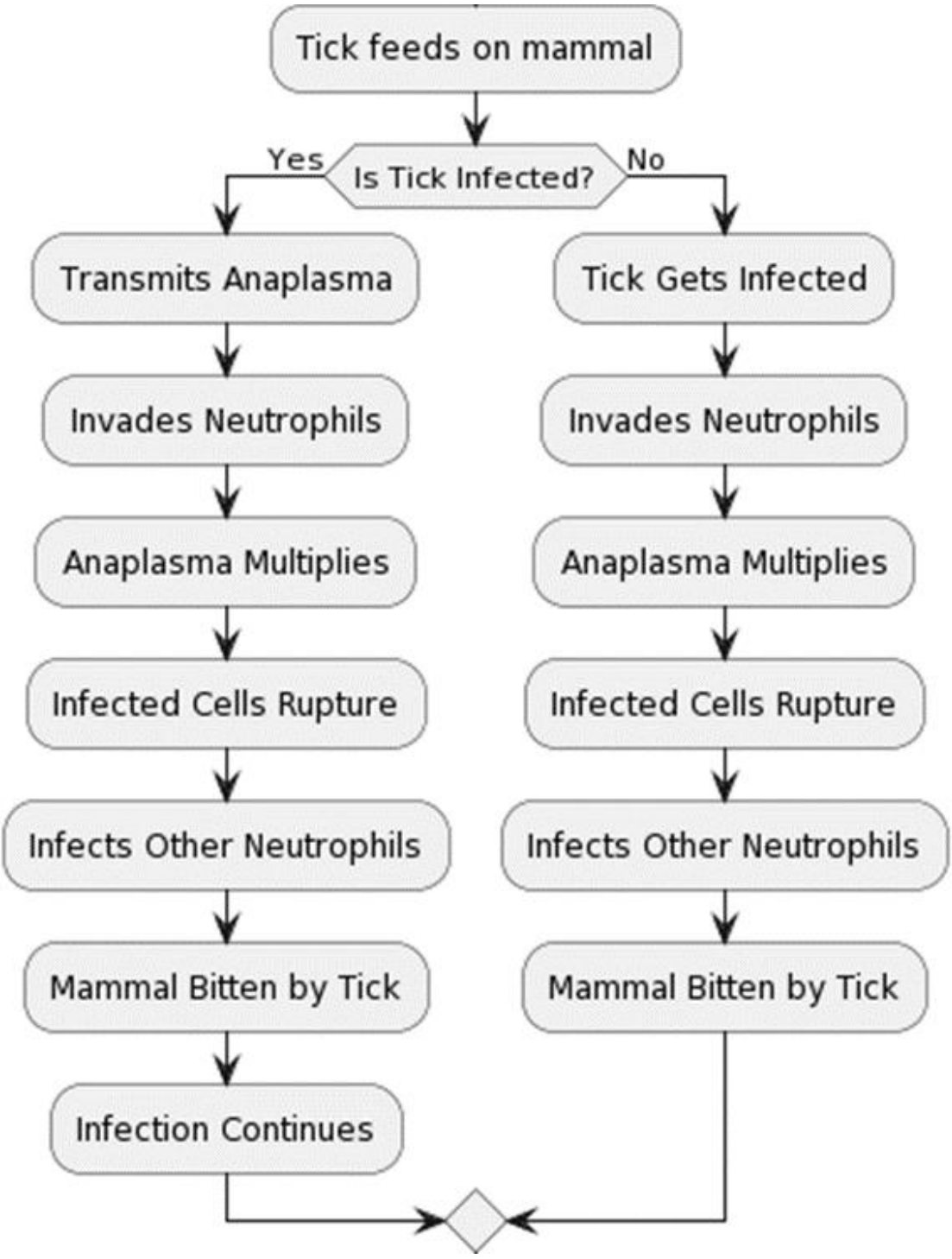

Fig. 1: Life cycle of *Anaplasma* spp.

monitoring, efficient diagnostic methods, and effective treatment protocols. Vaccination, where available, and vector control measures are essential components of a comprehensive prevention strategy. Collaboration between veterinary authorities, farmers, and researchers is crucial to develop and

implement policies that address the complexities of anaplasmosis in different regions and livestock populations (Aktas and Özübek 2017). Additionally, transfusion-transmitted *A. phagocytophilum* cases have been reported, emphasizing the importance of considering *Anaplasma* infections as potential complications in blood transfusion recipients (Annen et al. 2012).

5. DIAGNOSIS

The swift and accurate diagnosis of anaplasmosis is a paramount endeavor in ensuring effective treatment and preventing further transmission. Anaplasmosis has garnered increasing attention due to its impact on both human and animal health. As such, the development of robust diagnostic methods has become essential for timely intervention and disease control. One of the key challenges in diagnosing anaplasmosis lies in its nonspecific clinical manifestations. The symptoms exhibited by infected individuals or animals often overlap with those of other febrile illnesses, making it challenging to pinpoint the causative agent solely based on clinical grounds.

Therefore, diagnostic approaches rely on a combination of clinical evaluation, laboratory tests, and advanced techniques to unravel the presence of *Anaplasma* pathogens. A significant stride in the diagnosis of human granulocytotropic anaplasmosis (HGA) was the identification of *A. phagocytophilum* as the responsible agent. HGA was first recognized in 1990, shedding light on a previously unidentified pathogen that infiltrates neutrophils and elicits acute, nonspecific symptoms in infected individuals (Bakken and Dumler 2006). The clinical diagnosis of HGA is further complicated by its similarity to other tick-borne illnesses. This underscores the importance of integrating laboratory findings with clinical presentation to establish a conclusive diagnosis. Laboratory testing constitutes a cornerstone in the diagnostic process for anaplasmosis. Techniques like polymerase chain reaction (PCR) have emerged as powerful tools for detecting the genetic material of *Anaplasma* pathogens in blood samples. This molecular approach enables the identification of the pathogen's DNA, offering a rapid and accurate means of diagnosis.

Similarly, serological assays play a pivotal role in detecting antibodies produced in response to *Anaplasma* infections. Enzyme-linked immunosorbent assays (ELISAs) have been developed to target specific antigens associated with *Anaplasma* species, aiding in serodiagnosis and serosurveillance. A noteworthy advancement in the diagnostic landscape is the utilization of major surface proteins (MSPs) as serodiagnostic markers. Genetic diversity among *Anaplasma* strains has prompted the exploration of MSPs as targets for serodiagnosis and vaccine development. The implications of these findings extend beyond diagnosis, potentially paving the way for preventive measures through vaccine strategies (Kocan et al. 2010).

Canine anaplasmosis, caused by *A. phagocytophilum*, presents diagnostic challenges akin to its human counterpart. In dogs, clinical manifestations can include various nonspecific symptoms, such as fever and lethargy. To facilitate diagnosis, observation of neutrophilic morulae, distinctive inclusions within neutrophils, has been employed (Lester et al. 2005). This microscopic finding contributes to a conclusive diagnosis of canine anaplasmosis and guides appropriate management. The diagnostic landscape for anaplasmosis continues to evolve. Emerging research highlights the potential of whole organism–based immunofluorescent assays (IFAs) for serologic diagnosis. These assays, which target antibodies against *Anaplasma* and other tick-borne pathogens, hold promise for accurate and comprehensive diagnostic evaluations (Qurollo et al. 2021).

6. TREATMENT AND CONTROL

One of the primary goals in anaplasmosis management is the development of suitable treatment regimens. Tetracyclines, a class of antibiotics, have emerged as the treatment of choice for eliminating

Anaplasma infections. Notably, these antibiotics have shown efficacy against a spectrum of *Anaplasma* species, including both human and animal pathogens. The use of tetracyclines, such as doxycycline, has proven to be instrumental in mitigating clinical manifestations and reducing pathogen burden. This is particularly significant as *Anaplasma* infections can lead to serious health complications if left untreated. The symptoms of anaplasmosis can vary widely, ranging from mild fever and fatigue to more severe manifestations like organ failure. The effectiveness of tetracyclines in treating *Anaplasma* infections can be attributed to their broad-spectrum antimicrobial activity. They inhibit the synthesis of bacterial proteins by binding to the ribosomal subunits, thereby preventing the growth and multiplication of the bacteria. This makes them highly effective in targeting various strains of *Anaplasma*. However, the use of tetracyclines is not free of challenges. Resistance to these antibiotics has been reported in some cases, necessitating ongoing research and development to identify alternative treatment options or to enhance the existing ones. Additionally, the administration of tetracyclines must be carefully monitored, as overuse or misuse can lead to side effects such as gastrointestinal disturbances or photosensitivity (Dantas-Torres and Otranto 2017).

Anaplasmosis control, however, extends beyond treatment to encompass preventive measures. The concept of endemic stability comes to the fore, emphasizing the need to maintain *Anaplasma*-free herds. This entails meticulous breeding practices, robust biosecurity protocols, and identification of carriers to prevent the transmission of the pathogen within the herd (Atif 2015). The role of vectors, such as ticks, in anaplasmosis transmission underscores the significance of vector control strategies. The use of acaricides and tick control measures assumes paramount importance to curb the spread of pathogen. These measures, aimed at breaking the transmission cycle, prove pivotal in managing the disease. Vaccination offers a promising avenue for anaplasmosis control, an approach that has gained momentum in recent years with advances in research and technology. The identification of specific antigens, such as *A. phagocytophilum* Major Surface Protein 4 (MSP4), has opened new vistas for vaccine development, bringing hope to livestock industries across the globe. Vaccination trials targeting MSP4 have demonstrated potential in conferring protection against *Anaplasma* infections, reflecting an innovative approach to disease control. These trials have been conducted both in the laboratory and in field settings, with results showing significant reductions in clinical symptoms and transmission rates among vaccinated animals. The utilization of MSP4 as a key component in vaccines represents a targeted and refined strategy that addresses the unique characteristics of *Anaplasma* pathogens. This protein has shown to be highly conserved among different *Anaplasma* species, thereby increasing the potential for cross-protection against various strains of the bacteria (Miraballes and Riet-Correa 2018).

Collaborative efforts between researchers, veterinary scientists, and pharmaceutical companies are essential to further refine the vaccine, ensuring safety, efficacy, and scalability for commercial use. Challenges such as the adjuvant selection, dosage optimization, and delivery methods must be carefully addressed to develop a vaccination protocol that can be widely adopted. Public-private partnerships may also play a vital role in accelerating the development and deployment of the vaccine, ensuring that it reaches the farmers and regions where it is most needed. Education and training programs for farmers and veterinary professionals will also be crucial in maximizing the effectiveness of the vaccine, enhancing understanding of when and how to administer it, and monitoring its impact on herd health (de la Fuente et al. 2022).

7. PUBLIC HEALTH IMPACT AND ZOONOTIC POTENTIAL

The interplay between human-animal interaction necessitates a one health approach to anaplasmosis control. The potential zoonotic implications of *Anaplasma* infections further accentuate the importance

of cross-species disease management. Strategies that address animal reservoirs and vector control in tandem with human preventive measures will be pivotal in halting the progression of the disease. The management of anaplasmosis is critical to both veterinary and public health due to the potential economic losses in livestock and the zoonotic nature of certain *Anaplasma* species. Developing effective strategies for treatment and control is imperative to mitigate the impact of the disease. Early diagnosis and treatment are essential for effectively managing anaplasmosis. Administering antibiotics, particularly tetracyclines like doxycycline, plays a crucial role in treating the disease. Doxycycline's mechanism of inhibiting bacterial protein synthesis hinders the proliferation of *Anaplasma* organisms within host cells (Atif et al. 2021).

Vector Control is a pivotal aspect of anaplasmosis control due to the reliance of *Anaplasma* pathogens on vectors like ticks for transmission. Implementing efficient tick control measures disrupts the transmission cycle, protecting animals from infection and reducing the risk of transmission to humans (Waruri et al. 2021). Identifying carriers and isolating infected individuals helps to prevent the disease's spread within the herd. Strategies like culling or treating carriers are necessary to achieve and sustain endemic stability (Abdisa 2019). Challenges in treatment and control persist. The emergence of antibiotic-resistant strains of *Anaplasma* underscores the need for continuous research and innovative solutions. The complex behavior of vectors and the evolving nature of the disease demand adaptable strategies. Economic Considerations highlight the benefits of diagnostic testing. Accurate diagnosis enables timely treatment, reducing the severity of the disease and minimizing economic losses. Analyzing the cost-effectiveness of diagnostic testing provides valuable insights into managing anaplasmosis (Railey and Marsh 2021).

The zoonotic potential of anaplasmosis extends to the canine population, with diseases like canine infectious cyclic thrombocytopenia and granulocytic anaplasmosis being of particular concern. The detection of *A. platys* in dogs from Florida underscores the close relationship between animals and humans, necessitating a thorough understanding of these zoonotic diseases for effective prevention (Atif 2015). Genetic genotyping of *Anaplasma* strains offers valuable insights into their impact on public health. Continual discovery of novel tick-associated microbes with zoonotic potential has global implications for public health. As these discoveries reshape our understanding of zoonotic diseases, they underscore the need for surveillance and effective management strategies (Yang et al. 2017).

Equine granulocytic anaplasmosis (EGA), caused by *A. phagocytophilum*, is a tick-borne equine disease with zoonotic implications. EGA's veterinary and public health significance highlights the importance of comprehensive control measures to mitigate its impact. Similarly, the detection of *A. phagocytophilum* in olive baboons and vervet monkeys in Kenya raises concerns about the role of wildlife reservoirs in zoonotic pathogen transmission, necessitating effective management strategies (Masika et al. 2021). The transmission of zoonotic *Anaplasma* species across various host types, such as dogs, horses, and ruminants, emphasizes the need for coordinated efforts to minimize public health risks. The interconnectedness of human, animal, and environmental health is underscored by the impact of vector-borne zoonotic diseases like *A. phagocytophilum*, highlighting the importance of a one health approach that addresses both veterinary and public health concerns (Kulkarni et al. 2015).

Clinical awareness and surveillance play a critical role in managing zoonotic diseases like *A. phagocytophilum* infection in cats, serving to detect and manage these diseases in various animal species to prevent transmission to humans and promote overall health. Integrating regular veterinary check-ups, diagnostic testing, and public education helps in early identification and containment of the disease. Collaboration between veterinarians, healthcare providers, and public health authorities fosters a comprehensive approach, ensuring that both pet owners and professionals are informed and equipped to deal with the challenges of zoonotic infections. This alignment of efforts is crucial in safeguarding animal and human health (Schäfer and Kohn 2020).

8. CONCLUSION

The complex landscape of anaplasmosis, characterized by its diverse species, global distribution, intricate life cycle, clinical manifestations, and zoonotic potential, demands a comprehensive and collaborative approach for effective control and prevention. The intricate interplay between Anaplasma species, arthropod vectors, mammalian hosts, and the environment underscores the need for a One Health perspective that integrates veterinary and public health efforts.

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Recent Advances in Diagnosing of Human Cysticercosis

12

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ABSTRACT

Cysticercosis in humans is a parasitic disease caused by Cysticercus (C.) cellulosae, the larval stage (known as metacestode) of the Cyclophyllidean tapeworm Taenia (T.) solium. It is a highly prevalent infection in India, China, the Southern part of the African continent, South America, the Central American region of North America, and a few Eastern European countries. T. solium (neuro cysticercosis, NC) causes a zoonotic disease complex. According to many authors NC is considered as one of the most important food-borne zoonotic diseases respectively, caused by helminth parasite. Accurate and prompt diagnosis is essential for early detection and effective treatment of the disease. Diagnostic approaches including: Direct Detection of metacestodes and tissue lesions in CNS and soft tissues; Various Imaging Techniques like: CT, MRI, Ultrasonography (US) and X-ray; Classical and Rapid Serology tests and Molecular techniques. Neuroimaging by CT or MRI is critical in the diagnosis of neurocysticercosis. MRI is more expensive but is becoming more accessible in developing countries often provides a clearer picture of cysticerci and has greater sensitivity for multiple lesions. Serological testing provides important confirmatory data for patients with suspicious lesions on CT or MRI. Serological testing has improved, with a sensitivity of 98% and a specificity approaching 100%. The electro-immune-transfer blot (western blot) assay using lentil lectin purified glycoprotein antigens (LLGP-EITB) is preferable to the ELISA for the identification of anti-cysticercal antibodies in human serum. Low molecular weight metacestode secretion proteins, and especially glycoproteins, have shown the best performances in NC diagnosis assays. The LAMP (loop-mediated isothermal amplification technique) test would be a very useful tool to contribute to reducing the incidence of cysticercosis in developing countries, except when the cysticerci are calcified, because in that case no circulating antigens are available. Ideally both ways are used, combining the advantage of the higher sensitivity of MRI with CT detection of calcium. Identifying of human cysticercosis is possible by collecting and analyzing laboratory results and clinical/epidemiological data strictly following Del Brutto's revised diagnostic criteria.

Key words: human cysticercosis diagnosing, neuro cysticercosis, Cysticercus cellulosae, Taenia solium metacestode

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1. INTRODUCTION

Cysticercosis in humans is a disease caused by infestation with *Cysticercus* (C.) *cellulosae*, the larval stage (known as metacestode) of the tapeworm *Taenia* (T.) *solium* (Linnaeus 1758). It is a highly prevalent infection in India, China, the Southern part of the African continent, South America, the Central American region of North America, and a few Eastern European countries (WHO 2020). The *T. solium* metacestode is located in subcutaneous space, intermediate host internal organs (Chatuthanai et al. 2022) and muscles, but more often invades the central nervous system (CNS), resulting in a medical syndrome known as neurocysticercosis (NC). Separate intramuscular *C. cellulosae* cysticercosis without the involvement of the central nervous system is very rare (Dwipayana et al. 2022). Metacestodes affect predominantly the central nervous system (97.46%), followed by ocular localization (1.4%), rarely subcutaneously, and soft tissue infection (1.14%) (Gnanamoorthy and Suthakaran 2019). NC, a leading cause of adult-onset seizure disorder, is a major global public health concern from parasitic origin in many endemic areas. NC may cause neurological manifestations such as epileptic seizures and/or chronic prolonged headaches (Li et al. 2019). The intensity of presented clinical signs and their frequency depends on the size, number of growing cysts, predilection site, and inflammatory stage of the cyst in the brain (Mlowe et al. 2022). Parasite subcutaneous tissue location may provoke the formation of palpable nodules, on the other hand, diffuse intra-muscular cysticercosis may present as myalgia, or pseudo-hypertrophy (Gnanamoorthy and Suthakaran 2019). Clinical symptoms of pulmonary cysticercosis include cough, sputum production, constitutional disorders, pulmonary nodule/s, and pleural effusion (Savigamin et al. 2022).

T. solium (neuro cysticercosis) causes a zoonotic disease complex. According to many authors NC is considered as one of the most important food-borne zoonotic diseases respectively, caused by helminth. Annually, NC causes approximately 28,000 deaths and more than three million people are at risk of infection (Despommier et al. 2019). NC is becoming an emerging or re-emerging disease in industrialized countries from endemic areas. The presented problem is a result of the increased global traveling due to business trips or tourism (Chun-Seob et al. 2019). NC is one of the leading causes of human epilepsy in many hyper-endemic regions in Latin America, Asia, and sub-Saharan Africa. In endemic regions, NC accounts for 10%–12% of all hospitalizations in hospital neurology departments. The World Health Organization (WHO) reports an estimated 2.5–8.3 million cases of NC annually with a disability-adjusted life year burden of 2.8-5.0 million people and 3%–6% population in endemic regions, but as for all neglected tropical diseases these values are likely to be underestimated (Jobanputra et al. 2020; Butala et al. 2021). *C. cellulosae* metacestodes are rounded or oval cysts, up to 15 mm in diameter, whitish, filled with transparent fluid, and possesses a single invaginated scolex (bearing hooks and four suckers typical for *T. solium*) which can be seen as small eccentric solid granule. Occasionally, large, irregular, fluid-filled, and round or lobulated cysts, similar to a bunch of grapes known as racemose cysticercus (*Cysticercus racemosus*).

This infection thrives in areas with poor sanitary facilities, overcrowding, poor personal hygiene, and places, where pigs are reared commonly. Note that cysticercosis is acquired from the fecal-oral route (ingestion of eggs). This may happen when humans drink water or eat fresh vegetables or fruits contaminated with tapeworm eggs or put contaminated feces fingers in their mouths. People who live with someone who has a tapeworm infection in their intestines have a much higher risk of getting cysticercosis than other people. When the egg is ingested, the embryo (oncosphere) inside survives the action of gastric hydrochloric acid in the stomach and enters the small intestine. The egg hatches and the larva penetrate the intestinal wall and enters the bloodstream of the intermediate host. Eventually, the oncosphere penetrates into one of many tissues (e.g., striated muscles, heart, brain, eyes) and encysts there into a cysticercus-type metacestode, grows, develops, and creates a space-filling lesion within 2–3 months. Parasite life cycle maintenance between humans and pigs is attributed to poor sanitation, unhygienic

practices in food preparation, inadequate hygienic measures of the slaughter house personnel, improper handling of infected pig carcasses. *T. solium* cysticercosis positive pig carcasses are not properly treated (buried or incinerated), and poor pig husbandry practices in which pigs are left to scavenge on human feces and eat feeds and drinking water contaminated by *T. solium* infective eggs (Kungu et al. 2015).

2. DIAGNOSTIC APPROACHES FOR HUMAN CYSTICERCOSIS EVALUATION

2.1. DIRECT DETECTION OF METACESTODES AND TISSUE LESIONS IN CNS AND SOFT TISSUES

NC is usually revealed during necropsy and confirmed by further biopsies (postmortem). Ophthalmologic examination is of great use in cases of ocular cysticercosis. When cysticerci are located in muscles or subcutaneous tissue, palpation, biopsies, and fine-needle aspiration cytology are employed, with rapid onsite evaluation. A technique known as fine-needle aspiration (FNA) of a viable cysticerci cyst yields clear fluid and bladder wall fragments in a clear acellular background, whereas aspirates from necrotic lesions contain bladder wall fragments, including calcareous bodies and detached from rostellum single hooks. This approach is effective in cases of subcutaneous cysticerci. Diagnosis of cysticercosis is made on fine needle aspiration cytology (FNAC) only when fragments of metacestode tegument and parenchyma are identified, which were absent in the present case. Even to suspect a parasitic infection characteristic cell type host inflammatory reaction consisting of eosinophils, neutrophils, palisading histiocytes, and giant cells must be present in the aspirate from suspicious subcutaneous nodule (Koteeswaran et al. 2013). Cytochemical evaluation of cerebrospinal fluid is also of great help in extra parenchymal cysts, showing various stages of inflammatory cell reaction (increase of lymphocytes, proteins, and hypoglycorrhachia) that are important to perform prognosis before antiparasitic treatment.
Differential diagnosis (DD) from other pathogens and pathological conditions should be made necessary. All presented parasitological methods enable early parasitic infection detection, particularly when lesions are found in anatomically approachable superficial sites.

2.2. IMAGING TECHNIQUES

CT and MRI are key tools for the imaging diagnosis of NC, since they allow revealing the number, size, evolutionary stage, and specific location of the lesions (Nepal and Ojili 2021). Cysticerci of *C. cellulosae* have variable appearances in neuroimaging studies. Inside the brain parenchyma, metacestodes show different visual characteristics according to their evolutionary stage: cystic-like lesions without amplification (vesicular cysticerci), contrast based hyper enhancemented cystic or nodular lesions (colloidal and granular cysticerci), and small globular calcifications (calcified cysticerci).
The same parasites in the subarachnoid space may be present as multiple confluent cysts (racemose cysticerci) or may appear as focal or diffuse arachnoiditis that most often involves the Sylvian fissure or the basal brain cisterns, which is typical to the picture of obstructive hydrocephalus. Intra-ventricular metacestode cysts appear as lesions with different signal properties than the ventricular fluid, distorting the normal structure of the ventricular system and leading to asymmetric hydrocephalus. Some of the present cysts may contain parasite scolices (Pineda-Reyes and White 2022).
Spinal cord-located cysticerci appear as nodular or cystic lesions if they are present intramedullary or as focal or diffuse spinal arachnoiditis with or without cysts into the spinal subarachnoid space (Yang et al. 2022). Lesions in the ventricles and the cisterns (Garcia et al. 2020) are better visualized by using volumetric balanced steady-state gradient echo sequences (FIESTA, BFFE, or CISS).
Ultrasonography (US) and MRI findings allow for identifying intramuscular cysticerci even if solitary cysts are present. Imaging techniques have improved the detection of scolices and visualization of cysts in extra

parenchymal spaces (Del Brutto et al. 2017; Nash et al. 2020). High-resolution ultrasonography is very helpful in settling down the exact DD: Lipoma US findings are hyperechoic lesions with no evidence of cystic-like pathological changes. Neurofibromas are hypoechoic structures near the nerve, which are proximally and distally visualized by the US. Schwannomas respectively are hypoechoic lesions present eccentric to the nerves (Jeyakumar et al. 2022). High-resolution ultrasonography is the first choice in intra-parenchymal stages of cysticercosis in humans and various animal species inside abdominal or pleural cavities especially effective for rapid and inexpensive diagnosis in the acute migration phase (Kanchev 2013). Neuroimaging techniques are more helpful than serology in providing data about the number, size, predilection site, and stage of lesions, also in peri-lesion inflammation type and response (Garcia et al. 2020).

X-ray is not so sensitive and accurate IT method due to the visualization of already formed calcified foci (Maquera-Afaray et al. 2014). Radiography is a possible choice for visualization of cysticercus in predilection sites of parasites in internal organs parenchyma or skeletal muscles.

Computed tomography (CT) is more sensitive test in detection of calcified lesions but magnetic resonance imaging (MRI) is more accurate for the detection of the metacestode scolex, edemas, small parenchymal foci and abnormalities, lesions inside posterior fossa, and the involvement of the subarachnoid spaces and ventricles in infection with the parasite. The fluid attenuation inversion recovery (FLAIR) technique is particularly very helpful for identifying associated tissue edema and cysticercus scolex (Clinton et al. 2018). The advent of CT changed the landscape of NC diagnosis by revealing many clinical cases with mild disease, much more benign than the severe cases seen before, which were limited to those that could be detected by old, less-sensitive techniques. The introduction of MRI improved imaging definition and added the capacity to present images in different visual planes (Garcia et al. 2020). Imaging diagnosis of human cysticercosis needs precise CT or MRI scans and further image reliance by qualified and experienced radiologists. DD: cysticercosis must be differentiated from cystic-formed tumors that bear debris of neoplastic cells in the interior area of the cystic compound resembling a scolex (pseudo-scolices). Warning of patients with a single intra-parenchymal brain cyst (Del Brutto 2022).

The complexity of NC clinical signs and the difficulties in diagnosing, and identifying human cysticercosis is possible by collecting and analyzing laboratory results and clinical/epidemiological data strictly following Del Brutto's revised criteria (Table 1).

2.3. IMMUNO-DIAGNOSTIC TESTS

2.3.1. CLASSICAL SEROLOGY DIAGNOSIS

Neuroimages may be highly compatible with NC diagnosis. In many cases, the diagnosis is not conclusive. Serology plays a major role in confirming the diagnosis (Deckers and Dorny 2010).

Antibody detection is most frequently used because of its higher sensitivity, while antigen detection is very effective in cases where live parasites are available. The enzyme-linked immunosorbent assay (ELISA) for the detection of anti-cysticercal antibodies has been used for NC diagnosis and is still used in many countries where more advanced tests are not available. Recent studies have documented reliability problems with the use of ELISA, because of the involvement of crude and semi-purified antigenic extracts in reaction, which makes this test unreliable for cysticercosis diagnosis. The electro-immune-transfer blot (western blot) assay using lentil lectin purified glycoprotein antigens (LLGP-EITB) is preferable to the ELISA for the identification of anti-cysticercal antibodies in human serum. This test has a sensitivity of 98% in patients with more than one cysticercus and no cross-reactivity with antibodies induced by other infections appears. Unfortunately, LLGP-EITB sensitivity drops down to about 50%-70% when patients have a single parasite cyst or they are calcified (Del Brutto 2022). Several studies, as well as the Pan

American Health Organization, have recognized LLGP-EITB as the gold standard for NC. LLGP-EITB has 100% specificity and is confirmed overall sensitivity of 98%. The introduction of monoclonal antibodies-based antigen detection by ELISA is an encouraging approach for cysticercosis diagnosis (Ferrer and Perteguer 2022).

Table 1: Diagnostic criteria and degrees of diagnostic certainty for diagnosing of NC (described and revised by Del Brutto et al. 2017)

Absolute criteria:	Neuroimaging criteria:	Clinical/exposure criteria:	Degrees of diagnostic certainty:
• Histological demonstration of the parasite from a biopsy of a brain or spinal cord lesion • Visualization of subretinal cysticercus • Conclusive demonstration of a scolex within a cystic lesion on neuroimaging studies	Major neuroimaging criteria: • Cystic lesions without a discernible scolex • Enhancing lesions • Multi lobulated cystic lesions in the subarachnoid space • Typical parenchymal brain calcifications Confirmative neuroimaging criteria: • Resolution of cystic lesions after cysticidal drug therapy • Spontaneous resolution of single small enhancing lesions • Migration of ventricular cysts documented on sequential neuroimaging studies Minor neuroimaging criteria: • Obstructive hydrocephalus (symmetric or asymmetric) or abnormal enhancement of basal leptomeninges	Major clinical/exposure: • Detection of specific anticysticercal antibodies or cysticercal antigens by well-standardized immunodiagnostic tests • Cysticercosis outside the central nervous system • Evidence of a household contact with *T. solium* infection Minor clinical/exposure: • Clinical manifestations suggestive of neurocysticercosis • Individuals coming from or living in an area where cysticercosis is endemic	Definitive diagnosis: • One absolute criterion. • Two major neuroimaging criteria plus any clinical/exposure criteria • One major and one confirmative neuroimaging criteria plus any clinical/-exposure criteria • One major neuroimaging criteria plus two clinical/exposure criteria (including at least one major clinical/exposure criterion), together with the exclusion of other pathologies producing similar neuroimaging findings Probable diagnosis: • One major neuroimaging criteria plus any two clinical/exposure criteria • One minor neuroimaging criteria plus at least one major clinical/exposure criteria

Antigen detection is limited to patients with clinically presented infection and is possible for patients with multiple cysts, predominantly those located in the subarachnoid space or the ventricular system (Del Brutto 2022). The seven LLGP antigens used in the LLGP-EITB assay belong to three families, with low-molecular-weight antigens associated with active disease and appearing a few weeks or months after infection (Donadeu et al. 2017). Heavier-molecular-weight antigens appear first and are the latest to disappear after the patient is healed and all the parasites have died. Patients have circulating antibodies for months or years after successful therapy (Garcia et al. 2020).

Several studies have shown that the detection of low molecular weight proteins with subunits in 150kDa and 120kDa in blood serum or cerebrospinal fluid (CSF) is a good target for the diagnosis of active NC stages (Corstjens et al. 2014). High antigen levels are associated with extraparenchymal NC, whereas low or undetectable antigen levels are associated with the intraparenchymal type of cysticercosis (Chun-Seob et al. 2019). Serodiagnosis is able to differentiate those two types of NC which is crucial in low-living standard countries, where neuroimaging is not practically available outside of major

hospitals. ELISA kits vary from US$5 to US$30 per sample, whereas EITB tests range from US$22 to US$100 but can cost as much as US$347 per sample (Butala et al. 2021). Low molecular weight metacestode secretion proteins, and especially glycoproteins, have shown the best performances in NC diagnosis assays. Thus, 14- and 18-kDa antigens and 8kDa–30kDa protein fraction have been described as the best alternative for developing an antibody detection system ruled at detection of NC (Ferrer and Perteguer 2022).

Recent progress in NC serodiagnosis has resulted in two different types of antigenic platforms:

- Chimeric protein fused with defined molecules with different epitope specificities
- Multi-antigen print immunoassay that uses different antigens as a mixture (Hancock et al. 2006).

Detecting circulating blood parasite antigens is a difficult task because, unlike antibodies, antigens are limited in amount and can't be multiplied by the host immune system (resulting in decreased sensitivity), closely related helminths share many diagnostic epitopes (resulting in frequent cross-reactions). Antigen levels are used to monitor the efficacy of antiparasitic treatment (Garcia et al. 2020).

Diagnosing the acute NC is important because it allows treatment with specific chemotherapeutics especially anthelmintics, while chronic-phase or acephalic budding cysticercosis in the brain ventricles requires surgical techniques or symptomatic therapy for the control of intractable seizures (Chun-Seob et al. 2019). Based on the HP10 monoclonal antibody, a lateral flow assay (HP10-Ag-LFA) for the diagnosis of extraparenchymal neurocysticercosis (EP-NC) has been developed and successfully tested with CSF and serum samples, providing an encouraging field test for rapid identification of endemic human cysticercosis. The monoclonal antibody-based B158/B60 Ag-ELISA has been used for human cysticercosis diagnosis in several epidemiological studies (Kabululu et al. 2020). Some difficulties (biochemical purification, requirement of large parasite amounts, reproducibility) restrict their uses. Biotechnological approaches have been used to solve the insufficiency of *T. solium* parasitic material for the preparation and purification of diagnostic antigen applicants. Many genes have been studied for that purpose. Paramyosin, sHSP, TSA18/HP6, F18, TS14, TS18, T24, 50-kDa glycoprotein, TsAg5, and other molecules were cloned and expressed in prokaryotic and eukaryotic systems and evaluated with collections of serum and CSF samples. The recombinant products were checked by ELISA, western blot (WB), EITB, or multiplex bead-based assay, with good sensitivity and specificity for NC diagnosis. Three other new recombinant antigens of *T. solium* metacestode have been described for the immunodiagnosis of cysticercosis, TsF78 (filamin), TsP43 (peroxidase), and TsC28 (collagen XV), with diagnostic performances. Proteins with 8-kDa have been chemically synthesized (Ts18, Ts18 var1, Ts18 var3, Ts18 var4, Ts18 var6, TsRS2 var1 Ts14, Ts18 var8, and TsRS1) and evaluated successfully for cysticercosis testing by ELISA. Of all these proteins, TsRS1 has 100% sensitivity and 100% specificity, when tested with cysticercosis-positive sera (previously reactive with the 8-kDa proteins) on WB, and Ts18 var1 and Ts18 var3 show 97% sensitivity and 100% specificity were selected for a future diagnostic antigen mixture. Recently, NC41 synthetic peptide was evaluated for NC diagnosing with high diagnostic performance (Ferrer and Perteguer 2022).

2.3.2. RAPID SEROLOGY DIAGNOSIS

2.3.2.1. ANTIBODY DETECTION TESTS

Several tests have been developed targeting antibodies against the previously described recombinant T24H (rT24H) glycoprotein in serum for the diagnosis of cysticercosis. The rT24H-MICT test uses the same technology as rES33-MICT; therefore, the advantages and disadvantages are similar. The up-converting phosphor T24H lateral flow reporter assay (UCP-rT24H LFA) uses up-converting phosphor (UCP) particles

as a detection method. Results are read using a multi-lane reader after chromatography and when the strips are dry. In this format, this test is reported to detect a low level amounts of antibodies. Ease of use is reduced by the requirements for sample dilution, washing, conjugate application, and strip analysis. This limits its use to begin at the clinic level. The rT24H antigen has been used in other test formats with reported sensitivity and specificity as follows; Multi-antigen print immune assay (MAPIA), 97%-99%, and EITB 94%-99%, respectively (Mubanga et al. 2019).

Quick ELISA™ is adapted for serodiagnosis of cysticercosis based on the recombinant T24H and GP50 glycoproteins, as well as the synthetic peptide sTs18var1. It is suitable for surveillance of cysticercosis. The Quick ELISA™ is a high-throughput quantitative assay that can be performed on a benchtop but can also be automated. It is suitable for field studies but requires a basic laboratory due to; the number and type of samples, the buffers used, which require a cold chain, and equipment such as the absorbance reader, which requires electricity. Performance of rGP50 in other test formats, sensitivity, and specificity; EITB 90% and 100%, ELISA 95% and 94%, MAPIA 93% and 100%, is close to the minimum requirement of 90% and 98% respectively. Reported false positives for rGP50 have not been associated with any specific parasite infections. The performance, sensitivity, and specificity of Ts18var1 in other reported test formats are; EITB, 97%, and 100%, ELISA 95% and 85%, other ELISA 90% and 90%, RAPID ELISA 97% and 100%. No cross-reactivity has been reported in other test formats of rGP50 and sTs18var1. Quick ELISA appears to be a good test for use in a peripheral laboratory.

All antibody detection tests meet the minimum performance requirements for the diagnosis of cysticercosis with a sensitivity of 90% and a specificity of 98% proposed by WHO in the target product profiles for human cysticercosis, only tests based on sTs18var1 do not meet the requirements. Quick ELISA rT24H showed the best performance among the antibody detection tests, but for the detection of NC, sensitivity falls down when the cysticerci count is low (Lee et al. 2011).

2.3.2.2. ANTIGEN DETECTION TESTS

Rapid Slide/Latex Agglutination test has been standardized and evaluated for the detection of *T. solium* metacestode antigen in cerebrospinal fluid (CSF) and serum. It uses latex particles that have been sensitized from manufactured rabbits' hyperimmune antiserum against cysticercosis. Agglutination is performed on a glass slide where the latex suspension is added to a serum or CSF sample. A positive test is seen by agglutination. Cross-reactions with tuberculous meningitis have been reported in both serum and cerebrospinal fluid. One healthy control out of 25 serum samples tested positive. Test results were below the performance requirements proposed for cysticercosis and NC. Nevertheless, the simplicity of the test format makes it easy to diagnose a single case. Preparation of the latex suspension and sensitization of the latex particles is relatively easy, and they can be stored at 4°C for further use. This test can be used at the clinical level for serum, with the limitation being the expertise required to obtain CSF by lumbar puncture (Mubanga et al. 2019).

Another RDT reported for NC is HP10 LFA. The target analyte is the HP10 antigen in CSF, which is a circulating surface as well as an excretory-secretory antigen in *T. saginata, T. hydatigena, T. solium, and Echinococcus granulosus*. The primary objective of this analysis is a post-treatment follow-up in patients with extra-parenchymal NC as well as supportive diagnosis.

whereas imaging is less sensitive for extra-parenchymal NC. The HP10 antigen was used in an ELISA format for the diagnosis of NC using CSF and serum with a sensitivity and specificity of 91.3%-100%, and 84.8%-98%, respectively. The diagnostic efficiency meets the proposed requirements for the diagnosis of cysticercosis. Further evaluation is required in patients with multiple parasite infections. The biggest

limitation of this test is the sample type. Although the test is easy to perform, the lumbar sampling procedure is invasive and requires experience.

The results show that there is the development of RDTs for NC. Most of the serological tests developed are based on the same antigens, only changing the test formats. The tests developed, despite their limitations, are potentially able to be used as intervention mapping and monitoring tools, especially when integrated with conventional tests. Further evaluation of most of these tests is needed to provide sufficient information on their applicability in endemic areas, especially in low-resource settings. It is also necessary to determine the added value of these tests to the health outcomes of individuals. Further research is needed on the effect of test format on diagnostic outcomes (Mubanga et al. 2019).

2.4. MOLECULAR DIAGNOSIS

A new quantitative PCR (based on the highly repetitive Tsol13 sequence) was recently designed, showing high sensitivity and specificity for the diagnosis of subarachnoid and ventricular NC and for the assessment of response to antiparasite treatment (O'Connell et al. 2020). PCR systems have also been used to detect *T. solium* DNA in samples such as brain biopsy material, blood and urine samples (Goyal et al. 2020). On the other hand, CSF next-generation sequencing-based pathogen analysis has been reported for successful diagnosis and monitoring of NC patients (Garcia et al. 2020).

The LAMP (loop-mediated isothermal amplification technique) test would be a very useful tool to contribute to reducing the incidence of cysticercosis in developing countries, except when the cysticerci are calcified, because in that case no circulating antigens are available (Rodriguez et al. 2012). The LAMP technique requires further work with symptomatic patients to demonstrate its utility in the diagnosis. Cox1 sequence technique has ability to perform species differentiation between Taenia species (*T. solium, T. saginata* and *T. asiatica*), combined with its greater capacity to detect positive samples. The used protocols have been shown to be able to detect the different stages of cysticercus and have greater sensitivity than multiplex PCR (Avendano and Patarroyo 2020).

T. solium DNA detected by PCR or deep genomic sequencing using cerebrospinal fluid (CSF) of patients with subarachnoid NC. There are no reports of its use in parenchymal NC cases, much less in patients with a single brain lesion, where most diagnostic problems are available. Cell-free *T. solium* DNA has been demonstrated in the urine and serum of patients with NC, and recent data suggest that monocyte gene expression and serum mass spectrometry profiles can be used to identify NC cases. Unfortunately, molecular biology assays are not directly applied to routine case assessments (Garcia et al. 2020).

3. CONCLUSIONS

The diagnosis of cysticercosis is usually based on imaging tests and established criteria. Neuroimaging by CT or MRI is critical in the diagnosis of neurocysticercosis. Ideally both ways are used, combining the advantage of the higher sensitivity of MRI with CT detection of calcium. Because it is often not practical to biopsy cysticerci, consultation with a neuroradiologist is extremely helpful. CT scans of the cysts appear as hypodense images containing a small hyperdense nodule that represents the parasitic scolex. Calcium can sometimes be seen. The inflammation around the dead and dying parasites will provide the so-called ring enhancement in the presence of contrast material. In the natural history of neurocysticercosis, dead and dying cysts become calcified. MRI is more expensive but is becoming more accessible in developing countries often provides a clearer picture of cysticerci and has greater sensitivity for multiple lesions. Serological testing provides important confirmatory data for patients with suspicious lesions on CT or MRI. Serological testing has improved, with a sensitivity of 98% and a specificity approaching 100%. For patients

with a single lesion, sensitivity is much lower, possibly because of the small amount of parasite antigen available to the host immune system. In the acute care setting, ELISA requires considerable interpretation because antibody titers can remain high long after the parasites have died. Clinical practice guidelines that outline specific diagnostic criteria were updated in 2017.

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Fasciolosis: a Non-attended Zoonosis

13

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ABSTRACT

In this chapter, recent aspects of fasciolosis are analyzed. The manuscript focusses mainly in Fasciola hepatica rather than other species of the Fasciola genera. Its biologic cycle, world geographical distribution, economic importance and importance on human health are reviewed. Fasciolosis, is one of the most important parasitic diseases of economically productive animals, particularly cattle, sheep and goats. This disease is caused by a flatworm, one to four centimeters long, similar to a small brown leaf and is scientifically known as Fasciola hepatica. It is also known by the names of orejuela, moth or liver fluke. The adult worm lives in the bile ducts of the liver and can parasitize different animal species, including man. F. hepatica parasitizes cattle and humans since at least 4,500 years. At present, fasciolosis is recognized as an important parasitic disease which affects humans too (zoonosis). Due to the climate change, fasciolosis is a serious health threat for human beings. The authors suggest that this disease may be controlled on the basis of the One Health approach.

Key words: Fasciola hepatica, Zoonosis, ruminants, economic losses, humans

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1. INTRODUCTION

This chapter deals more with *Fasciola hepatica* rather than other species of the *Fasciola* genera. Among the most important parasitic diseases of economically productive animals, particularly cattle, sheep and goats, is Fascioliosis, a disease caused by a flatworm, one to four centimeters long, similar to a small brown leaf scientifically known as *Fasciola hepatica* (Sánchez Manzano et al. 1998, Rokni 2014). It is also known by the names of orejuela, moth or liver fluke. The adult worm lives in the bile ducts of the liver and can parasitize different animal species as indicated above, including man (Fig. 1). *F. hepatica* parasitizes cattle and humans since at least 4,500 years (Dittmar and Teegen 2003). At present, fascioliosis is known as an important parasitic disease which affects humans too (Carrada-Bravo 2007, Robinson and Dalton 2009, Mas-Coma et al. 2009, Nyindo and Lukambagire 2015, Mehmood et al. 2017, Caravedo and Cabada 2020, Bargues et al. 2022). In this context, and due the climate change, fascioliasis is a health threat for human beings (Anonymous 2020).

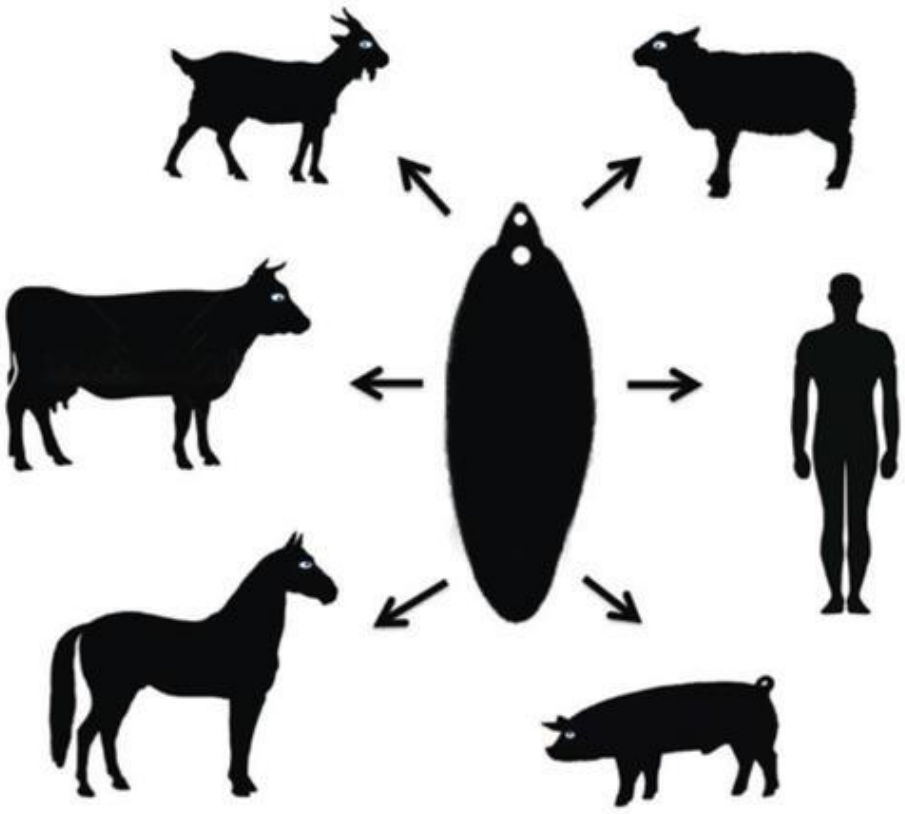

Fig. 1: Animal species of economic importance, including man, parasitized by *Fasciola hepatica* (Figure made by Carlos Ramón Bautista-Garfias).

The life cycle of *F. hepatica* is summarized in Fig. 2.

This cycle, which can last between four and six months, takes place in two hosts, one terrestrial vertebrate (mammal) and the other aquatic invertebrate (gastropod mollusk) as described in Fig. 2: 1. The eggs of the parasite hatch in the manure of the definitive bovine host, mature and hatch in a humid place. 2. A miracidium hatches from each egg and swims in the water, seeking out and infecting an intermediate host aquatic snail (*Lymnaea*) that lives on the banks of puddles, lagoons or irrigation canals. 3. In this, the parasite becomes a sporocyst, a mother redia and then a daughter redia that gives rise to another phase called the cercaria. 4. The cercariae is mobile, swims in the water and attaches itself to the surrounding vegetation or to the surface of the water, then loses its tail and encysts. 5. Now the parasite is called

metacercaria and constitutes the infective phase that can remain like this for several months waiting for the plant to which it is attached (grass, alfalfa, watercress) to be ingested. 6. The animal or man ingests the vegetation along with the metacercariae. 7. These pass through the digestive tract to the duodenum, where they are excysted, releasing newborn flukes that cross the intestine, travel through the peritoneal cavity and enter the liver, perforating the Gleason capsule and migrating through the parenchyma. 8. The parasite proceeds to the bile ducts where it matures and after eight to 10 weeks produces eggs. 9. These travel through the intestine in the bile along with the feces. 10. The eggs come out. A single fluke can oviposit up to 25,000 daily (Fig. 3).

2. GEOGRAPHICAL DISTRIBUTION OF *FASCIOLA* SPP

Fasciolosis in animals and man is distributed worldwide. The flatworms in the *Fasciola* genera originated in the African continent, then dispersed inside their hosts to other continents (Fig. 4).

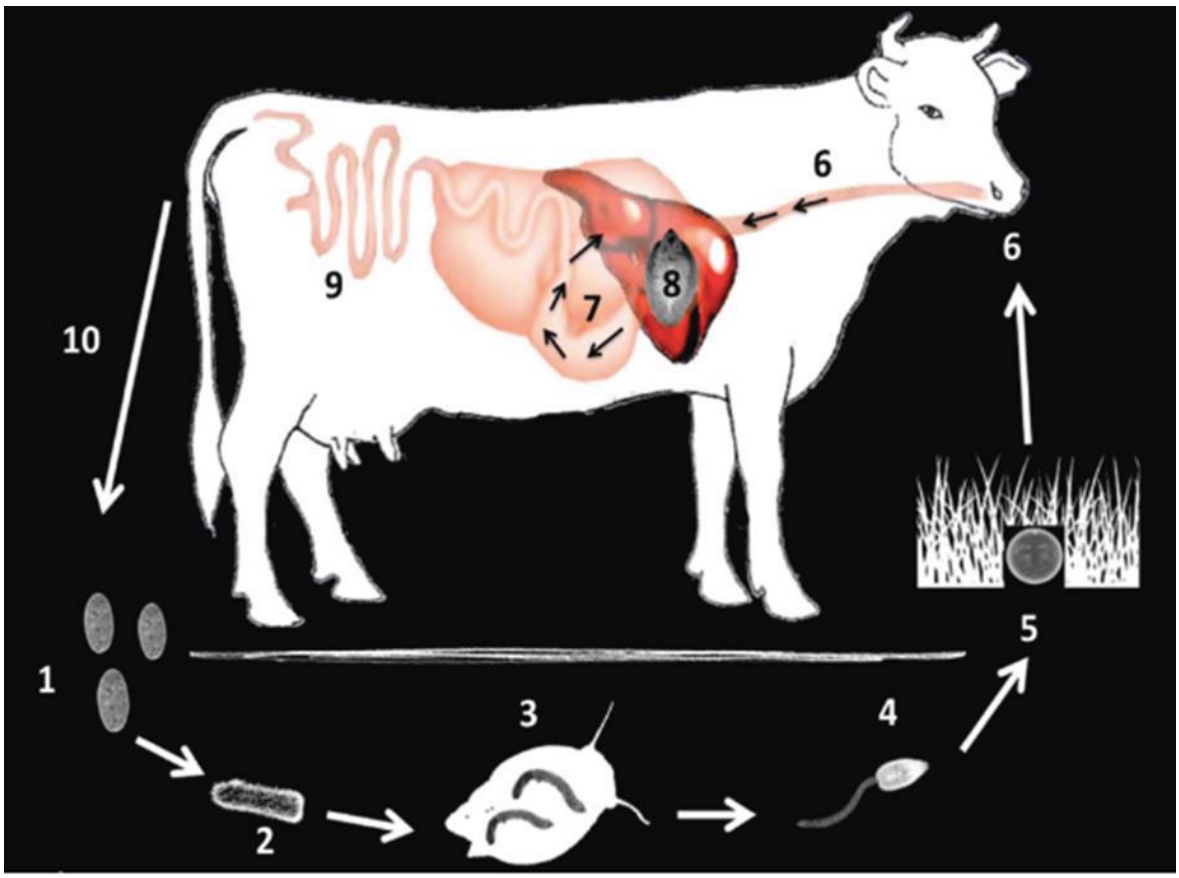

Fig. 2: *Fasciola hepatica* life cycle (Figure made by Carlos Ramón Bautista-Garfias)

3. SIGNS OF THE DISEASE IN RUMINANTS

As in most parasitic infections caused by worms, the signs of fasciolosis in ruminants are not specific. Thus, for example, weakness, anemia (blood deficiency), submandibular edema (soft swelling in the lower part of the jaw) can be observed (Fig. 5), sometimes jaundice (yellowing of the mucous membranes and eyes), diarrhea or constipation, abortions in pregnant females, chronic weight loss (Fig. 6) and sometimes death.

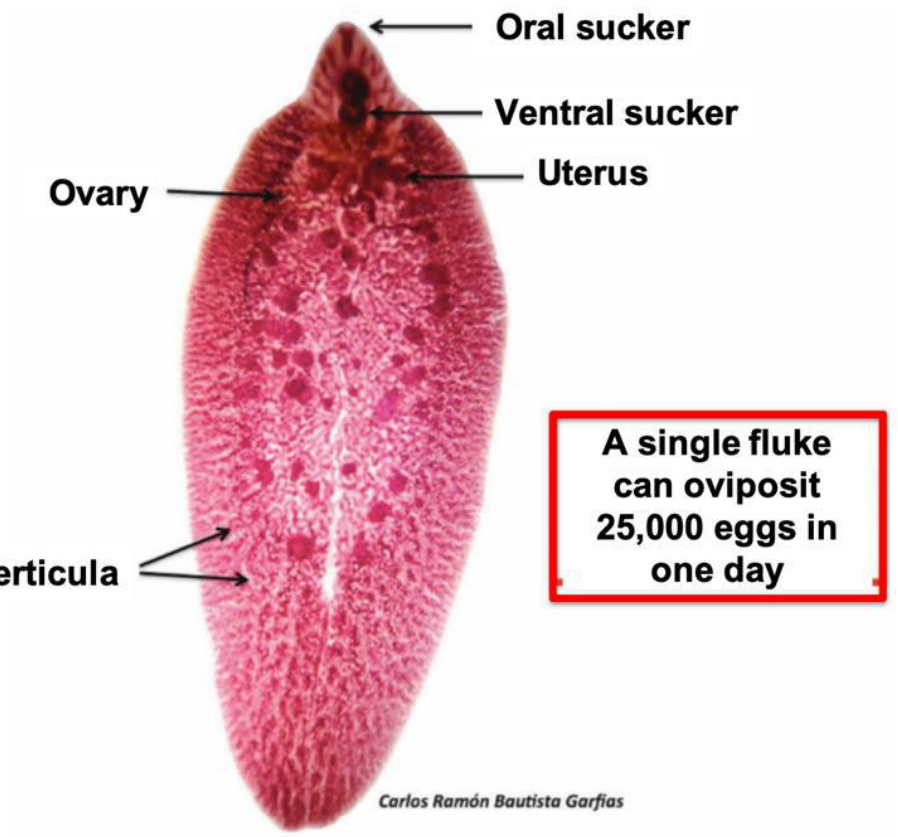

Fig. 3: Adult *Fasciola hepatica* (Figure made by Carlos Ramón Bautista-Garfias).

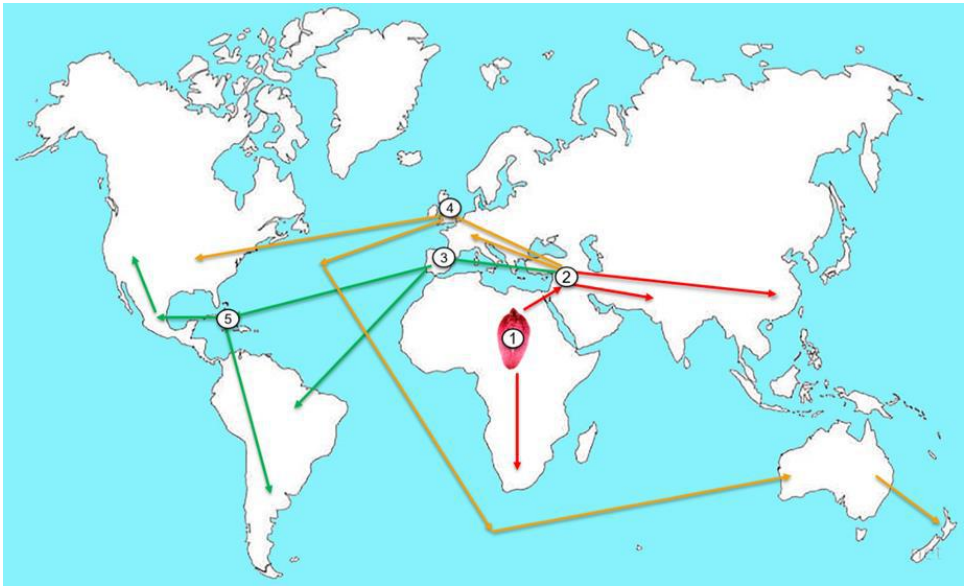

Fig. 4: Dispersion of *Fasciola* spp. 1. *Fasciola* genera originated in Africa. 2. Moved to the Middle East, then to Asia. 3, 4. Infected ruminants transported the trematode to Europe, and then, 5, to the American Continent. In the world. Based on the following references: Dittmar and Teegen 2003, Mas-Coma et al. 2009, Mehmood et al. 2017, Caravedo and Cabada 2020, Bargues et al. 2022.

4. ECONOMIC IMPORTANCE AND ZOONOSIS

In general, the losses caused by *F. hepatica* in production are not noticed by the producer and depend on the number of flukes that parasitize the animals, as well as their age, diet and health status. The main losses are due to: 1) delay in growth, 2) reduction in body weight, 3) decreased production of meat, milk

or wool, 4) reproductive disorders, 5) abortions, 6) predisposition to contract other diseases, 7) increased food consumption due to poor digestion. In short, fasciolosis negatively affects all sectors that make a livestock business profitable. In addition, we must add the risk of death of animals that are not adequately cared for and at the end of their reproductive life, the confiscation of the liver (loss) in the slaughter, since said viscera is not suitable for human consumption. In this context, it is estimated that in Mexico 100 million kg of beef production per year are lost due to fasciolosis (Bautista, unpublished data). Based on the price per kg of carcass meat, the annual loss in bovines amounts to 6,600 million pesos (393.6 million dollars, MDD). Worldwide, it is estimated that 46,958 million pesos are lost per year (2,800.5 million dollars). On the other hand, *Fasciola hepatica* is found on all continents with almost 180 million people at risk of acquiring the parasitosis and an estimated 17 million people already infected worldwide (World Health Organization 2007, Mas-Coma et al. 2009). Regarding Mexico, in Atlixco, Puebla, in 2013, of 865 examined children, a prevalence of fasciolosis was found that ranged between 2.94 and 13.33% (Zumaquero-Rıos et al. 2013).

5. FACTORS THAT FAVOR THE SPREAD OF *F. HEPATICA*

The most important are:

• The long life of the parasite in animals. In sheep it can live between eight and 11 years.
• The large number of eggs that each adult fluke is capable of producing daily (between 8,000 and 25,000).
• The biotic potential of the parasite inside the snail, since each egg can form between 400 or more cercariae.
• The great resistance of the infecting metacercariae. In the environment they can survive for up to 12 months and up to eight in wet harvested hay
• Vertebrate animal species in which *F. hepatica* can live include cattle, sheep, goats, horses, pigs, dogs, deer, rabbits, hares, squirrels, and wild rats, among others; which hinders the effective application of fasciolicide treatments.
• The high concentration of grazing animals per hectare in humid areas and the fertilization with recent or unfermented manure from sick animals are actions that contribute to the contamination of pastures with *Fasciola* eggs.
• The ease of reproduction of snails (Lymnaea) that are infected with Fasciola and the great number of eggs removed by each host vertebrate (up to 25,000).
• The great resistance of the snails that can stay alive, even when infected, for more one-year-old, buried in the dried mud.
• Inadequate drainage of the pastures, which gives rise to permanently humid areas, in which the snails live and reproduce easily.
• Periodic flooding that contributes to the spread of both snails and parasites.
• Temperatures, between 10 and 30ºC, and abundant rain that favor the development of the parasite, both in the external environment and in the intermediate host snails.

6. HOW ANIMALS AND MEN ARE INFECTED WITH *F. HEPATICA*

In animals, infection occurs by ingesting encysted metacercariae in grass, grass, and water. Most infections occur when animals graze in areas where snails have been or are eliminating parasites. However, they can also occur in the barn due to the ingestion of metacercaria attached to fresh or poorly ensiled fodder.

Man is infected mainly by ingesting raw plants (for example, watercress) or undercooked, contaminated with metacercariae

Fig. 5: Submandibular edema in a cow infected with *F. hepatica*. (Figure made by Carlos Ramón Bautista-Garfias).

Fig. 6: Fasciolosis signs in cattle: diarrhea, chronic weight loss. (Figure made by Carlos Ramón Bautista-Garfias).

7. PLACES AND TIMES OF GREATEST RISK FOR ACQUIRING THE INFECTION WITH *F. HEPATICA*

The most propitious places for acquiring *Fasciola* are those in which the aquatic snail (Lymnaea) lives, such as:

- Slow watercourses on whose banks different types of plants grow.
- Wet meadows or those that are periodically flooded.
- Muddy terrain with small holes filled with water.
- Surroundings of fountains and cattle troughs.
- Land with insufficient drainage.

The most dangerous times to acquire the infection are those in which the snails release cercariae as they encyst and rapidly transform into metacercariae. These are already capable of infecting animals and humans 24 hours after being encysted.

The studies carried out during the last 10 years in various slaughterhouses in Mexico indicate that fasciolosis in cattle and sheep has not diminished; rather it shows an increasing trend. It is important to take into account that environmental conditions are not the same throughout the country and that they vary from year to year. In the same way, important meteorological phenomena (climate change) that affect different countries should be considered, such as "El Niño" (Trenberth 1997), which occurs every two to seven years and causes droughts and areas of atypical high humidity. "El Niño" of 2015 was one of the worst in 15 years (Milton 2017), which it has caused intense rains in much parts of Mexico, which will favor parasites such as *F. hepatica*. Typically, “El Niño” events reach their maximum effect between October and January, but often continue to wreak havoc during the first four months of the year. In this context, it has been stated that climate change contributes to the emergence or re-emergence of parasitic diseases (Short et al. 2017).

8. HOW FASCIOLOSIS MANIFESTS ITSELF IN CATTLE AND SHEEP

The disease is classified as acute or chronic. The acute one occurs more frequently in sheep and occasionally in cows. It is due to a massive ingestion of metacercariae found in the green grass of irrigation canals. In the acute form, anemia, digestive disorders, loss of appetite, pale mucous membranes and little movement are observed in the animals; signs that evolve until death generally rapid. Chronic fasciolosis in cows and sheep results in listless and anemic animals. Weight loss, diarrhea, submandibular edema, wool loss, abortions and, at slaughter, increased liver volume are observed, a condition known as hepatomegaly. When the infection is light, only a decrease in production and in the general physiological state is observed. If there are no complications, the course of bovine fasciolosis is benign. However, in some young animals or those subjected to intensive production, considerable weight loss is detected. It's important pointing that some cattle with fasciolosis give negative reactions to tuberculin.

9. DIAGNOSIS OF FASCIOLOSIS

The clinical diagnosis, both in the acute and chronic forms, is carried out based on the signs described above. Additionally, in chronic fasciolosis, the diagnosis is made by examining fecal material (coproparasitoscopic examination), to verify the existence of *F. hepatica* eggs in it. However, it must be taken into account that eggs will only appear when the cow or sheep already have adult flukes in the liver, approximately three months after the first infection. For this reason, all animals in the herd should be examined, including the apparently healthy ones. Feces obtained directly from the rectum should be sent to the nearest diagnostic laboratory for analysis. In cases where no clinical signs or parasite eggs observed

in the feces, serological diagnosis can be used. In this case serum samples must be taken from the animals for analysis by means of immunological tests such as the standard immunoenzymatic assay (ELISA) and its variants (DIG-ELISA, Dot-ELISA), passive hemagglutination Test (Fig. 7) and others that are practiced in some research institutions (Arriaga de Morilla et al. 1989, Bautista-Garfias et al. 1989, Bautista Garfias 1991, Ruíz-Navarrete et al. 1993, Fernández et al. 1995, Arriaga and Bautista Garfias 1997, Álvarez Rojas et al. 2014) or the parasitological diagnosis can be practiced after the sacrifice of the animals. It is important to note that *F. hepatica* infection depress the immune response of the host (Bautista and Lebrija 2008) and dairy cattle infected with this parasite may not respond to the tuberculin test (Claridge et al. 2014). Today, by using molecular techniques it is possible to identify Fasciola species (Baran et al. 2017, Tang et al. 2021, Saadatnia et al. 2022, Levy et al. 2023).

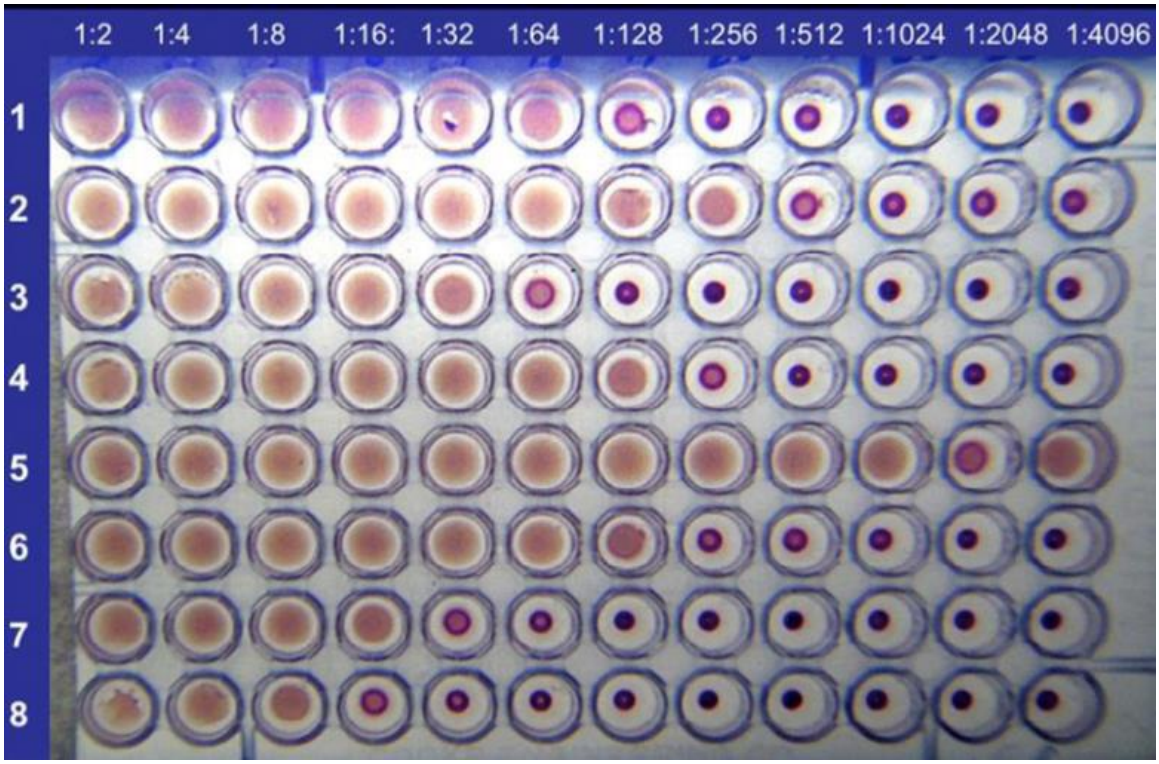

Fig. 7: Passive hemagglutination test for the detection of anti-*Fasciola hepatica* antibodies in sheep sera. The figures at the top indicate the serum dilution. The numbers on the left indicate the serum number of the animal examined. Number 5 is from a positive sheep with a very high titer (1:4096), while number 8 is from an animal negative to *F. hepatica* (1:8), the other sera are positive. (Figure made by Carlos Ramón Bautista-Garfias)

10. TREATMENT

Once the diagnosis has been made, fasciolicide treatment should be applied to the entire herd, including those animals that appear to be healthy or those that shed small numbers of parasite eggs. It is advisable to repeat the treatment every three months.

When cows are of reproductive age, dry cows and those that have not yet calved should be treated. It is not convenient to use fasciolicides in cows in production since the chemical products are eliminated in the milk and can harm the health of man and calves.

To determine the difference between what is spent and what is earned, when deworming is carried out, the following points must be taken into account:

1) Fasciolicide (route of administration, presentation, ease of obtaining it, side effects and cost).
2) Animals to be treated (cattle, sheep or goats and number to be treated, cost of handling them, production period).
3) Time of the year
4) Production system

To select the most effective fasciolicide (considering the increase in parasite resistance to fasciolicides such as Albendazole, Triclabendazole and Clorsulon) and its proper use, the Veterinarian should be consulted. It must be taken into account that *F. hepatica* has developed resistance to Albendazole and to Triclabendazole (Álvarez-Sanchez et al. 2006, Daniel et al. 2012, Brockwell et al. 2014, Kelley et al. 2016).

11. VACCINES AGAINST *FASCIOLA*

One of the more attractive technologies for controlling parasites is vaccination. In this context, several experiments have been carried out through the years to develop vaccines against *Fasciola hepatica* with different degrees of success (Bautista Garfias et al. 1987, Turner et al. 2016, Wesolowska et al. 2018, Silvane et al. 2020, Zafra et al. 2021, Cwiklinski and Dalton 2022, Cwiklinski et al. 2022)

12. SUGGESTIONS TO PREVENT FASCIOLOSIS

The animals must be kept away (even with the use of fences) from places that favor the presence of the aforementioned aquatic snail (*Lymnaea*).

Drain, when possible, the land to eliminate excessive soil moisture and achieve the eradication of the snail.

For the same reason, surround the troughs with a wide strip of material permeable to water such as sand, small stones, tezontle, etc.

The forage must be adequately tied, avoiding harvesting it with humidity. It is convenient to add two percent of common salt to the hay grass, or even delay its consumption for two or three months, to be sure that the metacercariae die.

It is advisable to ensile grass from wet areas to avoid the risk of infection of the animals. Do not allow animals with fasciolosis to graze

in irrigated meadows and alfalfa fields to avoid contamination with F. hepatica eggs.

The manure that is going to be used for fertilizing must first be dried, properly fermented or treated with copious amounts of lime, to destroy the eggs and miracidia of the parasite.

12.1. ONE HEALTH APPROACH CONTROL OF FASCIOLOSIS

Taking into account several factors, including climate change, the best system for controlling fasciolosis is by using the One Health approach (Cwiklinski et al. 2016, Mas-Coma et al. 2020).

12.2. PERSPECTIVES

At the present, man has effective tools for controlling fascioliasis such as simple practices for avoiding infection of cattle (pasture handling, draining accumulation of rainwater, avoiding to cattle pasture in water plants) promising vaccines, one Health approach, among others. Government health authorities (medical and veterinary) must cooperate to control Fasciolosis in animals and man. However, as is the

case of México, cattle Fasciolosis is a neglected parasitic disease. As an example, condemned cattle livers infected with F. hepatica during a period of 11 years (from 1977-1987), in one of the main abattoirs in the Country, showed a frequency similar each year, except in the years 1979, 1981, and 1982 (Encinas et al. 2020).

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Chagas Disease as a Neglected Zoonosis in the American Continent

14

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ABSTRACT

In this chapter, the authors analyze the epidemiology, associated risk factors, worldwide prevalence, host range, life cycle, and treatment of Chagas Disease or American Trypanosomiasis. This disease is caused by an infection to human and other mammals with the hemoprotozoan Trypanosoma cruzi, which is transmitted by infected insect vectors (Hemiptera: Reduviide, subfamily Triatominae). The presence of wild reservoirs and vector insects naturally infected with T. cruzi has only been reported from the American Continent. Some cases of natural transmission to the human have been recognized in the southern half of the United States. The transmission of T. cruzi to the humans was mainly associated with the presence of triatomine bugs in rural dwellings and poor sanitary conditions. However, in recent years, there have been changes in the epidemiological landscape, where infection in humans has increased by contact with reservoirs, wild vectors, climate changes and the migratory phenomenon. The presence of T. cruzi in the insect vector and its ability to experimentally infect rodents were described in 1908 by Carlos Chagas. Scientific researchers described the disease first, and the etiological agent and the mechanisms of transmission later. The discovery of Chagas disease has been so crucial in advancing science and enhancing public health benefits that it serves as an outstanding illustration to comprehend the intricate dynamics intertwining science and society. From 2019, the World Health Organization inaugurating World Chagas Disease Day on April 14, the date on which Carlos Chagas identified the first human case of a T. cruzi infection. Authors conclude that in the control of Chagas Disease, the climate change must be taken into account.

Key words: Chagas Disease, American Continent, Trypanosoma cruzi, Triatomines, zoonosis

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1. INTRODUCTION

Chagas Disease or American Trypanosomiasis is caused by an infection to human and other mammals with the hemoprotozoan *Trypanosoma* (*Schizotrypanum) (T.) cruzi*, transmitted by contamination with faeces and/or urine of infected insect vector. The presence of wild reservoirs and transmitting triatomine insects naturally infected with *T. cruzi* has only been reported from the American Continent (Fig. 1) (from the south of the United States to northern Chile and Argentina). Recently, some cases of natural transmission to the human have been recognized in the southern half of the United States (CDC 2020). Historically, the transmission of *T. cruzi* to the human was mainly associated with the presence of triatomine bugs in rural dwellings and poor sanitary conditions. However, in recent years, there have been changes in the epidemiological landscape, where infection in humans has been favoured by contact with reservoirs, wild vectors, climate changes and the migratory phenomenon (Guhl et al. 2020).

Fig. 1: Distribution of *T. cruzi* in the American Continent (in red). Figure made by Carlos Ramón Bautista Garfias

Curiously, the presence of *T. cruzi* in the insect vector and its ability to experimentally infect rodents were described in 1908 by Carlos Chagas. Historically the microbe hunters first described the diseases, later the etiological agent and the mechanisms of transmission (Reyes 2009).
The discovery of Chagas disease has been so crucial in advancing science and enhancing public health benefits that it serves as an outstanding illustration to comprehend the intricate dynamics intertwining science and society (Petraglia and Trindade 2022). From 2019, the World Health Organization inaugurating World Chagas Disease Day on April 14, the date on which Carlos Chagas identified the first human case of a *T. cruzi* infection (WHO 2023).
At present, the World Health Organization classifies this disease as the most prevalent of poverty-promoting neglected tropical diseases and the most important parasitic disease of humans in Latin America, which affects between six to eight million people. It is also true that climate change is a serious hurdle for controlling this zoonotic disease and the migratory phenomenon of people from endemic zones to areas where natural transmission occurs between reservoirs and Triatomine insects (Kreier and Baker 1987; Martín-Escolana et al. 2022).

2. EPIDEMIOLOGY

In addition to vector transmission of *T. cruzi*, it can also be transmitted transplacentally, through infected blood transfusions or organ donations, laboratory accidents, needle sharing among intravenous drug users (IVDU), and orally through food and drink contaminated with triatomine insect or their feces or urine. Due to increased population mobility over previous decades and the possibility of transmission without the participation of the insect vector, the infection has been increasingly detected in the United States of America, Canada, and many European, African, Eastern Mediterranean and Western Pacific countries. The presence of infected people means that there is transmission of the parasites in other continents as well. The transmission of *T. cruzi* to the vertebrate host can be considered as a fortuitous event, an accident that can occur due to the repeated contact of the host with the vector when the triatomine feeds and defecates on, near or in human food (WHO 2023).
Reservoir infection occurs mainly through the oral route, where the metacyclic trypomastigote present in faeces, urine, and the hindgut of the triatomine insect is ingested or by contact of the droppings with mucous membranes. While in man, oral infection is less frequent compared to transmission by contamination from exposed skin (wounds), mucous membranes (eye, mouth), blood transfusion, congenital transmission, organ transplants and by laboratory accidents. The detection of the distinctive DNA of T. cruzi in mummies dating back approximately 4,000 years in the region where the initial human settlements existed in Chile, which are at least 7,000 years old, suggests that human infection didn't solely result from the presence of the vector/host. This finding implies that various factors influenced and increased the likelihood of the parasite's transmission to humans (Guhl et al. 2020).

3. ASSOCIATED RISK FACTORS

3.1. HUMAN HOST

Poverty; which includes poor sanitary conditions and houses with crevices allowing the proliferation and hiding of the vector.
Lack of knowledge; Ignorance that triatomine insects are carriers of a pathological agent makes the presence of insects in dwellings seem natural and there is no attempt to eliminate them. This happens at all socioeconomic levels. Ecological tourism and staying in places where the natural conditions of the ecosystems are preserved as much possible way also favours the attraction of triatomine insects

to the light sources in the rooms of houses. Displacement of food sources of blood-sucking insects and the continuous invasion of human settlements in peri-urban areas to build houses scares away reservoirs and causes insects to move into peridomestic areas and even further into houses (De Fuentes-Vicente et al. 2023).

3.2. VECTOR

Their feeding in both sexes frequently occurs on less hardened skin sites such as the face, which is why they are commonly known as kissing bugs and barbeiros (barber). Adaptation to human habitation and movement from wild environments to peridomiciles and even more to homes, makes contact of blood-sucking insects with humans more likely. Once the insect vectors are infected, the infection with the parasite remains throughout their life, which in some species can be a year or more. This increases their probability of being infected after feeding, since the life cycle of some species can be one year or more. They can withstand long periods of fasting, have few predators and are attracted at night by the light of the houses and their flight. Although they are not the best flying insects if they are allowed short flights (Carbajal de la Fuente et al. 2022).

3.3. RESERVOIRS

Chagas disease is mainly an enzootic infection, where transmission originates via the oral route when the vertebrate host is infected by ingesting faeces or urine contaminated with trypomastigotes or by ingesting the vector. There is also the possibility of transplacental transmission route (De Fuentes-Vicente et al. 2023).

4. WORLDWIDE PREVALENCE WITH EMPHASIS IN MÉXICO

Chagas disease (or the presence of *T. cruzi* in mammals) is present in humans from South United States of America to Argentina, showing different degrees of prevalence (Carbajal de la Fuente et al. 2022; WHO 2023) (Fig. 1). Chagas disease is one of the most important human parasitic diseases in the American continent. In this context, approximately six million people are affected and that about 172,000 new infections occurred during 2019 in this continent (Rojas de Arias et al. 2021). Regarding infection scenario in Mexico, the disease is present in the whole country, showing the highest prevalence in states geographically located in the Neotropical area of the region (Dumonteil 1999; Carabarin-Lima et al. 2013; Ibañez-Cervantes et al. 2018).

5. HOST RANGE

5.1. MAMMAL HOSTS

Many mammal species are infected by *T. cruzi*, including farm animals, such as cows (Fujita et al. 1993; Correa et al. 2020), pigs, sheep, horses (Ruiz-Piña et al. 2018), goats (Correa et al. 2020), and wild animals, such as primates (Jansen et al. 2018) bats (Torres-Castro et al. 2021), opossums (Jansen et al. 2018; Cantillo-Barraza et al. 2020), and skunks (Galaviz-Silva et al. 2017). In a recent report, an American barn owl (*Tyto furcata*) was found to be infected with *T. cruzi* (Martínez-Hernández et al. 2022).

Dogs are especially important because they can die of heart failure caused by *T.cruzi* infection (Barr 2009; Hamer and Saunders 2022). In this context, It was found that dogs infected with *T. cruzi* had owners infected with the parasite too (Chan-Pérez et al. 2022).

5.2. VECTORS

There are 149 species of triatomine insects (order Hemiptera, family Reduviidae), distributed mainly in the American continent. In Mexico, there are 33 vector species. The epidemiological significance of vectors is notably high, primarily due to their infection indices and their ability to transmit *T. cruzi* (Chagas) (Carbajal de la Fuente et al. 2022).

6. LIFE CYCLE

The life cycle of *T. cruzi*, the protozoan parasite responsible for Chagas disease, unfolds through a complex interplay between insect vectors and mammalian hosts. Initiated when an infected triatomine bug feeds on a mammalian host, the cycle begins in the insect's midgut, where bloodstream trypomastigotes transform into epimastigotes. These epimastigotes multiply through binary fission and eventually transition into infective metacyclic trypomastigotes (Fig. 2). During subsequent blood meals, these metacyclic trypomastigotes are excreted with the bug's feaces, leading to their deposition near the bite wound or mucous membranes of the host. Entry into the mammalian host may occur through various routes, including breaks in the skin or mucous membranes. Inside the host cells, metacyclic trypomastigotes transform into amastigotes, multiplying within the host cell's cytoplasm. The host cell may rupture, releasing amastigotes into the bloodstream, where they transform into bloodstream trypomastigotes. Circulating in the bloodstream, these trypomastigotes can infect various cells and tissues,

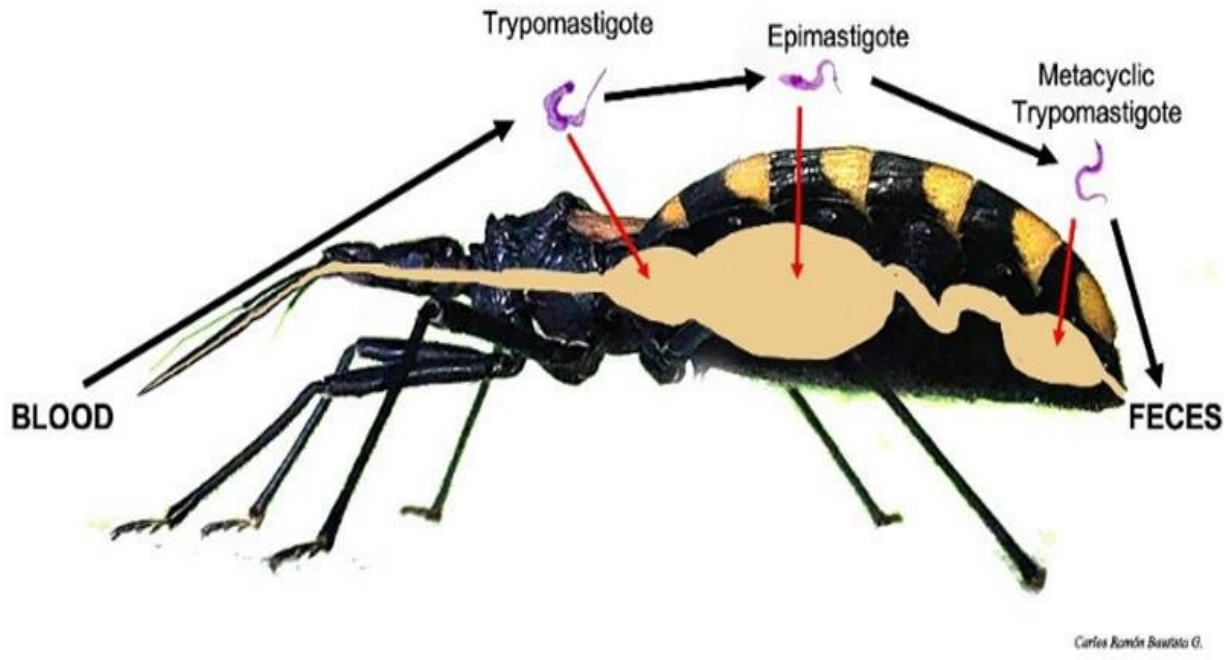

Fig. 2: *T. cruzi* life cycle in the vector *Triatoma pallidipennis*. Figure made by Carlos Ramón Bautista Garfias based on a photography of Benjamin Nogueda Torres.

perpetuating the infection (Fig. 3). The cycle may repeat when another triatomine bug feeds on an infected host, taking up bloodstream trypomastigotes and initiating the transformation back into epimastigotes in the insect's midgut. This intricate life cycle, involving both insect vectors and mammalian hosts, contributes to the persistence and transmission of Chagas disease in endemic regions of the Americas (Bern et al. 2019).

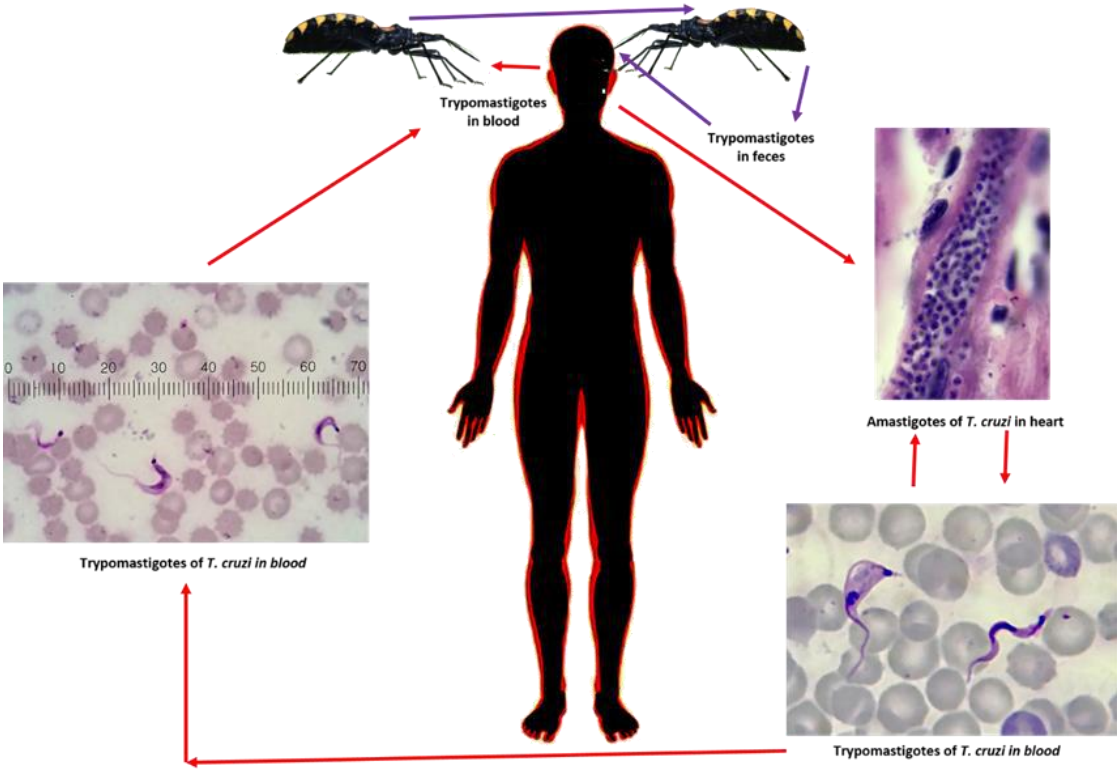

Fig. 3: *T. cruzi* life cycle in man (red arrows in man; purple arrows in vector) (Figure made by Carlos Ramón Bautista Garfias using photographs of Benjamín Nogueda Torres)

7. TREATMENT OF CHAGAS DISEASE

The parasitological treatment of Chagas disease is an unresolved issue. Currently, Benznidazole and Nifurtimox are the only two drugs that are available for the treatment of Chagas disease. There are factors that make the antiparasitic treatment of the disease unsatisfactory:

1. Very few patients receive antiparasitic treatment. Globally it is estimated that less than 1% of confirmed cases receive treatment (Arce-Vega et al. 2017).
2. The treatment is prolonged (up to 60 days), so treatment have toxic effects and induce parasitic resistance.
3. The curative efficacy of Benznidazole in the acute phase is 60 to 100% but in the chronic phase, it decreases to 8 to 20% (Reséndiz-Mora et al. 2022).

8. DISCUSSION

The first description of a wild reservoir was made by Carlos Chagas (1912) when he identified the parasite in the blood of an armadillo and in the faeces of a vector (Chagas 1912). Sometime later, it was shown

that the wild cycle between reservoirs and triatomine insects with oral transmission is much more frequent than the presence of the parasite in humans and that the transition from the wild cycle to the peridomestic/domestic cycle is favoured by factors such as reservoir displacement due to the construction of new man houses, causing deforestation and increasing the probability of contact of the transmitters with man (Barretto and Ribeiro 1979). This observation is consistent with what happens today.

9. CONCLUSION

Governments in different countries of the American continent are aware of the importance of Chagas Disease in the region; however, the disease has not been controlled yet, so there is the need to carry out effective control measures. The improvement of new techniques for the diagnosis of Chagas disease and the advancement in the knowledge of its epidemiology should improve the control of the disease in the coming years. Climatic change must also be taken into account to control the infection.

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Epidemiology of Toxoplasmosis in Iraq

15

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ABSTRACT

Toxoplasmosis, caused by the obligate intracellular parasite Toxoplasma gondii, is a globally prevalent zoonotic infection with varying epidemiological patterns across different regions. This abstract presents a comprehensive review of the epidemiology of toxoplasmosis in Iraq, shedding light on the prevalence, risk factors, clinical manifestations, and preventive measures against this parasitic disease. In Iraq, toxoplasmosis has been recognized as a significant public health concern. Studies conducted in various regions of the country have reported seroprevalence rates among different populations, indicating a widespread exposure to Toxoplasma gondii. Seropositivity rates have shown variability based on geographical locations, age groups, and socio-economic factors. Higher prevalence rates have been observed in rural areas, among pregnant women, and individuals with occupational exposure to soil or animals. The routes of Toxoplasma gondii transmission in Iraq encompass ingestion of contaminated food or water, contact with infected soil, consumption of undercooked meat containing tissue cysts, and vertical transmission from mother to fetus. The clinical spectrum of toxoplasmosis ranges from asymptomatic or mild flu-like symptoms to severe manifestations in immunocompromised individuals or congenitally infected infants. However, there remains a lack of comprehensive nationwide data on the burden of toxoplasmosis-associated morbidity and mortality in Iraq. Preventive measures and strategies for toxoplasmosis control in Iraq include health education campaigns to raise awareness about proper food hygiene, cooking practices, and minimizing contact with potentially contaminated sources. Additionally, antenatal screening programs for pregnant women and the implementation of serological testing in immunocompromised individuals could contribute significantly to early diagnosis and appropriate management. In conclusion, the epidemiology of toxoplasmosis in Iraq reflects a complex interplay of various socio-demographic factors influencing its prevalence and transmission dynamics. Further research efforts are warranted to establish a more precise epidemiological profile, enhance surveillance systems, and implement targeted interventions to mitigate the burden of toxoplasmosis on public health in Iraq.

Key words: Toxoplasmosis, Toxoplasma gondii, Epidemiology, Iraq, Seroprevalence, Risk factors, Clinical manifestations, Prevention, Public health

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1. INTRODUCTION

This book chapter aims to comprehensively the prevalence and epidemiology of toxoplasmosis in Iraq. For future reference, we ascertained the prevalence of *Toxoplasma* in the Iraqi population over different years. We systematically reviewed research articles published in Iraq from Google Scholar, Research gate, and PubMed. The seroprevalence of *T. gondii* against anti-toxoplasma IgG antibody among women by serological tests was higher than the IgM antibody. A high rate of infection among housewives was 35.2% and among employees was 28.5%, also the prevalence of infection was higher in women who live in rural areas compared to people who live in urban regions. This is the first comprehensive analysis of *T. gondii* infection epidemiology in Iraq. It recorded a high prevalence of toxoplasmosis among women of reproductive age. We passionately support more studies to enhance patient care, the creation of more effective diagnostic tools, and the development of preventative measures.

Toxoplasma (T) gondii is a single-celled, obligate intracellular parasite of blood and tissues. It is widely distributed among the human population and is considered the main cause of world morbidity (Murad and Eassa 2023). It belongs to the phylum Apicomplexa. The life cycle of toxoplasmosis is complex, in which, cats are considered the final host and humans and other mammals serve as their intermediate hosts as shown in Fig. 1 (Sundar et al. 2007). Humans and animals are infected with toxoplasmosis by ingestion of sporulated oocysts. After ingestion, excystion of oocysts occurs in the small intestine and sporozoites are released, which entered the epithelium of the gut and by asexual reproduction convert to tachyzoites stage. Tachyzoites are carried out by blood circulation to vital organs and cause necrosis, mainly in the placenta, and are the main causes of abortion in pregnant women. Under unfavorable conditions tachyzoites are converted to bradyzoites (Rostami et al. 2011). *T. gondii* can affect various organs and glands of the human body i.e., the thyroid gland. In a new study done in Duhok City, Kurdistan Region, Iraq, it was recorded that there is an association between autoimmune thyroid infection and toxoplasmosis which led to premature delivery, death, abortion, and new borne baby with low weight (Murad and Eassa 2023).

The disease is asymptomatic in adult and may have mild symptoms like flu, lymphadenopathy, and fatigue (Gagne 2001), while in pregnant women it causes abortion, death, and deformities in a fetus (John and Petri 2006; Rostami et al. 2011; Hoseini et al. 2014). *T. gondii* may cause neurological disorders such as encephalitis in immunocompromised patients due to acute toxoplasmosis or due to latent infection (Jones et al. 1997). If acquired as an acute infection during pregnancy, toxoplasmosis can have serious negative effects on mother, fetus, and newborn baby. Numerous pathologies are also brought on by latent toxoplasmosis, which has also been linked to harmful effects on pregnant women (Rostami et al. 2020). The risk of acquiring acute toxoplasmosis during pregnancy is critical and precautions should be strictly followed to prevent pregnant women from picking up the disease (Rostami et al. 2019).

a. The final host is Cat
b. Infective stage, the sporulated oocysts in the feces of a cat
c. Contaminated grasses with infective stage
d. Intermediate host (human or other mammals) infected by ingestion of sporulated oocysts
e. Intermediate hosts (mice, rate, domestic ruminants)
f. Infection occurs by ingestion of tissue cysts in undercooked meat
g. Human is an intermediate host for toxoplasmosis
h. The tachyzoite stage can transmit from mother to baby through placenta

i & j. Other ways of transmission are by blood transfusion and tissue transplantation

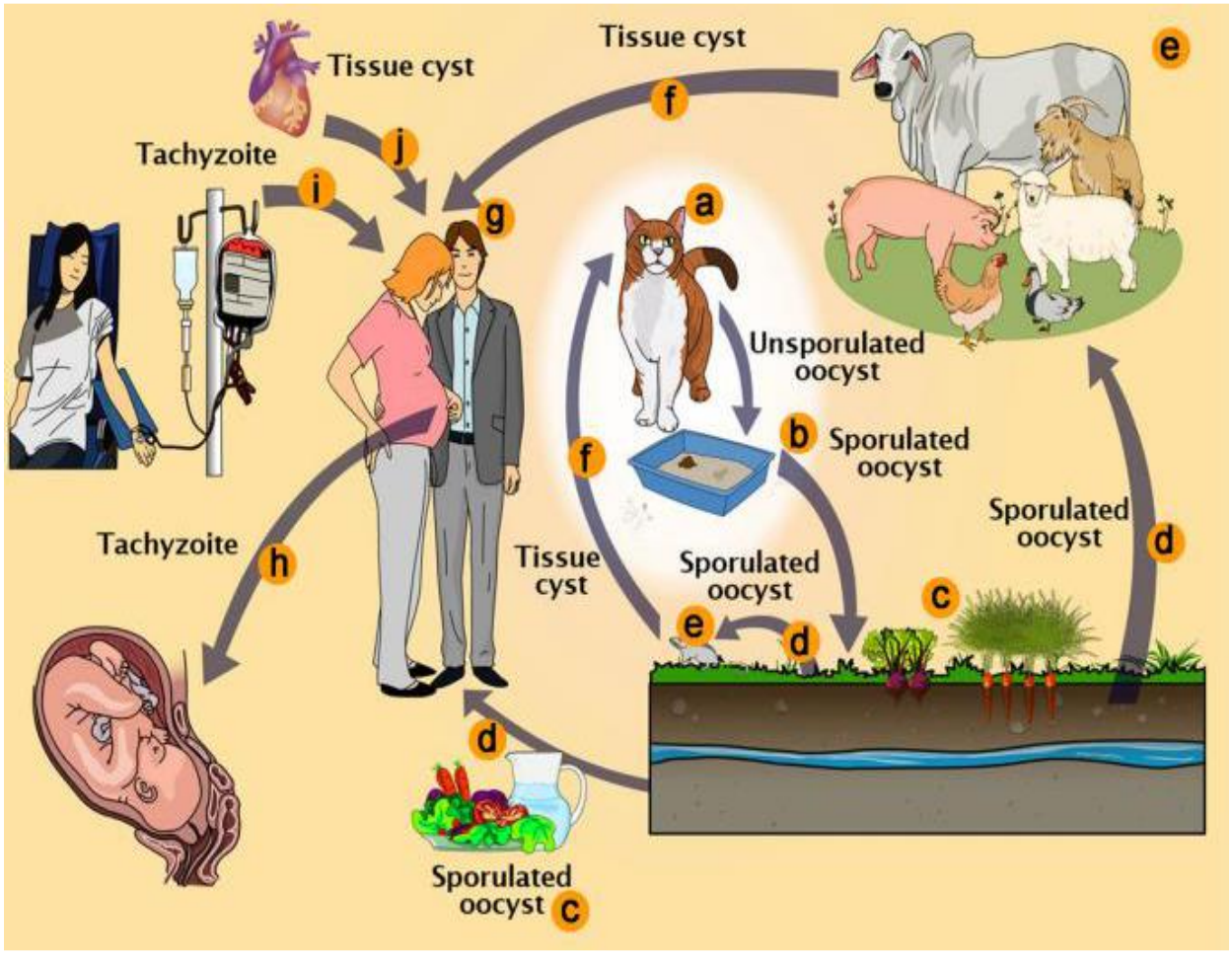

Fig. 1: The morphological stages of *T. gondii* and the routes of transmission (Attias et al. 2020)

T. gondii has three morphological stages: the trophozoite stage (tachyzoites), the tissue stage (bradyzoites) and the infective stage (oocysts) as mentioned in Fig. 1 (Attias et al. 2020). Sporulated oocysts are the infective stage for the intermediate hosts, and infection occurs by ingestion of sporulated oocysts, then excystation occurs in the gastrointestinal tract, and tachyzoites are produced which infect the intestinal epithelium, and pass to vital organs by blood circulation, and transfer to bradyzoites in tissues (Tente et al. 2000). There are several ways for transmission of toxoplasmosis, while the common ways are by ingestion of soporulated oocysts and by ingestion of undercooked infected organs or meat containing bradyzoite stage which is more popular way of transmission in Europe (Cook et al. 2000). This infection can have terrible effects on the fetus's eyes and nervous system if it is transmitted from mother to fetus. Chronic illness is linked to maternal mental illness, even though toxoplasmosis infections can have long-term effects on the fetus. The risk of congenital toxoplasmosis and the long-term effects of infection in the fetus can be decreased with effective treatment. This infection will go largely undetected if proper screening and education programs are not implemented (Deganich et al. 2020). Prevalence of toxoplasmosis in cats and other carnivorous may serve as a sign of the contamination of nature with oocysts and livestock animals (Ruminants) are infected by ingestion of sporulated oocysts from nature and humans are infected by ingestion of undercooked meat of ruminants (Skjerve et al. 1998; Shapiro et al. 2019). Domestic ruminants are

considered the main source of infection for humans. As farm animals represent a source of infection for humans and reservoirs of *T. gondii* for wildlife it has been proposed to reduce *T. gondii* infections in livestock as much as possible, particularly in pigs (Stelzer et al. 2019). A study was done in Hormozgan, Iran by Khademi et al. (2019), who reported that women who ingested undercooked red meat or have direct contact with domestic cats are the main sources of toxoplasmosis among pregnant women. The risk of getting *T. gondii* infection is higher in animals kept outdoor due to environmental contamination with an infective stage (oocysts), which is responsible for the difference in prevalence of Toxoplasmosis between animals kept indoor and outdoor, as has been reported in different species (Djokic et al. 2016). Additionally, the prevalence is known to rise with aging and the infection lasts the entirety of the host's life (Stelzer et al. 2019).

There are many factors linked to the prevalence and epidemiology of toxoplasmosis i.e., nutritional status, poor hygiene, poor economic condition, poor or lack of education, cultivating gardens, coming into contact with pets mainly domestic cats (Alvarado-Esquivel et al. 2017), contamination of water food, grasses and the environment with sporulated oocysts (Rostami et al. 2020). Climate and geographic factors can also affect the spread of toxoplasmosis (Spalding et al. 2005). Screening for toxoplasmosis in women of reproductive age is important because it helps to manage the innate toxoplasmosis and identifies those who are at risk of infection (Montoya et al. 2004).

Toxoplasmosis may be asymptomatic in normal people but may have life-threatening symptoms (Dubey and Jones 2008), and the risk of toxoplasmosis increases in case of pregnant women (Teweldemedhin et al. 2019). Many factors that can affect the virulence of symptoms such as the infective dose of ingested oocysts, strain variation of *T. gondii*, genetics, and host immune response (Montoya et al. 2004). General clinical signs of toxoplasmosis in normal people are headache, fever, general weakness, and enlargement of lymph nodes, while there are some dangerous defects like pneumonia, chorioretinitis, and encephalitis (Cantos et al. 2000). While pregnant women can cause the death of a fetus, epilepsy in infants, small or very large size of infant's head, blindness, and abortion (Khan et al. 2011). Generally, infection in infants is asymptomatic at birth, later, several complications may appear such as blindness, loss of hearing, and mental disorders (Fan et al. 2006). Clinical symptoms in immunocompromized patients are confusion, seizure, schizophrenia, ocular disorder, loss of intelligence and respiratory disorders such as difficulty in breathing (Dogruman Al et al. 2009; Odeniran et al. 2020; Dupont et al. 2021).

Prior epidemiological studies have found that different countries have different rates of toxoplasmosis in pregnant women, ranging from 9 to 67% in European countries (Nash et al. 2005), 34.1% in Sudan (Elnahas et al. 2003), 33% in New Zealand (Morris and Croxson 2004), and 70.9% in Cuba (González-Morales et al. 1995). Although the prevalence of toxoplasmosis was low (28.6%), seroconversion testing during pregnancy revealed that 9 out of 12 women had an acute infection, and 5 (41.6%) of those women had infants with congenital toxoplasmosis (Muñoz Batet et al. 2004).

The routine diagnosis of toxoplasmosis is done by two methods, first by detection of antibodies (IgM and IgG) against toxoplasmosis in blood samples by Latex Agglutination Test or Enzyme Linked Immunosorbent Assay (ELISA) (Montoya 2002; Molan and Rasheed 2016; Ibrahim 2018) and second by the direct detection of parasites in body fluid or tissue sections, which are done by histology, cell culture (Alfonso et al. 2009), conventional polymerase chain reaction (PCR), and real -time PCR (Montoya 2002; Su et al. 2010; Ybañez et al. 2020; Ismael 2021) or indirect methods by ELISA and biochemical tests (Montoya and Liesenfeld 2004). This chapter aims to determine the epidemiology of *T. gondi* in Iraq. It is based on information gathered from articles written by Iraqi researchers from the north to the south. These were collected from academic journals published in Iraq and Google Scholar. These studies were limited to looking at the epidemiology of toxoplasmosis in Iraq.

Toxoplasmosis is recognized as a significant contributor to perinatal morbidity. Acute infection during pregnancy can result in fetal infection, which can cause fetal loss or the birth of an infant who is clearly or latently infected. However, it is a disease that can be avoided. Significant differences have been observed in Europe, not only between nations but also within a single nation, indicating regional variations in the impact of epidemiological factors causing infection. As a result, numerous European nations have put prevention programs into place in proportion to their respective estimated risks of congenital toxoplasmosis. A preventative strategy and hygienic advice should be given to expectant women, in addition to identifying the specific populations at a higher risk of infection, who will then be specifically screened (Bobić et al. 2003).

2. EPIDEMIOLOGY OF TOXOPLASMOSIS IN DIFFERENT CITIES OF IRAQ

About one-third of the population in the world is infected with *T. gondii*. In North Europe, North America, Africa, and Southeast Asia, there was a decrease in the seroprevalence of toxoplasmosis from 10–30%. In South and Central European countries and in Latin American and tropical African nations, there were high prevalence rates (Dubey 2008; Robert-Gangneux and Darde 2012). *T. gondii* has a complex life cycle and can be transmitted vertically (from mother to baby) (Borna et al. 2013; Daryani et al. 2014) or horizontally by ingestion of undercooked meat containing bradyzoites or by ingestion of sporulated oocysts in food, grasses, and water (Kirby 2012; Torgerson and Mastroiacovo 2013). According to a study done in Western Romania by Mihu et al. (2022), *T. gondii* IgG seroprevalence is high (46.09%) in females of reproductive age. However, testing for IgA may increase the likelihood of detecting a recently acquired toxoplasmosis in people with demonstrable *T. gondii* IgG and IgM antibodies. According to estimates, 33% of blood donors worldwide carry the *T. gondii* infection and seroprevalence varies significantly between countries (Lupu et al. 2022).

Women who are exposed to primary toxoplasmosis after conception are at significantly higher risk of transmitting the infection from mother to child during pregnancy than those who were exposed to the infection prior to conception. As a result, diagnosing recent primary toxoplasmosis through laboratory testing is crucial for managing pregnant women who may have been exposed to toxoplasmosis (Boyer et al. 2005). Toxoplasma IgM detection is a sensitive marker for the presence of primary toxoplasmosis, but the specificity of the marker is limited because natural IgM antibodies can occasionally bind to Toxoplasma antigens even in the absence of an infection. Additionally, after the initial infection, Toxoplasma IgM can occasionally remain in blood serum for several months or years (Joynson and Guy 2001).

The type of toxoplasma is recognized as the most virulent factor (Rico-Torres et al. 2016; Sasai and Yamamoto 2019). There are three major types of *T. gondii* including type (I), type (II), and type (III), and each type causes varying degrees of severity (Sibley et al. 2009). Type I is the most severe and virulent type in humans, the second type causes a chronic infection in both humans and animals and may be very severe in immunocompromized patients, and the third type is found in birds with less severity (Sibley et al. 2009; Xiao and Yolken 2015).

In Iraq, the incidence rates of toxoplasmosis vary from region to region depending on the hygiene of personal and community sanitation, and climatic conditions. For example, in Duhok City, research was done by Mikaeel and Al-Saeed (2020), and they found that the seroprevalence of toxoplasmosis against anti-toxoplasma IgG antibody among women by ELISA technique was 28% and for IgM antibody was 0.46%. Another study was done in the same city by Al-Atroshi and Mero (2013), who reported different results for the seroprevalence of toxoplasmosis in women with prevalence rate of 27.7%. In 2020, in Akre, Duhok City, Iraq, the prevalence of Toxoplasma IgM and IgG among

pregnant women was 4.44% and 54.46 %, respectively (Shukri and Jumaa 2020). Recently, in Duhok City, Iraq, a high prevalence of toxoplasma IgG has been reported by Murad and Eassa, (2023) and was 31.8%. In Erbil City, a study was done by Husain et al. (2011), the researchers reported that the seroprevalence rates of both Toxoplasma IgG and Human cytomegalovirus (HCMV) IgM among 348 sera samples collected from pregnant women who had a history of abortion at the Rezan Private Laboratory were 2:9.05% and 45.25%, respectively. Another research done by Abdul Ameer Jaber and Noori (2021) in Erbil Province, Iraq, also found a high prevalence rate of *T. gondii* IgG (42.1%), and no IgM was found. A study was done by Ibrahim (2018) in Garmian, Kurdistan Region, Iraq, who reported that a high rate of infection among housewives was 35.2% and among employees was 28.5%. He also reported that the infection rate was higher in people who live in rural areas than in people who live in urban regions. Research work by Edrees and Ibrahim (2020) in Mosul City, Iraq, determined that the seroprevalence rate of toxoplasma IgG by ELISA Technique was 26.7% among pregnant women who attended the antenatal clinic, in Mosul City, Iraq from the period of November 2019 to January 2020. As reported by Addor (2011) in Salaha-Adden City and Mohammed (2011) in Baghdad City, who reported higher seroprevalence, rates of 29.2% and 28.77%, respectively, of *T. gondii* infection among women. However, some Iraqi Cities have reported much higher seroprevalence rates of toxoplasmosis, with rates of 55.26%, 52.6%, and 42.6%, respectively (Fatohi 1985; Al-Attar 2000; Al-Timimi 2004; Hadi et al. 2016). A high prevalence rate of toxoplasmosis in pregnant women in Al-Muthanna province during October, November, and December 2009 and January of 2010 was recorded by Al-Se'adawy and Hemza (2010). Higher rates of unemployed women than employed women were 75% and 25%, respectively.

Table 1: Prevalence rate of Toxoplasmosis among pregnant women in Iraq during 2008 -2023

No. of Pregnant women Examined	Year	Prevalence (%)		References
		IgM	IgG	
350	2008	8.3	58.3	Al-Mishhadani and Al-Janabi 2008
45	2009	33	94	Mossa 2009
81	2010	0	13.81	Al-Se'adawy and Hemza 2010
348	2011	45.25	29.05	Husain et al. 2011
51	2012	31.70	24.39	Al-Warid and Al-Qadhi 2012
100	2013	0.05	55	Al-Ethawi et al. 2013
62	2014	11.29	48.3	Hamad and Khdir 2014
96	2015	9.7	32.3	Bakre et al. 2015
120	2016	3.2	35.4	Hadi et al. 2016
263	2017	12.93	34.8	Abduallah and Mohmood 2017
150	2018	4.0	48	Darweesh et al. 2018
118	2019	20.6	.0	Raza et al. 2019
57	2020	2.7	96.7	Jwad et al. 2020
210	2021	3.33	9.5	Barzinij 2021
15	2022	51.0	8	Hamza et al. 2022
110	2023	0	22.75	Murad and Eassa 2023

Recently, results for the prevalence of toxoplasmosis were reported by several Iraqi researchers. Barzinij (2021) revealed that the prevalence of toxoplasmosis IgG was 9.05% and for IgM it was 3.33%, and these results were significant at a P value <0.05. Another study was done by Abdul Ameer Jaber and Noori (2021) in several rural and urban areas in Al-Najaf province, Iraq. They recorded the prevalence rate of IgM and IgG as 0.0% and 42.1%, respectively by both ELISA and the Rapid Diagnostic Immunochromatographic test.

Data mentioned in Table 1 shows a history of toxoplasmosis in pregnant women in Iraq from 2008 to 2023. The serological test revealed the prevalence of toxoplasma IgG and IgM as 75% and 25%, respectively. These are a critical percentage which is a risk factor among pregnant women in Iraq and needs continuous screening of women before pregnancy to prevent abortion and deformity in newborn baby.

3. CONCLUSION

The sharp increase in prevalence of toxoplasmosis led to an increase in the number of pregnant women who were exposed to the infection, which increased the risk of congenital toxoplasmosis. Based on the previously mentioned information, we suggest that, as an epidemiologically sound and financially viable alternative to a general screening-in-pregnancy program, all pregnant women should receive health education along with serological testing of those who have been exposed to infection predictors. Furthermore, a new viewpoint on risk factors gives a modern foundation for approaching preventive measures.

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The author thanks the researchers whose work on toxoplasmosis in humans in Iraq was used in this study.

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Control and Detection of Toxoplasma Gondii in Meat Chain

16

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ABSTRACT

Toxoplasmosis caused by infection with the Toxoplasma gondii which can infect all warm-blooded hosts including humans is estimated to be present in one-third of the world population. Human infection with T. gondii has different routes by ingesting sporulated oocysts present in water, soil, or vegetables. Food-borne toxoplasmosis in humans may result from exposure to different stages of T. gondii, especially from the ingestion of tissue cysts or tachyzoites contained in meat, offal, and meat-derived products of many different livestock animals. Although the prevalence of viable T. gondii in retail meat was very low, consumers can acquire T. gondii infection from ingestion of undercooked meat. Thus, raw or undercooked meat containing viable cysts has been suggested to be a major source of T. gondii infection in humans. Prevention of toxoplasmosis transmission in humans depends on meat safety strategies. Most realistic solutions to control T. gondii in the meat chain come in the form of a risk-based meat safety assurance system that incorporates risk-categorization of farms and slaughterhouses, as well as education of all interested parties. The difficulty of toxoplasmosis monitoring in farm animals is due to the asymptomatic and chronic expression of the disease. Vaccine development and vaccination of farm animals against T. gondii are traditionally being implemented. On the other hand, carcass surveys present several advantages and control plans to detect and take measures for this pathogen. After systematic meat inspection, some treatments must be applied to the meat found to contain cysts, such as freezing, cooking, irradiation, or high pressure. Laboratory analyses to detect T. gondii are very important in terms of protecting public health. The gold standard for detecting T. gondii in meat samples is a bioassay using either mice or cats. Molecular methods are used as fast and realiable in addition to conventional serological methods for the diagnosis of toxoplasmosis.

Key Words: Toxoplasma gondi, Farm Management, Meat Inspection, Vaccination.

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1. INTRODUCTION

Toxoplasma gondii is a protozoan parasite of the Apicomplexa Phylum (Opsteegh et al. 2011). Its biology, natural life cycle and epidemiology have many aspects. Everything depends upon the factors by which Toxoplasma is spreading and how it's affecting living beings, leading to the fatalities caused by it in worldwide cases (Tenter et al. 2000). *T. gondii* reproduction occurs solely in species of the Felidae family, its ultimate hosts, and ends up in the shedding of numerous oocysts following initial infection (Opsteegh et al. 2011). All warm-blooded animals, including livestock and humans, to will likely be its intermediate hosts (Tenter et al. 2000).

Toxoplasmosis caused by infection with *T. gondii* is estimated to be present in that one-third of the world's human population (Kijlstra and Jongert 2008a; Hussain et al. 2017) and is responsible for approximately 8% of all hospital stays and 24% of all deaths resulting from foodborne illnesses in America annually (Guo et al. 2015; Rani and Pradhana 2020). Recent assessments on foodborne diseases rank toxoplasmosis in the globe and it is on par with salmonellosis or campylobacteriosis (Kijlstra and Jongert 2008a). *T. gondii* is rated second place out of 14 pathogens transmitted by food in the United States and top in the Netherlands in terms of illness burden (Almeria and Dubey 2021).

Toxoplasmosis has a vast distribution in a range of cattle and wild species (Sousa et al. 2010). Because of this, the major transmission routes of toxoplasmosis to humans are the consumption of infected raw or undercooked meat harboring bradyzoites cysts (Rahdar et al. 2012; Zoua et al. 2017; Rani and Pradhana 2020). The largest proportion of specimens positive for the protozoa or antibodies was found in the EU, which was reported for sheep and goat meats (Opsteegh et al. 2016). Therefore, nowadays *T. gondii* is regarded as one of the most significant veterinary and medical parasites of food and water (Almeria and Dubey 2021).

Despite the fact that it can spread very easily by affecting many species and creating very important clinical findings in humans, toxoplasmosis continues to be seen as a minor and ignored illness. And also, vaccines for humans are not present and current specific antiparasitic treatment application is not at the desired level yet (Kijlstra and Jongert 2008b). Because of this fact, pre-harvest control programs to struggle with toxoplasmosis in farm animals and post-harvest detection and destruction methods to ensure/promise toxoplasma-safe meat are very important (Kijlstra and Jongert 2008a).

2. LIFE CYCLE OF *TOXOPLASMA GONDII*

T. gondii is an organism that lives inside cells and infects animals (Ragozo 2018). Its medicinal significance was unknown until 1939 and its life cycle was not found until 1970 (Dubey 2008). *T. gondii*'s life cycle is a heterogeneous structure alternating between sexuality and asexual phases (Tenter et al. 2000; Ragozo 2018) (Fig. 1). Felines and wild felines are the only definite hosts that play a significant role in *T. gondii*'s epidemiology (Dubey 1996; Roqeplo et al. 2011). Both pets and wild cats shed environmentally resistant oocysts in their feces for only 1-2 weeks after primary infection (Roqeplo et al. 2011; Opsteegh et al. 2016) and they frequently become resistant to oocyst re-shedding (Dubey 1996). Warm-blooded species and humans are mid-hosts (Tenter et al. 2000; Rani and Pradhana 2020), allowing parasites to multiply in two stages (Opsteegh et al. 2016).

T. gondii develops asexually in two stages in intermediate hosts (Tenter et al. 2000). Tachyzoite is the parasite's earliest, rapidly replicating stage. Tachyzoites transform into bradyzoites that are contained in tissue cyst (Opsteegh et al. 2011). Tissue cysts with a strong affinity for neurological and muscular tissues are primarily seen in areas such as the eye, the skeletal and cardiac muscles, and the lungs, liver and kidneys (Tenter et al. 2000).

T. gondii has three infectious stages: tachyzoites, bradyzoites located inside tissue cysts, and spores included in spore-forming oocysts. All three stages are infectious to intermediate and definitive hosts, who

can become infected with *T. gondii* primarily by one of the following methods as shown in Fig. 2. *T. gondii* can be transferred from definitive to intermediate hosts, interim to definitive hosts, and between definitive and intermediate hosts (Tenter et al. 2000).

Most of this parasite collected from humans as well as animals in North America has been classified as one of three clonal lineages, including types one, two, and three, and varies physiologically and genetically from isolates obtained in some other countries but are similar to isolates from Europe. Recent genotyping investigations of pig, lamb, and goat isolates show a Type II line prevailing in animals used for food in the United States, followed by Type III strains and atypical genotypes (Hill and Dubey 2013).

3. *T. GONDII* IN HUMANS

Toxoplasmosis is still a public health issue. An estimated 1 million persons are infected with *T. gondii* annually in the USA, (Dubey et al. 2011) and almost 400 cases of toxoplasmosis are clinical in England and Wales (Plaza et al. 2020). Daryani et al. (2014) reported a 39.3% total frequency rate of toxoplasmosis in Iran's population. According to a recent comprehensive review and a meta-anlysis in pregnant women, the worldwide incidence of latent disease in pregnant women is 33.8 % (Almeria and Dubey 2021). However, considering the lack of pathological indications in a lot of cases and the fact that they are not reportable, the real prevalence of toxoplasmosis is likely to be underestimated (Plaza et al. 2020).

Infection of humans with *T. gondii* can occur in a variety of ways, including the eating of uncooked or raw meat from chronically infected animals, the ingestion of sporulated oocysts found in water or on vegetables (Rani and Pradhana 2020), by handling contaminated soil or cat litter trays (Opsteegh et al. 2016) and by vertical transmission (congenital toxoplasmosis) (Rani and Pradhana 2020).

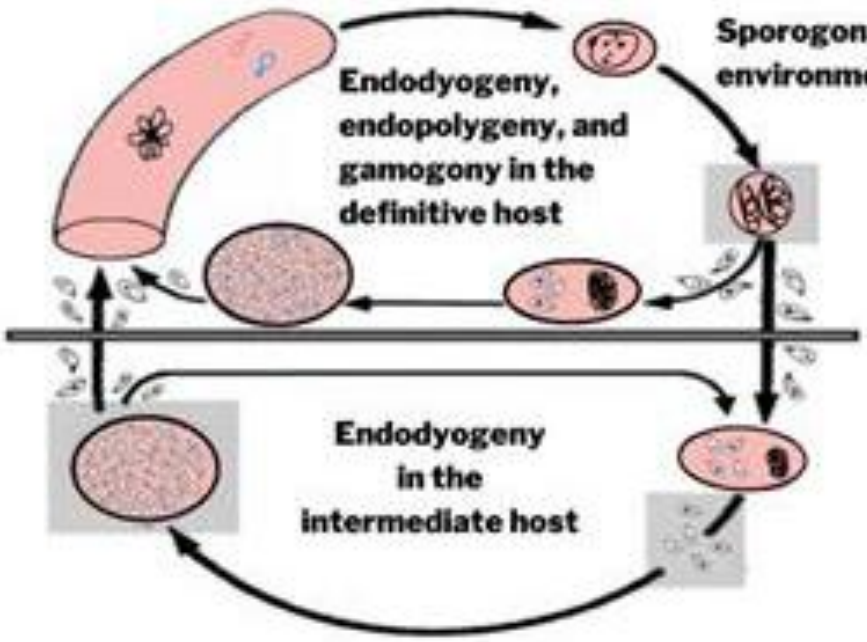

Fig. 1: Life cycle of *T. gondii*

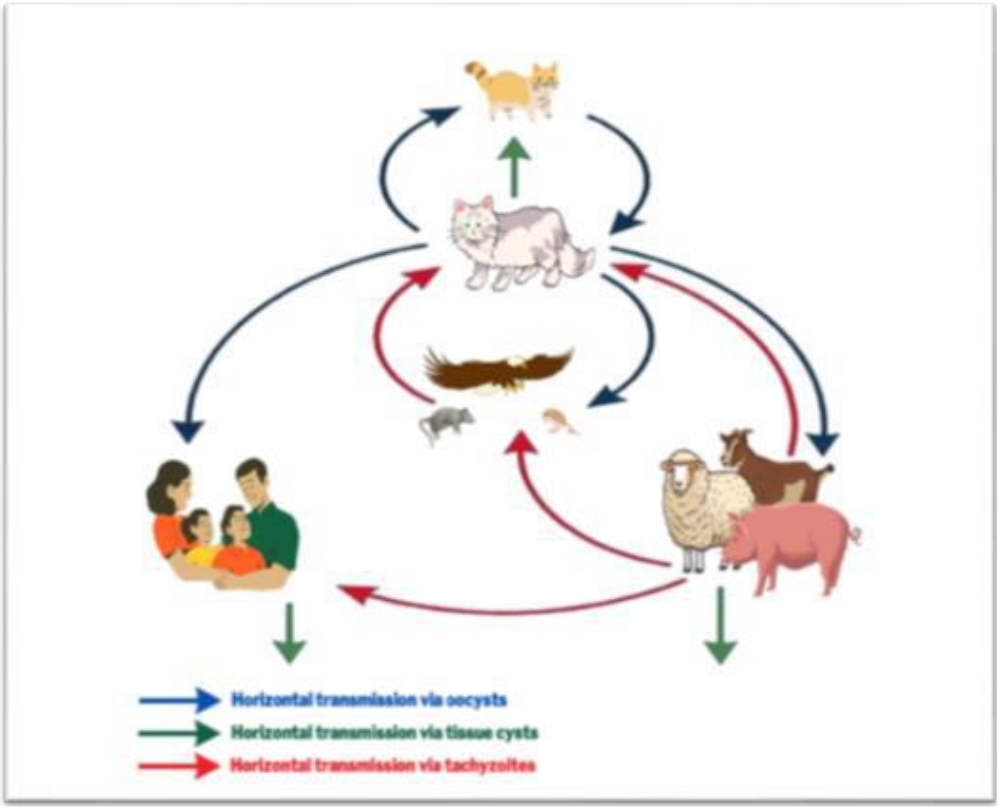

Fig. 2: Major routes of transmission of *T. gondii*

Inhalation of dust-containing oocysts has also been implicated as a transmission route (Kijlstra and Jongert 2008a). A European multicenter case-control research found that undercooked or raw meat caused 30% to 63% of acute infections in pregnant women in diverse European cities, while soil exposure caused up to 17% of infections (Opsteegh et al. 2016). Similar to these results, *T. gondii* infection in strict vegetarians demonstrates that oocyst invasion by dust still plays a key role in infection (Kijlstra and Jongert 2008a). Jones et al. (2009) evaluated 148 patients and reported that several raw or undercooked foods, as well as kittens, are hazards for *T. gondii* illness. Some important recommendations for pregnant women to prevent infection by *T. gondii* include: ingest well-cooked (67° C for 10 minutes) meat, do not experiment with the ingestion of raw or uncooked meat, freeze (-18°C for 7 days) all products, protect all food from flies and cockroaches, not feed the cat with raw meat and prevent the animal from hunting (Navarrol 2018).

In mankind, infection is typically asymptomatic or causes mild flu-like symptoms (such as fever, lymphadenopathy, headache, myalgia, stiff neck, sore throat, arthralgia, rash, confusion, nausea, eye pain, and abdominal pain) (Hill and Dubey 2002; Halos et al. 2010). Although most healthy people remain asymptomatic, severe clinical illness, including death, can be seen in infants and immunocompromised patients, such as HIV infection, long-term corticosteroid therapy, hematologic malignancies, and transplant recipients (Almeria and Dubey 2021). If an infection occurred at an early stage of pregnancy, toxoplasmosis can induce serious disease in the fetus (Tenter et al. 2000; Zoua et al. 2017) and can induce miscarriage or congenital abnormalities of the fetus's brain, eyes, or other organs (Halos et al. 2010). If an initial infection occurs 46 months or earlier before pregnancy, protective immunity typically prevents vertical transfer to the unborn on future exposures (Tenter et al. 2000). If the patient is under investigation or with a confirmed infection, spiramycin (under investigation and first trimester), pyrimethamine, sulfadiazine, and folinic acid (after the 18th week, until parturition) could be used (Navarrol 2018).

4. *T. GONDII* IN FOOD ANIMALS

Toxoplasmosis' symptoms differ based on the animal type. Early embryonic expiring and resorption, abortion, stillbirth, and infant death, in most ruminants, are the main symptoms (Almeria and Dubey 2021). *T. gondii* can be identified more frequently in the muscles of pigs, sheep, and goats compared to tissues from other edible animals. Although the incidence of *T. gondii* within sheep is unknown, alive *T. gondii* is detected in several edible tissues of *T. gondii*-infected lambs (Dubey 1996). Seroprevalence in pigs and chickens from nonbio-secure management systems is high. Clinical disease and high seroprevalence are seen in sheep and goats (Hill and Dubey 2013). In sheep up to 92% seroprevalence has been seen in some European countries (Tenter et al. 2000). According to a recent poll, 62.2% of sheep in the US are pasture-raised. Roaming sheep are surrounded by soil and water, both of which can be a source of *T. gondii* (Guo et al. 2015). *T. gondii* infection was found in hogs and wild boars reared and slaughtered in the Czech Republic (Slany et al. 2016). According to some researchs, cattle are immune to infection and are not thought to be the key carriers for *T. gondii*. (Sroka J et al. 2019).

5. TRANSMISSION OF *T. GONDII* WITH DIFFERENT KINDS OF MEAT

The use of undercooked/raw meat with viable tissue cysts (Plaza et al. 2020; Mancusi et al. 2023) is thought to be a significant cause of infection due to *T. gondii*'s propensity to spread to a wide range of animals and remain in their structures for years (Tenter et al. 2000; Kuruca et al. 2023). Human toxoplasmosis has been documented following the intake of raw and uncooked flesh and organs, and live parasites have been identified from fresh, frozen, and cured meat (Liu et al. 2015; Kuruca et al. 2023). Sheep and goats had the greatest proportion of positive samples for the pathogen or antibodies amongst every single one of the Member States in the EU. Beef, sheep, pork, and combined meat products account for 68%, 14%, 11%, and 7% of meat-borne diseases in the Netherlands, respectively (Opsteegh et al. 2016). Beef is one of the most popular foods in the United States, and diverse *T. gondii* seroprevalences were found in a cow study (Guo et al. 2015). *T. gondii* prevalence in cattle, on the other hand, did not match the incidence of cysts in tissues in cattle. Dubey et al. (2005) examined the incidence of live *T. gondii* in 6,282 data acquired from 698 retail meat outlets in 28 main geographic regions of the United States. They found no bioassay or ELISA-positive results that were positive in any one of the 349 pools or 2,094 specific edible beef samples. Rahdar et al. (2012) collected tongue, heart, and muscle samples from 50 lamb and 50 beef wholesalers, as well as 90 meaty product samples from local Ahvaz city merchants. They discovered *T. gondii* cysts in seven lambs (14%) and two beef (4%), however, the infection did not originate from a single of the meat samples. Plaza et al. (2020) acquired 300 meat samples from butchers, farmers' markets, farm stores, and supermarkets. They found DNA in 0/39 (0%) cattle samples, 19/67 (28.4%) venison samples, and antibodies to *T. gondii* in the meat and juice of 2/38 (5.3%) beef samples.

T. gondii seroprevalence in raw sheep meat ingested in France was examined by Halos et al. (2010). The proportion of French carcasses bearing live parasites was estimated to be 5.4%. They cautioned that all case-control investigations have indicated mutton/lamb meat intake as a significantly major danger factor among pregnant women. Likewise, Villena et al. (2012) stated that ovine meat has been linked to an increased risk of Toxoplasma infection in specific parts of France.

Little knowledge on the presence of viable *T. gondii* in goats' tissue is available (Villena et al. 2012). Although *T. gondii* has been isolated from caprine tissues in investigations, no enormous scale prevalence data on the detection of parasites in goat flesh and products are known (Kijlstra and Jongert 2008a; Dubey et al. 2011). Dubey et al. (2011) obtained the hearts of 234 goats from a market in the United States. They discovered the antibodies to *T. gondii* in 125 of 234 goat hearts.

Pig meat constitutes one of the most common forms of meat involved with human-caused toxoplasmosis, and consumer cooking temperatures may not be adequate for deactivating *T. gondii* cysts in carcasses and products (Guo et al. 2015). Hamilton et al. (2015) detected antibodies from meat juice in 55% of pig hearts. The transmission of *T. gondii* in pigs has been nearly eradicated, and the amount of *T. gondii* in pork products has reduced considerably (Tenter et al. 2000; Kijlstra and Jongert 2008a). As a result, in many regions of the world, pork meat is no longer considered the primary source of infection (Kijlstra and Jongert 2008a).

Zoua et al. (2017) collected tissue from muscle specimens of 414 poultry birds (257 chickens, 115 ducks, and 42 geese) and discovered that 32 (7.37%) examples of meat from poultry were *T. gondii* B1 DNA positive, with chicken having the highest *T. gondii* incidence (8.17%), followed by ducks (7.83%), and geese (4.76%). This means that out of 257 chickens, 8.17% were infected, and of the 115 ducks mentioned, only 7.83% were affected. At the end of the study, the lowest rate was found in the geese; with a flock of 42, only 4.76% of them were affected.

Silva et al. (2003), examined *T. gondii* frequency in allowed-to-roam hens and reported that seroprevalence of free-ranging chickens was up to 65% and the present parasite in flesh obtained from seropositive was 81%. Although *T. gondii* is prevalent throughout chicken meat, the danger of spreading from infected chicken meat to humans is minimal since chicken flesh is normally properly cooked, and purchasing frozen chicken flesh may lessen the risk (Almeria and Dubey 2021).

T. gondii DNA was detected in 43% of 231 horse carcass samples in France by Aroussi et al. (2015), who also reported that there was no strain solitude in mice obeying inoculation of over 100 horse meat examples suggesting a low prevalence of cysts in the muscles of the skeleton and a small chance of infection with *T. gondii* related to horse meat consumption.

6. CONTROL OF *T. GONDII*

6.1. PRE-HARVEST PREVENTION

Preventive medicine practices in the fight against diseases in livestock are very important for both economic and public health (Dubey 1996). Many studies have found risk variables that are cat-related, although those connected with risk factors include contamination of feed or water, having access to a severely polluted environment, age, gender, and geographic and regional characteristics (Stelzer et al. 2019). During pre-harvest production, to control all these risks and to lessen the propagation of *T. gondii*, there are two important strategies: scientific and realistic management practices and vaccination (Dubey 1996).

a- FARM MANAGEMENT

Management of a farm can greatly help in *T. gondii* prevention in animals from which we get the meat by decreasing the animals' interaction with infectious phases from the environment. Farm type (indoor or outdoor production systems), feeding practices, decontamination of animal feed and bedding, cat, rodent, and bird control methods, water source, and quality are important farm management practices (Guo et al. 2015; Hussain et al. 2017). It is also critical to develop biosecurity policies on traditional farms and to apply laboratory tests to various kinds of flocks to detect farms that may have too much risk and to utilize them all across the chain of food information systems to improve food safety (Guo et al. 2015).

One of the drawbacks of outdoor farming is the lack of biosecurity. As a result of being around infected rodents in particular nature, and animal feed, water, or ambient surfaces carrying infectious oocysts, *T. gondii* prevalence is greater in conventionally raised pigs, sheep, and fowl than in cattle (Guo et al. 2015).

In order to be successful in these matters or in reducing the levels of infection in animals, monitoring and surveillance programs must be implemented (Kijlstra and Jongert 2008a).
The incidence of *T. gondii* severity in swine varies according to the animal's age, farm management techniques, and farm location (Guo et al. 2015). Natural pig farms are gaining popularity in many regions of the world. Pigs usually eat infectious rodents or rodent cadavers, as well as small animals. Because of this fact to reduce *T. gondii* seropositivity in pigs, monitoring and surveillance programs are vital (Burrells et al. 2015). The rate of seroprevalence is high in pigs and hens from non-biosecure management practices (Hill and Dubey 2013). The quantity of smaller swine farms in the United States is dropping, which might be one of the primary causes for the reduction in *T. gondii* seroprevalence in pigs during the previous decade (Dubey 1996).

b- VACCINATION OF CATS AND LIVESTOCK

Vaccine applications are very important in protecting humans and animals, especially against various diseases that cannot be treated with drugs. Because of the fact that there is no medicine that can combat the *T. gondii* tissue cyst stage (Innes and Vermeulen 2006), vaccination of farm animals against *T. gondii* is traditionally being implemented (Kijlstra and Jongert 2008a) and have two objectives; to reduce abortions in small ruminants, especially goats and sheep, and to decrease the risk of mankind's exposure to infected meat (Dubey 1996).
Understanding the defensive immune system response throughout *T. gondii* penetration and infection will be critical to producing a safer, more effective vaccine for toxoplasmosis in both people and cattle (Innes et al. 2019). The first generation of vaccines was elaborated with live, live attenuated, or inactivated antigens, the second generation consisted of the subunit vaccines (recombinant), and finally in third generation immunogens are based on genetic vaccines (Garcia 2018). Live and inactivated vaccinations that the immune system perceives as alien cause the creation of antibodies that can prevent infection (Garcia 2018). This vaccine is a tachyzoite vaccine with a live mutant strain (S48) that is easily available commercially. A live vaccination employing a nonpersistent variant of *T. gondii* is being researched in the United States to prevent abortion in sheep as is available in the country of New Zealand, the United Kingdom, and Europe to minimize oocyst shedding by cats (Dubey 1996). The researcher believes that developing single or multi-epitope-based antigens expressing possible B or/and T cell epitopes of both tachyzoite and bradyzoite-specific particular antigens would significantly enhance *T. gondii* vaccination techniques (Bastos et al. 2016). Vaccines made of DNA have various advantages, including ease of production, ease of administration, immunogenicity, and the possibility for long-term protection (Kim et al. 2012). Manufacturing vaccines by eliminating gene mutants provides a unique strategy for toxoplasmosis control (Zhang et al. 2022).
Burrells et al. (2015) wanted to see how immunization (incomplete S48 strain) affected the production of infective tissue cysts in pigs. According to the findings, the parasite preferentially inhabits the brain and extremely vascular skeletal muscles (such as the tongue, diaphragm, heart, and masseter) of swine, whereas meat cuts used for human consumption, including chop, loin, left triceps, along with left semitendinosus, possessed a smaller percentage of *T. gondii* cysts in the tissue in pork.

7. POST-HARVEST PREVENTION

a- MONITORING

The World Health Organization has frequently recommended collecting reliable epidemiological information on *T. gondii*. However, only a few nations monitor toxoplasmosis in people on a regular basis,

and even fewer countries track the infection of *T. gondii* in animals (Tenter et al. 2000). To effectively prevent *T. gondii* infection in meat, an extensive carcass assurance system from 'farm to fork' is required (Felin et al. 2016). On the other hand, veterinary leadership is very important and adds value to the process (Huey 2012). Because toxoplasmosis surveillance in livestock farming is challenging due to the disease's silent and chronic manifestations, carcass surveys conducted by veterinary teams present several advantages and control plans for *T. gondii* (Villena et al. 2012). Despite the fact that monitoring systems for Salmonella and Campylobacter have begun in numerous nations, *T. gondii* in meat is not checked at the slaughterhouse because there are no defined benchmark sera or additional sources of information available, and there is no testing certification scheme (Kijlstra and Jongert 2008a). If a slaughterhouse monitoring program could be used for *T. gondii*, it offers an important opportunity to prevent transmission from meat to humans (Villena et al. 2012).

B- KILLING THE PARASITE

T. gondii tissue cysts are not resistant to environmental factors (Dubey 1996) and in some treatments, applied to the meat, such as freezing, and cooking, the elevated pressure is favorable for the elimination of *T. gondii* (Dubey 1996, Kijlstra and Jongert 2008a).

Dubey described the impact of cooling on *T. gondii* cyst survivability in 1966. Freezing to -12°C can kill tissue cysts in meat and the most feasible approach to risk control would be a freezing operation (Dubey 1996). *T. gondii* tissue cysts were alive for several days at -1°C and -7°C but were frequently declared unprofitable by freezing at -12°C by Kotula et al. (1991). Nevertheless, consumer attitudes towards freezing shows less demand. Garcia et al. (2021) found that dry-cured bacon contaminated with 4,000 *T. gondii* oocysts stored at -20°C for 7-14 days failed to inactivate *T. gondii*. Based on the results of the Monte Carlo computerized test, the cells in the cysts can survive at 4°C for at least 30 days (Rani and Pradhan 2021).

Heat treatment is an especially secure way to kill the parasite (Kijlstra and Jongert 2008a). Tissue cysts in meat can be killed by heating to an interior temperature of 67°C (Dubey 1996). The safe temperature for cooking is determined as 64°C (Rani and Pradhan 2021). Aroussi et al. (2015) recommended that cooking of horse meat is an important stage to avoid any risk of toxoplasmosis. Lunden and Uggla (1992) investigated the changing acts of microwave cooking over the infectivity of *T. gondii* and reported that the parasite remained infective in steaks processed in a microwave oven, maybe due to proper heat spreading. When tissue cysts are exposed to dosages of 0.4 to 0.7 kg of gamma radiation, the parasite can become inactive (Kijlstra and Jongert 2008a). *T. gondii* tissue cysts could also be inactivated utilizing a 300 MPa high-pressure (Kijlstra and Jongert 2008a).

8. DETECTION OF *T. GONDII*

a- MEAT INSPECTION

Meat inspection is an important stage in the healthy meat production chain. Monitoring animals during slaughter might be utilized to identify *T. gondii*-infected flesh and remove cysts from tissue in the meat using particular techniques, as well as to make farm management adjustments (Kijlstra and Jongert 2008a). The existence of this threat in asymptomatic animals during antemortem inspection impedes controlling it in the meat chain. Traditional meat inspection cannot identify minute cysts of *T. gondii* in slaughter animal tissues, and existing laboratory techniques are still not sensitive enough to identify the infection in individual carcasses (Kuruca et al. 2023). As a result, during the current post-mortem

assessment of pig carcasses, mainly *Trichinella* spp. are detected as meat-borne biological risks (Zdolec and Kis 2021).

Developing the Quantitative Microbial Risk Assessment model has helped to look into the presence and amount of feasible bradyzoites in cattle to implement farm management changes, to assess the impact of muttering flesh on bradyzoite amounts using real batch sizes, and to estimate the percentage of meat that has been still prior to purchase (Opsteegh at al. 2011). When compared to traditional slaughter and meat inspection, the meat factory cell allows for customized chilling regimes for various sections, focused cleaning or pathogen-killing procedures, and reduced energy usage, which will benefit public health (Alvseike et al. 2018).

Although numerous indirect and direct techniques for detecting *T. gondii* have been established (Rani and Pradhana 2020) yet three types of approaches for detecting *T. gondii* in flesh and other goods have been created: (i) mouse or cat bioassays, (ii) serological tests, and (iii) PCR-based approaches. While bioassays in mice and cats are regarded as the gold standard for detecting live *T. gondii* in animal products, but these are time-consuming, costly, and need an extensive number of animals (Rani and Pradhana 2020).

Gazzonis et al. (2020) sought epidemiological and molecular information on *T. gondii* contamination in small ruminants slaughtered and sold in Italy. They discovered antibodies in 28.6% of sheep and 27.5% of goats. *T. gondii* DNA was found in fifteen sheep and three goats based on DNA testing of positive muscle samples. Hosein et al. (2016) gathered samples of 305 animals slaughtered throughout the UK, and 1.6% animals tested positive for *T. gondii* after real-time PCR.

b- SEROLOGICAL DETECTION

The present approaches for detecting *T. gondii* in meat-producing livestock, animal products, or the environment are inadequate since they do not allow for the quantification of infectious phases (Tenter 2009). Most people with *T. gondii* infection have no or few clinical signs, and their diagnosis is based mostly on serological testing. To detect distinct antibody classes or antigens, a variety of serological tests have been developed, including dye tests, modified agglutination tests (MAT), enzyme linked immunosorbent assays (ELISA), immunosorbent agglutination assays, indirect fluorescent antibody tests, and indirect haemagglutination assays (Liu et al. 2015). Serological screening for toxoplasmosis in slaughterhouse has shown to be the most effective approach. Based on their findings, Felin et al. (2015) proposed that using meat juice serology during slaughter is a valuable technique for controlling this pathogen.

The indirect ELISA proved to be a simple, quick, low-cost, and more sensitive assay for detecting *T. gondii* in livestock juice, and it might be regarded as an attractive test to track toxoplasmosis in cow meat and meat products. The meat juice was used as an appropriate sample for detecting *T. gondii* antibodies using ELISA, and diaphragmatic tissues could be used as a matrix for parallelism seropositive diagnosis (Shaapan et al. 2021).

At a slaughterhouse, Villena et al. (2012) gathered flesh specimens and muscular secretions from 419 ovine carcasses. On cardiac fluids, an industrial ELISA plus a MAT were used. They discovered that ventricular fluids appear to be a useful matrix for toxoplasmosis detection in meat. Wang et al. (2012) collected 416 freshly slaughtered pork samples from various sites in Anhui province, Eastern China, and utilized ELISA to identify *T. gondii* antibodies in the fluid from the tissues. Similarly, Mecca et al. (2011) collected effusion from meat packaging owing to their blood content in order to identify infections that occur naturally in rabbit meat for sale. In addition, they acquired chops from Botucatu strain rabbits during slaughter and discovered 1.35% (1/74) positive samples in commercialized Brazilian rabbit meat cuts using this technique.

Sousa et al. (2010) collected blood samples from 11 free-range hens and fifteen pigs in order to isolate *T. gondii* genotypes. Serotyping findings from three chicken serum samples and two pig serum samples were consistent with genotyping. Roqueplo et al. (2011) collected samples (serum or meat juice) from 205 animals in New Caledonia and detected *T. gondii* in 2% of the pigs, 3.3% of the cattle, 13.8% of Rusa deer, 16% of the horses, 32.8% of the dogs, and 50% of cats.

c- MOLECULAR DETECTION

The gold standard bioassay methods for identifying *T. gondii* in samples of meat (Opsteegh et al. 2011; Rani and Pradhana 2020) are tedious and time-consuming approaches that are not suitable for screening large quantities of samples due to animal ethical concerns (Opsteegh et al. 2011). Toxoplasmosis is diagnosed using molecular approaches in addition to traditional serological methods (Liu et al. 2015). *T. gondii* may be readily defined by detecting distinct and unique genes, and the amount of *T. gondii* may even be counted using a real-time PCR experiment (Guo et al. 2015). Mancusi et al. (2023) set out to create a droplet digitally polymerase chain reaction-based test for detecting and quantifying *T. gondii* in meat samples, to achieve absolute quantification of the target DNA, QuantaSoft software was utilized for determining the PCR + and PCR - droplets. Each target's quantification values were represented as the number of genome copies per 1 l of reaction. They observed that this novel method might be highly beneficial for detecting tiny quantities of *T. gondii* in meats. If the sample had two drops, it was declared affirmative. Positive droplets were not seen in the negative control samples. The reaction produced droplets in the range of 8985 to 13,940, with a median of 11,384 droplets. For analysis, reactions with over 8000 accepted droplets per well were employed. The ddPCR results showed a strong separation of both positive and negative droplets with few interface droplets, indicating a high primer specificity and reaction efficiency. Drops were positively saturated at high concentrations (> 10,000 GC/l), rendering the Poisson method incorrect and resulting in a substantially lower variability than qPCR. Wang et al. (2012) attempted to provide the first study on the distribution and genetic type of *T. gondii* in pork in Chinese retail meat outlets. They discovered that 75 out of 416 specimens (18.03%) had a positive real-time PCR result. Aspinall et al. (2002) collected 71 meat specimens from retail establishments in the United Kingdom. Using particular primers that targeted the *T. gondii* SAG2 locus, they discovered the parasite in 27 of the flesh samples and 21 of the infected foods. Franco-Hernandez et al. (2016) used PCR to examine a total of 120 specimens from the first group of herbs and 60 samples from class II herbs in Colombia. They discovered that 79 (43%) of the samples were positive for B1 nested PCR tests (33 from chicken, 22 from beef, and 24 from pig) and advised that comprehensive PCR research can assist to implement effective methods to limit the risk of getting this parasite through meat intake.

Pastiu et al. (2015) gathered sera and cardiac tissue from 82 horses killed in the northern part of Romania for trade and human consumption. Antibodies anti-*T. gondii* were found in 32 (39%) of the serum and 31 (37.8%) of the cardiac tissues. *T. gondii* DNA wasn't found in any of the cardiac samples, contrary to the PCR results. Despite these breakthroughs in identifying *T. gondii* using PCR, it is stated that the substantial salt content in certain cured foods reduced the PCR assay's sensitivity. Warnekulasuriya et al. (1998) found alive *T. gondii* in one of 67 ready-to-eat meat cure samples, although they suggested that new methods for identifying the protozoa to infiltrate of cured meats were needed. It's quite a challenge to get rid of this protozoa easily and completely by using the old and known techniques.

9. CONCLUSION

Food-borne toxoplasmosis in humans can occur as a result of exposure to various stages of *T. gondii*, including eating or drinking cysts of tissue or tachyzoites found in meat, offal (viscera), or the intake of

sporulated oocysts found in the atmosphere and water. Because of *T. gondii*'s propensity to spread through a broad spectrum of livestock hosts and stay within their tissues for years raw or undercooked meat carrying live cysts has been acknowledged as an important cause of infection with *T. gondii* in humans. Although the percentage of live *T. gondii* in meat sold at retail was very low, customers can get *T. gondii* infection by eating raw meat. However, many people are unaware of ways to avoid *T. gondii* infection, particularly the hazards related to ingesting or handling undercooked or raw meat. Understanding these risk variables can assist to focus preventative efforts.

Monitoring programs for toxoplasma infection in livestock animals is desirable for human toxoplasmosis prevention. Good Farming Practices to develop efficient and sustainable control measures and better rodent management towards infection with *T. gondii* in farms, such as severe confinement housing and stringent biosecurity laws, is critical. Vaccine development and immunization of livestock on farms against *T. gondii* have traditionally been used to prevent miscarriages in small livestock and the danger of human exposure to contaminated meat. Despite the fact that toxoplasmosis is recognized as one of the most underappreciated biological dangers in the meat supply chain, there is no legal mandate for *T. gondii* surveillance or control in livestock-producing animals and their products. The presence of the parasite inside slaughtered animals suggested that the danger of food-transmitted toxoplasmosis remains. Unfortunately, routine meat inspection cannot identify minute cysts of *T. gondii* in slaughtered animal tissues, and modern laboratory techniques are still not sensitive or specific enough to detect contamination in individual carcasses. As a result, developing and standardizing assays for identifying the infection of *T. gondii* in meat-producing species and their products with equivalent specificity and sensitivity is critical.

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Role of Nanotechnology in Treating of Toxoplasma Gondii

17

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ABSTRACT

Toxoplasma gondii, an intracellular protozoan parasite, poses significant health risks globally, affecting humans and animals alike. Conventional therapies for toxoplasmosis often encounter limitations such as poor bioavailability, drug resistance, and systemic toxicity. Nanotechnology has emerged as a promising avenue in the development of innovative therapeutic strategies, offering enhanced drug delivery, improved efficacy, and targeted action against Toxoplasma gondii. This abstract provides an overview of the role of nanotechnology in combating Toxoplasma gondii infection. Nanoparticle-based drug delivery systems have shown considerable potential in overcoming the drawbacks associated with traditional anti-toxoplasmosis medications. Various nanoformulations, including liposomes, polymeric nanoparticles, solid lipid nanoparticles, and nanomicelles, have been engineered to encapsulate and deliver anti-parasitic agents effectively. Nanocarriers offer several advantages, such as sustained release of drugs, protection of payloads from degradation, increased cellular uptake, and selective targeting of Toxoplasma gondii-infected cells. Additionally, surface modification of nanoparticles enables specific ligand-receptor interactions, facilitating targeted drug delivery to the parasite, thereby reducing off-target effects and enhancing therapeutic efficacy. Moreover, nanotechnology-based diagnostic tools employing nanoparticles have been developed for the sensitive and rapid detection of Toxoplasma gondii antigens or DNA, enabling early diagnosis and timely intervention. Challenges in the application of nanotechnology for toxoplasmosis treatment include scaling up production, ensuring biocompatibility, and addressing potential toxicity concerns associated with nanomaterials. Further research endeavors focusing on refining nanocarrier design, optimizing drug loading and release kinetics, and evaluating long-term safety profiles are crucial for clinical translation. In conclusion, nanotechnology holds immense promise in revolutionizing the management of toxoplasmosis by offering novel therapeutic and diagnostic approaches. The synergy between nanotechnology and anti-toxoplasmosis therapies presents an encouraging pathway towards more efficient, targeted, and safer treatments for combating Toxoplasma gondii infection.

Key words: Nanotechnology, Toxoplasma gondii, drug delivery, nanoparticles, nanoformulations, targeted therapy, diagnosis, anti-parasitic agents, nanocarriers, toxoplasmosis treatment.

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1. INTRODUCTION

Toxoplasma gondii (*T. gondii)* is a protozoan parasite and is widely distributed throughout the world (Dubey 2021; Ismael 2021). *T. gondii* is an obligatory intracellular parasite under the phylum Sporozoa that may cause serious clinical symptoms particularly, in pregnant women and immunocompromised people (Deng et al. 2018). It has three morphological forms includes: trophozoite (Tachyzoites), Tissue cyst (Bradyzoites), and sporozoites which are found within the oocysts. The life cycle of *T. gondii* is complex and has two parts, the first part, is the asexual reproduction which occurs in the intermediate host (such as man and cow), and the sexual reproduction which occurs in the final host (cats and other carnivorous) There are three infectious development stages: tachyzoites, bradyzoites (in tissue cysts), and sporozoites (within oocysts) (Delgado et al. 2022). Toxoplasmosis can be transmitted in many ways such as from mother to baby (Tachyzoites pass to the fetus), by ingestion of infective sporulated oocysts, drinking of undercooked milk, ingestion of undercooked meat, and also can be transmitted sexually (Milne et al. 2020). Congenital toxoplasmosis, which can result in abortion, ocular disease, hydrocephaly, microcephaly, and mental retardation for the fetus, can be brought on by the re-activation of parasites during pregnancy (Elsheikha 2008). Immunocompromised patients may also develop severe diseases, such as encephalitis and pneumonitis. Some psychiatric illnesses such as schizophrenia, depression, and bipolar disorder have been associated with toxoplasmosis (Wang et al. 2017; Liu et al. 2022).

The classical treatment of toxoplasmosis, as usual, is pyrimethamine and sulfadiazine and is usually given with folic acid (Katlama et al. 1996; Dard et al. 2018). The severity of the side effects and the fact that this drug combination is only effective against the tachyzoite form, failing to eradicate latent forms like slow-diverging bradyzoites within tissue cysts, contribute to the low therapeutic adherence of this drug combination (Silva et al. 2021). These drugs have various side effects and can lead to an increase in the level of liver enzymes, an increase of serum creatinine, a decrease in the number of platelets (thrombocytopenia), and suppression of bone marrow (Ben-Harari et al. 2017).

Like other parasites, drug resistance has been observed in this parasite as well (Antczak et al. 2016). Additionally, attempts to develop a new toxoplasmosis vaccine have failed (Foroutan et al. 2019). Nevertheless, we require drugs that are more lethal for all stages of the *Toxoplasma* life cycle, including bradyzoites in tissues and less toxic for the host (Antczak et al. 2016). Now-a-days, there is a rapid development of nanoparticles (NP), and is used throughout the world for the diagnosis, prevention, and treatment of specific types of cells and tissues (Bala et al. 2004). Nanoparticles, serve as a tool for improving pharmacological information like drug release, tissue specificity, and even cell specificity because it can pass blood-brain barriers (Akerman et al. 2002). Nanoparticles are a strong option now for the prevention of most infections including toxoplasmosis, COVID-19, and Hepatitis B virus (Peplow 2021).

Drug crystals have been successfully utilized as nanocarriers in several cases (Sordet et al. 1998; Schöler et al. 2001). Polymer-based NP can be coated with molecules that give them specific surface properties to bind to and be taken up by specific cells, or they can be loaded with drugs that release in a controlled manner. Despite these attractive objectives and 40 years of research, polymeric nanoparticles are not currently being used in pharmaceutical applications (Lherm et al. 1992; Alyautdin et al. 1997; Kreuter 2001). Nanoparticles may be used to deliver medications across the blood–brain barrier (Kayser et al. 2003)

2. NANOTECHNOLOGY AND NANOMATERIALS

Nanotechnology is an advanced technology, which depends on the nanometer scale, usually ranged between 0.1- 100 nm. As a branch of nanotechnology, nanomedicine describes highly specific therapeutic impacts at the nanoscale (Saha 2009). Nanoscale devices are used for the management and treatment of

infectious diseases (Freitas 2002). Numerous ingredients, each having a measurement of less than 100 nm, are combined to form nanoparticles (Laurent et al. 2008). The general form indicates that those substances might have 0, 1, 2, or 3 dimensions (Tiwari et al. 2011). When scientists learned that dimension might influence the material's physiochemical properties, they realized the importance of these substances (Dreaden et al. 2012).

Due to their useful surface reactivity and nanoscale sizes, nanoparticles are used in a wide range of biomedical applications today. Additionally, due to their small size and ability to cross membrane barriers, NPs can produce free radicals that can kill infectious agents (Alajmi et al. 2019). Metal nanoparticles, such as silver and gold, are of particular interest for this purpose because they have bioactivities such as selective inhibition of some enzyme (Venkataraju et al. 2014), antimicrobial (El-Khadragy et al. 2018) and antiparasitic activity (Khan et al. 2013).

2.1 NANOPARTICLES CLASSIFICATION:

Nanoparticles are generally classified into different types depending on their dimension, origin, and materials (Pokropivny and Skorokhod 2007). The first classification depends on the dimension and is classified into four groups including Zero-dimension nanomaterials (0D), One-dimension nanomaterials (1D), Two-dimension nanomaterials (2D), and Three-dimensional nanomaterials (3D) (Zaheer et al. 2022). The second classification depends on their source and is classified into two groups natural and artificial (Kumar and Kumbhat 2016). The last classification is depended on the type of material used to prepare and is classified into four groups: organic, inorganic, Carbon-based, and composite-based (Verma et al. 2003; Jeevanandam et al. 2018).

2.2. ROLE OF NANOMEDICINE IN THE TREATMENT

Nanotechnology is the most effective way to deliver drugs. Because of increasing the solubility area, stability, dissolution rate, and surface of a drug, and by modulating therapy and the permeability of the drug action through absorption into membranes, a drug's bioavailability is increased, which lowers the dosages of the drug that are needed (Wan et al. 2014). Different techniques for creating nanomaterials have been developed, but decrease of chemical has emerged as the most practical technique for creating these kinds of materials (Assolini et al. 2017).

Nanomedicine is the term for the application of nanomaterials in healthcare, and more nanoparticles are being evaluated for use in a variety of diagnostic, therapeutic, and preventive applications. Nanomaterials are defined as organic or inorganic, amorphous or crystalline particles that range in size from tens to hundreds of nanometers (Assolini et al. 2017; Soares et al. 2018). Nanomaterials be arranged as single particles, powders, aggregates, or dispersed in a matrix to create emulsions, suspensions, or nanolayer films. They are much more reactive than larger particles due to their size, which also results in a surface area-to-volume ratio (Gaafar et al. 2014). Due to their propensity to adsorb biomolecules upon coming into contact with biological fluids, colloidal nanoparticles develop a layer on their surface known as the corona. Additionally, due to their size, they can enter cells and react with intracellular molecules. Because of their diversity, nanoparticles are very adaptable (Gaafar et al. 2014).

2.3. NANOMEDICINE FOR TREATMENT OF TOXOPLASMOSIS

Sulfadiazine and pyrimethamine are the two most frequently prescribed medications for treating human toxoplasmosis, and both of them have serious adverse effects that include allergy, and complications with

the kidneys and liver (Abou-El-Naga et al. 2017). Several antibiotics and anti-malarial medications have also been used, but they can also have harmful impact (Anand et al. 2015). By changing their pharmacokinetics, the distinct physicochemical properties of nanoparticles can be used to enhance drug delivery. According to Anand et al. (2015), this may lead to slow delivery of drugs, improved target specificity, increased efficacy, and a decrease in side effects. Using nanotechnology-based methods, drugs that are toxic, poorly soluble, or easily degraded in the gastrointestinal tract can be administered to the body for more effective treatment at lower doses. Both the efficacy of using nanoparticles to deliver current anti-toxoplasmosis treatments and their potential as standalone anti-microbial agents have been studied (Pissuwan et al. 2009; Teimouri et al. 2018).

Chitosan is a natural polysaccharide that has been demonstrated to have antibacterial, antimalarial, and anti-Toxoplasma properties. All sizes of nanoparticles were used to demonstrate anti-*T. gondii* activity in vitro, but low molecular weight nanoparticles killed the exposed tachyzoites faster. In an in vivo model, smaller nanoparticles also worked best. They significantly reduced the load of parasites compared to infected untreated mice, but they were not as effective as sulfadiazine treatment (Etewa et al. 2018).

Spiramycin is a safe drug and is used for toxoplasmosis during pregnancy, but due to its poor bioavailability and unable to cross the blood-brain barrier, it is not very effective. In comparison to spiramycin or chitosan nanoparticles alone, loading spiramycin into chitosan nanoparticles increased its absorption and permeation, extending mice's survival time and lowering parasite burden. The spiramycin-chitosan nanoparticles had a direct impact on the parasites themselves, as evidenced by the reduced inflammatory response to infection in the treated animals and morphological deformities in the parasites that were isolated (Khalil et al. 2013; Hagras et al. 2019). In 2017, a study was done by Abou-El-Naga et al. (2017), who discovered that by giving PLGA nanoparticles in combination with anti-retroviral lopinavi/ritonavir to infected mice can reduce parasitic burden.

Investigations have been done on nanoparticles. The key attributes of NPs are reduced toxicity, alteration of pharmacokinetics, enhanced bioavailability, and the capacity to transport pharmacological components (Khalil et al. 2013; Torres-Sangiao et al. 2016). Because of this capacity, the medicine can be administered directly to the intended target. Till now, the range of available treatments for toxoplasmosis is limited (El-Ashram et al. 2015). These include using antibiotics and anti-malarial medications, both of which frequently have disadvantages like allergies (rashes on the skin) and suppression of bone marrow (Wigginton et al. 2010; Adeyemi and Sulaiman 2015). Therefore, toxoplasmosis is characterized by a significant global burden that is made worse by the limitations of the available therapeutic options (Kamau et al. 2012). These components emphasize the need for improved anti-Toxoplasma medications and/or novel toxoplasmosis treatment methods.

The ideal anti-Toxoplasma medication should be safe, effective, and capable of curing latent infection (Das et al. 2013). According to research, nanoparticles could make up the majority of future biomedical treatment strategies for a variety of diseases as interest in using nanotechnology increases (El-Khadragy et al. 2018). Nanoparticles are currently employed in a wide range of biomedical applications due to their nanoscale dimensions and other advantageous surface reactivity. Additionally, because of their small size and ability to cross membrane barriers, NPs can produce free radicals that can kill infectious agents (Adeyemi et al. 2017). Nanoparticles may also accumulate in tissues, providing cysts in host tissues with a strong foundation (Adeyemi and Sulaiman 2015).

Liposomal carriers played a crucial role in the development of a new strategy for battling protozoans in the 1990s. Stearylamine-bearing liposomes were used for the treatment of RH strain of toxoplasma by Tachibana et al. (1990) both in the laboratory and in live animals during the tachyzoite phase. They

discovered that as liposome concentration is decreased, the in vitro viable activity of SA/PC liposomes gradually decreased and had both therapeutic and preventive benefits, according to in vivo results. Elsaid et al. (1999; 2001) investigated the impact of liposomes on toxoplasmosis. They investigated mouse-specific liposomal antigens against *T. gondii*. All mice that were given the *T. gondii* antigen had higher ELISA antibody levels after vaccination, but there was no statistically significant difference between the groups. However, immunization with liposomal-encapsulated total trophozoites and/or tissue cysts antigen and pure tachyzoite antigen (L/pTAg) increased the protective immunity (both cellular and humoral immune response), likely helping to reduce the transmission of toxoplasmosis and mainly decreased congenital transmission.

A study by Pissuwan et al. (2009) reported gold nanoparticles coated with anti-*T. gondii* antibodies were successful at treating the acute strain of *T. gondii* antigen by using the light of the laser. They came to the conclusion that while a specific laser dose boosted the mortality rate of tachyzoites in the laboratory (in vitro), the mortality rate changed remarkably when the light of laser was utilized as one of the primary methods of production for these materials. In a different study, Kunjachan et al. (2011) compared using Chitosan and silver nanomaterials separately or together to treat toxoplasmosis in experimental animals. Combining them demonstrated a notable decrease in the number of parasites in both the liver and spleen.

Azami et al. (2018) assessed the therapeutic benefits of curcumin nano-emulsion in infected mice with acute and chronic toxoplasmosis. They found that the survival period of mice treated with the emulsion was considerably longer than that of the control group during the acute phase of infection. The emulsion also markedly reduced the mean counts of tachyzoites in the peritoneum of acutely infected mice as compared to control untreated mice. In a separate work, Alajmi et al. (2019) found that the treatment of toxoplasmosis by using silver nanoparticles was more effective than traditional treatments in reducing liver toxicity. According to another study by El-Shafey et al. (2020), by using Curcumin as a treatment for chronic toxoplasmosis in infected rats significantly decreased the mean number of parasite cysts in rats' brains.

A further investigation by El-Shafey et al. (2020) revealed that the use of curcumin for the treatment of chronically infected rats (strain ME49) resulted in a considerable decrease in the mean number of parasite cysts in these rats' brains. Triclosan (TS) and liposomes loaded with triclosan (liposomal-TS) were tested by El-Zawawy et al. (2015) in Swiss albino mice against a potent strain of *T. gondii*. Oral medication was used to treat the intraperitoneal infection. After treatment, tachyzoites load was significantly reduced by both TS and liposomes-TS, but the latter was more efficient. Additional measures like mouse mortality and survivability, morphological modification, and infectivity of tachyzoites from infected mice revealed a similar profile when compared to non-infected mouse controls. The authors concluded that TS's activity in peritoneal fluid and living organisms was prolonged by its longer release phase when it was loaded in liposomal structures.

As previously mentioned, other researchers suggested testing a common medication, like pyrimethamine, after it has been modified by nanotechnology for the therapeutic use of toxoplasmosis. In 2014 a study was done by Pissinate et al. (2014) who compared the effectiveness of PYR-loaded lipid-core nanocapsules and SU-PYR (surfactant prepared) against *T. gondii*. In an in vitro experiment, they used the LLC-MK2 (kidney, Rhesus monkey, Macacamulata) strain. Mice were used in in-vivo experiments by utilizing intraperitoneal injections. Comparative formulations using only LNC (lipid-core nanocapsules) were created.

Selenium is required for good human health. When the body lacks this component, serious symptoms like deficiencies and immune system cognitive deficits may manifest (Shakibaie et al. 2011). Nanostructured materials have a variety of bioactive benefits because of their high surface-to-volume ratios. The fact that they can enter cells more easily than other particles is one of their biomedical benefits (Whanger 2004). Recent studies have demonstrated that SeNPs can stop the growth of several bacterial pathogens, such as *Leishmania* species and *Escherichia coli* (Yang et al. 2009; Kojouri et al. 2012). According to recent studies, these inorganic forms can cause membrane peroxidases to produce oxygen-free radicals like superoxide radicals (Shubar et al. 2011; Mohammadinejad et al. 2019).

Toxoplasmic encephalitis is one of the clinical signs of toxoplasmosis. If the patient doesn't get treatment, it could be fatal. The classical treatments have side effects that can results in allergy and change in the hematological parameters. To reduce these disadvantages, Shubar et al. (2011) used nanoscale suspensions formed by atovaquone and coated with sodium dodecyl sulfate (SDS) and poloxamer 188 (P188).

A new approach for the treatment of toxoplasmosis had been discovered by Costa et al. (2021) to decrease the disadvantages of the classical treatment and enhance infection control. They discovered that AgNp-Bio, independent of mediators in the chorionic villus, can reduce infection in trophoblast cells and villous explants by inducing inflammatory mediators in the cells. These findings led them to conclude that AgNP-Bio-based treatment is an effective way to treat toxoplasmosis.

2.3. TOXICITY AND SAFETY OF NANOMATERIALS

As they can lead to a chronic type of sickness, parasites are thought to be more dangerous to both animals and humans than bacteria (Gupta and Xie 2018). Each stage of development causes a distinct sensitivity to the same medicine, which allows them to survive for years in their environment and with their hosts due to their complicated life cycle stages (Sarangi et al. 2018). Due to their insolubility and short half-life, antiparasitic drugs have an extremely low bioavailability. Important antiparasitic medications like ivermectin and praziquantel, for instance, are more susceptible to enzymatic degradation and have poor cell membrane penetration. As a result, the drug's expected therapeutic effect is not realized and its bioavailability is decreased (Parish 2019). When treating these parasitic infections, doctors and other medical professionals face a significant challenge (Yang et al. 2018). Blind use of antibiotics, which may lead to a big issue is resistance to many antibiotics (Li et al. 2018). According to several researches, chemotherapeutic medicines are often used, which causes bacteria to change and become resistant to conventional medication. Therefore, effective antiparasitic therapy has been made possible by nanomedicine (Kashyap et al. 2018; Sun et al. 2019). There are currently a variety of nanocarriers that can be administered orally, intravenously, or through pulmonary route. Solid lipid nanoparticles, liposomes, and nanocrystals are some examples of these nanocarriers. They offer physical stability as well as targeted and controlled drug release (Adeyemi et al. 2018; Aziz et al. 2021; Jalil et al. 2021).

Because they aren't biodegradable as other substances, such as liposomes or chitosan, and they can accumulate in organs, metal nanoparticles can be harmful. However, according to research done so far, the nanoparticles studied are hazardous to the parasite but not to the host cells both in vitro or in vivo (Park et al. 2013). Due to the pharmacokinetic alterations brought about by packaging these drugs in nanoparticles, lower doses of drugs may still be efficacious. This raises the possibility that this strategy will result in more patient-friendly and side effects-free treatments. However, it is important to recognize that nanoparticles can interfere with pregnancy, which may limit their usefulness (Elsharawy et al. 2020).

3. CONCLUSION

T. gondii is an obligatory opportunistic parasite that affect humans and animals, mainly the immune-compromised patients. More research is needed to develop safe and efficient therapeutic agents due to more adverse effects of the old medication and medication deficiencies. Technological developments on a nanometer scale are referred as nanotechnology. The only physicochemical properties of nanomaterials are their extraordinarily small size, high surface area to mass ratio, and unusual activity. They have enhanced bioavailability and medication delivery.

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Role of Wildlife in Parasitic Zoonosis

18

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ABSTRACT

Zoonotic diseases are a group of communicable disorders caused by variety of pathogens of which 95% are helminthes. Zoonotic agents can spread by contaminated food and drink, direct contact with the animal, feces samples, animal excretions and secretions. The development of zoonotic spillover from the wildlife reservoir has been linked to a sharp increase in global human activity, human population growth, habitat encroachment, expanding deforestation and land use change, globalization of travel and trade, and rising need for an animal-based food system. Some of the zoonotic species are Echinococcosis spp, Trichinella spp, Trypanosoma cruzi, Dirofilaria spp, Cryptosporidium spp, Toxoplasma spp, Toxocara spp, Taenia multiceps, Strongyloides stercoralis, Fasciola hepatica, Fasciola gigantica, Toxoplasma gondii, Leishmania infantum, Baylisascaris procyonis, Giardia spp, Ancylostoma spp etc. Wild and domestic animals are host of zoonotic diseases. Gastrointestinal disorders, fever, weight loss, skin lesions, dysfunction of organs, white fluid filled cysts in infected tissues, and paralysis are the major clinical symptoms. People often use wildlife as a source of food and as a home for parasites that can spread disease to people. Certain zoonotic parasites that originate from wildlife are emerging and resurfacing, but they either have been disregarded or are not believed to pose a serious threat to human health at this time. The perspective has to change by informing the public about possible sources and possible countermeasures to lessen the risk of human infection. Workers with wildlife should be mindful of the possibility of disease transmission. It is possible to develop and implement detection, prevention, and control programs that work.

Keywords: Wildlife, Parasites, Zoonosis, Transmission, Zoonotic spillover

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1. INTRODUCTION

Zoonotic diseases are a class of contagious illnesses that affect the animals and humans and can propagate from humans to vertebrate animals and from vertebrate animals to humans (Franjić 2022). Zoonoses, pose a risk to public health. According to estimates, 71% of new zoonotic human infections originated from wildlife. Public health is at risk from zoonoses, infectious disorders spread from animals to people (Mafuyai et al. 2013). Wildlife has historically played a significant role in the dissemination of infectious agents to humans. Zoonoses with a reservoir in wildlife afflicting all continents, posing a prominent public health issue recently. These zoonoses are becoming more and more important, and there is a need for additional focus in this field. Although there are 1415 recognized human infections, 62% of them are zoonotic; hence, the actual number of zoonoses is unfamiliar (González-Barrio 2022). Wildlife serves as a significant zoonotic pathogen reservoir and wild animals can spread disease to domestic animals and humans directly or indirectly (Cilia et al. 2021).

Numerous pathogens, such as parasites, viruses, bacteria, and fungi can be the source of zoonoses. Approximately 95% of helminthes, 40% of fungi, 50% of bacteria, and 80% of viruses that infect people are zoonotic (Morse et al. 2012; Recht et al. 2020). Cryptosporidiosis, balantidiosis, and taeniasis are some examples of parasitic zoonoses (Atawodi et al. 2013). Emerging zoonoses like leishmaniasis, *Bordetella bronchiseptica* infections, arthropod-transmitted rickettsioses, brucellosis and bartonellosis and re-emerging zoonoses like onchocercosis, leptospirosis, sporotrichosis, influenza, rabies, salmonellosis, and echinococcosis have been announced universally (Gado et al. 2023). The study of parasites in wild animals is crucial because they may have significant zoonotic indications (Liatis et al. 2017; Hewavithana et al. 2022). Animal excretions and secretions, fecal samples, contaminated food and water, and direct contact with the animal are all possible ways that zoonotic agents can circulate (Jannat et al. 2020). The proportion of wild species that are carriers for zoonotic disease is rising, raising concerns about human safety and management. Wildlife is frequently utilized by humans and serves as both a source of food and a host for parasites that can infect humans with diseases (Okoye et al. 2015).

The prevalence of zoonoses with a wildlife source may also be influenced by demographic factors and human behavior. Activities like hunting, camping, and hiking may increase the risk of contracting some zoonotic agents that have wildlife origin, like tularemia and tick-borne zoonoses. Eating patterns may also be important. For instance, consuming meat from unusual animals like bears raises the risk of contracting trichinellosis (Schellenberg et al. 2003; Kruse et al. 2004). However, certain other human-specific infections were first discovered in wildlife earlier. For instance, the parasite that causes malaria, *Plasmodium falciparum*, is most likely a descendant of *Plasmodium* of western gorillas (*Gorilla gorilla*) (Sharp et al. 2020; Wegner et al. 2022). Indirectly, through detrimental effects on host fitness, parasitic infection can harm wild animal populations irreparably, further threatening species that are already threatened with extinction. High parasitic infection would decrease fitness and renders animals more susceptible to predators and random environmental events (Hewavithana et al. 2022). The purpose of this chapter is to evaluate studies on the function of wild animals as reservoirs and dispersers of etiological agents of human infectious diseases in order to assemble information on the primary wild animals and etiological agents engaged in zoonotic outbreaks (Cupertino et al. 2023).

2. ZOONOTIC PARASITES

The four types of zoonotic parasites are sapro-zoonotic, cyclo-zoonotic, meta-zoonotic, and direct zoonotic. *Strongyloides stercoralis* and *Ancylostoma caninum* are two sapro-zoonotic parasites that can infect people through water or soil. The vertebrate intermediate hosts of cyclo-zoonotic parasites involve *Taenia saginata*, *Taenia solium*, and *Echinococcus granulosus*. *Schistosoma* spp. and *Fasciola* spp. are

examples of meta-zoonotic parasites that can infect people from invertebrate intermediate hosts. *Cryptosporidium parvum*, *Toxoplasma gondii*, *Sarcoptes scabiei*, and *Entamoeba histolytica* are examples of direct zoonotic parasites that transmit infection directly from animals to humans (Youssef and Uga 2014).

3. ROLE OF WILDLIFE IN TRANSMISSION OF PARASITIC INFECTION

All the animals including helminths to mammals are included in wildlife (Kruse et al. 2004). A sharp and growing rise in global human activity including habitat encroachment and human population growth, globalization of travel and trade, expanding deforestation and land use change, rising need for animal consumption based food system, has been associated with development of zoonotic spillover from the wildlife reservoir (Hilderink and de Winter 2021). Dietary, vector borne and environmental factors can all contribute to parasite transmission. Toxoplasma, a soil and foodborne parasite, and *Plasmodium*, *Leishmania* (both spread by blood-sucking arthropods), are some of the most common human protozoan parasites on a globe scale. The helminthes (parasitic worms) *Dirofilaria* (spread by mosquitoes), *Toxocara*, *Echinococcus*, and hookworms, which are all soil borne, are important for human health. Because of their behavior of feeding on blood, varieties of arthropods are implicated in the spread of infection. They play a key role in zoonotic infections, which are cycles of animal to human transmission (Franjić 2022). Whether it is legal or unlawful, using wildlife for commercial purposes brings a variety of wild species into proximity with people (Watsa 2020). Because of the frequent or extended contact required for husbandry, wildlife farms—also known as establishments that raise non-domesticated species for commercial purposes—can increase the risk of disease transmission between wild animals and the people who care for them (Kimman et al. 2013). Additionally, the conditions found frequently found in wildlife farms, such as a dense population of wild animals housed in the same territory, stress brought on by captivity, and poor sanitation can lower the risk for pathogen resistance and raise the likelihood of disease transmission (Mukarati et al. 2013; Whitehouse-Tedd et al. 2015; Green et al. 2020).

3.1. ECHINOCOCCOSIS SPP

One of the most ignored zoonotic illnesses identified by the World Health Organization (WHO) is echinococcosis, often named as hydatid disorder (Guo et al. 2022). In Asia, echinococcosis is endemic (Ito and Budke 2017). Cystic echinococcosis (CE) and Alveolar echinococcosis (AE) are both caused by *Echinococcus granulosus* sensu lato and *Echinococcus multilocularis*, respectively (Guo et al. 2022). Alveolar echinococcosis involves rodents (primarily arvicolids) as intermediate hosts in its life cycle. Wild carnivores like raccoon dogs (*Nyctereutes procyonoides*), golden jackals (*Canis aureus*), arctic fox (*Vulpes lagopus*), red fox (*Vulpes vulpes*), wolves (*Canis lupus lupus* and *Canis latrans*), and domestic dog (*Canis lupus familiaris*) serves as definitive hosts (Khan et al. 2021).

Canids are the definitive hosts of the larval stage metacestodes of *Echinococcus granulosus* (sensu lato), while a large variety of domestic, ungulates primarily serve as intermediate hosts in the development of Cystic echinococcosis (CE) (Ohiolei et al. 2019). About hundreds to thousands of adult *Echinococcus* spp. worms, which range in length from three to seven (mm), grow in the intestines of their chosen hosts. As each worm reaches sexual maturity, its proglottid releases eggs into the environment through the excretion of the carnivore. Then, after being consumed by humans or intermediate hosts, the eggs hatch in the intestine to release oncospheres that travel through the lymphatic and portal vessels and eventually reach the liver, where they usually develop as larvae (hydatid cysts or metacestodes). Less frequently, however, they may also travel to the bones, brain, lungs, and any other organ of intermediate host or humans (Wen et al. 2019).

3.2. TRICHINELLA SPP

Nematode worms of the genus Trichinella are one of the most widespread zoonotic pathogens worldwide (Pozio 2007). The primary factor favoring human infection is cultural eating practices that involve eating undercooked or raw meat from diseased animals (Pozio 2013). Universally, 66 countries have records of Trichinella infections in wildlife, compared to 43 countries for domestic animals. Trichinella may be carried by a diverse range of animal species birds, reptiles, and mammals. One of the most significant foodborne zoonoses, *Trichinella* spp. larvae have been found in weasels, wild boars, raccoon dogs, foxes, bears and a variety of rodents in China (Wang et al. 2007) where it is one of the most common causes of outbreaks and fatalities each year. As a result, human infections from animal hunting pose a continuing threat to domestic foci and are increasing (Thompson 2013; Chhabra and Muraleedharan 2016).

3.3. TRYPANOSOMA CRUZI

The parasite *Trypanosoma cruzi* (*T. cruzi*) causes the zoonotic infectious disorder known as Chagas disease (CD) (Ibarra-Cerdeña et al. 2020). *T. cruzi* is a vectorborne stercorarian trypanosome with considerable genetic variability that affects changes in host specificity in both the vector and a wide variety of wildlife hosts, causes the disease (Zingales et al. 2012). Triatomine bugs, which contract the disease through blood-feeding on an infected mammal, spread *T. cruzi*. The parasite's infectious stage is transmitted through the bug's feces, contaminating successive hosts' bite wounds or surrounding mucosal membranes. In addition, oral transmission in animals occurs when they consume infected insects (Barr 2009; Dorn et al. 2012; Rocha et al. 2013; Desquesnes 2017; Hodo et al. 2018).

Congenital transmission, blood transfusions, and organ transplants are additional methods of transmission. Raccoons, wood rats, opossums, skunks, armadillos, packrats are just a few of the mammalian species that have been discovered to be *T. cruzi*-infected and acting as disease reservoirs in the US (Paniz Mondolfi et al. 2020). The acute phase of disease typically lasts 8 to 10 weeks and manifests as either asymptomatic or mild flu-like symptoms (CDC 2007; Montgomery et al. 2016). The chronic indeterminate phase, which affects people with chronic CD, is marked by prolonged periods without symptoms that might last years or decades. Only 30% of those with CD are thought to progress to the determining phase and experience intriguing gastrointestinal and cardiac symptoms (Kruse et al. 2019). The condition is challenging to treat, and the few available medications are frequently hazardous (Keenan et al. 2013).

3.4. DIROFILARIA SPP

One of the new zoonotic parasite diseases, dirofilariasis is brought on by filarial worms of the genera Dirofilaria and Nochtiella, which unintentionally infect humans (the dead-end host). Canines are the primary reservoir hosts, and it naturally infects a variety of domestic and wild animals. They have a history of accidentally infecting people. The most common species found in India is *Dirofilaria repens* (*D. repens*). In order to obtain a blood meal, mosquitoes of the genera Mansonia, Armigeres, Anopheles, Culex, Aedes deposit hemolymph on the wound, which contains infectious "larvae 3" stage that enters the host's skin on their own. Coughing, an intolerance to strenuous activity, dyspnea, hemoptysis, cyanosis, ascites, epistaxis, and syncope are among the clinical symptoms (Vivekanandhan et al. 2019).

The zoonotic parasite *Dirofilaria repens*, which affects dogs and other animals, is spreading through vectors (Alsarraf et al. 2023). The zoonotic parasite *D. repens*, which affects dogs and other animals, is spreading through vectors. The prevalence of *Diroflaria immitis* has been extensively studied in wildlife

and reported frequently in a wide range of carnivorous species, including grey wolves, red foxes, raccoon dogs, golden jackals, wild cats, and domestic ferrets, in contrast to the relatively few studies on reservoir hosts of *D. repens* (Kido et al. 2011; Penezić et al. 2014; Hiedari et al. 2015; Moroni et al. 2020; Gomes-de-Sá et al. 2022; Villanueva-Saz et al. 2022).

3.5. CRYPTOSPORIDIUM SPP

One of the most intestinal protozoa known as Cryptosporidium spp. causes diarrhea in wild animals, domestic animals, and people (Khan et al. 2019). Wild mammals particularly rodents can accompany in human made habitats and poses a threat to the public's health because they serve as reservoirs for several zoonotic parasites, bacteria, viruses (Meerburg et al. 2009), including some species of Cryptosporidium (Zhao et al. 2010; García-Livia et al. 2020). Due to the parasite's wide range of hosts, it is less common in poultry and more prevalent in wild birds (Li et al. 2021). The three most common species of Cryptosporidium found in birds are *C. galli*, *C. meleagridis*, and *C. bailey* (Javed and Alkheraije 2023). Cryptosporidium is one of the numerous zoonotic infections that wild rats (*Rattus* spp.) carry (Zhao et al. 2019).

3.6. TOXOPLASMA SPP

The parasite *Toxoplasma gondii* (*T. gondii*) is one of the most common in the world. Wildlife is acknowledged as a significant *T. gondii* reservoir and source of infection (Trisciuoglio et al. 2015). Because they are the only source of oocysts, the parasite life stage that allows overall *T. gondii* transmission, wild felid animals and domestic animals are crucial to the epidemiology and ecology of *T. gondii* (Zhu et al. 2023). Because of their strong dispersal ability, wild birds are particularly significant intermediate hosts for *T. gondii* (Wilson et al. 2020). Migratory birds can transport infectious disease pathogens across oceans while flying (Sandström et al. 2013). Additionally, because herbivores consume intermediate hosts of *T. gondii* and wild birds' forage on the ground, both domestic and wild birds provide good sentinels for environmental contamination with *T. gondii* oocysts (Dubey et al. 2020; Iemmi et al. 2020). As a result, a wide range of wild bird species with various habitats and diets are susceptible to contracting this parasite (Wilson et al. 2020; Dubey et al. 2020). *T. gondii* prevalence rises with trophic level in the terrestrial environment, consistent with transmission of main cyst tissue, but it decreases with trophic level in the aquatic environment, reflecting a significant amount of watery exposure to oocysts (Wilson et al. 2020).

3.7. TOXOCARA SPP

Toxocara spp. nematodes are the primary cause of the global anthropozoonotic disease toxocariasis. The disease is reported to be highly prevalent in underdeveloped nations, particularly in areas with low hygienic conditions. (López-Osorio et al. 2020). The source of zoonotic parasitic nematodes is wild animals. More than 66% of samples of feces from boars, hares, deer, and fallow deer living in the territories of the Pozna Province had developmental forms of parasites from the genera *Trichostongylus* spp., *Capillaria* spp., *Toxocara* spp., *Eimeria* spp., and *Trichuris* spp. (Gałęcki et al. 2015).

Toxocara canis (*T. canis*) is a worldwide nematode parasite that uses domestic and wild canids as its primary hosts (Richards and Lewis 2001). Large number of unembryonated, non-invasive, *T. canis* eggs are excreted in canine feces (Glickman and Schantz 1981), and after a number of weeks, in the right environmental settings, these eggs can mature into an embryonated level that can infect paratenic and definitive hosts (Keegan and Holland 2013; Overgaauw and Nederland 1997). Avian species, pigs, rodents,

Table 1: Parasitic species involving wildlife.

Parasitic species	Disease	Pathogen class	Animal host	Major clinical symptoms	References
Trichinella spp.	Trichinellosis	Helminthes	Cats, pigs, dogs and other wild species	Gastrointestinal disorder (Vomiting, nausea, abdominal pain, diarrhea)	(Rahman et al. 2020)
Echinococcus granulosus and *Echinococcu smultilocularis*	Echinococcosis	Helminthes (Cestode)	Domestic and wild animals (Foxes, sheep, dogs)	Dysfunction of organs (lungs, liver, brain, kidney, spleen)	(Rees et al. 2021; Wen et al. 2019)
Taenia multiceps	Coenurosis	Cestode	Wild and domestic animals (foxes, jackals, dogs)	White fluid filled cyst in infected tissues	(Sikandar et al. 2018; Varcasia et al. 2022)
Strongyloides stercoralis	Strongyloidiasis	Nematode	Non human primates, cats, dogs, wild canids, rodents	Respiratory and gastrointestinal issues	(Eslahi et al. 2022; Unterköfer et al. 2022; Kusumarini et al. 2022)
Fasciola hepatica Fasciola gigantica	Fasciolosis	Trematode	Wild and domestic animals (camelids, monkeys, horses, donkeys)	Fever, hypereosinophilia, Obstructive symptoms (acute pancreatitis and cholecystitis)	(Rayulu and Sivajothi 2022; Levy et al. 2022; Webb and Cabada, 2018)
Toxoplasma gondii	Toxoplasmosis	Apicomplexan Protozoan	Domestic and Wild felines (lions, cheetahs, and leopards)	Asymptomatic infection in the immune competent host, Abortions in goats and sheeps	(Bokaba et al. 2022)
Leishmania infantum	Leishmaniosis	Intracellular protozoan	Rodents, lagomorphs, carnivores	Progressive weight loss, skin lesions, muscular atrophy, generalized lymphadenomegaly, Epistaxis, ocular lesions, onychogryphosis, diarrhea and vomiting	(Abbate et al. 2019; Edo et al. 2021)
Baylisascaris procyonis	Baylisascariasis	Roundworms	Wild and domesticated animals (rodents, foxes, dogs, woodchucks and primates)	Paralysis, death, blindness in intermediate hosts	(Pope et al. 2021; Sorvillo et al. 2002)

humans and many other hosts can serve as paratenic hosts (Strube et al. 2013). Although infective larvae can survive in host tissue for a long time and serve as a *T. canis* reservoir for canids, eggs ingested by paratenic hosts cannot mature into the adult stage (Parsons 1987). When a defensive host consumes prey that has been infected with stalled tissue larvae, the life cycle is complete (Brunaská et al. 1995; Krupińska et al. 2023).

3.8. GIARDIA SPP

There are eight recognized species of *Giardia*. These include *Giardia duodenalis* (*G. duodenalis*), which affects both animals and humans. *G. ardeae*, *G. agilis*, *G. muris*, *G. psittaci*, *G. microti*, *G. cricetidarum*, and *G. peramelis* which affect non-human hosts like rodents, marsupials, birds and amphibians. Eight assemblages (A-H) make up the species complex *G. duodenalis*, with assemblages A and B predominating in people (Ryan and Zahedi 2019). Giardiasis is a significant protozoan illness that affects both adult and children and causes diarrhea. It is widely spread around the world and is frequently transmitted by the fecal-oral route. *Giardia* spp. also infects wild and domestic birds, which can serve as asymptomatic mechanical carriers of Giardia cysts. There are six different species of Giardia. A complex species with several assemblages, *G. lamblia* (syn. *G. intestinalis*) is linked to human disorders via the assemblages A and B (Malik et al. 2021). Throughout the world, giardia infects a wide variety of animal hosts, including birds, reptiles, fish, amphibians and mammals, and causes asymptomatic or moderate to severe gastrointestinal sickness in its host species (Caccio` et al. 2018; Feng and Xiao 2011; Ryan and Caccio` 2013).

3.9. ANCYLOSTOMA SPP

Ancylostoma hookworm infections in wild species, cats, and dogs result in the zoonotic illness of Ancylostomiasis, frequently seen in tropical areas and Asia (Kladkempetch et al. 2020). One of the most significant soil-transmitted helminth parasites that affect a number of animal species, including humans, is the Ancylostoma species. The family Ancylostomatidae includes several species of Ancylostoma. The third-stage larvae of Ancylostoma species may penetrate the host's skin or infect them through the fecal-oral pathway (Palmer et al. 2007). Intestinal hypersensitivity and eosinophilic enteritis are symptoms of *Ancylostoma caninum* (*A. caninum*) infection. *Ancylostoma caninum* mostly affects dogs, with very little exposure to cats or people. Humans frequently get cutaneous larva migrans or enteric infections when they have eosinophilic enteritis (Daba et al. 2021).
In humans, follicular dermatitis is also typical (Colon and Patton 2012). In Asia, the small intestine is frequently inhabited by the parasitic worm *A. ceylanicum*, which may cause iron-deficient anemia in affected individuals. This is the first record of *A. ceylanicum* in wild canids; it was previously seen in domestic dogs in Australia. *A. ceylanicum* has been found in wild felids such as the civet (*Felis temminchii*), the leopard cat (*Felis bengalensis*), and the Asian golden cat (*Viverricula malaccensis*) (Smout et al. 2013). Table 1 highlights the parasites that may infect wildlife.

4. CONCLUSION

There are zoonotic parasites that are developing and reemerging that are acquired from wildlife sources but have thus far been ignored or are not thought to be of major consequence for human health. By educating the public about potential sources and the steps that may be taken to reduce the danger of human infection, the viewpoint has to shift. People who work with wildlife need to be aware of the risk of

illness transmission. Effective programs for identification, prevention, and control may be created and put into practice.

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Lyme Disease: Etiology, Transmission, Impact and Control

19

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ABSTRACT

Lyme disease is an infection caused by bacteria known as Borrelia burgdorferi sensu lato, is the most widespread vector-borne disease in the United States, and transmitted to human beings through the bite of Ixodes tick. However, significant portion (10-30%) individuals may experience ancillary non-specific symptoms that last months after the completion of therapy. The group of symptoms known as post-treatment Lyme disease syndrome (PTLDS), a form of the more general term "chronic Lyme disease," includes tiredness, cognitive impairment, and musculoskeletal discomfort. These symptoms are linked to disability and last longer than six months. Patients with nonspecific symptoms assumed to be caused by a supposed persistent Borrelia burgdorferi infection and may or may not exhibit signs of Lyme disease are referred to as having chronic Lyme disease. The diagnosis of PTLDS and chronic Lyme disease has become more and more important in immunologists' practices because it is difficult to diagnose. ELISA and western blot testing are useful in diagnosing Lyme disease. The cornerstone of care is still antibiotic therapy. This chapter reviews the evidence that supports current understanding of the life cycle, historical biogeography, and evolution of Ixodes spp., the ticks that carry the Lyme disease, as well as their methods of dispersal, and disease-transmission mechanisms, and the efficacy of vector control interventions.

Key words: Lyme disease, Borrelia burgdorferi, Tick-borne diseases, PTLDS, Lyme Arthritis

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1. INTRODUCTION

Lyme disease is a vector borne zoonotic disease that is usually caused by bacteria known as *Borrelia burgdorferi* sensu lato. A tick vector of the disease, *Ixodes scapularis*, spreads in different regions of North America and became a major cause of the upsurge of Lyme disease in the above mentioned region of America between 2004 and 2016 (Pitrak et al. 2022). The disease shows the symptoms like arthritis in the beginning of the infection. Later on, different kinds of rashes appear on the body and in some sever cases it associated with the different kinds of disorders involving the nervous and cardiac systems (Coburn et al. 2021). The first clinical manifestation of the Lyme disease was elaborated by Buchwald in 1883 and referred the formation of lesions as diffused idiopathic skin atrophy. Afterwards, the continuous study revealed many interesting facts about the Lyme disease such as the serum analysis of the patient affected with the disease had very high number of antibodies titers which supported the argument that the disease caused by a microorganism (Cardenas-de la Garza et al. 2019).

Lyme disease is a kind of arthritis. In its initial stages it causes skin rash, fever, fatigue, and headache. If it remains untreated for a long time, it penetrates to the joints, heart, and nervous system as well. Lyme disease is a multisystem zoonotic bacterial vector borne disease caused by *Borrelia burgdorferi* sensu lato that is transferred due to the bite of infected *Ixodes pacificus* or *Ixodes scapularis* ticks. *Borrelia Burgdorferi* supported in nature in enzootic cycle containing numerous vertebrate species; humans are accidentally infected when manifested by infected ticks in highly forested areas. Almost 30,000 outlined cases and approximately 300000 cases occurring yearly. *Borrelia burgdorferi* is frequently outlined vector borne disease in the United States of America. It constitutes about 62.6 percent of increasing outlined vector borne disease cases. Most cases of Lyme disease arise in Northeastern and North Central States where number of endemic and highly prevalent countries is continue to inflate (Bisanzio et al. 2020).

Lyme disease was first identified in 1976 when a bunch of juvenile arthritis cases was identified. Many of these patients also have cutaneous skin lesions that are identical to those of outlined in Europe that were formerly related with tick bites. There was a strong impression that infectious agent was fundamental cause of both of the cases in old Lyme, CT and also in Europe. But later in 1982 the spirochete presented in Ixodes ticks was recommended to be the cause. The part of this bacterium as infectious agent named *Borrelia burgdorferi* as the causative agent of Lyme disease was rapidly developed as the bacterium was retrieved from both patients and as well as from reservoir host, such as in white footed mice. While it was first identified because of its relation with arthritis, it became obvious that arthritis was a later stage indication and that acute infection was personified with a distinctive erythema migrans rash, and in some cases with the involvement of cardiac and neurological system (Coburn et al. 2021).

2. HISTORY

The Midwest focus for Lyme disease historically focused on forested areas of Wisconsin and Minnesota, which present habitats that are extremely worthy for *Ixodes scapularis* populations and *B. burgdorferi* hosts in both regions and where first Midwestern cases of Lyme disease were stated (Gardner et al. 2020).

Lyme disease appears in North America, Europe, and Asia. However; the majority of cases occur in certain endemic locations. It is the most prevalent vector-borne illness in Europe and North America. Vector tick species differ substantially according to their geographic location. The most common vector in the United States is *Ixodes scapularis*. *I. pacificus* is without a doubt the most significant vector in the western United States. From 1993 to 2013, the prevalence in the United States nearly tripled. The geographic region has expanded in tandem with the rise in incidence. Inadequately discussed Lyme

disease is a major problem, and it is predicted that the real frequency is eight times higher than that reported (Cardenas-de la Garza et al. 2019).

3. POST-TREATMENT LYME DISEASE SYNDROME

Even after receiving correct therapy, some persons (5% to 15%) may endure persistent weariness, achiness, or headaches. This is referred to as post-treatment Lyme disease syndrome (PTLDS). The symptoms do not indicate that you are still infected. Additional antibiotics are unlikely to help PTLDS. The majority of persons in this category experience symptoms that subside during the next six months after the diagnosis of Lyme disease (Doshi 2022).

4. CHRONIC LYME DISEASE

Chronic Lyme disease is a phrase that is used to describe a situation in which a person has Lyme disease as well as the symptoms of PTLDS. Some individuals believe that chronic Lyme disease and PTLDS is the same thing. However, some persons are diagnosed with chronic Lyme disease without first being diagnosed with Lyme disease. This word has been used to describe symptoms in individual who have neither diagnostic nor clinical evidance of previous infection with *B. burgdorferi* because some individuals believe they get Lyme disease without being bitten by a tick. There is insufficient evidence that mosquitoes may spread Lyme disease. Many specialists disagree with the use of phrase "chronic Lyme disease" due to the ambiguity and lack of well-established clinical description (Lantos et al. 2021).

5. TICK PHYSIOLOGY

From the perspective of the vector, physiological variations across tick species are likely to produce an effect on vector ability. Ixodes species are three-host ticks in common and biotic and abiotic conditions dictate wherever they may thrive. On-host feeding periods are brief in comparison to off-host periods, which include genetically designed diapause or quiescence due to adverse environment (Waldman et al. 2023). Temperature, humidity and day length are all abiotic elements that influence tick physiology, influencing host seeking phenology, egg hatching success, and immature and adult overwintering. Hot circumstances during off-host seasons might cause cold and overheating in chilled temperatures or dryness in dry environments. The circumstances necessitate ticks seeking shelter for cooling or rehydration. The suitable habitats with sufficient plant or leaf trash layer are important in the tick's life cycle (Udobi 2023).

The seasonal synchronization of nymphal and larval feeding patterns was found in Ixodes scapularis populations in the Midwestern United States. Investigations into the infection pattern of Borrelia burgdorferi sensu lato indicated that lineages carrying 16S-23S rRNA intergenic spacer restriction fragment lead to lengthen the polymorphism sequence of type 1 which is more commonly identified in Northeastern Ixodes populations (Paulauskas 2023).

6. TICK IMMUNITY

The idea that tick immunity contributes to varying vector competence seems appealing. The intricacy of the tick's immune system has been better understood in recent years (Margos et al. 2022). The presence of immune effectors and modulators in Ixodes species has been demonstrated. These include recognition molecules, such as thioester-containing proteins (T-TEPS that act as lectins labeling cells for immune assault, defensins, phagocytotic hemocytes, lysozymes, the antimicrobial peptides, a dityrosine

network, and signaling pathways. Toll, Immunodeficiency, and JAK-STAT are three of the signaling pathways that control the immune system. There is also an indirect, cross-species signaling pathway that detects the cytokine interferon gamma in the blood of the vertebrate host (Sri-In et al. 2023). Therefore, it is quite likely that immune effectors are crucial in deciding whether an Ixodes species is capable of attacking a Borrelia species and vice versa.

7. MICROBIOME OF TICKS

Tick's micro biome has received a lot of research in the last 10 years with increased focus. These researches have demonstrated that the gut microbiome, endosymbiontic bacteria, and microorganisms connected to the tick's exterior surface make up the tick microbiome (Wiesinger et al. 2023). In the majority of research, bacterial taxa including Spiroplasma, Coxiella, Lariskella, Midichloria, Francisella, Wolbachia, Francisella, Arsenophonus, and Rickettsia that are recognized tick symbionts were discovered. This demonstrated that Rickettsia and *B. burgdorferi* sensu lato were the dominant bacteria in the gut microbiome of *I. scapularis* in the majority of adult patients. Only a small percentage of tick samples had very diverse microbiomes, with bacteria from the families Enterobacteriaceae and the genera Bacillus and Pseudomonas present in their midguts (Remmal et al. 2023).
In areas where these species coexist in sympatry, the hybrid progeny of numerous tick vector species such as *Ixodes persulcatus and Ixodes ricinus* may present a chance for vector switching. The hybrid ticks inherit genes from both parents, and immune system or mid gut receptor molecules may phenotypically resemble both parents. The hybrid ticks might assist in the survival and adaptation of an invasive Borrelia species to a new vector (Rana et al. 2022).

8. SIGN AND SYMPTOMS

Clinically, Lyme disease frequently presents with a variety of dermatological or viral-like signs and symptoms during the acute phase including intermittent fever, chills, malaise, sweats, fatigue, and achiness which can lead to neurologic, joint, and cardiac involvement in advanced stages of the infection as the causing bacteria disperse homogenously. In addition to these physical indicators persistent and recurring symptoms such as tiredness, arthralgia, myalgia, sleep disturbance, and headache are common throughout the late stages of untreated Lyme disease and are responsible for the majority of the patient complaints. Patients with intermittent attacks of late Lyme arthritis are more likely to experience similar symptoms in the interim. Symptoms without any physical, laboratory, or other objective evidence are sometimes the main or only indication of untreated Lyme disease. The use of direct tests such as polymerase chain reaction (PCR), culture test, or antigen detection for *B. burgdorferi* to assist doctors in diagnosis is fairly restricted because *B. burgdorferi* cannot be cultivated in non-research materials. A two-tier antibody test is available internationally and can be useful despite its severe sensitivity limits, particularly in the early stages of illness and following antibiotic treatment of primary Lyme disease (Rebman and Aucott 2020).

9. LYME DISEASE ETIOLOGY

Borrelia burgdorferi is a member of the phylum spirochetes and the class spirochete. Many families in this class include spirochaetaceae and leptospiraceae. Other human bacteria in this family include Leptospira interrogans, *Treponema pallidum* and *Borrelia recurrentis* are responsible for producing leptospirosis, relapsing fever borreliosis, and syphilis. *B. burgdorferi* is a gram-negative bacterium which is 20 to 30µ in length and 0.2 to 0.3µ in breadth. This can be grown in Barbour-stoenner-kely media however; it is only

rarely retrieved from human samples (Chuma et al. 2020). *B. burgdorferi* is a diverse group of 18 bacterial species, just three of which are primarily related with Lyme disease: *B. burgdorferi sensu stricto, B. garinii,* and *B. afzelli*. In addition to this, six additional species, including *B. spielmanii, B.valasiana,* and *B.bissettii*, are human pathogenic and have been implicated in human illnesses (Barriales San Miguel 2021).

The specific clinical spectrum of many Borrelia species is yet unknown. Different species differ in their vectors and reservoirs, and they also differ in their geographical distribution. *Borellia burgdorferi* sensu stricto is the most common species in North America. The enzootic cycle is quite complex (Bernard et al. 2020).

The ticks of *Ixodes ricinus* are the primary vectors of Lyme disease. In America, *I. scapularis* and *I. pacificus* predominate whereas *I. ricinus* and *I. persulcatus* predominate in Eurasia (Wodecka and Kolomiiets 2023). Many animals play a part as intermediate reservoir, including white footed mouse known as *Peromyscus leucopus* is considered to be dominant. A number of species of small mammals like rats, shrews, squirrels as well as birds are additional reservoirs that could aid in the dissemination of disease. The ticks have a life cycle of three stages that obtained the bacteria by nourishing on infected reservoir. Earlier cases reports tend to suggest that ticks are responsible for transferring the spirochete to humans principally during nymph stage. Despite the fact that deer are inadequate hosts for Borrelia species, but they play an important part in Lyme disease because they are principal feeding hosts for Ixodes ticks (Cardenas-de la Garza et al. 2019).

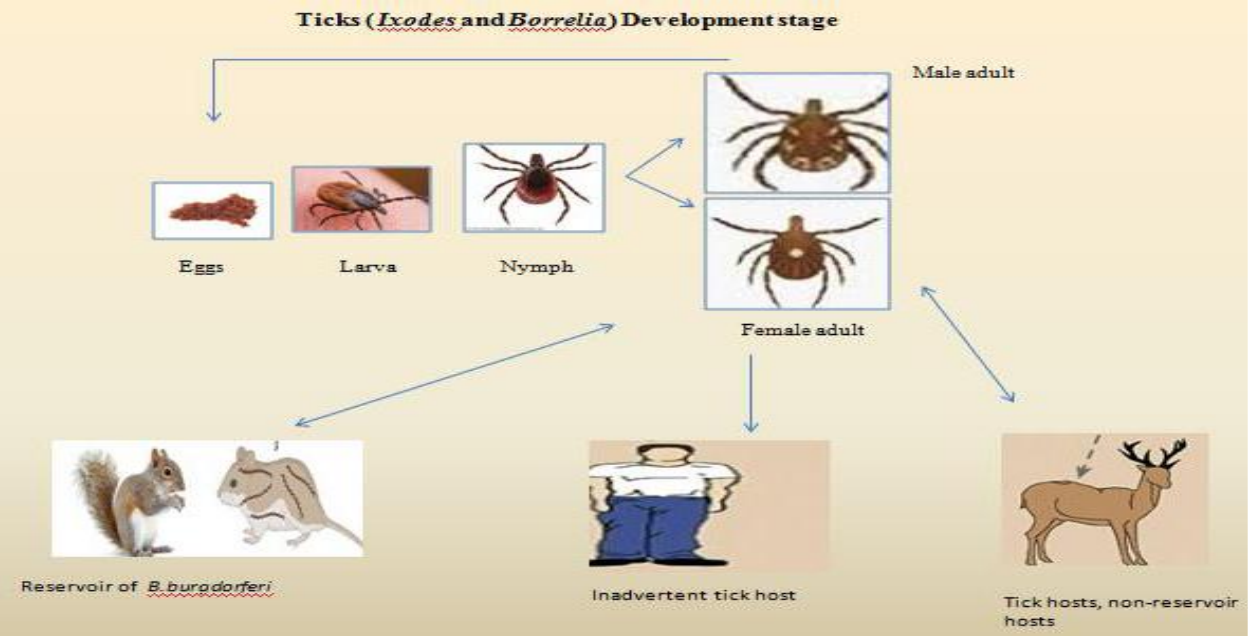

Fig. 1: Transmission of *Borrelia burgdorferi*

10. TRANSMISSION OF LYME DISEASE

Ixodes ticks transmit a broad range of infections, containing Lyme disease to animals as well as human (Fig. 1). Lyme disease is an important tick-borne illness that promotes extensive distribution and caused by group of spirochetes related to *Borrelia burgdorferi* sensu lato complex. Latterly new virulent strains of Borrelia pathogens have been discovered, containing new clinical isolates *Borrelia burgdorferi* sensu lato, *B. bissettii, B. miyamotoi* and *B.mayoni* are related with severe human disorders and are also

transmitted by Ixodes ticks. Globally, the Borrelia species that are frequently related with Lyme disease include *B. burgdorferi* sensu stricto which is wide spread throughout the US and Europe, and *B. garinii* and *B.afzelii* which are dispersed throughout Eurasia (Rollins et al. 2023). In Europe and North America, Lyme disease spirochetes commonly are supported in nature by a complex tick rodent infection cycle. Humans and household animals that acquire Lyme disease are secondary hosts and are not involved in natural transmission cycle (Pal et al. 2021).

11. THE SPIROCHETE

The spirochete host interfaces the outermost membrane like all other spirochetes. *B. burgdorferi* is a diderm comprising an outer membrane surrounding the periplasmic space, the cytoplasmic membrane, the peptidoglycan, and the protoplasmic cylinder. The organs for motility are flagella contained completely in the periplasmic compartment. Additionally to propagating a planer wave that allows spirochete to invade collagen matrices as well as in connective tissues and endothelial junctions, the flagella also provide a cytoskeletal function.
B. burgdorferi containing the host pathogen interface in all milieus by which the spirochete transported or in which spirochete takes up final residence; it is not astonishing because this structure are of great importance from past. Because of its double membrane structure *B. burgdorferi* frequently referred to Gram negative bacteria (Radolf et al. 2021).

12. THE *B. BURGDORFERI* LIFE CYCLE IN TICKS

B. burgdorferi persists throughout the mammalian infection life cycle of ticks. In the absence of vertical transmission, the pathogen needs to be obtained during one of its vector's life stages, namely during a downpour on infected creatures, primarily wild rodents. After attachment of tick to the host, the arthropod tends to prepare itself for its blood meal ingestion during first 12 hours. The transfer of the spirochete to the vector has already started but transmission of *B. burgdorferi* between vertebrate and arthropod host remains unclear. The transfer of spirochete occurs either by active chemotactic dispersion or by passive transfer together with host fluid. During possession, spirochetes enter the tick's stomach from the dermis and fluid of the infected host and are likely to continue migrating until the tick becomes fully engorged, which normally occurs in 72-96 hours. *B. burgdorferi* becomes informally associated with the mid gut tissues during 48 hours of feeding which remains in the gut throughout the whole life of arthropod (Narasimhan et al. 2022). When the tick consumes a successive blood meal, the spirochete tends to increase in the gut and at this time, an unspecified fraction of B. burgdorferi population present in the gut that penetrates the hemocoel and then disperse towards the salivary gland, and it is transferred by the salivary stream to new mammalian host (Pal et al. 2021).

13. RISK FACTORS

Almost all states in the United States have recorded instances, despite the fact that there is a spatial risk within endemic areas. But in 2012, 13 states—Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Vermont, Virginia, and Wisconsin—reported 95% of all instances. The risk of infection is highest in the late spring and summer because *B. burgdorferi*-transmitting ticks are vulnerable to desiccation and require continual high relative humidity.

Ticks can be brought into homes by pets and onto lawns by animals. Ticks typically live in overgrown brush and the border regions between lawns and woodlands (Merryweather 2023).

14. CLINICAL MANIFESTATIONS

The clinical manifestations of Lyme disease are classified into three stages to make easier for the diagnosis.

1. Early localized stage: This is the first stage, which is distinguished by erythema migrans that appear at tick bite site. It happens within the first several weeks following vaccination.
2. Early disseminated stage: This is the second stage, which appears weeks to months after tick contact and is characterized by many erythema migrans lesions, Lyme neuroborreliosis, carditis, borrelial lymphocytoma, and in rare cases Lyme arthritis.
3. Late disseminated stage: This is the third stage, which is distinguished by acordermatitis chronica atrophicans, Lyme arthritis, and Lyme neurological symptoms (Cardenas-de la Garza et al. 2019).

These warning signs and symptoms might be present in different geographic areas and in different animals.

14.1. DERMATOLOGIC MANIFESTATIONS

The most prevalent dermatologic symptom of Lyme disease is erythema migrans, which Azfelius first described as erythema chronicum migrans. During the first three weeks after vaccination, it is prevalent in 70–95 percent of the infected patients. Children experience it more frequently than adults do. It appears as a circular or oval, red to bluish-red area with centrifugal growth and the possibility of center clearing. The head and neck, as well as the extremities or pelvic area, are the most often impacted areas in both adults and children. Sometimes, a burning feeling or pruritus is mentioned. Erythema migrans may be accompanied by broader symptoms such fever, lymphadenopathy, and malaise. The lesions might last for weeks or months if untreated. Rarely, individuals will have many erythema migrans lesions at this stage (Strle and Wormser 2022).

14.2. LYME ARTHRITIS

The second most typical clinical manifestation in patients previously termed antibiotic refractory Lyme arthritis in them. is Lyme arthritis. Untreated EM patients develop arthritis in over 60% of cases within 6 months. Transient or recurring bouts of asymmetrical, monoarticular, or oligoarticular arthritis can occur. The knee joint is most frequently impacted. Periarticular involvement is common, and each episode typically affects fewer than five joints with synovitis. Typically, the pain is low to moderate while the joint swelling is considerable. While inflammatory indicators are often high and serum white blood cell (WBC) count typically falls within the average range (Arvikar and Steere 2022).

White blood cell (WBC) counts in synovial fluids vary from range 10,000 to 25,000 cells/mm3. Treatment is necessary if anti-Borrelia antibodies are present or prior history of erythema migrans. Even though most cases of arthritis respond effectively to antimicrobial treatment and Nonsteroidal anti-inflammatory drugs (NSAIDs). Treatment for these complex instances may be comparable to that given for other forms of chronic inflammatory arthritis, such as methotrexate and hydroxychloroquine (Cardenas-de la Garza et al. 2019).

14.3. LYME CARDITIS

Lyme carditis is an uncommon occurrence with an estimated frequency of 4-10% in United States and 0.3-4% in Europe in individuals who are untreated. However, more recent statistics indicate that the incidence may be as low as 1% in the United States perhaps as a result of earlier diagnosis and rapid treatment of Lyme disease. The existence of *B. burgdorferi* sensu lato genospecies in Europe which are less cardiotropic such as *B. garinii* and *B. afzelii* opposed to *B. burgdorferi* are the most prevalent species in United States. Due to the behavior of the vector, reservoir, and host chances of Lyme carditis to take place are more commonly ranges from June to December. After a tick bite or the development of erythema migrans, symptoms might appear anywhere from a few days and seven months later. However, cutaneous (erythema chronicum migrans), joint (arthritis), or neurologic (neuroborreliosis) signs are more frequently present in addition to cardiac involvement (Radesich et al. 2022).

14.4. LYME NEUROBORRELIOSIS

The early disseminated stage of *B. burgborferi* infection is often when the neurologic symptoms first appear. The three main ways that Lyme borreliosis damages the neurological system are mononuclear cell meningitis, cranial neuropathies, and radiculoneuropathies, the latter of which is a catch-all term for painful radiculopathies and unifocal or multifocal peripheral nerve involvement. Much has been discovered about the interplay between the pathogenic infection and the brain system, and diagnostic methods have been greatly improved, including enhanced peripheral blood and CSF on the basis of diagnosis of serum (Halperin et al. 2022). Clinical manifestations of Lyme disease have been enlisted in Table 1.

15. DIAGNOSIS OF LYME DISEASE

Erythema migrans might be used to make a clinical diagnosis of Lyme disease, as can a combination of clinical symptoms and serologic tests. Patients may also experience non-specific manifestations like pain in muscles, headache, and weakness. Infected persons have clinical findings of Lyme disease like arthritis, erythema migrans, heart block, or cranial nerve paralysis. In the USA, there is a significant prevalence of non-specific subjective symptoms, with up to 40% of persons experiencing chronic pain and 10% to 15% of people expressing exhaustion (Wong et al. 2022). Although patients with Lyme disease frequently have vague symptoms, many patients also simultaneously display objective Lyme disease symptoms. Serologic test results for Lyme disease in people with subordinate pretest likelihood of infection show weak positive predictive value (Kobayashi and Auwaerter 2022).

The Centers for Prevention and Control of Diseases advise using a two-step test. Either an immunofluorescence test (IFA) or an enzyme immunoassay (EIA) should be carried out first.

- If IFA test is positive, Western blot analysis should be performed during which patients with symptoms less than 30 days will be supposed to check for IgG and IgM and those with symptoms more than 30 days will be supposed to test for IgG isotype. Assays for antibodies are often negative in the first few weeks following infection.
- Histopathology is frequently used to rule out other comparable disorders and may aid in the diagnosis.
- Additionally, biopsy analysis by PCR or Borrelia culture may be used to verify the diagnosis although the sensitivity of these tests varies greatly.

Table 1: Clinical manifestations of Lyme disease (Cardenas-de la Garza et al. 2019; Miller and Aucott 2021)

Clinical manifestations	Signs and symptoms
Dermatologic manifestations	• Erythema migrans • Borrelial lymphocytoma • Lymphoma • Lichen sclerosus • Parry–Romberg syndrome • Acrodermatitis chronica atrophicans
Lyme Arthritis in them.	• Asymmetrical, monoarticular, or oligoarticular arthritis in them. • Swelling of joint • Pain in joint • Elevated level of inflammatory markers
Lyme Carditis	• Atrioventricular (AV) block • Angina pectoris • Acute heart failure • Palpitations • Dyspnea • Infrequently pericarditis and myocarditis
Lyme Neuroborreliosis	• Lymphocytic meningitis • Encephalitis • Bannwarth's syndrome • Myelitis • Intracerebral hemorrhage • Sleep disturbances • Headache • Subarachnoid hemorrhage • Fatigue or stroke • Paresthesia

 Serologic testing should only be carried out in accredited labs that adhere to the Centers for Disease Control and Prevention's (CDC) guidelines for immunoblot interpretation in order to prevent the misdiagnosis of Lyme disease.
 The diagnosis may be aided by histopathological examination, PCR, or tissue culture; however high-titer tests are frequently enough (Simon et al. 2022).

16. PREVENTION AND TREATMENT OF LYME DISEASE

Depending on the clinical presentation, there are several approaches to Lyme disease prevention and therapy. The cornerstone of care is still antibiotic therapy, however there are few reliable data indicating the most efficient and economical antibiotic, dosage, route, and duration.
To avoid Lyme disease, it is recommended that tick bites be avoided. The use of protective clothes, inspect skin and cloth frequently. The use of tick and bug repellent, and the timely removal of attached ticks are all recommended. The antibiotics are recommended after tick bite. New and current antibiotics with potential against bacterial cells from diverse bacterial genera including Staphylococcus and E. coli have been identified to either directly kill or reactivate persister cells. Most research involving antibiotics and small molecule medications for LD and PTLD employ innovative synthetic pharmaceuticals or homeopathic extracts that have not yet obtained FDA clearance, or they use pre-approved drugs that

have been repurposed alone or in conjunction with existing antibiotics (Adkison and Embers 2023). The recommended dosage of various drugs in different ages has been enlisted in Table 2.

Table 2: Recommended oral dosage of Lyme disease (Nguyen et al. 2022)

Age	Recommended dosage
Pediatric	4 mg/daily in 2 divided doses Doxycycline + 30 mg/kg in 2 divided doses Cefuroxime + 50 mg/kg in 3 divided doses Amoxicillin
8 years children	4 mg/kg Doxycycline
Adults	Single 200 mg Doxycycline

Lyme meningitis is a rare but devastating clinical symptom of Lyme disease. The first-line therapy for Lyme meningitis was intravenous ceftriaxone, but it is linked with a high prevalence of complications. Although studies on oral doxycycline's efficacy and effectiveness (or real-world evidence) are sparse, practice recommendations have recently been modified to prescribe either oral doxycycline or ceftriaxone as first-line therapies for Lyme meningitis (Nigrovic et al. 2023). Borrelial lympochytoma, erythema migrans, and cranial nerve paralysis should be treated with 14-day antibiotic treatment orally whereas ACA should be treated with a 21-day regimen. Lyme arthritis without involvement of the neurological system treated with a 28-day course of oral antibiotics.

17. CONCLUSION

Estimation of persistent or chronic Lyme disease in an immunology may be complex. The tiredness and anxiety are unexplained symptoms which are common in the broad-spectrum population but definite relationship between these symptoms and Lyme disease has not been established. Furthermore, no clear standard for the diagnosis of chronic Lyme disease has been established. The individuals with Lyme disease have arrived with a wide range of symptoms and histories. There is scant evidence that *B. burgdorferi* cause symptoms or result in a down regulated immune response. At this time, no conclusions about the etiology and pathophysiology of these individuals' ongoing symptoms can be evaluated till now. There is also no evidence about the further use of antibiotic treatment in individuals report with chronic Lyme disease symptoms

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Main Causes and Control of Cyclozoonosis in Humans

20

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ABSTRACT

One of the most severe threats to worldwide public health is Cyclozoonosis, a complicated set of illnesses that are spread from animals to humans. In order to lessen the impact of cyclozoonotic infections on human health, this chapter presents a thorough investigation of the main factors causing these diseases. It also suggests practical control techniques. Exploring the complex relationships between zoonotic organisms and their animal hosts, the chapter explores the various factors that contribute to Cyclozoonosis. In order to provide insight into the various ways that these infections manifest in human populations, it looks at how environmental factors, behavioral patterns, and biological mechanisms contribute to the spread of these diseases. Using a variety of data from case studies and in-depth research, the chapter emphasizes how important different animal reservoirs are to the dynamics of cyclozoonotic pathogen transmission. It emphasizes how critical it is to increase monitoring and learn more about the interactions between these hosts and the viruses they contain. The chapter also examines efficient control techniques, stressing the value of integrated methods. In order to reduce the prevalence of Cyclozoonosis, it promotes preventive measures like vaccination campaigns, better hygiene standards, and focused measures in animal populations. It also emphasizes how important it is to support behavioral changes through education in order to reduce the chance of contracting these illnesses. Policymakers, researchers, and public health professionals will find this chapter to be a useful resource as it synthesizes current knowledge and offers workable recommendations. With a view to protecting human health, it provides a framework for all-encompassing control and prevention methods that will inform and direct future efforts in the fight against cyclozoonotic diseases.

Keywords: Pathogens, Hosts, Echinococcosis, Symptoms, Virus, Respiratory.

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1. INTRODUCTION

1.1. CYCLOZOONOSIS

The term "Cyclozoonosis" describes the spread of infectious diseases that affect humans and animals. These diseases spread through pathogens which include bacteria, viruses, and parasites. Without necessarily including humans in the transmission process, few diseases include a cycle of transfer between different animal hosts. When one animal species acts as a carrier for a certain infection, which can subsequently be transmitted to another animal species, Cyclozoonosis may occur (Chomel 2009). There are some Cyclozoonotic diseases in human as mentioned in Fig. 1.

1.2. MAIN CAUSES AND CONTROL OF CYCLOZOONOTIC DISEASES IN HUMAN

Cyclozoonosis in humans and their control might differ depending on the pathogen involved. These cyclozoonotic diseases caused by different pathogens, which include bacteria, viruses, and parasites that can affect both humans and animals (Blancou et al. 2005). There are the main causes and control measures of different cyclozoonotic diseases.

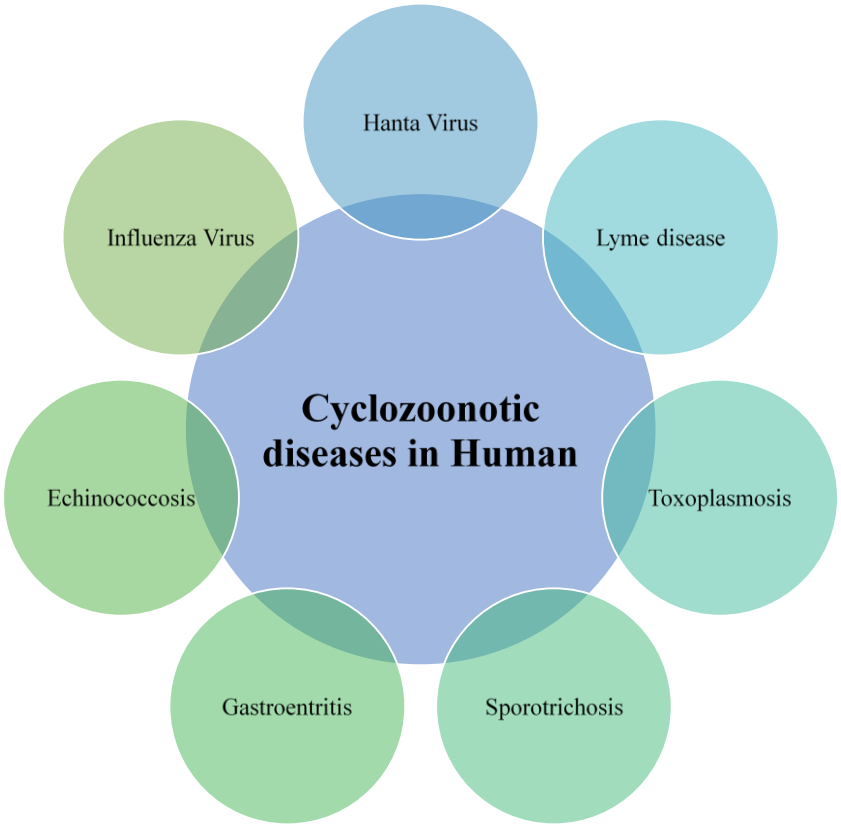

Fig. 1: Cyclozoonotic diseases in Human.

2. ECHINOCOCCOSIS

A significant helminthic zoonotic disease that affects both humans and numerous animal species is echinococcosis (Pal and Purohit 2005; Pal 2007). Adult tapeworms are carried sub clinically by hosts such as hyenas, dogs, wolves and wild predators. Dogs play a significant role in zoonotic transmission because

of their closeness to humans. Worldwide, there could be 2–3 million human cases, according to estimates (Pal and Boru 2012). A cestode is Echinococcus granulosus, also referred to as the dog tapeworm or hydatid worm. It is responsible for cystic echinococcosis, commonly known as hydatid disease. (Khuroo and Mohammad 2002). Water contamination is a rare cause of infection (McManus and Thompson 2003). Fig. 2 shows the main causes of echinococcosis.

2.1. CAUSES

Several species of Echinococcus, small cestode parasites in the Taeniidae family, are responsible for disease. Echinococcus vogeli, Echinococcus granulosus sensu lato, Echinococcus oligarthrus, Echinococcus multilocularis, and probably Echinococcus felidi and Echinococcus shiquicusare among the currently known species (Pal 2007; CFSPH 2011). Alveolar echinococcosis-causing E. multilocularis is less frequently exposed to humans. Poly cystic echinococcosis is caused by the unusual species E. oligarthrus and E. vogeli.

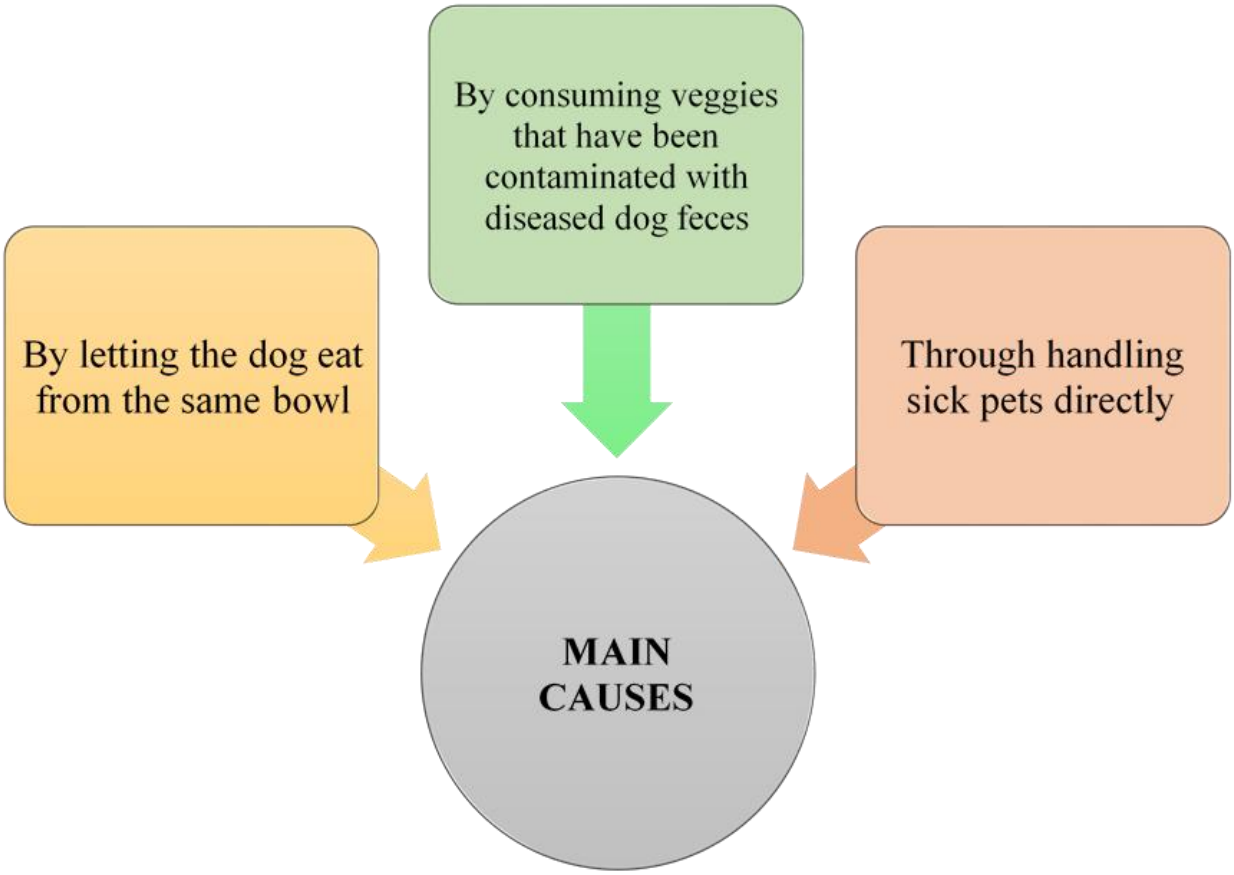

Fig. 2: Main causes of Echinococcosis.

Humans become an incidental host for cystic echinococcosis and usually get affected by handling an infected dog. The most frequently affected organs are the liver and lungs. Younger people appear to have a higher prevalence of pulmonary illness. It is passed on by larvae, the tapeworm Echinococcus' metacestode. The eggs may be consumed in meals like fruits, vegetables, herbs or with contaminated water (Pal 2007).

When a person handles a wild animal, pets an infected dog or cat, or comes in contact with contaminated vegetation and soil, the parasite may also stick to their hands (CFSPH 2011). Infected dogs excrete eggs, and humans become infected by fecal or oral contact, especially during jocular and close contact between dogs and children. Eggs cling to the hairs around a diseased dog's anus, as well as on face and paws. Infection to humans can also occur by the indirect egg transmission, such as via arthropods, contaminated water and uncooked food (Moro and Schantz, 2009).

2.2. CONTROL

The control of disease can undoubtedly be aided by preventive measures such as

- ✓ Avoiding contact with dog or fox feces
- ✓ Reducing dog or fox populations
- ✓ Hand washing
- ✓ Improved sanitation
- ✓ Incinerating infected organs
- ✓ Treating dogs with praziquantel-impregnated baits, arecoline Hydro bromide and using praziquantel
- ✓ Promoting good health (Pal 2007; Pal and Boru 2012).
- ✓ The carcasses from livestock at the slaughterhouse should not be fed to the dogs. Cats and dogs should not be permitted to kill wild animals or be given any tissues from these species since they are susceptible to parasites from wildlife cycles. Animals that go outside frequently in endemic areas should be tested and/or treated (CFSPH 2011).

Table 1: Control of types of echinococcosis:

Cystic echinococcosis	Alveolar echinococcosis
Cystic echinococcosis can be controlled by reducing the spread of parasites. Two preventive steps include: 1. Limiting the locations where dogs are permitted and 2. Banning animals from consuming meat that has been contaminated with cysts (Budke et al. 2017).	By avoiding contact with wild animals like foxes, wolves, and dogs and their feces as well as by restricting contacts between dogs and rodent populations, alveolar echinococcosis can be avoided (Budke et al. 2017).
➢ Control stray dog numbers and stop dogs from eating contaminated sheep carcasses.	➢ Do not let dogs consume rodents or other animals that roam for food.
➢ Limit the slaughter of sheep and other animals at home.	➢ Avoid interacting with stray dogs, coyotes, and other wild animals.
➢ Don't drink or eat anything that might have been contaminated by dog feces.	➢ Shouldn't pet or bring wild animals into your yard.
➢ Wash your hands with warm water and soap: ✓ After dealing with pets and ✓ Before eating food.	➢ After touching dogs or cats and before eating and cooking food, wash your hands with: ✓ soap and ✓ Warm water.
➢ Teach children the importance of washing their hands to prevent disease.	➢ Teach Kids the necessity of cleaning their hands frequently to avoid the illness.

3. GASTROENTRITIS

Cyclozoonotic diseases can be caused through certain microorganisms. For instance, Salmonella species can be spread directly from animals, especially livestock, to people via infected food. Another illustration

is the Campylobacter bacteria, which are frequently, found in poultry and can cause gastroenteritis in people (Cohen 2016).

3.1. CAUSES

The common disease known as gastroenteritis causes the vomiting and diarrhea. Bacterial, viral or tummy bug causes the gastroenteritis disease. Viral gastroenteritis can be caused by many different viruses (Moore et al. 2015).

Norovirus is one of the most typical causes of viral gastroenteritis. The most typical cause of viral gastroenteritis is norovirus. People of all ages contract illnesses from norovirus (the 'winter vomiting bug'). Astrovirus, adenovirus and rotavirus typically harm infants and young children, while they can also infect adults.

Viral gastroenteritis can occur at any time of the year. The most frequent times of year for rotavirus, astravirus and norovirus illnesses are the winter months (Gelaw et al. 2019). Diarrheal illnesses are frequently caused by Salmonella and Campylobacter germs.

Under cooked poultry is the most common way to get both diseases, while unpasteurized milk can also be a source. Dogs or cats with diarrhea can infrequently transmit Campylobacter. Consuming uncooked eggs and coming into contact with reptiles, birds, or amphibians are two ways that salmonella can be spread (Bányai et al. 2018).

Although Foodborne epidemics do happen, Shigella species are another typical bacterial cause of diarrhea. They are typically spread from person to person. Cryptosporidium bacterial toxins are just a few examples of the many things that can cause gastroenteritis. The bacteria do not directly cause disease, but they can contaminate food with their toxic metabolites. Some staphylococcal bacterial strains create toxins that can result in gastroenteritis.

The most frequent cause of bacterial enteritis in humans is Foodborne campylobacteriosis. The two main Campylobacter species that cause intestinal disease are Campylobacter jejuni and Campylobacter coli, but numerous additional species, including rare ones, are also responsible. The growing international movement of goods, cattle, and people has led to the emergence of zoonotic infections in humans, including these unusual Campylobacter species (Desselberger and Gray 2009). Because patients may experience discomfort in the right lower quadrant, Yersinia enterocolitica can produce gastroenteritis or a condition that mimics appendicitis. Under cooked pork, unpasteurized milk, or tainted water are the main carriers of the disease.

Consumption of raw seafood, certain Vibrio species (such as V. parahaemolyticus) might result in diarrhea. In areas where people lack access to clean drinking water and sanitary methods of disposing of human waste, V. cholerae can occasionally produce severe dehydrating diarrhea. This is a particular issue following natural disasters or in refugee camps.

Rarely can Listeria lead to Foodborne gastroenteritis, but it more frequently leads to bloodstream infection or meningitis in elderly persons, pregnant women, and newborn.

Swim in contaminated fresh or brackish water or consume contaminated water to get Aeromonas. Patients who have consumed raw shellfish or visited tropical low-resource areas are at risk of developing diarrhea brought on by Plesiomonas shigelloides (Wilhelmi et al. 2003).

3.2. CONTROL

The simplest daily hand washing with soap and water is still the most efficient way to stop the spread of the cyclozoonotic disease. Since gastroenteritis is highly communicable, the following general precautions can be performed to lower the infection risk:

When you are ill, stay home until 48 hours after your symptoms have subsided. See your doctor if your symptoms don't go away.
There are two live-attenuated oral rotavirus vaccines on the market that are secure and efficient against the vast majority of disease-causing strains. The recommended baby vaccination schedule includes rotavirus vaccine (Glass et al. 2001).
Thoroughly clean hands with water and soap:
After using the restroom or changing a baby, smoking, using a tissue
After handling pet animals, thoroughly wash hands with water and soap.
Disposable paper towels should be used to dry your hands rather than cloth towels because bacteria might remain on surfaces for a while.
Use different tools (tongs, knives, and cutting boards) to handle raw and cooked meals unless they have been well cleaned between uses. Keep all kitchen appliances and surfaces spotless.
Cleanse hands completely with water and soap before preparing food or eating. To stop the growth of bacteria, keep:
cold food below 5 °C and
Hot food above 60 °C.
Make sure food is cooked all the way through. Clean toilet seats, toys, kitchen counters, faucets and baby changing tables thoroughly to avoid transmitting the disease to family members.
People with diarrhea should avoid swimming to prevent recreational waterborne illnesses. Baby and toddler diapers should be checked frequently, and they should be changed in a restroom rather than next to the water. Swimmers should refrain from ingesting water. Only consume bottled water when visiting foreign nations where hygiene is uncertain. Remember to use bottled water to clean your teeth as well. Avoid food buffets, raw or peeled produce, uncooked foods, and drinks with ice.

4. INFLUENZA VIRUS

Influenza virus, which can spread between humans and animals (such as pigs and birds). Human-infected influenza viruses are the source of influenza, also referred to as the flu (Shinya et al. 2006). The three sub types of influenza virus are:

1. Influenza Virus A
2. Influenza Virus B
3. Influenza Virus C

Influenza A and B viruses are the cause of human seasonal flu outbreaks.
The term “bird flu “or "avian influenza" refers to the illness brought on by an infection with type A viruses. These viruses can infect both wild and domesticated mammals and birds. They are a naturally occurring in group of aquatic wild birds all over the world (Hutchinson 2018).

4.1. CAUSES

Avian influenza A (bird flu) viruses can spread from diseased birds to other animals and even Human being in two main ways:

1. Directly from diseased birds or areas where the avian influenza A virus has been introduced through a different animal or a second host (Thomas and Noppenberger 2007).
2. A direct infection can happen if you come into contact with infected birds' saliva, mucous, or feces.

Bird flu infections in humans are rare, but they can happen if enough virus gets inhaled or enters the body by the eyes, nose and mouth (Li et al. 2019). Without appropriate eye and respiratory protection, people

may be more likely to receive the bird flu virus if they have close or prolonged contact with sick birds or areas that they have contaminated with their mucus, salivaor faeces. Direct contact with infected live or dead poultry has been the main risk factor for avian influenza infection in humans; however, a few cases have also been linked to the ingestion of raw chicken products, the killing of infected wild swans, or close contact with other human cases.

In China and Laos, the Eurasian H5N6 HPAI virus has been implicated in 84 laboratory-verified instances of human infection. In human H5 instances, respiratory infection has been the most common clinical symptom (Peteranderl et al. 2016). The spread of this virus between humans is relatively rare. People who come into contact with sick birds are susceptible to the bird flu. A person might, for instance, handle a sick hen, get chicken poop on their hands, and forget to wash their hands before eating. They will consume the diseased bird flesh. This is the most frequent method through which a human receives the avian flu. Although it could persist in uncooked poultry flesh as well, the virus is killed by conventional cooking (Khanna et al. 2008).

Through direct contact with or inhalation of secretions (saliva, mucus, or excrement) from sick birds, humans may get avian influenza viruses. Any avian influenza virus that develops mutations that allow it to bind to receptor sites in the respiratory tract particular to humans is likely to be able to infect people with influenza (Kuiken and Taubenberger 2008).

4.2. CONTROL

Controlling the influenza virus in humans involves a range of measures aimed at preventing its transmission and reducing the impact of influenza outbreaks (Fig. 3).

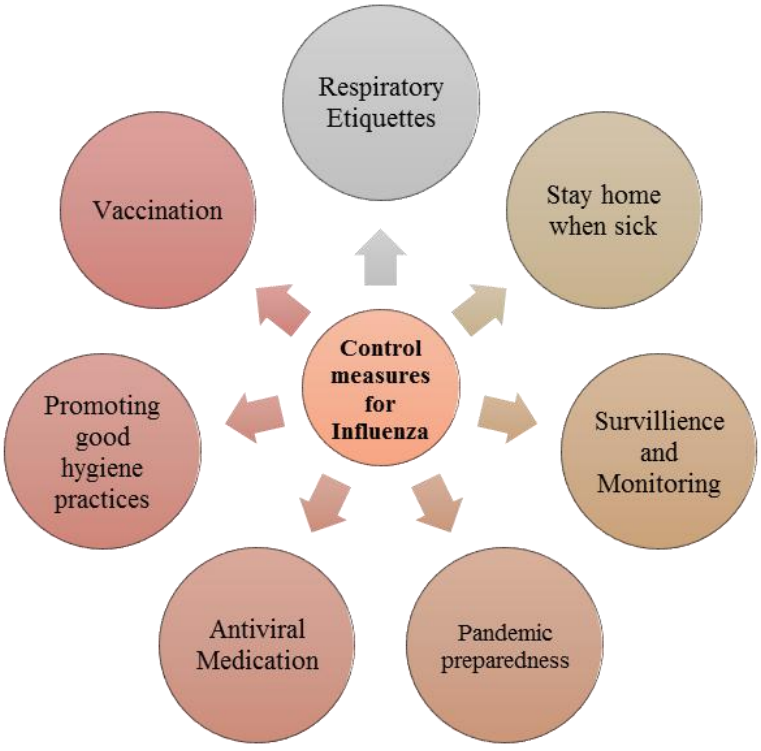

Fig. 3: Key Control Measures for Influenza

In addition to antiviral therapy, personal preventive measures such as: "Regular hand washing with a proper drying of the hands" is part of public health management.

Good respiratory hygiene includes using tissues properly and protecting your mouth and nose when you cough or sneeze.
Isolation of people who are feverish, sick and exhibiting other type of influenza signs as soon as possible.
Keeping distance from unhealthy persons.
Avoid touching your eyes, nose and lips.
When performing aerosol-generating procedures, health care professional workers should adopt airborne precautions. The appropriate personal protective equipment should be made available during epidemics and utilized in conjunction with customary contact and droplet precautions. (Peiris et al. 2007).
When possible, it is advised that citizens and visitors of countries with avian influenza outbreaks avoid going near interacting with animals in live entering locations where poultry may be slaughtered, poultry farms and touching any surfaces that appear to have been contaminated by the excrement of other poultry or animals. It is advised to adhere to good hygiene practices and food safety such as washing your hands with water and soap. Returning visitors from impacted areas should contact their local health services if they experience any respiratory symptoms that could indicate a zoonotic influenza virus infection (Capua and Marangon 2006).

5. LYME DISEASE

Vectors like ticks, and mosquito or fleas can spread several diseases to both humans and animals (Steere et al. 2004). The first case of Lyme disease, also known as borreliosis, was discovered in 1975 when numerous kids with juvenile rheumatoid arthritis were given the diagnosis in Lyme, Connecticut, and two other areas. Deer tick bites were found to be the cause of the spread of arthritis, according to researchers (Kilpatrick et al. 2017).

5.1. CAUSES

The bite of an infected deer tick, commonly referred to as a black-legged tick, is how the bacteria that causes Lyme disease, Borrelia burgdorferi, and is transmitted. Neither common “wood ticks” nor "dog ticks" transmit the disease. These bacteria can be carried by black-legged ticks, sometimes known as deer ticks. Ticks of some species can't transport these germs (Radolf et al. 2021). Juvenile ticks or Deer tick nymphs, are the most common Lyme disease carriers. Their size is comparable to a pinhead. When nymphs eat little animals like mice that have been exposed to B. burgdorferi, they catch the infection. Since the Lyme deer tick is so little, you might not even see it (Coburn et al. 2021). An anesthetic-like chemical found in the tick's saliva numbs the skin so that the bite may not be felt. The Lyme disease-causing ticks are often scarcely bigger than a sesame seed. Nymphs are assumed to infect humans more frequently than adult ticks because they are more difficult to spot borreliosis (Ostfeld and Keesing 2000).

5.2. CONTROL

A minimum tick exposure is the best defense against Lyme disease, Rocky Mountain spotted fever and other tick-borne diseases (Marques 2010). To stop and treat Lyme disease, follow these steps:
Protect your skin and tuck your jeans into your socks before going outside.
Use insect repellent on your skin and clothing; DEET-containing solutions work best.
Keep to well-lit paths whenever you can.
Wear light-colored clothing to make ticks more visible and easier to remove.
During the summer, take extra precautions and wherever you can, stay away from locations with tall grass. If you do stroll or climb in these places, take precautions to avoid getting bitten by ticks.

Wear clothing that is light-colored so that ticks may be seen and removed from you if they settle on you. Wear long sleeves and long, tucked-in pants with your socks on.
When you get home, take off your clothing and check your entire body, including your scalp. Take a shower as soon as you can to remove any hidden ticks (Ostfeld 2011).

6. HANTA VIRUS

Hantavirus is a rodent-borne virus that can cause severe respiratory disease in humans (Zhang et al. 2010). Hantaviruses have been detected in wild rodents such as mice and rats all over the world. Hantaviruses can cause Hantavirus Pulmonary Syndrome, a potentially fatal lung condition. (Muranyi et al. 2005). Non-Pulmonary Hantavirus infection is a milder type of the disease that might develop. In 1993, human Hantavirus infections were discovered in the Southwest (Avšič-Županc et al. 2019).

6.1. CAUSES

Hantaviruses are eliminated by rats in their urine, feces, and saliva, and human infection occurs mostly through inhalation of virus-contaminated rodent excreta. As a result, rodent-infested dusty areas pose a concern (Krautkrämer et al. 2013). Hantaviruses are viruses that can cause serious sickness in humans. The deer mouse is the most common viral carrier. The rice rat, cotton rat, and white-footed mouse are also carriers. The rice rat and the vesper mouse are rodent carriers (McCAUGHE and Hart 2000). An individual can get a Hantavirus if they:
When mouse waste is stirred up by vacuuming or sweeping, it is possible to inhale virus particles that have been released into the air as a result of rodent urine, droppings, or saliva (Dearing and Dizney 2010).
Eat food that has been contaminated by the saliva, urine, or droppings of infected rodents.

6.2. CONTROL

Reduce or eliminate rat contact in your place of business, home or campsite. If rats don't think where you are is a decent place for them to be, you're less likely to encounter them.
Mice may fit through gaps that are as narrow as 1/4 inch (6 millimeters). Store your food, especially pet food, in rodent-proof containers and wash your dishes immediately. Also, keep your counters and floors clean. Garbage lid should be secure (Bi et al. 2008).
Keep debris, grass, and brush away from the foundation of a building.
Set spring-loaded traps around the baseboards (Fig. 4). Use caution while utilizing poison-bait traps because the poison can be harmful to both people and animals. avoiding regions where they leave their droppings and avoiding wild mice and rats. When exposed to mouse and rat droppings, using rubber gloves and a mask that covers your mouth and nose is recommended. sanitizing regions with disinfectant after they have been cleaned of mouse or rat droppings (Krüger et al. 2001).

7. SPOROTRICHOSIS

Some fungi can also cause cyclozoonotic infections. For instance, the fungus Sporothrix schenckii, which causes sporotrichosis, can be transferred from animals, particularly cats, to people. A sub-acute/chronic mycosis is sporotrichosis. The fungus sporothrix causes a disorder known as sporotrichosis or "rose gardener's disease". This fungus can be found all around the world in soil and on plants including rose bushes, hay and sphagnum moss (Gremião et al. 2021).

7.1. CAUSES

Exposure to fungus spores in the environment is the process that causes sporotrichosis. Cutaneous infections or Skin infections are the most frequent kind of infection. Usually after contacting contaminated plant material, the fungus enters through a small cut or scratch on the skin. Skin on the hands or arms is most usually affected (Mahajan 2014). The sporotrichosis-causing fungus Sporothrix is found in soil and on plant materials like sphagnum moss, rosebushes, hay, and wood. Skin scrapes or tiny incisions allow the microscopic fungus to penetrate. In rare instances, inhaling the fungus can result in a lung infection. Sporothrix brasiliensis, which causes sporotrichosis, is spread by animal bites or scratches, mainly from cats (Schechtman 2010). Through contacts with cats, people have contracted sporotrichosis. The fungus that

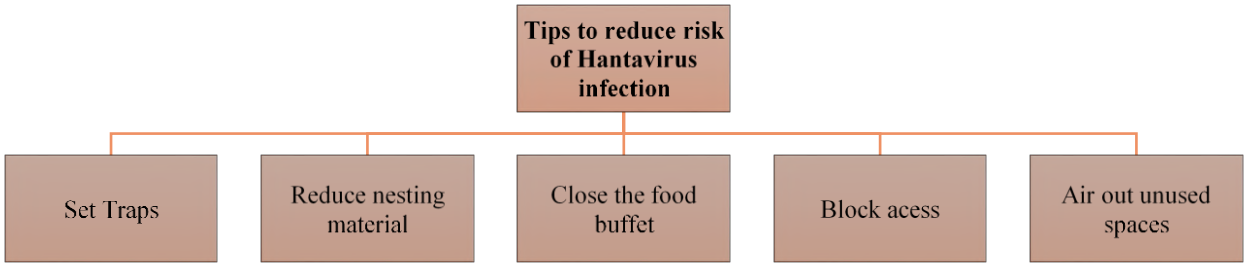

Fig. 4: Tips to reduce risk of Hantavirus infection.

causes sporotrichosis and other diseases can spread through cat bites and scratches. Sporotrichosis often affects the skin, but it can also spread to the lungs, bones, and joints, as well as other body organs (Chakrabarti et al. 2014). The following are some typical causes and risk factors for sporotrichosis in people:
Contact with contaminated material
Animal scratches and bites
Skin Injuries
Occupational exposure
Although the infection can start in skin that seems to be unbroken following contact with hay or moss containing the mold, rose gardners disease atypically starts when the mold spores are driven under skin by a sharp stick or rose thorn (Fichman et al. 2021).

7.2. CONTROL

Preventing mold spores from entering the skin is the most crucial step in preventing sporotrichosis. Anyone who works with roses, sphagnum moss, hay or roses should cover any skin cuts or scratches. To avoid puncture wounds, they should also wear thick gloves and boots. When handling objects like rose plants, pine seedlings, hay bales, or other objects that could break or scratch the skin, these precautions include: donning gloves, boots, and clothing that covers the legs and arms. Avoiding skin contact with sphagnum moss is also a good suggestion (Fig. 5) (Lyon et al. 2003).
Since the fungus often occurs in organic matter, such as soil and plants, it's important to avoid coming into close touch with these sources of infection. When touching soil, plants, or other items that could contain the fungus, wear gloves, long sleeves, and pants.
Keep your skin clean, and steer clear of any activity that could cause skin damage. When working with sharp objects or in areas where the fungus may be present, wear the proper protective equipment, such as gloves and strong footwear.

If you have a cut, scrape, or puncture, make sure to clean it up right away and cover it with a sterile bandage to stop fungus from entering. To reduce the risk of infection, adhere to the wound care instructions provided by medical specialists.

Encourage those at risk, such as farmers, gardeners, and veterinary professionals, to learn about sporotrichosis, its causes, and how to prevent it. Information regarding the illness, its modes of transmission, and the significance of taking personal safety precautions can be included in this.

If you have pets, especially cats, make sure they get the veterinary care they need to stay healthy and to treat any potential sporotrichosis infections. Keep children from having unrestricted access to areas where plant materials could be contaminated. Seek immediate veterinary care if your pet develops any wounds or skin sores. Early detection and treatment with antifungal drugs can aid in limiting the spread of the illness to other body regions.

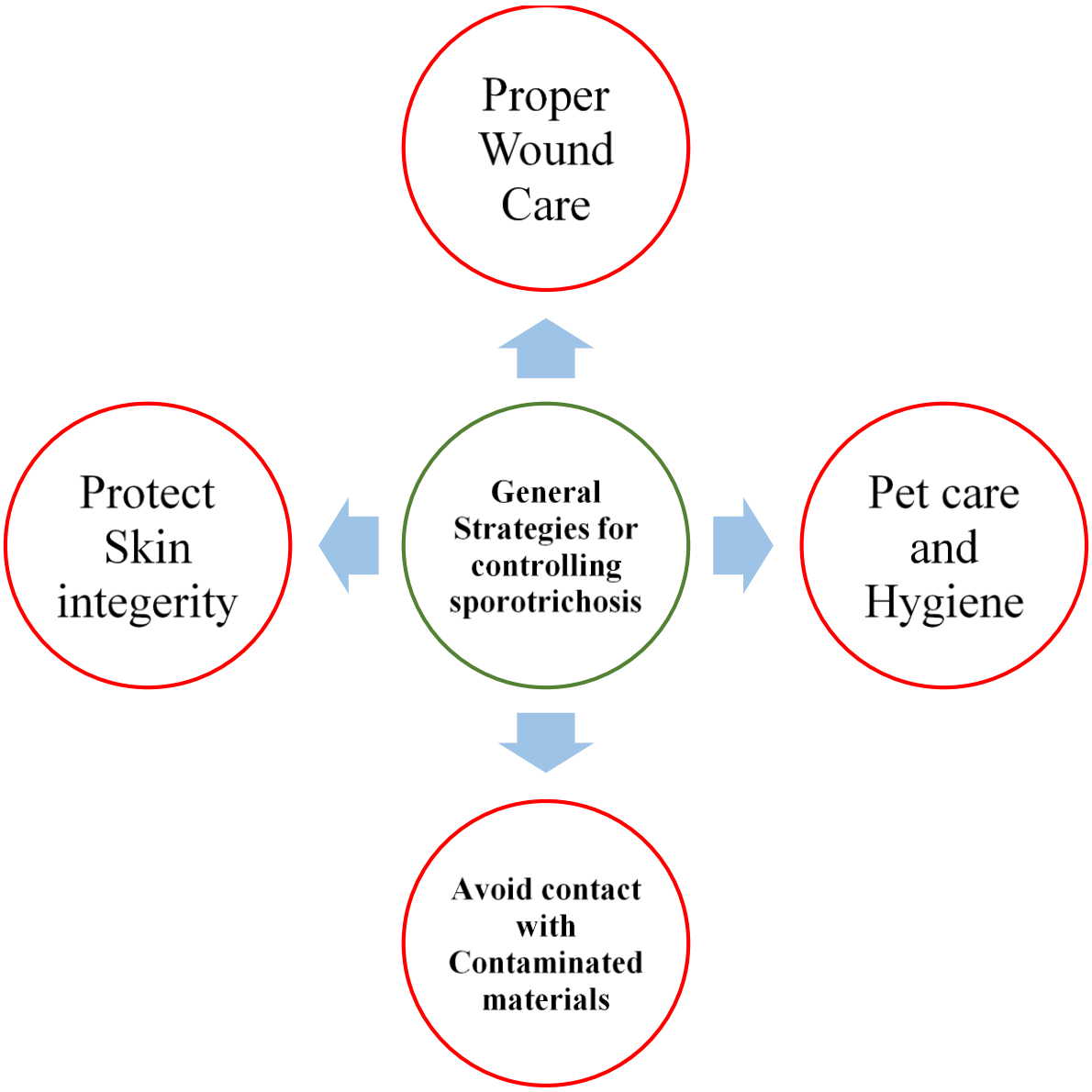

Fig. 5: General Strategies for Controlling Sporotrichosis.

8. TOXOPLASMOSIS

Toxoplasmosis is passed on by the parasite Toxoplasma gondii (T. gondii) (Hill and Dubey 2002). The parasite reproduces in the gastrointestinal tracts of cats. Humans can catch the illness by eating under cooked meat or by coming into touch with cat excrement either directly or indirectly (Dalimi and Abdoli 2012).

8.1. CAUSES

People who unintentionally eat (ingest) something contaminated with the parasite develop toxoplasmosis. Some patients experience flu-like symptoms at the time the parasite first enters the body (Weiss and Dubey 2009).

However, the initial infection is often cured by the immune system without any signs or symptoms appearing. While immune cells are fighting it off, T. gondii creates microscopic sacs (cysts) in your body. Inside these cysts, it can stay dormant (inactive) for a very long time. The entire reproductive cycle can only be completed by domestic and wild cats (Vallochi et al. 2005). These are the main hosts of the parasite.

Immature eggs, a stage of reproduction, may be found in cat feces. This immature egg allows the parasite to climb the food chain. Through soil and water, it can spread to animals, plants, and Human. After acquiring a new host, the parasite's reproduction cycle continues, which results in an infection (Djurković-Djaković et al. 2019).

8.2. CONTROL

Toxoplasmosis can be prevented with the following measures:

Eat no meat that is raw or under cooked. Make sure the meat is cooked through by using a meat thermometer. Whole meats and fish should be cooked to at least 145˚F (63 C) and rested for at least three minutes. Cook ground meat to a minimum internal temperature of (71˚C) 160 ˚F. Cook poultry, both whole and ground, to at least 165 ˚F (74 ˚C) (Smith et al. 2021).

Keep your cat inside to prevent it from contracting the parasite. Feed only canned or dry cat food to your pet. Keep your distance from stray or outdoor cats. While you are expecting, have someone else take care of your cat and clean the litter box. Put on gloves if you have to change the kitty litter by yourself.

After finishing, thoroughly wash your hands in warm water and soap. Every day, clean the litter box. Boil some water on it for five minutes to disinfect it (Kijlstra and Jongert 2008).

Avoid consuming raw goat milk. Steer clear of goat milk and items containing unpasteurized goat milk. Cover playground sandboxes. Avoid drinking unclean water while pregnant to stop outdoor cats from utilizing them as litter boxes.

Avoid consuming raw shellfish. Avoid consuming raw clams, mussels, or oysters, especially while pregnant.

Wash all of your kitchen equipment thoroughly. Knives, cutting boards, and other implements should all be cleansed in soapy water after coming into contact with, uncooked meat, unwashed produce or uncooked eggs. Whenever you prepare food and thereafter, wash your hands thoroughly (Aguirre et al. 2019).

All produce should be washed. Before eating, peeling, or cooking, wash fresh fruits and vegetables. When handling soil or gardening, put on gloves. When working outdoors, put on gloves. After that, wash your hands with soap and water.

9. CONCLUSION

Animals are the primary source of almost all of infectious cyclozoonotic diseases that harm humans. These infections pose a serious risk to human health in addition to infecting animals with diseases. It frequently

happens that alterations in climatic change, dietary patterns and environmentally unfavorable human activities have an impact on the establishment of various cyclozoonotic diseases as a result of increased human–wildlife contact. Because of the significant relation between animals (pet, wild), human, and the environment, research focusing on the one health approach needs to be given priority in order to identify key processes in the transmission of infections due to Cyclozoonosis.

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Cryptosporidiosis: Neglected Zoonosis of Global Importance

21

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ABSTRACT

Cryptosporidiosis is an important parasitic food and water born disease that distributed globally. It caused by protozoan pathogen belong to the genus Cryptosporidium, that infect human and different animal species Including mammals, fish, birds, rodents, reptiles, and amphibians. Although the parasites provide morphological similarity, recent molecular data identified 45 species and more than 120 genotypes of Cryptosporidium in different hosts. Some identified species are host specific while others have been isolated from different hosts. Among the valid species 15 of them can be source for human infection. Species belong to the genus Cryptosporidium spp. have monoxenous life cycle, and the feco-oral route is the common transmission mode. Within host Cryptosporidium parasitize intestinal epithelial cells. The parasites pass through complex cycle including sexual and asexual reproduction within a single host, finally two types of oocysts are formed the thin-walled oocysts are source of reinfection, and the thick-walled oocysts are shed in the feces to the environment, which are infective directly after being shed and cause infection. Cryptosporidiosis mainly associated with gastrointestinal illness, watery diarrhea and weakness are the obvious clinical symptoms, although respiratory cryptosporidiosis rarely occurs in immunocompromised individuals. The available techniques for diagnosing of Cryptosporidium infection were based on the detection of the oocysts including conventional and recent molecular procedures. Currently nitazoxanide has been approved for treatment of cryptosporidiosis. Cryptosporidiosis denotes the major public health concern especially in developing countries. Animals including livestock and carnivores are significant reservoir for Cryptosporidium species with zoonotic potential. The resistant oocyst can survive in the environment for months under cool and wet conditions. Watter supply management can reduce the risk of Cryptosporidiosis out breck, also practicing of personal hygiene is a proper control measure.

Keywords: Cryptosporidiosis, Zoonotic, Protozoa, Gastrointestinal, Human, Animals

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1. INTRODUCTION

Cryptosporidiosis is a water and foodborne zoonotic disease affecting various mammals, birds, fish, and reptiles (Fayer 2010). *Cryptosporidium* is an intracellular protozoan parasite inhabiting the epithelial cells of different hosts' digestive and respiratory tracts (Greene 2006).

Various animal species are supposed to be reservoirs for *Cryptosporidium* species, including livestock, pets, rodents, and reptiles, and become human infection sources (Tzipori and Ward 2002). Recent studies identified about 45 *Cryptosporidium* species and more than 120 genotypes (Xu et al. 2022). Based on molecular studies, nearly 15 species can cause human infection, *C. hominis, C. meleagridis, C. felis*, and *C. canis* are the most common species (Zakir et al. 2021). Also, *C. parvum* is an anthroponotic species associated with zoonotic transmission, while other species are less frequently cause zoonotic infection (Collinet-Adler and Ward 2010).

Various species were also isolated from different hosts, in livestock animals for example cryptosporidiosis in cattle is caused by C. *parvum, C. bovis, C. andersoni,* and *C. ryanae*; sheep are infected with *C. parvum, C. ubiquitum, C. xiaoi* (Ryan et al. 2014), *C. agni* (Xiao et al. 2004a), *C. bovis, C. scrofarum*, and *C. suis*; C. *andersoni* and *C. hominis* are reported in goats (Kaupke et al. 2017).

Cryptosporidiosis etiology in other hosts includes *C. felis* in cats, *C. canis* in dogs, *C. galli, C. baileyi* and *C. meleagridis* in birds, *C. wrari* in guinea pigs, *C. molnari* (Fayer 2010), and *C. nasorum* in fish, *C. crotali* in reptiles (Levine 1984), *C. serpentis* in snake (Bogan 2019), and *C. saurophilum* in lizards (Koudela and Modry 1998). Also, *C. muris* in rodents, *C. cervine* in deer, *C. suis* (Xiao 2010), and *C. scrofarum* in pigs (Kváč et al. 2013). Furthermore, *C. anserinum* in geese, *C. cuniculus* in rabbits, *C. rhesi* in monkeys, and *C. garnhami* in humans have been identified (Xiao et al. 2004a). According to their geographic range and public health significance, other species have also been isolated from various animals (Baroudi et al. 2018).

Numerous identified species might be moderately host-specific. For example, reported species in birds and reptiles cannot infect mammals (Xiao 2010). However, studies on the cross-transmission of *Cryptosporidium* isolates from different animals demonstrated that the parasites could be transmitted from one host species to another, which preventing naming species according to their hosts (Xiao et al. 2004a). For instance, *C. hominis* was first believed to infect humans and pigs only (Morgan-Ryan et al. 2002), but studies indicate that it can infect other animal species (Baishanbo et al. 2005).

The life cycle of *Cryptosporidium* is monoxenous, meaning it is completed within one host. During the entire cycle, parasitic stages were confined to the gastrointestinal (GI) cells' apical surfaces (Barta and Thompson 2006). So, cryptosporidiosis is usually a gastrointestinal illness, although some species and hosts also reveal respiratory cryptosporidiosis (Robertson et al. 2020).

The oocysts are shed with an infected host's feces, and these oocysts are directly infective (Putignani and Menichella 2010), and contain four sporozoites. They survive environmental conditions for months and are infectious for susceptible hosts (Krauss et al. 2003). Infection is transmitted through the fecal-oral route, aerosolized droplets, or indirectly through contaminated water or food or contact with fomites contaminated by coughing (Putignani and Menichella 2010).

The human infectious dose is approximately nine oocysts, and 50 oocysts for calves (Moore et al. 2003). However, one infected host can shed 10^{10} oocysts (Helmy and Hafez 2022). Diarrhea is the most prominent clinical sign of cryptosporidiosis in humans and animals (Ryan et al. 2016), and the disease severity relies mainly on host factors such as immune status (Chalmers and Davies 2010).

Cryptosporidium infection in animals is associated with economic losses due to mortality as well as retarded growth, production loss, and the cost of treatment (de Graaf et al. 1999).

In developing countries, people develop diarrhea by taking the organism with the contaminated public water supply (Greene 2006). Poor hygienic conditions create dirty and muddy surroundings that favor

oocysts' survival and environmental contamination. Also, contact with susceptible animals increases the probability of *Cryptosporidium* infection (Castro-Hermida et al. 2002). Hand sanitization, education, and water treatment reduce infection rates significantly (Fewtrell et al. 2005).

2. HISTORY

Cryptosporidium parasites were first described in 1895. After a period in 1907, Ernest Edward Tyzzer recognized *C. muris* in the peptic glands of laboratory mice (Xiao et al. 2004a). Later, more explanations about the parasite's life cycle were described, and the second species was recognized from laboratory mice (Tyzzer 1912).

Formerly the apicomplexan parasite was classified as a coccidian, and then it was classified in class Gregarinomorphea, subclass Cryptogregaria (Ryan et al. 2016). About 50 years after the initial discovery of the *Cryptosporidium* organism, the parasite was still mistaken for other apicomplexan genera, particularly *Sarcocystis*, a coccidian genus. This confusion was because many species of *Sarcocystis* have thin-walled oocysts that frequently rupture to release sporocysts, and each sporocyst carries four sporozoites similar in shape to the oocysts of *Cryptosporidium* (Xiao et al. 2004a).

The parasite's clinical significance was not realized for 70 years until it was distinguished as a cattle pathogen when it was detected in an 8-month-old calf suffering from chronic diarrhea in 1971 (Panciera et al. 1971). Later on, it was discovered in lambs suffering from diarrhea in Australia (Barker and Carbonell 1974).

Furthermore, the medical importance of *Cryptosporidium* was not even recognized until the first biopsy-diagnosed from a human case in 1976 (Nime et al. 1976). Additionally, in 1980s, *C. parvum* was recognized as a cause of diarrhea in acquired immunodeficiency syndrome (AIDS) patients. Nowadays, the parasite is also known as a cause of diarrhea in immunocompetent individuals (Cox 2015).

3. CLASSIFICATION

The taxonomic status of *Cryptosporidium* has been aligned with the coccidian parasites as follows: (Kerie 2019).

- Phylum: Apicomplexa
- Class: Conoidasida
- Subclass: Coccidiasina
- Order: Eucoccidiorida
- Suborder: Eimeriorina
- Family: Cryptosporidiidae
  - Genus: *Cryptosporidium*

Based on the genetic variances, several new species of *Cryptosporidium* have been defined in different hosts (Xiao et al. 2004a).

4. MORPHOLOGY

The oocyst is a diagnostic stage of *Cryptosporidium* spp., and the parasite can produce two types of oocysts, the thin wall type that has one layer matrix of protein-lipid and carbohydrate, and the thick wall that is provided with the inner and outer oocyst walls (Pumipuntu and Piratae 2018).

The *Cryptosporidium* oocyst wall has a four-layered wall comprising glycocalyx, lipid hydrocarbon, protein, and structural polysaccharide layers. The internal layer is a cysteine-rich proteinous wall responsible for

the wall's rigidity. The cysteines form disulfide bonds, preventing liquid intrusion and withstanding mechanical forces. Hence, the protein layer is responsible for the oocyst wall's rigidity (Jenkins et al. 2010). Oocysts are colorless ovoidal or spheroidal in shape, measuring about 4.6–5.4µm × 3.8–4.7µm (Upton and Current 1985). Mature oocysts contain no sporocysts, but four nucleated, spindle-shaped sporozoites (5.0µm × 0.5µm). The apical complex facilitates the gliding motility to reach target cells, and the nucleus is located centrally in the cell (Pumipuntu and Piratae 2018). The sporozoites can recognize and penetrate target cells in the host, like stomach and intestinal cells (Fayer 2010). They appear as bright red granules following their demonstration in Ziehl-Neelsen-stained fecal smears (Crawford et al. 1988).

5. TRANSMISSION

The fecal-oral route is responsible for *Cryptosporidium* spp. transmission, commonly related to contaminated drinking water and food, but person to person (Cacciò et al. 2005), zoonotic transmission might also occur (Leav et al. 2003). Drinking contaminated water can transmit *Cryptosporidium* through recreational water, including swimming pools and playgrounds (Widerström et al. 2014).

The parasite cannot be removed from the water and clear the contamination using a traditional method for water treatment, like flocculation, sedimentation, and filtration. Additionally, chlorination is insufficient to clear *Cryptosporidium* oocysts from water sources (Betancourt and Rose 2004).

Cryptosporidium is also transmitted through aerosolized droplets from infected hosts or via contact with contaminated fomites by coughing (Putignani and Menichella 2010). Unpasteurized raw milk from infected animals becomes another source for transmission of *Cryptosporidium* (Harp et al. 1996). Viable oocysts have been recognized from raw milk, shedding in the feces of infected animals and contaminating the udder, and subsequently, the milk (Dixon 2009). Rodents are a possible reservoir for livestock infection, and birds, insects, and humans via mechanical transmission may be another source of infection (Bajer 2008).

6. EPIDEMIOLOGY

The epidemiology and transmissibility of *Cryptosporidium* spp. is influenced by environmental conditions. The oocysts are infective immediately after being shed with the feces of reservoir hosts into the environment. They can survive in the animal manure and environment for months under cool and wet conditions. However, adverse environmental conditions inactivate the oocysts before finding an appropriate new host (Collinet-Adler and Ward 2010).

Temperature is essential in the oocysts' infectivity and survival after they are shed into the environment. Higher temperatures stimulate the oocyst's metabolic activity, depleting carbohydrate energy stores and decreasing autonomous survival and infectivity (King et al. 2005). Sunlight exposure for more than 10 hours completely inactivates oocysts in mouse models (McGuigan et al. 2006). The oocysts are susceptible to desiccation (Deng and Cliver 1999), also their infectivity decreases with age (Rochelle et al. 2001).

The incidence of cryptosporidiosis exhibits seasonal variations. Oocysts are at their highest concentrations in agricultural runoff at the onset of storm seasons (Miller et al. 2008).

Pathogen-laden manure is a favorable reservoir for *Cryptosporidium* oocyst to survive for an extended period in the environment. When infected manure is used as fertilizer in grazing areas, there is an increased risk of environmental contamination and animal exposure. Infected manure used as fertilizer for vegetable cultivation is also a public health threat (Berhanu et al. 2022).

The oocyst is also transmitted through land-applied manures or leaching through the soil to groundwater and drinking or recreational water. Polluted fields are a source of the spread of *Cryptosporidium* oocysts

through runoff water, which may enter water sources. Hence, cattle farms could significantly a source for human and animal infections with *Cryptosporidium* spp. (Ogendo et al. 2017).

Calves and lambs are susceptible to cryptosporidiosis, accompanied by the shedding of millions of oocysts, causing massive contaminations of the environment and increasing the chance of infection in humans and other animals (Kerie 2019). Symptomless adults and weaned bovines might also shed oocysts into the environment. It was suggested that one infected adult cattle could shed more than 3.6×10^7 oocysts daily. Infected ewes subclinically also become an infection source for lambs, particularly around the peri-parturient period (Ye et al. 2013).

Furthermore, cryptosporidiosis is common in animals during the rainy season, among large-sized herds, and in farms comprising multiple maternity facilities (de Graaf et al. 1999).

7. LIFE CYCLE

Cryptosporidium spp. have a complex life cycle, including sexual and asexual reproduction within a single host (Quadros et al. 2006). The life cycle initiates when susceptible hosts take viable oocysts. Excystation occurs, and four infectious sporozoites are liberated (Tzipori and Ward 2002). The progression of excystation in the GI tract is activated by pancreatic enzymes, acidic pH of bile salts, CO_2, and temperature of 37 °C (O'Donoghue 1995).

The released sporozoites mostly invade the ileal epithelial cells but can infect the GI tract from the abomasum to the colon (Fig. 1). The apical surface of the intestinal epithelium engulfs the infective sporozoites after they attach to the surface receptors. Then, the sporozoites are enclosed in parasitophorous vacuoles in the microvillous surface of enterocytes as trophozoites (Deng and Cliver 1999). The following endogenous stages occur intracellularly, but the parasite rests on the villar epithelial cell surfaces (Xiao and Fayer 2008).

Cryptosporidium is an intracellular and extra cytoplasmatic protozoan that reproduces asexually by two merogony cycles and sexually by gamogony. The trophozoites grow asexually by merogony, producing two types of meronts within 24 hours. Type I meronts contain eight merozoites that exit the parasitophorous vacuole and enter other epithelial cells. Inside the new cells, the liberated merozoites multiply to form more type I meronts or differentiate to type II meronts that contain four merozoites (Greene 2006).

Type II meronts initiate the sexual developmental cycle and infect more enterocytes. Then, they differentiate into micro- and macro-gametes. The spheroid immature microgamonts are 5µm × 4.5µm and comprise about sixteen nuclei located peripherally. Mature micro-gametes leave the cell and fertilize the macrogametes. The spherical macrogametes are 5µm × 5µm and comprise granulated cytoplasm and wall-forming bodies located eccentrically (Tandel et al. 2019).

Zygote is formed after fertilization or gametogony between male microgamonts and female macrogamonts and undergoes meiosis, producing oocysts. Two types of oocysts are formed (Collinet-Adler and Ward 2010). The thin-walled oocyst excysts inside the intestine and reinfects the host, and the thick-walled oocysts are shed in the feces to the environment and cause infection if taken orally. Thick-walled oocysts are infective directly after being shed from the host (Kerie 2019)

8. PATHOGENESIS

Cryptosporidium species cause damage and changes in the intestinal epithelial cells. Sporozoites and merozoites interact with host cells to initiate signaling cascades with proteases and hemolysin molecules, resulting in cell damage, increasing fluid secretion, and malabsorption (Robertson et al. 2020).

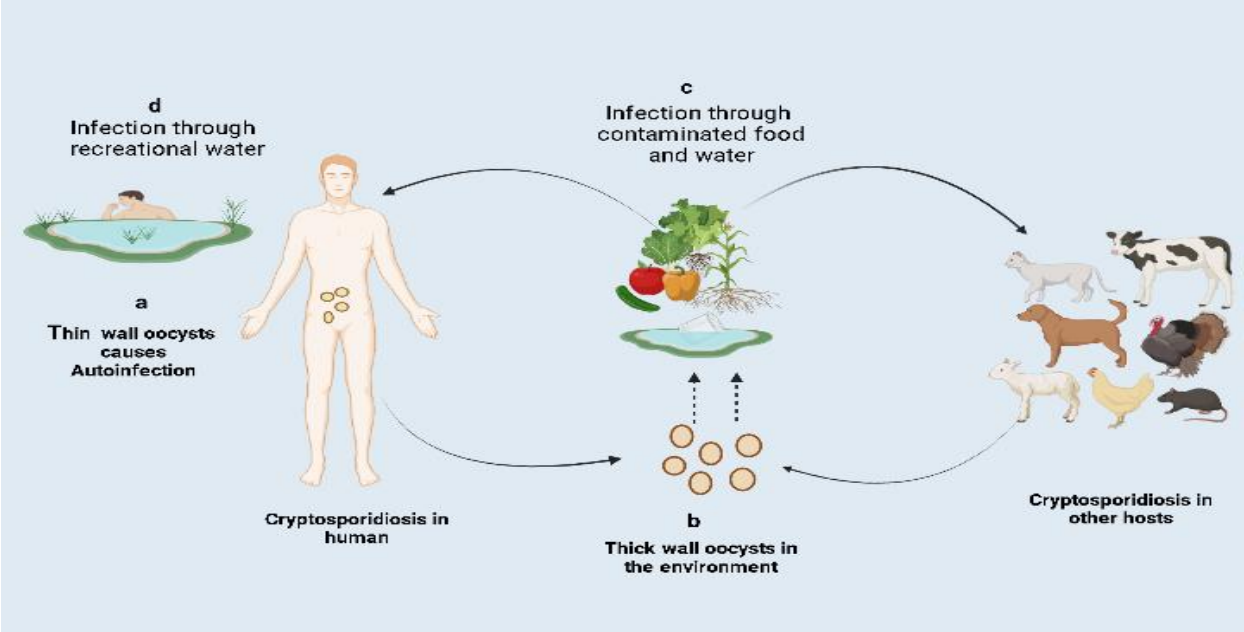

Fig. 1: Life cycle of *Cryptosporidium* spp.

The parasite continues to reside just below the intestinal epithelial mucosa's luminal cell membrane and accompanying by morphological changes in the intestinal mucosa, such as inflammatory cell infiltration, villous atrophy, and crypt hyperplasia. These effects impair glucose-stimulated sodium and water absorption (Greene 2006). Variable degrees of villous atrophy impair digestion and absorption processes accompanied by subsequent diarrhea (Heller and Chigerwe 2018). Infection in children under one year might result in psychomotor developmental stunting, and its effects are still noticeable several years after infection (Checkley et al. 2008).

9. CLINICAL SIGNS

Clinical signs vary significantly depending on the infected host's immune status (Collinet-Adler and Ward 2010). Symptoms are variable in immune-competent individuals but usually include diarrhea, which is self-limited. The diarrhea is foul-smelling, profuse, and watery but does not contain blood or leukocytes (DuPont et al. 1995). Possible accompanying symptoms include fever, nausea, vomiting, and abdominal cramps (Khan et al. 2004). Other signs, like anorexia, dehydration, headache, myalgia, weakness, and weight loss, might also be observed (DuPont et al. 1995). Additionally, *Cryptosporidium* infection causes malnutrition (Kirkpatrick et al. 2006). Asymptomatic carriage with oocyst shedding has been detected up to two months after symptom resolution (Jokipii and Jokipii 1986). Children are frequently vulnerable to acute and chronic infection with cryptosporidiosis (Checkley et al. 2008). In immunodeficient individuals, such as AIDS patients, infection becomes chronic and severe, causing cachexia and even death (Vakil et al. 1996).

There is also respiratory involvement, but in immunocompetent individuals, respiratory cryptosporidiosis rarely occurs (Mor et al. 2010), extending from no symptoms to dyspnea with bilateral infiltrates. In AIDS patients, upper respiratory infections, including tracheobronchial and nasal cryptosporidiosis, are familiar with severe small intestinal cryptosporidiosis. Lower respiratory infection might also occur, leading to interstitial pneumonia (Travis et al. 1990). Extraintestinal manifestations are higher in

immunocompromised individuals, such as sclerosing cholangitis, acalculous cholecystitis, and pancreatitis (Vakil et al. 1996).

Malnutrition due to *Cryptosporidium* infection secondarily impairs cell-mediated immunity, increasing susceptibility to other infections. Long-term infections also cause reduced mental growth and school performance and increased risk of cardiovascular and metabolic diseases (Sudfeld et al. 2015). Neonatal diarrhea in livestock animals is profuse and yellowish and causes metabolic acidosis, loss of electrolytes, and exsiccosis (Kasle et al. 2008).

Cryptosporidiosis causes low fatality rates in young animals unless complicated by other conditions such as concurrent infection, loss of energy from insufficient colostrum and milk intake, and adverse weather conditions. Cryptosporidiosis frequently occurs with other enteropathogens, particularly rotavirus and coronavirus, resulting in intestinal damage and severe diarrhea (Xiao and Fayer 2008).

In cows, a decrease in milk production has been reported during oocyst shedding, with no obvious clinical signs (Fayer et al. 2006).

10. DIAGNOSIS

Generally, the *Cryptosporidium* diagnosis is based on identifying oocysts in the fecal sample. Several techniques are available for detecting *Cryptosporidium* infection (Venu et al. 2013).

Conventionally *Cryptosporidium* oocysts have been detected by applying various concentration techniques, like formaldehyde-ether sedimentation and flotation techniques using Sheather's sugar, saturated sodium chloride, and modified zinc sulfate (Pumipuntu and Piratae 2018). Fecal concentration approaches are frequently essential to reveal the presence of oocysts (Greene 2006).

Various procedures can be used for oocyst staining from stool, including auramine-rhodamine, fluorescent acridine orange, Giemsa, modified Ziehl-Neelsen, and safranin-methylene blue stains (Varea et al. 1998).

Although oocysts can be identified microscopically by stool inspecting, experience is needed to separate *Cryptosporidium* from debris, other protozoa, and yeasts. So, the method lacks sensitivity and specificity, and misdiagnosis frequently occurs (Pumipuntu and Piratae 2018). Several samples must be examined when the infection is highly suspected (Van Gool et al. 2003). Furthermore, oocyst identification through a modified Ziehl-Neelsen staining procedure requires 30 to 40 minutes and intensive experience to interpret the results (Ayana et al. 2009).

Immunological-based techniques are alternative methods to detect cryptosporidiosis, such as enzyme-linked immunosorbent assay (ELISA), indirect ELISA, and direct fluorescent antibody (Fayer et al. 2000). Antigen-based ELISA has significant advantages of being more sensitive and specific and saving cost, labor, and time (Pumipuntu and Piratae 2018), also can be used as a large-scale screening tool in epidemiological studies (Kerie 2019), and with excellent sensitivities and specificities over other methods for detection of *Cryptosporidium* oocysts. Seroprevalence rates might detect asymptomatic cases. Hence, serology is not an excellent tool for diagnosis because it does not inevitably denote active infection (Collinet-Adler and Ward 2010).

DNA-based molecular techniques are implemented to diagnose *Cryptosporidium* species with high sensitivity and specificity depending on the 18S rRNA gene (Oskouei et al. 2014). *Cryptosporidium* species and genotypes in different samples can be differentiated via PCR, real-time quantitative PCR, RFLP (Xiao et al. 2004b), nested PCR, and multiplex PCR (Rochelle et al. 2001).

PCR techniques are accurate and rapid diagnostic techniques, and the results are easily interpreted. Nevertheless, the technical complexity challenges its use. Also, inhibitors, such as bile salt, bilirubin, and complex polysaccharides, may interfere with results, causing reduced sensitivity. Oocyst purification from

the fecal sample by density gradient concentration techniques has been used to eliminate inhibitors (Ahmed and Karanis 2018).
Other methods can also be applied for detecting *Cryptosporidium* oocysts from the environment, including colorimetric and fluorescent in situ hybridization (Xiao 2010).

11. TREATMENT

Cryptosporidiosis is typically self-limiting in young and immunocompromised individuals, and full recovery soon follows. Treatment of cryptosporidiosis relies chiefly on the host's immune status. Hydro electrolytic resuscitation is the most common therapy when possible, or intravenous resuscitation when essential (Rossignol 2010).
More than 100 therapeutic agents have been screened for treating cryptosporidiosis. Until now, no anti-cryptosporidial agent has been developed to overcome *Cryptosporidium* parasites (Chavez and White 2018). The United States Food and Drug Administration approved nitazoxanide as an anti-cryptosporidial compound. This drug reduces the symptoms' duration and severity in immune-competent individuals (Rossignol 2010).
Immune status restoration using antiretroviral therapy is also crucial in AIDS patients. Ritonavir, indinavir, and saquinavir are protease inhibitors with anti-cryptosporidial action and can be considered in these patients (Hommer et al. 2003).
The use of albendazole in high doses or probiotics exhibited a considerable reduction in the number of shed oocysts in the faces, and the severity of diarrhea. Similar results occurred following the administration of macrolides, such as azithromycin, erythromycin, and roxithromycin (Diptyanusa and Sari 2021). In animals, nitazoxanide reduced the number of *Cryptosporidium* oocysts excreted with feces (Ollivett et al. 2009).

12. PREVENTION AND CONTROL

Improvements in water treatment are essential by applying special physical and chemical processes for eliminating *Cryptosporidium* oocyst. These techniques include disinfection procedures, like ozone treatment or ultraviolet light irradiation, for reducing the oocyst viability and infectivity since *Cryptosporidium* is highly resistant to chlorine disinfection, even at high concentrations and for long contact times (Betancourt and Rose 2004). Furthermore, the oocysts are small (4–6µm), making filtration difficult. Pore sizes of less than one micron are required since the oocysts are pliable and plastic (Méndez-Hermida et al. 2007). So, pressure-driven membrane microfiltration or ultrafiltration is adequate for their elimination (Betancourt and Rose 2004).
Effective water supply management should also be provided so that no secondary contamination occurs during transport, storage, and final use (Méndez-Hermida et al. 2007). Also, food must be washed, boiled, or cooked before consumption (Rossle and Latif 2013).
Practicing proper personal hygiene and handwashing before food preparation and consumption, after using a toilet, and after contact with patients having diarrhea, children, livestock, or some animals reduces the probability of infection (Rossle and Latif 2013).
Preventive hygienic measures are essential tools to control cryptosporidiosis in farm animals. These preventive measures aim to destroy the parasite's external forms and reduce their spread between animals, the environment, and the host (Aboelsoued and Abdel Megeed 2022).
In livestock husbandry, control measures against cryptosporidiosis are also at pens and buildings utilized for parturition, including the destruction of oocysts by applying suitable disinfectant, using clean straw

beds abundantly, and avoiding high density in the parturition area and during cryptosporidiosis outbreaks (de Graaf et al. 1999).

Limiting animal stocking in the farms and keeping young or susceptible animals apart from adult animals reduce the risks of *Cryptosporidium* spp. transmission in the herds. Finally, colostrum supply protects neonatal animals against *Cryptosporidium* infection (Aboelsoued and Abdel Megeed 2022).

13. CONCLUSION

Cryptosporidiosis is a globally emerging parasitic disease and one of the water and foodborne conditions. A wide range of hosts become infected with various species. Livestock animals and poultry became the primary source for environmental contamination by *Cryptosporidium* oocyst, as they shed large numbers following their infection and have zoonotic concerns, capable of disease induction in humans. Good animal management practices might ensure a reduction in cryptosporidiosis prevalence rate through the reduction in oocyst shedding besides environmental and surface water contamination. Applying different molecular techniques will help in the epidemiology and transmission pattern of *Cryptosporidium* species, which are essential for following appropriate control measures. Identifying different *Cryptosporidium* subtypes permits understanding the zoonotic potential and their affection on public health ratings.

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Epizootiology of *Blastocystis* spp.: A Parasite with Zoonotic Concern

22

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ABSTRACT

Blastocystosis is an enteric infection caused by globally distributed unicellular Protista Blastocystis. Various animals' species as well as human can be parasitized by Blastocystis species. Due to the existence of genetic diversity between Blastocystis isolates in different hosts recently the organisms were defined as Blastocystis spp. or subtypes, although they are morphologically alike. About 34 valid subtypes have been reported in different hosts. Dissimilarity in Blastocystis genotypes have significant effect on their pathogenicity. The organism is poly morphic, and several distinct morphological forms have been observed including vacuolar, granular, cyst, and amebic forms.

Blastocystis spp. are transmit through feco- oral rout via contaminated food and water. The risk for human infection might be higher in the existence of infected animals with Blastocystis, as well as in poor hygienic conditions. Although most of reported cases are asymptomatic, Blastocystis infection can associated with gastrointestinal disorders and appearance of nonspecific symptoms of nausea, abdominal pain, bloating, and diarrhea which might be self-limiting or severe. Blastocystosis also accompany with extra-intestinal urticaria signs such as palmoplantar pruritus. The organism also reported from cases of irritable bowel syndrome. Diagnosis of Blastocystis infection can be done conventionally based on the parasitological methods including microscopic examination of fecal smear. In vitro cultivation of fecal samples in supplemented medium was another detection method. The development of molecular techniques provides a sensitive, and rapid detection procedures for Blastocystis spp., also aid in genotypes differentiation. Metronidazole is the treatment of choice for Blastocystosis. Control measures include improvement in hygiene, and sanitation conditions, increased health awareness also essential in preventing enteric parasites.

Keywords: Blastocystis, Zoonotic, Polymorphic, Vacuolar form, Enteric protozoa.

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1. INTRODUCTION

Blastocystis is a globally distributed unicellular, anaerobic, eukaryotic protist (Tan 2008) belonging to the phylum Stramenopila inhabiting various hosts' gastrointestinal tracts, including humans and animals (Villalobos et al. 2014). Infection with *Blastocystis* is known as blastocystosis.

Blastocystis has been detected in various domestic and wild animals, including cattle, dogs, cats, rats (Clark et al. 2013; Ramírez et al. 2014), birds, reptiles, and even insects (Rauff-Adedotun et al. 2020). Similarly, it has been reported in pigs and monkeys (Parkar et al. 2007), also recovered from fish (Gantois et al. 2020), indicating a possible threat of zoonotic transmission to humans.

Due to the morphological similarity between *Blastocystis* isolated from humans and animals, it is challenging to differentiate isolates based on morphology. However, broad genetic diversity exists among isolates of human and animal origins. The organism is no longer mentioned as *B. hominis* based on such diversity. Instead, it is called *Blastocystis* spp. or *Blastocystis* subtype (Tan 2008).

The description of *Blastocystis* subtypes depends upon assessing small ribosomal RNA subunit genes (Stensvold et al. 2007a). The genetic diversity among human and animal isolates of *Blastocystis* was interpreted using gene sequences (Baek et al. 2022). There are 38 subtypes (STs), numbered ST1 to ST38 (Maloney et al. 2022). Thirty-four of these subtypes are validated, but ST18–ST20 and ST22 are potential PCR amplification artifacts (Stensvold and Clark 2020).

Variability in *Blastocystis* genotypes plays a significant role in pathogenicity (Souppart et al. 2009). Studies showed that ST3 is the most common isolate in patients suffering from gastrointestinal disorders (Rajamanikam and Govind 2013). Approximately 90% of the human isolates belong to ST1–ST4 (Alfellani et al. 2013a). Some STs are shared between animals and humans, studies demonstrating that zoonotic STs occur frequently in livestock and pet animals (Higuera et al. 2021).

Human infection with *Blastocystis* spp. is around 50% in developing countries compared to 23.1% in developed countries (Alfellani et al. 2013b; Osman et al. 2016). An estimated one billion people are infected with *Blastocystis* worldwide (Alfellani et al. 2013b), most of whom are asymptomatic carriers (Andersen and Stensvold 2016).

The pathogenicity of *Blastocystis* remains debatable, while its association with different gastrointestinal disorders like inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) (Jimenez-Gonzalez et al. 2012) is established. Infection severity relies on the number of parasites discovered in stool samples (Roberts et al. 2011), and the parasitic subtype (Yan et al. 2006).

The prevalence estimates of *Blastocystis* rely on the applied diagnostic methods. Molecular methods based on PCR have better sensitivity and specificity (Stensvold et al. 2007b). SSU rRNA gene fragments analysis can identify the presence of *Blastocystis* and genetically characterize the organism (Gong et al. 2019). Metronidazole is commonly the first-line therapy, if it is ineffective, trimethoprim-sulfamethoxazole or nitazoxanide is the second-line treatment (Coyle et al. 2012).

Following hygienic stranded, proper waste clearance, contact with infected animals, and consuming contaminated food and water can alter the prevalence rates (Alfellani et al. 2013b). Poor hygiene increases the opportunity for *Blastocystis* transmission (Gong et al. 2019). However, in recent years awareness has been developed about the extensive prevalence of the *Blastocystis* species as a common emerging condition in humans and animals (Li et al. 2018).

2. CLASSIFICATION

The nomenclature of *Blastocystis* has remained indefinable for a long time, classified as flagellate, yeast or fungus, and protist based on its morphological structure (Zierdt 1991). Subsequently, it was suggested to classify *Blastocystis* in the subkingdom of protozoa, subphylum sporozoan, in a separate suborder Blastocystina based on the morphological and physiological criteria (Boreham and Stenzel 1993).

The species name was granted according to the host; the isolated organism from human isolates is called *B. hominis,* and the rat isolate is called *B. ratti*. *Blastocystis* was subsequently isolated from various hosts (Noël et al. 2005), and two other non-human species were described, *B. galli* from chicken and *B. lapemi* from sea snakes (Teow et al. 1991).
Meanwhile, due to its massive diversity, the parasite is no longer mentioned as *B. hominis;* instead, it is called *Blastocystis* spp. or *Blastocystis* spp. subtype n. The n is the number of subtypes based on the classification by Stensvold (Tan et al. 2008).

3. MORPHOLOGY

Previous studies indicated that *Blastocystis* was a polymorphic organism with several distinct morphological forms (Kukoschke et al. 1990). Four primary morphological forms exist: vacuolar, granular, cyst, and amebic. Other morphological forms were also reported by electron microscopy, including vacuolar and multi-vacuolar forms, which are small in size and rarely present. Moreover, the organism can also undertake strange forms like medusa head form on exposure to oxygen and chestnut burr cell in aging culture (Zierdt 1991). The organism can reproduce by five distinct modes: binary fission, budding, endodyogeny, plasmotomy, and schizogony (Zhang et al. 2007).

3.1. VACUOLAR FORM

This spherical form contains a large central vacuole, occupying approximately 90% of the cell space and limiting the cytoplasm and intercellular components in a thin peripheral layer (Lee and Stenzel 1999).
Following morphological identification of vacuolar form by wet mount microscopy, a central space has been observed and labeled as a vacuolar form. Later, the space was found to be a membrane-bound body containing fine granular materials (Yoshikawa et al. 1995).
The membrane-enclosed vacuole contains carbohydrates, fats, and proteins, accumulating due to the Golgi apparatus's action via clathrin-mediated endocytosis (Mehlhorn et al. 2012). The central body is a storage organelle called the central body form (Stenzel et al. 1989).
The nuclei are distributed at the periphery throughout the cytoplasm. More than one nuclease is present; typically, two nuclei are situated at the opposite end of the cell. More than two nuclei were reported rarely (Do Bomfim and Do Couto 2013). There is variation in the size of vacuolar form ranging from 3µm to 120µm in diameter, with an average of 5µm to 15µm, commonly observed in asymptomatic carrier individuals with *Blastocystis* (Sekar and Shanthi 2015).
Mitochondria and other organelles surround the nucleus as a rosette, making thickened pods in the cytoplasmic rim. Occasionally, a surface coat or capsule of various thicknesses surrounds the vacuolar form, especially in fresh clinical isolates, which protects the organism from osmotic shock. It was also assumed to take part in bacterial tapping for nutrition (Zaman et al. 1997).

3.2. GRANULAR FORM

This form is like the vacuolar form but contains centrally situated granules within the central body and cytoplasm (Cassidy et al. 1994). The granular form has two nuclei; at maximum, four are present. Based on the size, it is smaller than the vacuolar form (Do Bomfim and Do Couto 2013), and exhibits a lower degree of pleomorphism than the vacuolar form. The average diameter is 15–25µm, while the largest might reach 80µm (Zierdt and Williams 1974).
Based on electron microscopy studies, three kinds of granules have been identified: metabolic, reproductive, and lipid granules. The first type is mainly found in the cytoplasm and is involved in various

metabolic pathways in the organism. Reproductive granules are only observed in the central body and were proposed to have a role in schizogony (Tan and Stenzel 2003). Lipid granules are a storage space in the central body and cytoplasm (Tan and Zierdt 1973).

3.3. AMEBOID FORM

This form is highly uncommon, and it is irregular in shape, provided with one to two pseudopodia, and has strong adhesive properties that permit attachment to the bowel mucosa. The cytoplasm contains a large vacuole or multiple smaller vacuoles (Tan and Suresh 2006). It is non-motile and measures about 10µm in size (Tan and Zierdt 1973).

The ameboid form converts into a cystic form, and because of its small size and morphology, it looks like neutrophils and macrophages. In standard stool examination, it can easily escape recognition. Zierdt proposed staining an unfixed smear with Gram's stain for identification since the ameboid form of *Blastocystis* lyses when exposed to air, but leukocytes remain intact (Zierdt 1991). The ameboid form is progressively recognized in patients with diarrhea and has been reported to be pathogenic (Zhang et al. 2012).

3.4. CYSTIC FORM

This form is round to oval in shape and smaller in size, measuring 3–6µm, and it is surrounded by a thin, multilayer wall with or without a surface envelope (Do Bomfim and Do Couto 2013). The number of nuclei is from one to four, and the cytoplasm is dense and comprises several mitochondria and storage vacuoles produced from lipids or glycogen. The cystic form can remain viable for about a month even when exposed to air and at 25°C, enabling further infection spread (Tan 2008). The cysts are infective in a proper host and develop into vacuolar forms (Yoshikawa et al. 2004b).

Other forms, like the a vacuolar and multi-vacuolar, gained importance lately. These forms are about 5–8µm in diameter, with no size variations. A vacuolar form lacks the central body, and the multi-vacuolar has several different-sized small vacuoles interlinked or lying separated in the cytoplasm. Both forms are commonly uninucleate, but two nuclei are present sometimes (Parija and Jeremiah 2013).

4. TRANSMISSION

The cystic form of *Blastocystis* is the only transmittable form transmitted by the fecal-oral route (Yoshikawa et al. 2004b). Appropriate hosts may get infected infection by ingesting cysts from drinking contaminated water or eating raw aquatic plants (Lee et al. 2012). Unclean hands can serve as a fomite for transmitting cysts from suspected individuals on direct contact or from contaminated soil (Anuar et al. 2013). Close contact with animals is another source of *Blastocystis* infection (Javanmard et al. 2018).

Human-to-human and inter-species transmission between humans and animals might occur due to poor host specificity (Parkar et al. 2007). However, only 14 subtypes (ST1–ST10, ST12, ST14, ST16, and ST23) were found in humans, with different frequencies (Osorio-Pulgarin et al. 2021), and ST9 has only been described in humans (Andersen and Stensvold 2016). The globally available epidemiological data in several countries represented that > 90% of human subtypes were ST1–ST4 (Alfellani et al. 2013a). Consequently, the four subtypes are transmitted among humans, and the remaining subtypes found infrequently in humans are of animal origin as they predominate in particular mammal or bird groups (Hublin et al. 2021). The existence of these STs in humans explains zoonotic transmission, and isolates exhibit the same gene sequence of SSU rRNA in both animals and humans (Wang et al. 2014; Greige et al. 2018).

The ST1 occurs in several species, like cattle, pigs, primates, rodents, and birds (Yoshikawa et al. 2004b; Thathaisong et al. 2013). ST2 was isolated from pigs and monkeys (Tan 2008), while ST3 is cosmopolitan in humans, non-human primates, and other mammals, like cattle, pigs, dogs, rodents, and horses (Stensvold et al. 2009; Thathaisong et al. 2013). ST4 has been found in rodents (Stensvold et al. 2009), and ST5 occurred in dogs, cats, and pigs (Rauff-Adedotun et al. 2020). ST6 and ST7 are dominant avian subtypes. ST5 and ST9 seldom occur in human (Stensvold et al. 2009), and ST10 is reported in cattle, goats, and deer (Rauff-Adedotun et al. 2020).

5. EPIDEMIOLOGY

Blastocystis spp. have been observed worldwide with various prevalence rates between areas and populations (Yan et al. 2006). Variation in the prevalence rate might be due to poor hygienic conditions, animal exposure, and intake of contaminated water or food (Tan 2008). People in developing countries have a higher incidence of blastocystosis due to a lack of sanitary conditions and contaminated water and food. A seasonal impact on *Blastocystis* prevalence is also reported, with a higher incidence in summer (Yan et al. 2006).

Most of the isolated human subtypes have also been reported in animals, bringing up the issue of the role of animal reservoirs in the parasite's epidemiology (Hublin et al. 2021).

The opportunity for human infection might increase when there are infected animals with *Blastocystis*. Animal feces might contaminate streams and rivers via surface overflow after heavy rain, causing water contamination and spreading in a wide geographical area (Gong et al. 2019). Non-human primates, mammals, and birds are the primary reservoirs for most subtypes (Stensvold et al. 2007a). Regardless of the country of study, the prevalence of different subtypes shows the absence of geographic limitation of these subtypes' distribution (Rauff-Adedotun et al. 2020).

Both zoonotic and enzootic STs have been reported in farm animals (Hublin et al. 2021). Zoonotic STs (ST1, ST3–ST5, ST7) and enzootic STs (ST10, ST12, ST14) usually occur in ruminants (Alfellani et al. 2013b; Song et al. 2017). In cattle zoonotic STs (ST1–7 and ST12) and enzootic STs (ST10, ST14, ST17, ST21, ST23–ST26) are documented (Suwanti et al. 2020; Hublin et al. 2021).

Horses become host for different ST such as ST1, ST3–6, ST10, ST14, ST24–26, ST33 and ST34 (Baek et al. 2022). Eight STs were reported in pigs, which are ST1–ST5, ST7, ST10, and ST15. All these STs, except ST10 and ST15, are considered zoonotic and reported in humans (Hublin et al. 2021).

Ten *Blastocystis* STs (ST1–ST5, ST7, ST8, ST10, ST13, ST17) were described in rodents (Hublin et al. 2021). In monkeys, ST1–ST5 and ST8 have also been identified (Rauff-Adedotun et al. 2020).

Dogs and cats, could be reservoirs for enteric parasites in humans, including *Blastocystis*. wandering stray dogs and cats may contaminate soil, water, and food with zoonotic STs and contribute to transmitting *Blastocystis* (Hublin et al. 2021).

Eight subtypes, including ST1–ST6, ST8, and ST10, were identified in in dogs (Nagel et al. 2012). All subtypes except ST10 are described in humans, implying that dogs might play a role in transmitting *Blastocystis* to humans, or humans could be a source of infection for dogs. Also, ST1, ST3, and ST4 have been reported from cats, which are often found in humans and regarded as zoonotic STs (Hublin et al. 2021).

Birds can harbor enzootic and zoonotic subtypes, and have a critical role in transmitting the protozoa between wildlife, livestock, and humans (Chandrasekaran et al. 2014). *Blastocystis* was reported globally in wild and domestic birds, with prevalence rates of 2.1%–100% (Hublin et al. 2021). ST6 and ST7 are common subtypes, but other STs are also reported, like ST1, ST2, ST4–ST5, and ST8, and enzootic STs (ST10, ST13, ST14, ST20, ST24, ST27–ST29) (Ramírez et al. 2014; Greige et al. 2018; Maloney et al. 2021).

Subtypes 1–4, the primary subtypes infecting humans, have been identified in cockroaches, which implies that cockroaches could be a possible source of infection in humans (Rauff-Adedotun et al. 2020).
Infection with *Blastocystis* is higher among immunocompromised people, immigrants, and travelers who visit developing tropical countries (Tan 2008). Also, a higher prevalence was observed among patients taking immunosuppressive drugs (Rao et al. 2003). Furthermore, infections are widespread in occupations requiring animal exposure, like food handlers, animal handlers, and abattoir workers (Parkar et al. 2010).
Blastocystis was detected in various water sources like rivers and sewage, implying that human and animals infection can occur from contaminated water. The protozoa can occur as high as 50% in tap water and 100% in household water storage tanks and containers, further confirming the possible transmission of this parasite through contaminated water. *Blastocystis* identification in water supplies implies that water can be a source of parasite transmission to humans and animals (Hublin et al. 2021). Studies also identified *Blastocystis* in raw vegetables, and eating unwashed fruit was related to an eight fold increase in an infection rate (Canete et al. 2012).

6. LIFE CYCLE

The life cycle for *Blastocystis* was first proposed by Alexeieff in 1911 (Boreham and Stenzel 1993). Infection occurs upon an intake of cysts (Lee et al. 2012). In a suitable host, the cyst becomes vacuolar inside the large bowel lumen via cyst excitation. Further life cycle continuation relies on subtype-host compatibility (Sekar and Shanthi 2015). After a period of infection, other forms can also develop. The vacuolar forms can convert into other forms. The ameboid, a vacuolar, and multi-vacuolar forms are often observed in diarrhea, suggesting that these forms may have a role in the pathogenesis (Zhang et al. 2012). The encystation of the vacuolar form happens throughout the lumen of the large intestine, and the cysts are shed with feces (Fig. 1) (Vdovenko 2000).
Blastocystis can pass through a complex cycle to form primary cysts, comprising various reproduction modes, binary fission of a binucleate stage plasmotomy, and autogamy, a sexual phenomenon. These cysts produce spores by multiple budding, and the spores, or secondary cysts, are the resistant form. Studies demonstrated that *Blastocystis* can also reproduce through multiple fission, endodyogeny, and schizogony, but the vacuolar form's binary fission is the most common mode (Zhang et al. 2007).

7. PATHOGENESIS

Although the potential of *Blastocystis* spp. subtypes to be pathogenic is yet arguable, some authors showed its connection with gastrointestinal disorders, whereas others have rejected such involvement (Abedi et al. 2022). The pathogenicity of *Blastocystis* is suggested to be dependent on the subtypes and parasite burden. However, individuals may show symptoms even with small numbers of cysts (Coyle et al. 2012).
The proteases are the most virulent enzymes excreted by the ameboid form (Abdel-Hameed and Hassanin 2011; Scanlan 2012). Demonstrating numerous ameboid forms in a patient with severe diarrhea supports the idea that this form is virulent (Zierdt and Tan 1976).
Vassalos et al. (2010) reported an intriguing observation in favor of the pathogenicity of ameboid forms while they studied ST3 intra-subtype variations. In that study, a patient was a carrier of vacuolar and granular forms of ST3 without symptoms but became symptomatic quickly, shedding ameboid forms in his stool.
Proteases can result in secretory IgA splitting and supporting the parasite's survival through immune evasion (Parija and Jeremiah 2013), and provoke inflammatory cytokines (Puthia et al. 2008). Additionally, other hydrolytic enzymes have been detected through electrophoresis. For example, Lysates lead to cytoskeletal changes and stimulate apoptosis in epithelial cells, increasing bowel permeability. Cysteine proteases induce interleukin-8 production by mucosal cells, causing fluid loss and bowel inflammation in infected individuals (Parija and Jeremiah 2013).

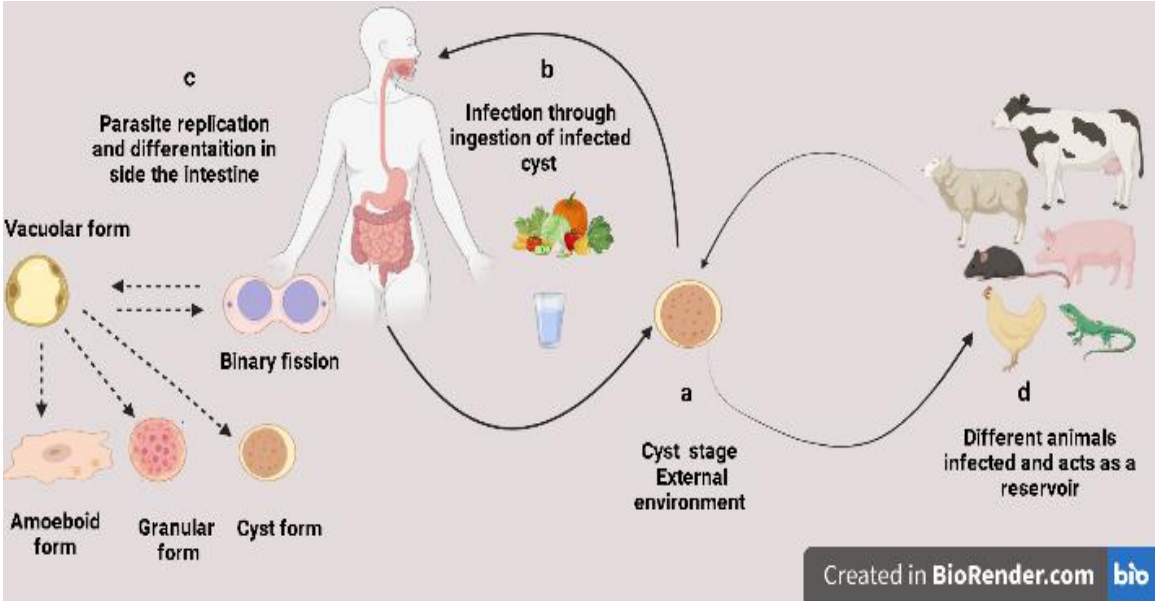

Fig. 1: Life cycle of *Blastocystis* spp.

Preliminary studies for defining the subtype pathogenicity stated a strong association of ST3 with symptomatic disease (Tan 2008). An investigation of ST3 from symptomatic isolates discovered that it typically possessed protease activity, particularly at 32 kDa, assumed to be a virulent element (Abdel-Hameed and Hassanin, 2011). Later, subtypes ST1, ST2, ST4, and ST6 were isolated from symptomatic patients (Nagel et al. 2012).

Intra-subtype pathogenicity variations have also been notable, meaning that some subtype strains can be nonpathogenic (Scanlan 2012), and subtyping alone cannot predict pathogenicity (Nagel et al. 2012).

Preceding studies reported *Blastocystis* association with IBS (Nourrisson et al. 2014), a complex functional bowel disorder (Longstreth et al. 2006). It has been proposed that IBS may be influenced by low-grade inflammation brought on by current immunological activation with *Blastocystis* infection, which provides prolonged antigenic exposure (Stark et al. 2007).

Blastocystis infection might also be associated with skin disorders, the ameboid form of ST3 was detected in acute urticaria cases, and it was believed that a disturbance in the immune homeostasis could trigger cutaneous symptoms (Katsarou-Katsari et al. 2008).

Furthermore, it has been proposed that luminal protozoa can cause allergy-like cutaneous lesions through activating specific cytokines by parasite molecules, such as interleukin 3 (IL-3)-, IL-4-, IL-5-, or IL-13 (Pasqui et al. 2004). According to previous theories, *Blastocystis* antigens induce T-helper 2 cells resulting in an IgE-mediated allergic reaction (Valsecchi et al. 2004; Yavasoglu et al. 2008). Eosinophils were assumed to have a direct role in urticaria pathology (Staumont-Sallé et al. 2006). Another theory supposed that *Blastocystis* might also trigger the complement pathway and production of anaphylatoxins C3a and C5a., These molecules interact with mast cells and basophils to stimulate histamine release and subsequently bring together skin-related disorders (Valsecchi et al. 2004).

8. CLINICAL SIGNS

Blastocystis-associated symptoms continue for 1–14 days and are usually self-limiting, but some untreated infections may last for months. The symptoms may be mild, moderate, severe, acute, or chronic (Roberts et al. 2011).

The host's immune status appears to be the primary risk factor for transmission of *Blastocystis* (Wawrzyniak et al. 2013). Immunodeficient/immunocompromised individuals are more vulnerable to *Blastocystis*, demonstrating opportunistic pathogenesis. Also, the occurrence and severity of clinical symptoms are related to the pathogen's density in the gut and the subtype's virulence (Matiut and Hritcu 2015).

Studies revealed that some individuals are more susceptible to infection. The parasite has been detected in HIV-infected individuals, cancer patients, immunodeficient patients, and frequent travelers. A higher prevalence of infection occurs in children in underdeveloped countries (Sekar and Shanthi 2015).

Intestinal colonization by *Blastocystis* is sometimes connected to asymptomatic conditions (Vassalos et al. 2008). Otherwise, nonspecific symptoms have been observed, such as nausea, anorexia, abdominal pain, bloating, and flatulence. Acute or chronic diarrhea, which may be mild and self-limited, and acute gastroenteritis, have been reported (Tan et al. 2010). There is also ulcerative colitis associated with inflammatory bowel conditions (Cekin et al. 2012). In addition to constipation and fatigue (Öner et al. 2022), vomiting and weight loss are other observed symptoms (Skotarczak 2018).

Reports imply the correlation of *Blastocystis* infection with IBS. When the intestine undergoes modifications, it favors the progression of the parasite (Sekar and Shanthi 2015). Also, its association with other conditions, such as nonspecific colitis and chronic IBD (including Crohn's disease and ulcerous colitis), has been documented (Özyurt et al. 2008; Basak et al. 2014).

There are extra-intestinal symptoms associated with *Blastocystis* infection, including acute or chronic urticaria (Gupta and Parsi 2006), palmoplantar pruritus (Kick et al. 2002), chronic angioedema (Micheloud et al. 2007), and joint pain (Cassano et al. 2005). However, *Blastocystis* infection is usually unrelated to animal clinical manifestation (Hublin et al. 2021).

9. DIAGNOSIS

Several diagnostic methods have been applied to detect *Blastocystis* infection, like direct examination of fecal smear, examination of iodine or trichrome-stained smear, concentration techniques using formalin-ether, *in vitro* cultivation, PCR, and sequencing (Wang et al. 2014).

9.1. MICROSCOPIC EXAMINATION

Conventionally *Blastocystis* detection was based on the clinical parasitological methods used to detect enteric parasites, including direct smear microscopic examination (Kukoschke et al. 1990).

Since the polymorphic forms of *Blastocystis* spp. can be present and range from 2µm to > 200µm in size (Celik et al. 2006). Variations in size make microscopic diagnosis in fecal samples challenging and require significant expertise. Furthermore, only the cyst and vacuolar forms can be detected in feces, while other forms are frequently lost during the processing of fecal samples (Navarro et al. 2008).

Ethyl acetate (formol-ether) concentration techniques have also been defined for diagnosing of *Blastocystis* infection (Suresh and Smith 2004). Nevertheless, incorrect identification can occur due to *Blastocystis* polymorphic structure and misinterpretation as fungi, *Cyclospora* spp., and fat drops (Sekar and Shanthi 2015).

Several staining procedures, including Giemsa, Gram, Wright, and iron hematoxylin, can be applied to detect *Blastocystis* forms (Stenzel and Boreham 1996). Staining of direct fecal smears with trichrome is a commonly used staining procedure. It is sensitive and provides permanent records for the sample (Tan 2008).

9.2. CULTURE METHOD

Culturing *in vitro* is a helpful diagnostic method for low parasite numbers (Suresh et al. 2005). This method was previously considered the gold standard for detecting *Blastocystis* (Popruk et al. 2013). Fecal samples are cultivated in supplemented Jones medium with 10% horse serum and incubated at 37 °C for 48h (Termmathurapoj et al. 2004). Following cultivation, the suspected colonies are examined through light microscopy (Basak et al. 2014). The culture method is more sensitive than direct microscopic examination for diagnosing *Blastocystis* from stool samples (Yakoob et al. 2010).

Blastocystis cells can be cultured on a solid medium, and the parasite clones resemble bacterial colonies microscopically. They can survive up to two weeks and longer in liquid or solid media (Tan et al. 2000). The cell densities reach maximal level around four days post-inoculation, move into the death phase at day five, and are challenging to subculture later on (Tan 2008).

Blastocystis isolates' pure cultures are vital for molecular and biochemical research. Antibiotic combinations can be added to the culture to obtain an axenic culture by eliminating bacteria and fungi. However, some isolates require bacterial existence for survival, and removing all bacteria can lead to the parasite's death (Nourrisson et al. 2014).

Although culture is a sensitive method, it is time overwhelming (2–3 days), and usually not performed in all laboratories. Additionally, culture methods may favor the growth of one subtype, leading to biased results (Navarro et al. 2008; Popruk et al. 2013).

9.3. SERODIAGNOSIS

Blastocystis infection can stimulate IgA, and IgG immune responses. Indirect immunofluorescent (IFA), and enzyme-linked immunosorbent assay (ELISA) can be used to detect antibodies (Mahmoud and Saleh 2003).

Secretory IgA, and serum IgA, and IgG levels in *Blastocystis*-positive people with and without symptoms were looked into by ELISA, and the data revealed that patients with symptoms had significantly higher IgA, and antibodies reactive to *Blastocystis* (Mahmoud and Saleh 2003). Furthermore, (Tasić et al. 2017) reported that symptomatic cases have elevated IgG titers and IgA response is weak or absent in asymptomatic infections.

9.4. MOLECULAR TECHNIQUES

The detection of *Blastocystis* has become more accessible with the advance of molecular methods (Sanggari et al. 2022). PCR targeting the SSU rRNA detects *Blastocystis* spp. effectively from stool (Yoshikawa et al. 2004a).

It has become prevalent owing to its capability to identify *Blastocystis* and its STs after sequencing (Wang et al. 2014). It provides high sensitivity and specificity for detecting the organism's DNA, and the SSU rRNA gene holds highly variable regions allowing phylogenetic analys*is* for *Blastocystis* (Rivera and Tan 2005; Dogruman-Al et al. 2008).

The advancement of real-time PCR for the sensitive and rapid detection of *Blastocystis* spp. and effective differentiation between the genotypes helps with screening and epidemiological studies (Tan 2008).

PCR-RFLP analysis is another technique for detecting of *Blastocystis* SS rRNA gene, which is professional for prevalence studies, and different primers have been described for parasite recognition from unpreserved stool samples (Stensvold et al. 2007a).

10. TREATMENT

In cases where diarrhea is persistent and *Blastocystis* is the only pathogen identified in stool specimens, treatment should be considered. Although infection might be self-limiting, several antimicrobial agents are available to treat *Blastocystis* infections (Sekar and Shanthi 2015).
Metronidazole is the first-line drug of choice for treatment. Nevertheless, *Blastocystis* elimination with this drug is between 0% to 100% (Sekar and Shanthi 2015). Trimethoprim-sulfamethoxazole is a second-line treatment in patients who may not tolerate or respond to metronidazole (Ok et al. 1999).
Treatment is not inevitably necessary in asymptomatic individuals, and detection of *Blastocystis* in symptomatic individuals demands a thorough investigation of other gastrointestinal disorders' causes. Initiating an antimicrobial therapy trial is justifiable in patients with persistent diarrhea, gastrointestinal symptoms, and many cysts in the stool, which might be a candidate for therapy. Furthermore, for those with *Blastocystis* in the stool and with an associated skin eruption, treatment should be considered after eliminating other causes (Coyle et al. 2012).
Paromomycin is a broad-spectrum antibiotic indicated for acute and chronic intestinal amebiasis. It is a successful therapy for *Blastocystis* cases linked to skin lesions, predominantly urticaria (Valsecchi et al. 2004). Other drugs like cotrimoxazole and nitazoxanide may also be applied (Tan 2008).

11. PREVENTION

The fecal-oral route is a major responsible route for the transmission of most *Blastocystis* infections. Hence, improved hygiene and sanitation conditions help prevent infections. Also, increased health awareness is essential in preventing parasitic diseases (Popruk et al. 2013).

12. CONCLUSION

Molecular epidemiological studies identified *Blastocystis* subtypes in different hosts globally. Contact with infected animals is a risk factor for the spread of zoonotic enteric protozoan parasites, including *Blastocystis*. Owing to the genetic diversity of the organisms, developing molecular techniques for genomic studies is essential for identifying genes responsible for the organism's virulence. Studies for establishing transmission outline and the host's specificity are also critical. Due to its zoonotic potential, investigating different subtypes in various hosts, including domestic and wild animals in different areas, is vital for recognizing possible animal reservoirs and finding a novel subtype. Health education, including good personal hygiene and improved sanitation, is highly recommended to prevent *Blastocystis* infection.

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Zoonotic Parasites and Food Safety: the Case of *Taenia Solium*

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ABSTRACT

This study provided a comprehensive overview of the zoonotic parasite Taenia solium, focusing on its biology, transmission, public health impact and control measures. Taenia solium, responsible for diseases such as taeniasis and cysticercosis, is significant due to its role in foodborne transmission and its status as the major global cause of acquired disease. We explored the broader context of zoonotic diseases, highlighting how changes in the environment, agriculture and urbanization contributed to their spread. It emphasized the importance of understanding the biological aspects of T. solium, including its habitats, food sources and transmission dynamics, to develop effective control strategies. The symptoms and diagnosis of taeniasis and cysticercosis were detailed, along with the epidemiological challenges in determining the true global burden of the disease. The role of food safety in controlling T. solium transmission was centered. We discussed the importance of pig rearing, pork handling and public education in breaking the parasite's life cycle. We also examined the traditional and modern control methods, including agricultural practices, sanitation improvements, use of pork and public health interventions. The use of antiparasitic drugs, diagnostic advancements, and potential of vaccines were explored as contemporary strategies to combat the parasite. Public health policies and regulations were highlighted as key elements in controlling T. solium, focusing on pork production, transmission control and community education. The study concluded by reflecting on the future of zoonotic parasite control, acknowledging the challenges posed by socio-cultural factors, resource constraints, and climate change.

Key words: Cysticercosis; Parasitism, Pork; Taenia solium; Taeniasis, Zoonosis.

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1. INTRODUCTION

In the intricate, interconnected web of life, the symbiotic and parasitic relationships between species form an intricate, often imperceptible matrix. When they result in the transmission of diseases, particularly zoonotic diseases, which are transmitted from animals to humans, the significance of these intricate relationships increases (Harms and Dehio 2012). Surprisingly, zoonotic diseases account for approximately 60% of emergent infectious diseases, with a significant proportion transmitted through foodborne routes. *Taenia solium* is the most prominent of these zoonotic parasites due to its global public health implications and close association with food safety (Gabriël et al. 2023).

Taenia solium is a cestode parasite whose lifecycle is dependent on two hosts: pigs, which serve as an intermediate host, and humans, which serve as the definitive host. This interspecies relationship not only aids the parasite's survival but also gives rise to two distinct diseases: taeniasis and cysticercosis. Taeniasis is a relatively innocuous intestinal infection caused by the consumption of raw or undercooked pork infected with cysticerci, the parasite's larval stage. In contrast, cysticercosis manifests as a severe condition when humans accidentally consume parasite ova. This ingestion causes the formation of cysticerci in human tissues, including the brain, resulting in neurocysticercosis, and a major cause of acquired epilepsy around the globe (García et al. 2003).

Geographically, the infection is more prevalent in developing regions with inadequate sanitation, where pigs may come into contact with human faces containing *T. solium* eggs due to their free-roaming nature. These regions include portions of Asia, Sub-Saharan Africa, and Latin America. With the advent of increased global travel and migration, however, sporadic cases have also emerged in more developed regions (Rahantamalala et al. 2022).

To effectively manage and control *Taenia solium* infection, a comprehensive understanding of its biology, transmission dynamics, and disease burden is required. To halt the spread of this formidable zoonotic parasite, it is necessary to implement stringent food safety measures, effective veterinary public health initiatives, and robust community education (Bethony et al. 2011). This parasite has a global footprint and thrives predominantly in regions where close human-pig interactions and pork consumption are common. Its impact is truly pervasive and far-reaching (Kabululu et al. 2023).

2. UNDERSTANDING ZOONOTIC DISEASES

The intersection of human and veterinary medicine, zoonotic diseases are infectious diseases that are transmitted naturally between vertebrate animals and humans. The pathogens that can cause these diseases include bacteria, viruses, fungi, and parasites, among others. The potential for zoonotic disease transmission has increased substantially within the context of an increasingly globalized world in which humans, animals, and the environment interact more closely. Changes in the environment, agricultural practices, and growing urbanization all contribute to the spread of these diseases (Rahman et al. 2022).

3. INFLUENCE OF FREQUENT ZOONOTIC PARASITES ON HUMAN HEALTH

A subset of these pathogens, zoonotic parasites significantly contribute to global disease burdens. *Toxoplasma gondii*, which causes toxoplasmosis, Plasmodium species, which causes malaria, and *Taenia solium*, the swine tapeworm, are notable examples. Among others, these parasites can cause severe morbidity and mortality, especially in immunocompromised people (Idro et al. 2022).

Toxoplasmosis, for example, typically causes moderate symptoms in healthy individuals, but can result in severe neurological complications in those with compromised immune systems. Malaria, which is transmitted by the bite of an infected mosquito, remains one of the world's deadliest diseases, afflicting

young children in Sub-Saharan Africa in particular. The pork tapeworm, *Taenia solium*, can induce neurocysticercosis, a major worldwide cause of acquired epilepsy (Furtado et al. 2011) (Table 1).

4. ROLE OF FOOD IN ZOONOTIC DISEASE TRANSMISSION

Dietary products derived from animals, play a crucial role in the transmission of zoonotic diseases. Many of these diseases are foodborne, which means that they are transmitted to humans via contaminated food. For instance, pork that is undercooked or raw and infected with *Taenia solium* cysticerci can cause taeniasis. Similarly, consuming contaminated food or water with *Toxoplasma gondii* oocysts can cause toxoplasmosis (Table 1). Food safety practices are essential for regulating and preventing zoonotic diseases given the significant role food plays in disease transmission. These practices include proper animal husbandry, hygienic slaughtering procedures, and cooking food at safe temperatures to eliminate pathogens (Rahman et al. 2020).

5. BIOLOGICAL ASPECTS OF *TAENIA SOLIUM*

5.1. LIFE CYCLE

As with many parasites, lifecycle of *T. solium* involves two hosts and multiple developmental stages. The adult tapeworm, which lives in the small intestine of a human host (definitive host), excretes ova in the feces. If these eggs are consumed by a pig (intermediate host), they hatch in the intestines of the pig, unleashing oncospheres. These oncospheres permeate the intestinal wall, infiltrate the bloodstream, and are transported to various tissues where cysticerci develop.

When a person consumes raw or undercooked pork harboring these cysticerci, the cysticerci larvae are released in the intestine. Attaching to the intestinal wall, they mature into adult tapeworms and begin a new life cycle. Uniquely, if a human ingests *T. solium* eggs (via fecal-oral route), the human can serve as the intermediate host, resulting in the development of cysticerci in human tissues and causing the severe disease known as cysticercosis (Flisser et al. 2010) (Fig. 1).

5.2. HABITATS AND FOOD SOURCES

T. solium is a cosmopolitan parasite whose distribution is influenced by cultural and agricultural practices, specifically pig farming and pork consumption. Pigs are able to consume the parasite's eggs because they have access to human feces in areas with inadequate sanitation.

The definitive host is humans, where the adult tapeworm resides in the small intestine. As the intermediate host, pigs harbor cysticerci in their tissues. In uncommon and accidental instances, humans can serve as intermediate hosts for *T. solium* by ingesting its eggs, resulting in cysticercosis (Prasad et al. 2007).

5.3. TRANSMISSION DYNAMICS

The transmission of *T. solium* is intrinsically linked to the organism's lifecycle and interaction with its hosts. Transmission to humans typically occurs when fresh or undercooked pork containing cysticerci is consumed. It can also occur via fecal-oral transmission when a human ingests *T. solium* ova, often due to poor hygiene practices or contaminated food or water.

Pigs become infected by consuming contaminated vegetation or water containing *T. solium* ova from human feces. Pigs that roam freely, which are prevalent in many endemic areas, are particularly susceptible to this mode of transmission (Del Brutto 2013).

Sociocultural factors, such as dietary practices, hygiene and sanitation levels, pig-rearing practices, and availability and utilization of healthcare and veterinary services, also influence the transmission dynamics. To effectively control and prevent *T. solium* infection, it is essential to comprehend these dynamics (Mlowe et al. 2022).

6. SYMPTOMS AND DIAGNOSIS OF TAENIASIS AND CYSTICERCOSIS

Due to their distinct phases of parasitic infection, taeniasis and cysticercosis, both caused by the zoonotic parasite *T. solium*, exhibit distinct symptoms and necessitate different diagnostic approaches.

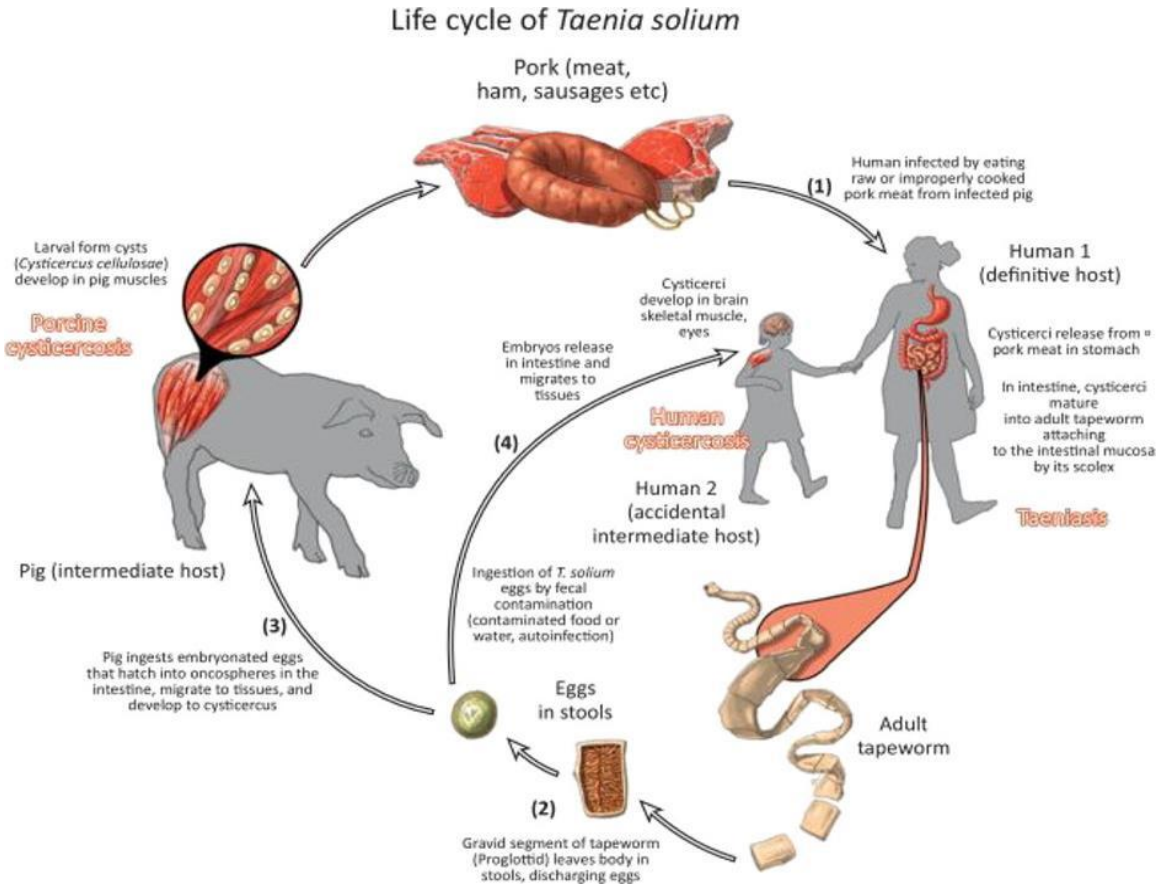

Fig. 1: Life cycle of *T. solium* (Aung and Spelman 2016).

6.1. TAENIASIS AND CYSTICERCOSIS SYMPTOMS

Taeniasis is an intestinal infection induced by adult tapeworms of the species *T. solium*. Those infected with taeniasis are frequently asymptomatic, exhibiting no obvious indications of infection. When symptoms do occur, however, they may include:

- Mild abdominal soreness
- Nausea
- Diarrhea or bowel irregularity
- Weight reduction
- Passage of proglottids in the stool (Kandi and Moses 2022).

Cysticercosis is caused by the ingestion of *T. solium* eggs and the subsequent establishment of larvae in tissues throughout the body. Depending on the location of the cysticerci, the symptoms vary. When the infection affects the central nervous system, it is known as neurocysticercosis, which can be severe and is characterized by the following symptoms:

- Headaches
- Seizures
- Visual impairments
- Hydrocephalus (brain fluid accumulation)
- Psychological disorders (Gripper and Welburn 2017).

Table 1: Common Zoonotic Parasites and their impact on human health

Parasite	Disease(s) Caused	Transmission Method	Impact on Human Health
Taenia solium	Taeniasis, Cysticercosis	Eating undercooked/raw pork	Digestive issues, neurocysticercosis, epilepsy
Echinococcus granulosus	Hydatid Disease	Ingesting contaminated food/water	Organ damage, potentially fatal complications
Trichinella spiralis	Trichinellosis	Eating undercooked meat	Digestive issues, fever, muscle pains, itchy skin

6.2. TAENIASIS AND CYSTICERCOSIS DIAGNOSIS

6.2.1. TAENIASIS

The identification of tapeworm ova or proglottids by microscopic examination of stool samples is frequently required for the diagnosis of taeniasis. However, because *T. solium* ova are indistinguishable from those of other Taenia species, a positive stool test cannot specifically identify *T. solium*.
Cysticercosis is more difficult to diagnose due to the fact that the symptoms are non-specific and the cysts can occur anywhere on the body. Methods of diagnosis may include:

6.2.2. IMAGING

MRI or CT scans are utilized to visualize lesions, especially in cases of neurocysticercosis.
Blood assays can detect antibodies against *T. solium*, indicating an active or past infection. In rare instances, a tissue biopsy may be required to corroborate the diagnosis (Mayta et al. 2000).

7. EPIDEMIOLOGY: DISTRIBUTION AND PREVALENCE OF *TAENIA SOLIUM*

T. solium is found worldwide, predominantly in areas with poor sanitation and close contact between pigs and humans. This includes portions of Asia, Sub-Saharan Africa, and Latin America. Due to increased global travel and migration, however, cases have also been reported in non-endemic areas.
T. solium, also known as pork tapeworm, is a parasite that is found worldwide, with the greatest prevalence observed in developing regions where pigs are raised in close proximity to humans and sanitation practices are inadequate (Galipó et al. 2021).

7. 1. LATIN AMERICA

T. solium infection is prevalent in Latin American rural communities. Mexico, Guatemala, Peru, and Bolivia are among the nations with a significant disease burden. The prevalence of the disease in these regions is influenced by the ingestion of pork that has not been fully cooked, the practice of open defecation, and the raising of pigs in a free-ranging environment (Hernández-Chea et al. 2023).

7. 2. SUB-SAHARAN AFRICA

T. solium is endemic to a number of countries in Sub-Saharan Africa, including Tanzania, Zambia, and Mozambique. Due to limited surveillance and reporting, the precise prevalence in many African countries is not well-documented, but the presence of suitable conditions for transmission (including small-scale, free-range pig farming and lack of sanitation infrastructure) suggests the disease burden could be substantial (Gulelat et al. 2022).

7. 3. ASIA

In Asia, *T. solium* is endemic to India, Nepal, China, and Vietnam, among others. High population densities, traditional pig-rearing practices, and dietary behaviors such as raw or undercooked pork consumption all contribute to the transmission of *T. solium* (Rajshekhar et al. 2003).

7. 4. NORTH AMERICA

Infections with *T. solium* are less prevalent in developed nations, such as North America and Europe. However, they are not unheard of and typically affect immigrant populations or travelers returning from endemic regions.

Despite significant efforts to contain the spread of *T. solium*, the parasite continues to affect millions of people around the world. The WHO identifies *T. solium*-caused neurocysticercosis as the primary cause of acquired epilepsy worldwide.

Notably, the precise global distribution and prevalence of *T. solium* remain difficult to ascertain due to underreporting, misdiagnosis, and limitations in surveillance systems, especially in resource-poor settings (Laranjo-González et al. 2017).

8. *TAENIA SOLIUM'S* INFLUENCE ON PUBLIC HEALTH

The effects of *T. solium* on public health are significant. Neurocysticercosis, the primary global cause of acquired epilepsy, can result in chronic illness, disability, and death. The economic burden resulting from treatment expenses and lost productivity is also substantial. Additionally, it influences agricultural economies because infected pigs are worth less on the market. Controlling and eliminating *T. solium* infections is crucial for public health, economic growth, and sustainable development (Butala et al. 2021).

9. ROLE OF FOOD SAFETY IN CONTROLLING T. SOLIUM TRANSMISSION

Ingestion of raw or undercooked pork contaminated with the parasite's larval stage, cysticerci, is a primary transmission route for *T. solium*, making food safety a crucial factor in preventing the spread of the disease. Food safety measures must be comprehensive, comprising everything from agricultural practices to consumer education.

9. 1. STRICT INSPECTION AND REGULATION

Regulatory agencies must enforce stringent inspection standards for swine. Regular testing for the presence of cysticerci in swine at slaughterhouses and the prevention of the sale and consumption of infected meat are crucial.

9. 2. EDUCATION AND PUBLIC AWARENESS

Public awareness campaigns emphasizing the risks of consuming raw or undercooked pork, the significance of thorough preparation, and safe food handling practices to prevent cross-contamination can significantly reduce transmission.

9. 3. SANITATION STANDARDS

Improving sanitation standards in environments and communities where pigs are raised and discouraging practices such as open defecation can prevent pigs from ingesting human feces containing *T. solium* ova.

9. 4. FOOD SAFETY POLICIES

The formulation and effective implementation of food safety policies at the local, national, and international levels can facilitate efforts to control and prevent the transmission of *T. solium* (Møller et al. 2022).

In addition, educating the public about the dangers of eating pork that is undercooked or raw and promoting safe food handling and preparation practices can significantly contribute to the control of *T. solium* infection. Despite the simplicity of these interventions, their implementation can be difficult and calls for the collaboration of farmers, food handlers, health authorities, and consumers.

Due to stringent regulations governing swine production and meat inspection, the risk of *T. solium* infection is typically low in developed areas. However, infection can still occur in immigrant populations and returning travelers from endemic regions. This demonstrates that food safety is a global issue, not just a local one.

We can reduce the incidence of taeniasis and cysticercosis by disrupting the lifecycle of *T. solium* by enhancing food safety measures. In this chapter, we will examine in detail the role of food safety in controlling *T. solium* transmission, as well as the steps required for safe swine production and the significance of public education and awareness (Saelens and Gabriël 2020).

10. ROLE OF PORK IN *T. SOLIUM* TRANSMISSION

T. solium is a zoonotic parasite known to induce taeniasis and cysticercosis in humans. Pork plays a central role in the transmission cycle of this parasite. Given that pigs are the primary intermediate carriers of this parasite, it is essential to comprehend their role in order to implement effective control strategies.

Pigs become infected with *T. solium* when they consume contaminated vegetation, soil, or water that contains *T. solium* ova from human feces. Once inside the pig, these ova develop into larvae, which then penetrate the intestinal wall and migrate to various tissues, including the muscles, where they form cysticerci - the parasite's larval stage. The cysts remain dormant in the tissues of the swine until the meat is consumed by humans.

When humans consume raw or undercooked porcine meat containing these dormant cysts, the risk of infection becomes significant. The cysticerci can develop into adult tapeworms in the human intestine, a condition known as taeniasis. When swine come into contact with feces containing tapeworm eggs, the transmission cycle is perpetuated.

Herein lays the heart of the issue: the close connection between pork consumption and the transmission of *T. solium*. In regions where pigs are raised in close proximity to humans and where sanitation facilities are insufficient or nonexistent, *T. solium* is likely to proliferate. This is exacerbated in regions where undercooked or uncooked pork is commonly consumed, a culinary practice that directly contributes to human infection.

To break the cycle of transmission, a multifaceted strategy is required. First, we must address the conditions under which swine are raised. Improving sanitation and preventing pigs from entering areas contaminated with human feces can substantially reduce the risk of infection among pigs. On the other hand, it is crucial to alter human behavior regarding swine consumption. By ensuring pork is prepared to a safe temperature, cysticerci can be killed and human infection can be prevented (Dixon et al. 2021).

11. SAFE PRODUCTION AND HANDLING PROCEDURES FOR PORK

Pig Rearing Methods: Improving conditions in pig farms, such as sanitation and feeding methods, can lower the risk of *T. solium* infection in piglets. The regular administration of anthelmintics to swine can eliminate any ingested *T. solium* eggs or larvae.

11. 1. INSPECTION OF MEAT

Thorough inspection of swine in slaughterhouses for the presence of cysticerci is essential. This includes physical inspection and laboratory testing to ensure that no infected meat reaches consumers (Sarti et al. 2017).

11. 2. SAFE PORK HANDLING AND PREPARATION

Educating food handlers and consumers on safe pork handling and preparation is essential. This includes proper storage, preventing cross-contamination in the kitchen, and cooking pork thoroughly to eliminate any cysticerci that may be present.
Adopting these practices can substantially reduce the risk of *T. solium* infection and contribute to the public health objective of eradicating this parasitic disease (Jayashi et al. 2012).

12. STRATEGIES FOR PREVENTION AND CONTROL

12.1. TRADITIONAL METHODS OF CONTROL AND THEIR EFFECTIVENESS

Traditional methods for regulating *T. solium* primarily involve modifying agricultural practices, enhancing sanitation, and administering anthelmintics (Table 2).

12. 2. AGRICULTURAL PRACTICES

This includes measures such as confining pigs to prevent them from having access to human feces and enhancing feeding practices.

12. 3. SANITATION

Sanitation improvements, particularly in endemic regions, can prevent swine from ingesting *T. solium* eggs present in human feces.

12.3.1. TREATMENT

Regular treatment of human populations with anthelmintics can reduce the prevalence of adult tapeworms and, as a result, reduce egg production (Ngwili et al. 2022).

Table 2: Strategies of prevention and control of *T. solium*

Strategy	Traditional/Modern	Effectiveness	Challenges
Improved Agricultural Practices	Traditional	Moderate	Implementation in remote areas
Vaccination of Pigs	Modern	High	Vaccine accessibility and affordability
Public Health Interventions	Both	High	Depend on political will and funding

Multiple strategies targeting various aspects of the parasite's lifecycle are required for effective *T. solium* infection prevention and control. These strategies involve the collaborative effort of healthcare providers, veterinarians, policymakers, and local communities, and span from traditional methods to modern techniques and scientific advances (Gilman et al. 2012).

Traditional methods of control frequently emphasize fundamental hygiene practices and changes in pig-rearing methods. The transmission of *T. solium* eggs can be substantially reduced by practicing good hand hygiene, particularly after using the lavatory and prior to handling food. Additionally, appropriate containment of human stool, especially in regions where open defecation is prevalent, is essential to prevent pigs from consuming the eggs. Modifying pig-rearing practices, such as confining piglets and providing them with clean food and water, to prevent their exposure to human stool can also be effective in breaking the transmission cycle (Hobbs et al. 2020; Kajuna et al. 2022).

Utilizing antiparasitic drugs for treatment and preventive chemotherapy, vaccinating swine, and enhancing meat inspection procedures are contemporary techniques for controlling *T. solium*. By eliminating adult tapeworms in humans, antiparasitic treatment can prevent the release of eggs that could infect swine. In endemic regions, routine preventive chemotherapy can also be an effective strategy. Vaccinating swine against *T. solium* has shown promising results in reducing infection prevalence in pig populations. In addition, enhancing meat inspection procedures to detect and discard infected pork can help prevent human infection (Hobbs et al. 2020).

The control of *T. solium* is largely dependent on public health policies and regulations. These may include policies promoting access to sanitation facilities and pure water, as well as laws regulating pig-rearing and meat inspection practices. Importantly, for these policies to be effective, they must be adequately enforced. Community education and awareness are among the most important aspects of disease prevention and control. Community education about the risks of *T. solium* infection, the significance of proper hand hygiene, the dangers of consuming undercooked or uncooked pork, and the advantages of improved pig-rearing practices can empower individuals to take preventative measures (Sakai et al. 2018).

The prevention and control of *T. solium* necessitate a multifaceted approach that addresses the lifecycle of the parasite, environmental factors, and human behaviors. These strategies can substantially reduce the burden of *T. solium* infections and improve public health despite the obstacles they face (Thomas et al. 2019).

12.3.2. MODERN CONTROL METHODS AND SCIENTIFIC DEVELOPMENTS FOR *TAENIA SOLIUM*

Modern techniques and scientific advancements have provided *T. solium* control with more robust and targeted instruments.

1. The development of TSOL18 and other vaccines for the prevention of porcine cysticercosis has shown promising results. This strategy can assist in breaking the parasite's life cycle.
2. Advanced diagnostic techniques, such as ELISA and PCR, can assist in the detection of *T. solium* infection in humans and animals with greater precision and sensitivity.

Due to scientific advancements, contemporary methods for controlling *Taenia solium* infections have evolved significantly. These strategies, which include mass drug administration, enhanced diagnostic tools, and possible vaccinations, demonstrate the progress made in addressing this public health concern.

The use of antiparasitic drugs, such as praziquantel and niclosamide, is one of the most extensively employed modern methods of control. These are used to treat taeniasis, which is an intestinal infection caused by adult *T. solium* tapeworms in humans. By treating infected individuals, the discharge of eggs in their feces is halted, preventing further environmental contamination and pig transmission. In areas where *T. solium* is endemic, mass drug administration (MDA) programs have been implemented to achieve a greater impact. These involve administering antiparasitic treatment to entire communities, regardless of whether or not specific individuals are afflicted. In areas where taeniasis prevalence exceeds a certain threshold, the World Health Organization recommends MDA (Lightowlers 2004).

In addition, the development and improvement of diagnostic instruments have contributed significantly to the management of *T. solium* infections. For example, serological assays such as Enzyme-Linked Immunosorbent Assay (ELISA) are used to detect antibodies against *T. solium* in human and pig populations, thereby assisting in determining the extent of the infection in a particular region. Advanced imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), have enhanced the diagnosis of neurocysticercosis in humans, allowing for prompt and effective treatment.

Vaccines represent one of the most promising developments in *T. solium* control. For piglets, a vaccine named TSOL18 has been developed, with promising results in trials. It prevents pigs from developing cysticercosis by inducing an immune response against the larval stage of *T. solium*, thereby preventing cysticercosis. The use of this vaccine in combination with MDA in humans and swine could potentially interrupt the lifecycle of the parasite and reduce the prevalence of *T. solium* in endemic regions.

Enhanced livestock inspection procedures, fueled by scientific advancements, also play a crucial role in *T. solium* control. Modern meat inspection techniques include meat biosensors and serological testing to detect *T. solium* cysticerci, allowing for more precise and dependable detection of infected swine. However, the implementation of these technologies requires resources and trained personnel, which may not be readily available in environments with limited resources.

Lastly, genetic studies of *T. solium* are ongoing in an effort to comprehend the parasite's genetic diversity and population structure. These studies could shed light on the transmission dynamics of the parasite and inform the creation of more effective control strategies.

Scientific advancements and modern techniques are transforming the landscape of *T. solium* control. While obstacles persist, especially in implementing these strategies in resource-poor, endemic settings, these developments offer promising avenues for reducing the burden of *T. solium* infections. In order to effectively combat this zoonotic parasite, a multifaceted strategy incorporating these modern techniques with traditional control methods and public health interventions will be essential (Samorek-Pieróg et al. 2018).

12.3.3. FUNCTION OF PUBLIC HEALTH REGULATIONS AND POLICIES

The function of public health policies in controlling *T. solium* is crucial (Table 3). This consists of:

12.3.4. REGULATION OF PORK PRODUCTION AND SALES

Policies that regulate swine breeding, meat inspection, and the sale of pork can aid in controlling the spread of infected meat.

12.3.5. TRANSMISSION CONTROL

Controlling the spread of the parasite requires government-led interventions such as mass drug administration campaigns, pig vaccinations, and sanitation development programs.

Table 3: Current Studies in Zoonotic Parasite Management

Research Area	Application	Future Prospects
Diagnostic Tools	Early detection of *T. solium*	Development of rapid, affordable tests
Vaccination	Prevention of porcine cysticercosis	Development of a vaccine for humans
Genomic Research	Understanding parasite's biology	New targets for drugs and vaccines

12.3.6. IMPORTANCE OF COMMUNITY EDUCATION AND SENSITIZATION

Community education is crucial for the prevention and control of *T. solium*. Awareness of the risks associated with poor sanitation, unsafe pork consumption, and the need for routine deworming can result in a change in behavior, thereby interrupting the cycle of *T. solium* transmission. Success requires regular community workshops, educational programs, and the incorporation of parasitic disease education into public health campaigns (Pray et al. 2020).

Regulations and policies pertaining to public health play a crucial role in the promotion and protection of community health. They guide the collective efforts of healthcare providers, communities, and governments towards enhancing health outcomes and reducing disease incidence.

In the context of zoonotic diseases such as *T. solium*, public health policies and regulations serve a number of essential purposes. First, they develop guidelines for best practices in sectors that have a direct impact on disease transmission. To reduce the likelihood of hogs ingesting *T. solium* eggs, for instance, policies could stipulate sanitation requirements in pig breeding facilities. Similarly, regulations could mandate meat inspection procedures to prevent the distribution of infected swine to consumers.

In addition, public health policies provide a structure for the implementation and coordination of large-scale interventions. The mass drug administration programs used to control *T. solium* in endemic areas are an outstanding example of this. The logistics of such programs, including who should be treated, how frequently, and how the medicines should be administered, are determined by policies.

Food safety standards enforcement is another essential function of public health regulations. These may include handling, preparation, and storage regulations for culinary products. In the case of *T. solium*, ensuring that pork is adequately prepared prior to consumption is essential for preventing taeniasis. Consequently, regulations may be enacted to educate food handlers on safe culinary techniques and ensure that restaurants adhere to certain food safety standards.

In addition, public health policies frequently direct research and development initiatives. They can priorities research areas, promote scientific collaboration, and fund studies aimed at enhancing disease control strategies. For example, policies may support research into novel diagnostic tools or potential *T. solium* vaccines.

Health education and awareness campaigns may be influenced by public health policies. These campaigns can educate the public about the dangers posed by *T. solium*, the significance of sanitation, the dangers of eating pork that has not been thoroughly cooked, and the significance of appropriate pig-rearing practices. For community participation in disease control efforts, education and awareness are indispensable.

Public health regulations and policies serve multiple purposes in preventing the spread of diseases such as *T. solium*. They provide a framework for best practices, direct large-scale interventions, enforce food safety standards, support research and development, and promote awareness and education. In doing so, they facilitate coordinated efforts for disease control and ultimately safeguard community health (Nyangi et al. 2022).

13. FUTURE OF ZOONOTIC PARASITE CONTROL: INNOVATIONS AND CHALLENGES

Future zoonotic parasite control holds both promise and difficulty. The increasing use of advanced technologies and integrated strategies promises more effective control and even eradication of parasites such as *T. solium*. However, significant obstacles persist.

13. 1. RESOURCE CONSTRAINTS

Many regions with a high prevalence of *T. solium* and other zoonotic parasites have limited resources, making it difficult to implement control measures.

13. 2. SOCIO-CULTURAL FACTORS

Socio-cultural factors, such as dietary customs and farming practices, can be formidable obstacles to zoonotic parasite control. For example, the practice of free-range pig husbandry and the consumption of pork that is not fully cooked can aid in the spread of *T. solium*.

13. 3. PUBLIC HEALTH INFRASTRUCTURE

Inadequate public health infrastructure and regulatory systems in certain regions may impede the effective control and surveillance of zoonotic parasites.

13. 4. CLIMATE CHANGE

Changes in climate can affect the distribution of parasites, while increased global travel and trade can facilitate the spread of these parasites to new regions.

13. 5. ONE HEALTH APPROACH

Addressing these challenges necessitates One Health approach, recognizing that the health of humans, animals, and our shared environment are interconnected. We can create more effective strategies for zoonotic parasite control and ensure a secure future for all by integrating efforts across multiple sectors and disciplines (Elsheikha 2014).

14. CONCLUSION

The intertwined nature of human, animal, and environmental health highlights the urgent need for comprehensive strategies to combat zoonotic diseases, especially parasites such as *T. solium*. These parasites pose a significant threat to public health, particularly in regions with limited resources, and highlight the profound implications of our food systems and behaviors for the emergence and transmission of disease. Despite advances in our understanding of the biology of *T. solium* and development of a variety of control measures, obstacles remain. Future efforts should concentrate on integrating sanitation improvements, secure food handling procedures, robust public health policies, community education, and continued research. As we face challenges such as socio-cultural factors, resource constraints, and global phenomena such as climate change and globalization, One Health

approach - recognizing the interconnectedness of human, animal, and environmental health - is essential for achieving sustainable control of zoonotic parasites and ensuring a healthier future for all.

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Cryptosporidium Transmission Dynamics: Bridging the Gap between Wildlife and Urban Environments

24

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ABSTRACT

Cryptosporidium, a coccidian apicomplexan protozoan, causes cryptosporidiosis, a widespread intestinal infection in humans and animals through zoonotic and anthroponotic transmissions. This parasite exhibits a complex life cycle, with both sexual and asexual stages, adapting morphologically to complete its cycle. Cryptosporidium comprises various species infecting a multitude of hosts, including mammals, birds, reptiles, amphibians, and fish. Notable species like C. hominis and C. parvum are frequent causes of human cryptosporidiosis. The parasite's oocysts are highly resilient, remaining viable for months in various environments. Cryptosporidiosis manifests differently across hosts, leading to symptoms such as diarrhea, vomiting, and weight loss. Zoonotic potential exists, with transmission routes encompassing waterborne, foodborne, and direct contact. Wildlife serves as a reservoir for zoonotic pathogens, including Cryptosporidium, with potential transmission to humans. Urban environments, especially those with inadequate sanitation, may foster Cryptosporidium transmission through contaminated water, food, and direct contact with infected animals. Understanding transmission dynamics is crucial, especially in regions with high population density and potential outbreaks. Effective water treatment is pivotal for preventing waterborne cryptosporidiosis, highlighting the importance of maintaining and improving sanitation facilities in urban areas. Travelers to developing countries face elevated risks, emphasizing the need for public health measures to mitigate Cryptosporidium infections.

Keywords: Cryptosporidium, Cryptosporidiosis, Zoonotic transmission, Waterborne transmission, Urban environments

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1. INTRODUCTION

Cryptosporidium is one of the most common intestinal parasites (Shirley et al. 2012). It is coccidian, oocysts-forming apicomplexan protozoa, which completes their life cycle in humans and animals through zoonotic and anthroponotic transmissions and causing mild to severe disease (Putignani and Menichella 2010). One of the most significant illness that causes an intestinal infection in both humans and animals is cryptosporidiosis (Bamaiyi and Redhuan 2017). It was first discovered in 1907 by Tyzzer (Tzipori and Widmer 2008). At the start it was considered as nonpathogenic until two human cases of cryptosporidiosis were reported (Khalil et al. 2018). It has been stated that 60% of them were the most prevalent protozoan parasites responsible for food and waterborne disease outbreaks globally between 2004-2010. Animal become infected by ingesting contaminated food and water containing oocyst of parasite (Baldursson and Karanis 2011).

Cryptosporidium is a microscopic parasite that causes the diarrheal disease collectively (parasite and disease) called as “Crypto”. From a variety of vertebrates, including humans, wildlife, mammals, domestic livestock, birds, reptiles, amphibians, and fish, a total of 23 species and 61 genotypes of *Cryptosporidium spp.* have been described (Ryan et al. 2014). *Cryptosporidium (C.) canis, C. meleagridis, C. suis, C. muris, C. andersoni*, and *C. felis* are few species that have been isolated from immunocompromised people (Fayer 2010) while *C. hominis, C. parvum*, and *C. meleagridis* are the three species of cryptosporidium that infect humans most frequently; however, these two species account for more than 90% of cases of cryptosporidiosis (Xiao and Ryan 2004).

It's fascinating to note that as of early 2013, 155 different species of mammals were known to be non-human sources of *C. parvum* (Slapeta 2013). This shows how these parasites are evolving and adapting to infect a variety of hosts and pose a serious threat to zoonotic diseases. Animal and human cases of cryptosporidiosis are frequently reported from a number of states, including Vietnam, Indonesia, Malaysia, Cambodia, Thailand, the Philippines, and Laos (Mahdy and Surin 2013). In Thailand, 11% river water and 6% ocean water has been contaminated with *Cryptosporidium spp.* (Koompapong and Sukthana 2012). However, some species (*C. parvum, C. muris, C. felis, C. meleagridis, C. hominis, C. canis*) have been isolated from HIV/AIDS cases in Bangkok (Srisuphanunt et al. 2011) with the prevalence rate of 19-34% from 1996-2009 (Berger 2017).

Although the prevalence of cryptosporidiosis is increasing in Europe due to climate change, such as heavy rains or floods that contaminate drinking water, the disease is also becoming more common among children and immune-compromised adults in many American countries, including Costa Rica, Brazil, Argentina, and the United States. However, *C. hominis* was found to be the most prevalent pathogen, and over time, reports of cryptosporidiosis have increased (Bamaiyi and Redhuan 2017). In Africa, cryptosporidium species are linked to severe diarrhea, mortality, and slow child growth. Additionally, the high frequency of the contagious disease in this region may be attributed to HIV/AIDS epidemic and the nutritional situation in some African regions (Squire and Ryan 2017).

Due to rising veterinary service and labor costs, cryptosporidiosis in livestock is becoming a serious issue for both animal health and financial losses (Santin 2013). Thailand's livestock previously had cryptosporidiosis rates of 31.5%, 5.7%, and 8.7% in dairy farms, individual animals, and dairy herds, respectively (Jittapalapong et al. 2016). Additionally, *C. parvum* was the most prevalent species in livestock animals, and 30% of buffalo farms had *Cryptosporidium spp.* infections (Inpankaew et al. 2014). It (cryptosporidiosis) was discovered in 2.1% of dogs and 2.5% of cats in other animals. There are about 1% of long-tailed macaques in Thailand that live in close proximity to people, but despite the low prevalence of oocysts, they pose a serious risk to humans (Sricharern et al. 2016).

2. CLASSIFICATION AND TAXONOMY

Domain: Eukaryota
Clade: Diaphoretickes
Clade: Alveolata
Phylum: Apicomplexa
Class: Conoidasida
Order: Eucoccidiorida
Suborder: Eimeriorina
Family: Cryptosporidiidae
Genus: *Cryptosporidium* (Chalmers et al. 2019).
Cryptosporidium has been found in a variety of animals and total 14 species seem to be most widely distributed that infect animals and humans. In the past, it was believed that *C. parvum* was the primary cause of infections in wild mammals (Xiao et al. 2004).

3. MORPHOLOGY AND LIFE CYCLE

Cryptosporidium has a complex life cycle having both sexual and asexual stages with monoxenous cycle but it changes to many morphological forms to complete its life cycle. It's life cycle begins when oocysts, or infective eggs, are ingested by a host. The oocysts can be found in contaminated water, food or feacal matter. Once inside the host, the oocysts release sporozoites, which then penetrate the host's intestinal lining and begin to reproduce asexually. During this phase, the parasite exists as a trophozoite, which is an active, feeding stage. The trophozoites multiply by binary fission, producing large numbers of meronts, which are specialized reproductive cells. These meronts then undergo further development to produce more oocysts, which are then shed in the host's feaces to start the cycle again. The oocysts of Cryptosporidium are highly resistant to environmental stressors and can remain viable for months in water, soil and other environments. This makes them highly effective at spreading the infection, as contaminated water or food can infect new hosts. When host ingest the infective oocyst; excystation occurs to release the four sporozoites (Fig. 1) (Bouzid et al. 2013).
The size of cryptosporidium oocysts is around 4-6µm and are rounded. Mature oocysts contain 4 sporozoites but no sporocyst. The sporozoites are spindle shaped and nucleated. The apical complex is the unique feature of apicomplexan parasites which mediates host penetration and attack. Intestine and stomach cells are among the host cells that sporozoites can recognize and enter (Fayer 2010). The sporozoites that have been invaded come from parasitephorous vacuoles, where they can develop into the trophozoite stage. The trophozoites initiate three merogoney-style mitotic divisions to produce type I meronts (Cacciò and Widmer 2013). It can produce type I (8 merozoites) or type II (4 merozoites) meronts through asexual reproduction. Microgamont (male) and macrogamont (female) are the sexually reproducing forms of type II merozoites. 16 rod-shaped, non-flagellated microgametes measuring 1.4 mm by 0.5 mm are produced by each microgamont. It get fertilized with a unicellular adjacent macrogamont, which is a spherical to oval structure with a large central nucleus and a diameter of 4-6 µm. After two mitosis divisions, the zygote either forms a thin-walled oocyst with a single layer of membrane or a thick-walled oocyst with two layers of membrane present. Contrary to thick-walled oocysts, which are released through faeces and can endure an unsuitable environment for months, thin-walled oocysts can cause reinfection with the gastrointestinal tract of the same host by rupturing and releasing infectious sporozoites (Tzipori and Ward 2002).

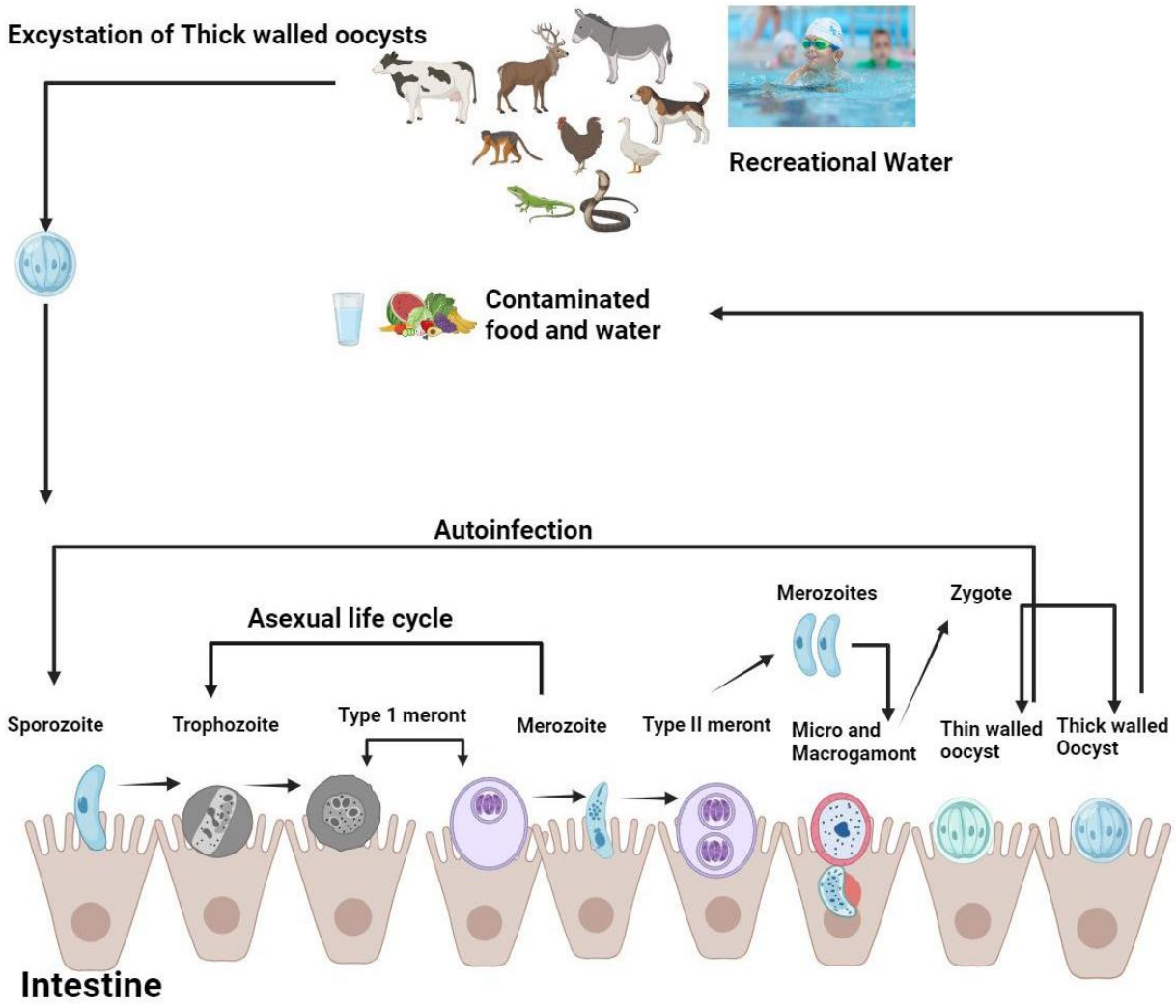

Fig. 1: Life cycle and development stages of Cryptosporidium in wildlife and humans

4. HOST ASSOCIATIONS OF CRYPTOSPORIDIUM/SYMPTOMS IN WILDLIFE

Cryptosporidium is a microscopic parasite that can infect a wide range of animals, including wildlife. The symptoms of cryptosporidium infection can vary depending on the severity of the infection and the species of animal affected. These are just a few examples of many host associations of Cryptosporidium. The parasite is widespread in the environment and can infect a wide variety of hosts, including both warm-blooded and cold-blooded animals. Host association refers to the relationship between a parasite and its host, which can range from a highly specific association to a broad range of hosts (Pinto and Vinayak 2021).

4.1. *CRYPTOSPORIDIUM ANDERSONI*

C. andersoni infects the digestive system of cattle and other ruminants. It is commonly found in the feaces of infected animals and can be transmitted through contaminated water or food. As a host-specific parasite, *C. andersoni* has a limited range of hosts that it can infect. Its main host is cattle but it

has also been found in other ruminants including sheep and goats. In cattle, its infection can cause diarrhea, weight loss and reduced feed intake. Calves are especially vulnerable to infection and in severe cases, the infection can lead to dehydration and death. Diarrhea caused by *C. andersoni* can be profuse and watery, often containing blood or mucus. Other symptoms of infection in cattle include abdominal pain, fever and decreased milk production in lactating cows (Jiang et al. 2014).

4.2. *CRYPTOSPORIDIUM BAILEYI*

C. baileyi infects birds particularly poultry and is a significant cause of respiratory and enteric diseases in these animals. It has been reported in a variety of domestic and wild bird species, including chickens, turkeys, ducks, geese, quails, pheasants, pigeons and sparrows. It is also commonly found in free-living waterfowl, such as ducks and geese and is a common cause of mortality in young waterfowl (Wang et al. 2011). The disease caused by *C. baileyi* infection is known as avian cryptosporidiosis. In birds, infection can cause diarrhea, weight loss and reduced egg production. The diarrhea caused by the infection can be watery and contain mucus or blood. Birds infected with this may also show signs of abdominal pain, decreased appetite and lethargy (Wang et al. 2021).

4.3. *CRYPTOSPORIDIUM CANIS*

C. canis infects wide range of host species, including dogs, cats and humans. The main host of this species is the domestic dog and the parasite is commonly found in puppies and young dogs that have not yet developed a strong immune system. However, *C. canis* has also been reported in other animals, including cats, pigs, cattle and horses. In dogs, *C. canis* infection cause diarrhea, vomiting and weight loss. The infection may also cause abdominal pain, decreased appetite and lethargy. In puppies, the infection can be severe and can lead to dehydration. *C. canis* infection can also be zoonotic in nature that can be transmitted from infected dogs to humans (Gonzalez-Díaz et al. 2016).

4.4. *CRYPTOSPORIDIUM FELIS*

C. felis infects cats and other feline species and the disease caused by *C. felis* is known as feline cryptosporidiosis. In cats, *C. felis* infection can cause diarrhea, vomiting and weight loss. Diarrhea can be watery and may contain blood and mucus. The infection may also cause abdominal pain, decreased appetite and lethargy. In kittens, the infection can be severe and can lead to dehydration. *C. felis* infection can also be zoonotic in nature (Fayer et al. 2006).

4.5. *CRYPTOSPORIDIUM HOMINIS*

C. hominis is common cause of cryptosporidiosis (a diarrheal disease) in humans. It can infect a variety of hosts, including livestock, wildlife and companion animals. Infection with *C. hominis* typically occurs through ingestion of contaminated water and food, and can cause symptoms such as diarrhea, stomach cramps and fever in humans. It is a common cause of diarrhea in both developing and developed countries especially in people with weak immune system such as those with HIV/AIDS or cancer. The infection can be more severe and prolonged. It can also lead to a more serious form of diarrhea known as chronic diarrhea which can cause malnutrition, weight loss and further complications. It can also be transmitted from infected humans to animals and vice versa. However, human-to-human transmission is the most common route of infection (Morgan-ryan et al. 2002).

4.6. *CRYPTOSPORIDIUM MELEAGRIDIS*

It primarily infects birds, including domestic turkeys and chickens, as well as wild birds such as pheasants and quail. It has also been found in other animals such as cattle and humans. Infection in birds can cause diarrhea and other digestive symptoms, while infection in humans can cause similar symptoms as well as other complications in individuals with weakened immune systems. Birds infected with *C. meleagridis* may also show signs of abdominal pain, decreased appetite and lethargy (Silverlås et al. 2012).

4.7. *CRYPTOSPORIDIUM MURIS*

C. muris infects rodents including mice and rats. In the case of *C. muris* the parasite has a relatively narrow host association, as it mainly infects rodents, but it has also been found in other animals such as pigs and humans. Infection in rodents may cause diarrhea which contains blood and mucus. Infection can occur through ingestion of contaminated food, water or direct contact with infected rodents or their faeces (Wang et al. 2023).

4.8. *CRYPTOSPORIDIUM NASORUM*

C. nasorum infects cattle, specifically affecting the nasal cavity and sinuses. In this case parasite has a narrow host association, as it primarily infects cattle and has not been found to infect other animal species or humans. Infection can cause symptoms such as nasal discharge and inflammation and can be transmitted through contact with contaminated materials, such as feed or water, or direct contact with infected animals (Appelbee et al. 2005).

4.9. *CRYPTOSPORIDIUM PARVUM*

C. parvum can infect a wide range of mammalian species, including humans and livestock. In the case of *C. parvum*, the parasite has a broad host association, as it can infect multiple animal species and is one of the most common causes of waterborne disease in humans (Delafosse et al. 2015). In wildlife, cryptosporidiosis can cause diarrhea, dehydration, weight loss, and lethargy. Animals may also show signs of abdominal pain and discomfort, decreased appetite and a rough or unkempt coat. In severe cases, the infection can lead to death. In some species, such as deer and elk, cryptosporidiosis may not cause any visible symptoms making it difficult to detect and control the spread of the disease (Davis et al. 2022).

4.10. *CRYPTOSPORIDIUM MOLNARI*

C. molnari infects fish specifically affecting the gastrointestinal tract. The parasite has a narrow host association as it primarily infects fish and has not been found to infect other animal species or humans. Infection can cause symptoms such as intestinal inflammation, diarrhea, difficulty swimming and can be transmitted through ingestion of contaminated water or infected fish (Couso-Perez et al. 2022).

4.11. *CRYPTOSPORIDIUM SAUROPHILIUM*

C. saurophilium infects reptiles, specifically affecting the gastrointestinal tract. In this case, parasite has a narrow host association, as it primarily infects reptiles. Infection can cause symptoms such as diarrhea, weight loss and lethargy. The diarrhea caused by the infection can be watery and may contain mucus or

blood. Infected reptiles may also show signs of abdominal pain, decreased appetite and difficult moving. The severity of the symptoms in wild reptiles may vary depending on the species (Appelbee et al. 2005).

4.12. *CRYPTOSPORIDIUM SERPENTIS*

C. serpentis infects gastrointestinal tract of snakes. This parasite has a narrow host association. Infection can cause symptoms such as diarrhea and weight loss. The diarrhea caused by the infection can be watery and may contain mucus or blood. Infected snakes may also show signs of abdominal pain, decreased appetite, and difficult moving (O'Rourke and Lertpiriyapong 2015).

4.13. *CRYPTOSPORIDIUM WRAIRI*

C. wrairi infects South American camelids such as llamas and alpacas, specifically affecting their gastrointestinal tract. Infection can cause symptoms such as diarrhea and can be transmitted through ingestion of contaminated food or water, or direct contact with infected South American camelids or their faeces. Infected mammals may also show signs of abdominal pain, decreased appetite, and difficult moving (Nazifi et al. 2010).

4.14. *CRYPTOSPORIDIUM GALLI*

C. galli mainly infects the birds. The parasite has a narrow host association. In birds, *C. galli* infection can cause diarrhea, weight loss and lethargy. Infected birds may also show signs of abdominal pain, decreased appetite, and difficult moving (Wang et al. 2021).

4.15. *CRYPTOSPORIDIUM SUIS*

C. suis infects pigs specifically affecting the gastrointestinal tract. Infection can cause symptoms such as diarrhea and weight loss. The diarrhea caused by the infection can be watery and contains mucus and blood. Infected pigs may also show signs of abdominal pain, decreased appetite and difficult moving. Young pigs, especially those less than three weeks old, are more susceptible to severe infection and may experience more severe symptoms and complications, such as dehydration and secondary bacterial infections (Bodager et al. 2015).

5. WILDLIFE AS A SOURCE OF ZOONOTIC INFECTIONS

Wildlife can serve as a reservoir for many zoonotic pathogens, including viruses, bacteria and parasites, which can be transmitted to humans through direct contact, consumption of contaminated food, water or through the bite of an infected animal. Some examples of zoonotic diseases that can be transmitted from wildlife to humans include (Kruse et al. 2004).

5.1. RABIES

Rabies is a viral infection that is transmitted to humans through the bite of an infected animal, such as a bat, raccoon or fox. Wildlife, particularly bats, are common reservoirs for the virus (Bilal 2021).

5.2. LYME DISEASE

Lyme disease is caused by a bacterium and transmitted to humans through the bite of an infected tick. Ticks can become infected by feeding on infected wildlife such as deer and rodents (Ogden et al. 2008).

5.3. HANTAVIRUS

Hantaviruses are a group of viruses transmitted to humans through contact with the urine, faeces or saliva of infected rodents. Deer and mice are a common reservoir for hantaviruses (Avsic-Zupanc et al. 2019).

5.4. AVIAN INFLUENZA

It is a viral infection that is mainly found in birds and it can be transferred to humans. Wild birds, such as waterfowl are a common reservoir for the virus (Kim et al. 2016).

5.5. CRYPTOSPORIDIOSIS

Cryptosporidiosis is a parasitic infection that is often associated with contaminated water sources, but can also be transmitted through contact with infected animals. Wildlife, such as deer and other ungulates are known to carry the parasite (Baldursson and Karanis 2011).
Wildlife can harbor Cryptosporidium in their digestive systems and shed the parasite in their faeces, which can contaminate the environment and potentially infect humans who come into contact with the contaminated material. This can happen through direct contact with wildlife i.e., when people handle or consume infected meat or indirect contact i.e., when contaminated water sources are used for drinking or recreational activities (Kruse et al. 2004).

6. UNDERSTANDING THE TRANSMISSION DYNAMICS FROM URBAN ENVIRONMENTS TO HUMANS

There are 31 species of cryptosporidium that have been recognized while *C. hominis* and *C. parvum* are most common species that cause infection in humans (Zahedi et al. 2016). Cryptosporidium is commonly found in urban environments, particularly in areas with inadequate sanitation and water treatment facilities. In urban environments, it can be transmitted through contaminated water, such as untreated or poorly treated drinking water, recreational water and wastewater. Contaminated water sources can become infected with Cryptosporidium when the parasite is shed by infected animals, including domestic livestock and wildlife, and then enters the water system. The manure from farms and runoff from urban areas can carry Cryptosporidium into rivers, lakes and groundwater sources, which can then contaminate drinking water supplies. It can also be transmitted through contaminated food, especially if the food has been grown or prepared in unsanitary conditions or comes into contact with contaminated water (Xiao et al. 2022).
In addition to water sources, Cryptosporidium can also be transmitted in urban environments through direct contact with infected animals, such as through exposure to their faeces or by ingesting contaminated food products. Because of high population density in urban areas and the potential for large outbreaks, it is important to ensure that urban water treatment facilities are properly designed and maintained to effectively remove or inactivate Cryptosporidium from drinking water supplies (Xiao et al. 2022). Cryptosporidium can be transmitted from animals to humans and person to person through the faeco-oral route. This can happen when an infected person doesn't wash their hands after using the bathroom and then comes into contact with another person or a surface that another person touches. This is particularly common in settings where animals are kept close to humans, such as farms or zoos. Travelers to developing countries are at a higher risk of Cryptosporidium infection due to poor sanitation and contaminated food and water sources (Ungar 2018). Table 1 shows the valid species of cryptosporidium, their hosts and reports in humans.

7. DIAGNOSIS AND DETECTION IN HUMANS

Accurate diagnosis of cryptosporidiosis is necessary to initiate appropriate treatment. Although the infection is self-limiting in healthy individuals, severe and prolonged symptoms can occur in those with HIV/AIDS or undergoing chemotherapy. Treatment with specific antimicrobial agents, such as nitazoxanide, can be effective in reducing the duration and severity of symptoms. Early diagnosis of an infected individual can help prevent further transmission. Cryptosporidiosis can be a significant cause of morbidity and mortality in certain populations, particularly in developing countries. Accurate diagnosis and monitoring of the incidence and prevalence of infection can help guide public health policies and interventions aimed at reducing the burden of disease (Smith 2007).

7.1. MICROSCOPY

The diagnosis of cryptosporidiosis can be made through microscopy, which involves visualizing the parasite in a stool sample. To diagnose cryptosporidiosis by microscopy, a fresh stool sample should be collected and processed using a concentration technique such as formalin-ethyl acetate sedimentation or zinc sulfate flotation. The concentrate is then examined under a microscope using a specialized staining method such as acid-fast staining, which allows the Cryptosporidium oocysts to be easily distinguished from other faecal particles (Smith 2007).

7.2. ELECTRON MICROSCOPY

Electron microscopy (EM) is a highly specialized and sensitive diagnostic tool that can be used in the diagnosis of Cryptosporidium infection. EM uses a beam of electrons to create highly magnified images of the specimen at a much higher resolution. This allows for the visualization of much smaller structures such as the internal structures of Cryptosporidium oocysts which cannot be seen with conventional light microscopy. EM is particularly useful for detecting low-level infections or for confirming the presence of Cryptosporidium in cases where other diagnostic tests such as acid-fast staining or immunological assays have produced ambiguous or inconclusive results (Ahmed and Karanis 2018).

To use EM for the diagnosis of Cryptosporidium, a stool sample is collected and processed using a concentration technique, such as formalin-ethyl acetate sedimentation or sucrose gradient centrifugation, to enrich the number of oocysts in the sample. The concentrated sample is then fixed in a chemical solution, such as glutaraldehyde to preserve the morphology of the oocysts. The fixed sample is then dehydrated, embedded in a resin and sliced into ultrathin sections, which are stained with heavy metals such as lead citrate and uranyl acetate to enhance contrast and create a detailed image of the oocysts. Under the EM Cryptosporidium oocysts appear as small, round to oval-shaped structures with a characteristic double-layered wall and internal structures are known as sporozoites (Khurana and Chaudhary 2018).

Although EM is a powerful diagnostic tool yet it is expensive and requires specialized equipment and expertise which makes it less widely available than other diagnostic methods. It is typically reserved for use in research or specialized diagnostic laboratories (Ahmed and Karanis 2018).

7.3. IMMUNOLOGICAL METHODS

Immunological methods are also used to diagnose Cryptosporidium infection particularly in cases where traditional microscopy techniques have produced ambiguous or inconclusive results. These methods rely on the detection of Cryptosporidium-specific antigens or antibodies in patient samples i.e., stool or serum.

Table 1: Valid species of cryptosporidium; hosts and reports in humans

Sr. #	Species Name	Author	Host type	Major Host	Reports in Humans
1.	*C. muris*	Tyzzer (1907, 1910)	Mice	Rodents	Numerous reports (Feng et al. 2011b)
2.	*C. wrairi*	Vetterling et al. (1971)	Guinea pig	Guinea pigs	None reported
3.	*C. felis*	Iseki (1979)	Cat	Cat	Many reports (Lucio-Forster et al. 2010)
4.	*C. serpentis*	Levine (1980)	Lizards, snakes	Lizards, snakes	None reported
5.	*C. meleagridis*	Slavin (1955)	Tuckey	Humans, birds	Commonly reported in humans
6.	*C. parvum*	Upton and Current (1985)	Cattle	Ruminants	Commonly reported in humans Tyzzer (1912)
7.	*C. baileyi*	Current et al. (1986)	Chicken	Birds	Not reported
8.	*C. varanii*	Pavlasek et al. (1995)	Lizards	Lizards	Not reported
9.	*C. andersoni*	Lindsay et al. (2000)	Cattle	Cattle	Leoni et al. (2006); Morse et al. (2007); Waldron et al. (2011); Agholi et al. (2013); Liu et al. (2014)
10.	*C. canis*	Fayer et al. (2001)	Dog	Dogs	Many reports (Lucio-Forster et al. 2010)
11.	*C. molnari*	Alvarez-Pellitero and Sitja-Bobadilla (2002)	Fish	Fish	Not reported
12.	*C. hominis*	Morgan-ryan et al. (2002)	Humans	Humans	Most common species in humans
13.	*C. galli*	Re: Ryan et al. (2003c): Pavlásek (1999) a	Birds	Birds	None reported
14.	*C. suis*	Ryan et al. (2004)	Pig	Pigs	Xiao et al. (2002a); Leoni et al. (2006); Cama et al. (2007); Wang et al. (2013)
15.	*C. bovis*	Fayer et al. (2005)	*Cattle*	Cattle	Khan et al. (2010); Ng et al. (2012); Helmy et al. (2013)
16.	*C. xiaoi*	Fayer et al. (2010)	Sheep	Sheep	Adamu et al. (2014)
17.	*C. ryanae*	Fayer et al. (2008)	Cattle	Cattle	Not reported
18.	*C. macropodum*	Power and Ryan (2008)	Kangaroo	Marsupial	None reported
19.	*C. fragile*	Jirku et al. (2008)	Toad	Toads	None reported
20.	*C. fayeri*	Ryan et al. (2008)	Kangaroo	Marsupial	Waldron et al. (2010)
21.	*C. ubiquitum*	Fayer et al. (2010)	Cattle	Rodents, ruminants and rodents	Commonly reported (Fayer et al. 2010; Elwin et al. 2012a)
22.	*C. cuniculus*	Re: Robinson et al. (2010)	European rabbit	Rabbits	Chalmers et al. (2009a); Anonymous (2010); Chalmers et al. (2011a)
23.	*C. tyzzeri*	Ren et al. (2012)	Mouse	Rodents	Raskova et al. (2013)
24.	*C. erinacei*	Kvac et al. (2014b)	European hedgehog..	Hedgehogs	Insulander et al. (2013)
25.	*C. scrofarum*	Kvac et al. (2013b)	Pig	Pigs	Kvac et al. (2009a); Kvac et al. (2009b)
26.	*C. viatorum*	Elwin et al. (2012b)	Humans	Humans	Insulander et al. (2013)

(Ryan et al. 2014)

The two main types of immunological methods used to diagnose Cryptosporidium are enzyme-linked immunosorbent assays (ELISAs) and immunofluorescence assays (IFAs) (Chalmers et al. 2011).

ELISAs are based on the binding of Cryptosporidium-specific antibodies to antigenic proteins or enzymes, which produce a colorimetric signal when an appropriate substrate is added. Several commercially available ELISA kits are available for the detection of cryptosporidium antigens in stool samples including the Meridian Bioscience Crypto-Giardia Rapid Test and the TechLab Cryptosporidium Antigen ELISA. These tests are highly sensitive and specific with a reported sensitivity of up to 98% and specificity of up to 99% (Kang et al. 2008).

IFAs are based on the binding of fluorescently labeled Cryptosporidium-specific antibodies to antigenic proteins on the surface of Cryptosporidium oocysts. The labeled antibodies produce a fluorescent signal when exposed to specific wavelengths of light allowing for the visualization of the oocysts under a fluorescence microscope. IFAs can be performed on a range of patient samples including stool, tissue and environmental samples and are highly sensitive and specific with a reported sensitivity of up to 99% and specificity of up to 100%. Both ELISAs and IFAs are rapid, highly sensitive and specific methods for the diagnosis of Cryptosporidium infection. However, these require specialized equipment and expertise and are not always readily available in all clinical or laboratory settings (Chan et al. 2000).

7.4. HISTOLOGY

Histology specifically the staining of tissue samples with special stains can be used to diagnose Cryptosporidium infection in humans. However, it is not commonly used in clinical settings due to the invasive nature of obtaining tissue samples and the availability of other non-invasive diagnostic methods such as stool microscopy, antigen detection and PCR. In cases where a tissue biopsy is obtained, Cryptosporidium infection can be diagnosed through histological examination of stained tissue sections. Hematoxylin and eosin (H&E) staining can be used to visualize the characteristic oocysts of Cryptosporidium, which are round or oval structures with a distinctive pale blue, refractile appearance. However, H & E staining alone may not provide a definitive diagnosis as other parasites or artifacts may resemble Cryptosporidium oocysts (Fayer et al. 2000).

Additional staining methods, such as modified acid-fast stains or immune-histochemical stains, may be used to improve the specificity of the diagnosis. Modified acid-fast stains, such as the Kinyoun or modified Ziehl-Neelsen stain, use a combination of acid-fast and counterstains to selectively stain Cryptosporidium oocysts. Immuno-histochemical staining uses specific antibodies to target Cryptosporidium antigens in tissue sections and can provide a more definitive diagnosis of Cryptosporidium infection (Fayer et al. 2000).

8. TREATMENTS

Recent studies have highlighted the importance of cryptosporidium as a factor in both morbidity and mortality associated with childhood diarrhea but, the progress in the treatment is very slow. In young children, cryptosporidium causes severe diarrhea (Kotloff et al. 2013), malabsorption, intestinal injury, excess mortality and stunting that is associated with malnutrition (Korpe et al. 2016) and its drugs are desperately needed (Striepen 2013). Although nitazoxanide is approved for the treatment of cryptosporidiosis, it is not better than a placebo in immunocompromised patients living with HIV and cryptosporidiosis (Amadi et al. 2009). Clofazimine (CFZ) which has been used to treat leprosy for over 50 years and is now used to treat multidrug-resistant tuberculosis (TB). It was recently shown to be effective against cryptosporidium in vitro (Love et al. 2017).

Fluid and electrolyte replacement are crucial in the treatment of cryptosporidiosis in addition to symptomatic therapy. Anti-motility medications are another crucial component of treatment. Narcotic substances are also used in majority of patients. According to literature, opium tincture (paregoric) may be more effective for AIDS patients. Because cryptosporidiosis is typically self-limiting in immunocompromised hosts, immune reconstruction in response to effective combination antiretroviral therapy has been linked to positive clearance as well as decreased long-term morbidity and mortality associated with the disease in AIDS patients (Masur et al. 2014). Nevertheless, chronic diarrhea is lined to a higher risk of passing away even when receiving effective antiretroviral therapy (Dillingham et al. 2009). According to earlier studies, using anti-motility and anti-parasitic medications as part of initial treatment has been linked to better outcomes (Masur et al. 2014), but there is no conclusive proof of this. Unexpectedly, some HIV protease inhibitors have activity against cryptosporidium both in vivo and in vitro (Mele et al. 2003)

9. PREVENTION AND CONTROL

As there are few effective treatments for cryptosporidiosis, prevention and risk reduction are the most crucial interventions (Rossignol 2009). Due to the high number of excreted oocysts and low infection dose, cryptosporidiosis is highly contagious and requires strict personal hygiene. The guidelines for preventing person-to-person transmission must be followed (Anon 2004). These include frequently washing your hands, disposing of excreta properly and washing soiled items like bedding and clothing. For 48 hours following the last episode of diarrhea, individuals with cryptosporidiosis should avoid being around food handlers and staff at healthcare facilities (Anon 2004). Washing hands before and after handling animals, properly treating non-potable water and washing product before eating are all general precautions against cryptosporidium infection. On open farms, handwashing facilities should be accessible and used. To properly rehydrate, it might be necessary to consume boiled water and use salts. To eliminate any potential Cryptosporidium contamination, avoid touching human or animal waste, dispose it properly, and thoroughly cook meat, poultry or fish. Infections can be severe and difficult to treat in the high-risk immune-suppressed populations that were previously mentioned. Although the goal of treatment is to lessen symptoms, eliminating the parasites won't be possible unless the underlying immune deficiency is addressed. One alternative measure is to use the appropriate point-of-use filters to remove the cryptosporidium oocysts (Hunter and Nichols 2002).

10. CONCLUSION

Cryptosporidiosis is in the list of top 10 food and water borne diseases which cause severe diarrhea in humans as well as in animals. Faeco–oral transmission of the oocyst stage has resulted in outbreaks through contamination of drinking water, food and recreational water. As cryptosporidium is a zoonotic protozoan parasite it can be transmitted from wild and domestic animals to humans. Immunocompromised people and children are more susceptible to cryptosporidiosis. Currently 26 species and genotypes are recognized from which *C. hominis* and *C. parvum* cause infections in humans. Cattle is one of the most significant reservoirs for zoonotic infections in livestock. Numerous Cryptosporidium species or genotypes that have a limited host range and therefore have no significant public health implications can infect both domesticated and wild animals. Microscopy, immunological, and molecular techniques for oocyst detection and identification are all constantly improving.

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Trichinellosis: A Hidden Threat in Meat Consumption

25

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ABSTRACT

Trichinella spiralis, the roundworm that causes trichinellosis, is a major global health issue. The main way that the disease is spread is by eating raw or undercooked meat from infected livestock, and wild boars are increasingly playing a role in spreading epidemics. Numerous symptoms, such as myocardial infarction, stomach discomfort, and neurological involvement, are present with the condition. Trichinella species have a complicated life cycle that includes an enteral phase, and a migratory. Trichinellosis is not as common worldwide, it is still a cause for concern, particularly in less developed nations where eating raw or undercooked meat is common. Trichinella species are distributed differently over the world, with T. spiralis being more common in Europe. phase, and a muscle phase. This causes tissue damage and severe inflammation. Due to the small size of larvae and limits in testing methods, difficulties in recognizing contaminated meat continue even in the absence of recorded cases. Trichinellosis vaccines are being developed using a variety of techniques, including DNA, synthetic peptide, live attenuated, and recombinant protein vaccines. The selection of antigens, adjuvants, and variations in immune responses among animal species present challenges in the production of vaccines. Future work should concentrate on developing genetic engineering tools, and comprehending immune evasion mechanisms.

CITATION

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1. INTRODUCTION

Trichinellosis is a parasitic infection caused by Trichinella spiralis (T. spiralis) (roundworm) (Wu et al. 2022). Through the consumption of contaminated meat, primarily through hunting or scavenging of meat from an infected animal, Trichinella spp. are transmitted to and survive in a variety of hosts (Sgroi et al. 2023). Trichinella can infect more than 150 different species of animals and humans. When consumers eat undercooked or raw meat that has Trichinella infective larvae, they become infected (Hady et al. 2023). Wild animals that are omnivorous and carnivorous serve as the natural reservoirs for Trichinella spp. Trichinella species in domestic and wild animals are not necessarily linked to human diseases (Murakami et al. 2023). The host's dietary habits play an essential role in transmission. The major source of human infection is pigs, particularly those reared in the backyard. However, for the past 30 years, wild boar meat has been a significant factor in epidemics. The worldwide distribution of Trichinella spp. and various cultural food patterns are the major factor that favors human infection in non-industrialized and industrialized countries (Pavel et al. 2023). Higher incidence is frequently observed where eating raw or undercooked meat from domestic animals and wild animals is common.

Compared to other foodborne parasites, Trichinella spp. the infection has a modest global burden. The global disability-adjusted life years (DALY) for trichinellosis were calculated at 76 per billion people per year, yet in recent years, 5751 cases and 5 mortalities have been recorded in 55 countries (Rostami et al. 2017). T. spiralis, a historically acquired species from Eastern Asia, is common in Spain, Poland, Lithuania, and the Balkan different countries of Bulgaria, Romania, Serbia, and Bulgaria (Veronesi et al. 2023). From 1986 to 2009, there were 65,818 incidents reported worldwide, with 56,912 of those incidents occurring in Europe. In the past 20 years, trichinellosis cases have not been documented in nearly half of the EU nations, including Luxembourg, Cyprus, Portugal, and Malta (Pozio 2019). The European Union has identified 5518 cases of trichinellosis over the past 16 years (2002–2017), with a declining trend. In the genus Trichinella, 9 species and 3 genotypes have been identified (Table 1).

Table 1: Trichinella species, biological characteristics, and hosts and distribution.

Species (genotype)	Larval form	Distribution	Pathogenicity to humans	Major hosts	References
T. spiralis (T1)	Encapsulated	Worldwide	High	Carnivores, wild boar, pigs, rats	Bruschi and Dupouy-Camet 2014
T. nativa (T2)	Encapsulated	Europe, and America, areas of Asia, the Arctic and Subarctic	High	Dogs, wild carnivores, Rare in pigs	Bruschi et al. 2002
T. britovi (T3)	Encapsulated	Asia, middle east countries, Europe,	High	Jackal, dog, Wild Boar,	Foreyt and Abbott 2013
T. pseudospiralis (T4)	Nonencapsulated	Australia, Thailand, Nearctic, and palearctic regions, New Zealand,	High	Birds and Mammals	Foreyt and Abbott 2013
T. murrelli	Encapsulated	Canada and USA	Moderate	Carnivores	Gottstein et al. 2009
T. nelsoni	Encapsulated	Southern Eastern- Africa	Low	Carnivores	Mitreva and Jasmer 2006
T. papuae	Nonencapsulated	Thailand, New Guinea, Papua	Moderate	Reptiles and Mammals	Pozio 2007
T. zimbabwensis	Nonencapsulated	South Africa, Ethiopia, Mozambique, Zimbabwe,	Unknown	Reptiles and Mammals	Pozio 2001

2. THE LIFE CYCLE OF TRICHINELLOSIS

The life cycle of Trichinella spp. occurs when muscle tissue carrying first-stage larvae is consumed by the new host (Pozio 2022). The larvae move from the intestines to the lymphatic system, then to the circulation, where they enter skeletal muscle cells and become contagious to the next host. Severe inflammation is the main cause of disease and includes encephalitis, myositis, and myocarditis, the severity of which is determined by the amount of parasites consumed. *T. spiralis* has minimal host specificity in mammals, lives its entire life cycle in one host, lacks a free-living stage, and exists as an intracellular parasite inside a single striated muscle cell (Shinn et al. 2023).

3. ENTERAL PHASE

The infection is passed on by eating meat that is either uncooked or undercooked and carrying the nurse cell-larva combination. The columnar epithelium is at the base of the villus where the young parasites enter (Bonis et al. 2021). They are referred to as intra multi-cellular organisms since they reside there in a row of columnar epithelial cells. The larvae go through four rounds of molting before becoming adults. After mating, the young larvae pass using the bloodstream to the muscles that are regulated voluntarily, where they encyst (Nthiga 2022). Acute immune-mediated inflammation caused by the adult stage, which lives in the epithelial layer of the host's small intestine, results in physiological structural and cellular alterations. These changes are linked with notable changes in epithelial cells, the release of inflammatory mediators, and increasing in inflammatory cells (Muthumalage et al. 2019). A little infection causes minimal harm. Severe infection, on the other hand, can induce serosa petechiae, hyperemia increased, enlarged Peyer's patches, mucous secretion, and intestinal loop dilatation (Bandyopadhyay et al. 2022). In the jejunum, histopathology of the small intestine indicates an intense inflammatory reaction with diverse cellular infiltration primarily of neutrophils, lymphocytes, and eosinophils. T.spiralis can also produce trophic modifications in the longitudinal smooth and circular muscle layers of the ileum and jejunum with crypt hyperplasia and villous atrophy. These are correlated with significant changes in epithelial cells and an increase in mediators and inflammatory cell types (Robinson et al. 2019).

4. MIGRATORY PHASE

The pathology in the migratory phase is produced by larvae discharged into the intestinal mucosa, then migrate to the blood vessels. They transmit through the body until they reached the striated skeletal muscles. Transferring Trichinella larvae and their byproducts generate an instantaneous reaction, resulting in pathological, immunological, and metabolic abnormalities, as well as the different clinical manifestations seen during the acute stage of infection (Saha and Saroj 2022) shown in Fig. 1.

5. MUSCULAR PHASE

Larvae develop a major set of cell physiological changes after invading skeletal muscles. These modifications cause the completely differentiated muscle cell to convert into a nurse cell, helping in the development and growth of the larva (Huang et al. 2015). This stage is connected with allergic and inflammatory reactions generated by the invasion of the muscles by wandering larvae. This process can directly or indirectly harm muscle cells by encouraging the infiltration of inflammatory cells, particularly eosinophils (Bruschi and Gómez-Morales 2014). In trichinellosis patients, there was a link between eosinophil levels with serum muscle enzymes, implying that these granulocytes may be involved in muscle

injury (Bruschi and Dupouy-Camet 2022). Thus, increasing eosinophilia is the most important clinical finding of Trichinellosis muscular phase. The infiltration of the accessory muscles and digraph of respiration by the pathogen results in dyspnea.

6. NEUROLOGICAL INVOLVEMENT

Neurotrichinellosis can affect either white or gray matter of the brain, spinal cord, pons, and cerebellum (Tanabe et al. 2021). Damage to the central nervous system can occur directly or indirectly as a result of the release of tumor necrosis factor (TNF), immune-mediated processes, vascular damage, and toxin reactions which result in eosinophil toxicity. Trichinella larvae can move into the CNS and cause common lesions, blood vessel blockage, and inflammatory infiltrates (Garcia et al. 2019). Different pathologic changes may be mediated by larval and muscle breakdown components. The larvae either cause pathological symptoms in tissues before returning to circulation, or they can be trapped and destroyed, resulting in inflammatory reactions (Hady et al. 2023). Punctuate hemorrhages, hyperemia, and Edema occur commonly in the brain tissues. Vascular changes enclosing the larvae are thought to be the primary processes causing neurological injury.

7. CARDIAC INVOLVEMENT

The liver damage can be caused by larval damage directly or indirectly by immune reactions and eosinophils (Leal-Silva et al. 2021). The liver is usually enlarged in these situations due to dystrophic pathologies such as fatty degeneration. Hypoproteinemia is prevalent and can be attributed to hepatic dysfunction, allergic capillaropathy caused by eosinophils, and protein digestion and absorption deficits caused by changes in the intestinal mucosa (Wen et al. 2022). The decrease in total protein is predicated on a decrease in the albumin fraction.

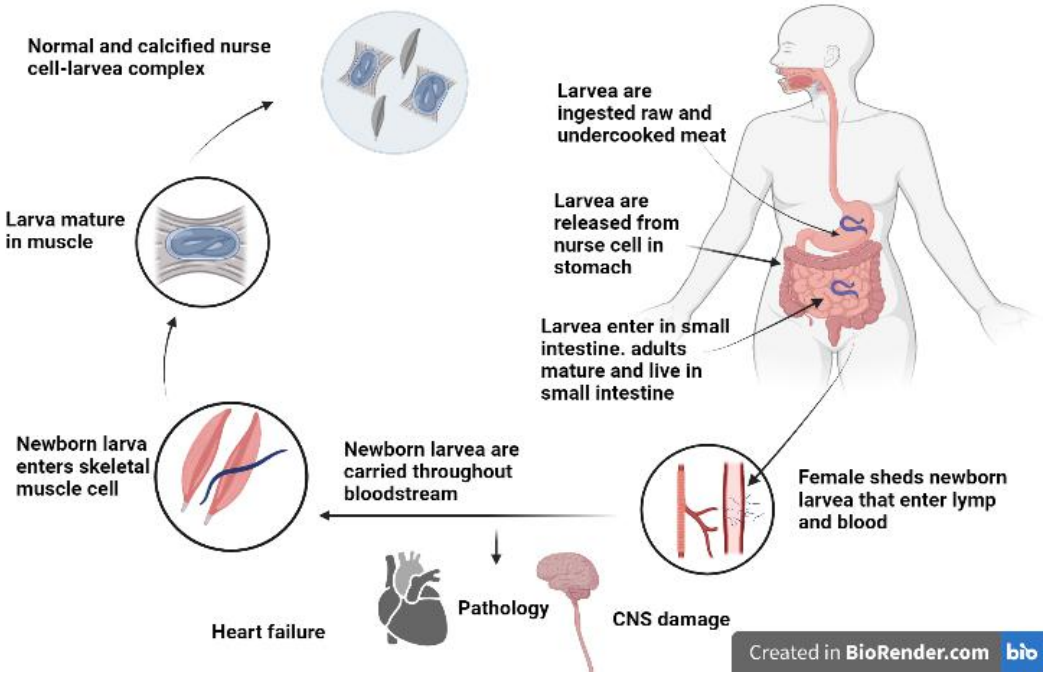

Fig. 1: Life cycle of Trichinellosis (Retrieved from biorender).

8. SYMPTOMS AND SIGNS

The severity of symptoms is determined by the degree of infection and is proportional to the number of larvae per gram of muscle. Infections are divided into three types based on the number of larvae. In subclinical, up to 10 larvae are involved in light infections. In moderate 50 to 500 larvae are involved and in severe infection (Bruschi and Murrell 2020), more than 1000 larvae are involved which can be lethal. In symptomatic situations, symptoms appear in three stages: enteric (intestinal invasion), invasive (laser migration), and encystation in the muscles (Bogoch et al. 2021). The major clinical symptoms are myocardial infarction, abdominal pain, allergic reactions, encephalitis, fever, myalgia, intestinal diarrhea, and facial swelling (Dupouy-Camet et al. 2021).

9. CHALLENGES IN IDENTIFYING TRICHINELLA-INFECTED MEAT

To recognizing Trichinella-infected meat provides multiple challenges due to the parasite's microscopic size, the spread of larvae in the meat, and the limitations of accessible testing procedures. The complicated nature of these problems can make detecting Trichinella contamination in meat difficult, increasing the danger of consuming contaminated products (Chalmers et al. 2020). Trichinella larvae can be found in muscle tissues in comparatively small numbers, rendering ocular inspection insufficient to detect their existence. The larvae are spread throughout the flesh, therefore a tiny sample may not fully represent their distribution (Gabriël et al. 2022). Trichinella larvae are enclosed in cysts in muscular tissues, adding another layer of defense. Because the cysts shield the larvae from external variables like frying and freezing, reaching the larvae during testing is difficult (Álvarez-Guerrero and Alba-Hurtado 2011).

Due to the irregular distribution of Trichinella larvae in the meat, obtaining representative samples for testing can be difficult. A single tiny sample of meat may not adequately represent the full batch. Traditional diagnostic approaches, such as artificial digestion, necessitate a significant amount of time and specialized devices (Bergwerff and Debast 2021). These procedures may not be practicable for large-scale testing or in resource-constrained places (Yang et al. 2022). Some diagnostic procedures may have sensitivity limitations, particularly when it comes to detecting low amounts of Trichinella larvae in meat samples. This may result in false-negative results, underestimating the level of contamination. Rapid and accurate identification of Trichinella is critical for preventing diseased meat from the food supply chain (Thanh et al. 2014). Some methods of testing may take a long time, causing delays in finding contaminated meat. Some advanced testing procedures are costly and may not be feasible for periodic inspection of all beef products, particularly in resource-constrained areas. There is a potential for cross-contamination between samples during testing, which could result in false-positive results or incorrect identification of contaminated meat (Haiminen et al. 2019).

10. TRICHINELLOSIS IN A GLOBAL ECONOMY

Global trade increases the risk of trichinellosis outbreaks from ready-to-eat pork foods, demanding urgent attention (Bintsis 2017). As a result, certain nations have unique rules for qualifying pigs or pork foods for importation. The European Union (EU) requires inspection of horse and pork meat before they can be imported into EU member countries. Despite these limitations, the eating of imported horse meat has resulted in an upsurge of trichinellosis in the EU (Bruschi and Dupouy-Camet 2022). Outbreaks caused by consuming examined meat occur worldwide as well. The reason for the ineffective

testing is probably due to inadequate quality control measures. The aggregated digestion assay, as recommended by the EU and others, is thought to be able to detect corpses with the smallest larvae load that would induce clinical sickness in humans (Thrastardottir et al. 2021). Use established protocols for meat inspection to ensure accuracy and reliability. To ensure precise test results, it is important to incorporate other elements of a quality assurance program, such as proficiency exchange, document management, critical point control analyst certification, sampling, and trace-back systems. Controlling Trichinella in cattle and food items globally is critical to reverse the pattern of emerging and re-emerging human trichinellosis. Alternative approaches to ensure Trichinella-free pork are now being examined in regions where pig infection has been almost completely eradicated and human trichinellosis is rare. Create efficient methods for managing farms to protect pigs from trichinellosis (Gamble 202). Rodent control and Bio-security are examples of sound management practices, as is the evasion of feeding garbage to pigs. Although the effectiveness of control in many nations, trichinellosis continues to trigger human disease in some areas, and the parasite's biology and epidemiology still need to be studied further to develop consistent, practical, and standardized control programs for all parts of the world.

11. VACCINES AGAINST TRICHINELLOSIS

The antigens for the *T. spiralis* vaccine are typically obtained from excretory-secretory products and basic extracts of whole worms (Zhang et al. 2018). It is widely accepted that inactivated and live attenuated vaccines are 1st generation vaccinations. Various approaches have been used to identify potential antigens for vaccines against trichinellosis, including immunoproteomics, genome, transcriptome, and proteome screening (Tang et al. 2022). Established on these techniques 2nd and 3rd generation vaccines have been accomplished in swine and rodents to investigate their shielding effects such as recombinant protein vaccine, DNA vaccine, and synthetic peptides (Khalid and Poh 2023).

12. RECOMBINANT PROTEIN VACCINES

Some development in the production of recombinant protein-based vaccines against *T. spiralis* disease has been accomplished with the quick progress of genetic engineering (Xu et al. 2020). Applicant antigens were mostly selected from functional proteins, ES products, and antigens implicated in *T. spiralis* attack pathways (Tang et al. 2022). The constituents that are crucial in ES products for *T. spiralis* infection are protease and protease inhibitors. To suppress *T. spiralis* infection, a great quantity of protein vaccine investigation has been conducted in recent years on deoxyribonuclease, serine proteases, cystatins, and serine protease inhibitors.

13. PROTEASES AND PROTEASES INHIBITOR

The serine proteases found in ES products assist *T. spiralis* in invading host cells and evading attacks from the immune system. The protein superfamily known as serine protease inhibitors is responsible for inhibiting the actions of serine proteases, and plays a part in inflammation, complement activation and blood coagulation (Sofronic-Milosavljevic et al. 2015). Worm serpins shield them from host serine proteolysis, helping parasites evade immune response. Mice immunized with recombinant rTsSP had 62.10 and 71.10% lower worm loads of ML and AD, respectively (Song et al. 2018). Mice vaccinated with rTspSP-1.2 had worm burdens of ML and AD reduced by 52.24 and 34.92 %, respectively. For pigs vaccinated with rTs-Adsp, the worm load of ML was reduced by 50.9% (Tang et al. 2022). Mice immunized with

recombinant rTsSPI had 57.25 and 62.2% decreased ML and AD worm loads (Grzelak et al. 2020). At ten days post-infection, animals inoculated with rTs-Serpin showed a 59.95% decrease in mature worms and a 46.41% decrease in larvae muscle (Xu et al. 2017b). The cystatin protein superfamily has the ability to inhibit the action of cysteine proteases. Cystatins serve a significant part in immune elusion and the regulation of the host immunological reaction during parasitic infection in nematodes (Stachyra, and Wesołowska 2023). At 5 days post-inoculation, mice immunized with a cystatin-like protein 64.28% drop in mature worms and 61.21% decrease in larvae muscle. Cysteine proteases are important enzymes that are existing in most living animals, including parasites and viruses. Parasitic cysteine proteases have a significant impact on the attack of host tissue and the survival of parasites within the host. As a result, they are a key focus for the growth of parasite vaccines (Stachyra et al. 2019).

14. DEOXYRIBONUCLEASE II

DNase II is found mostly in nuclei and lysosomes and plays an essential part in pathogen evasion and invasion of the host's immunological reaction (Kumari et al. 2020). T. spiral DNase II protein group is substantially larger than those of other species. Furthermore, investigations have revealed that *T. spiralis* DNase enzymes may play an important role in host-parasite contacts through infection, implying that they could be exploited as applicant antigens to regulate and avoid trichinellosis (Cui et al. 2019). Subcutaneously vaccinated mice with rTs-DNase II-7 and rTsDNase II-1 demonstrated 34.86 and 40.36 % decreases in mature worms at five dpi, respectively, 42.33 and 50.43 % decreases in larvae muscle (Tang et al. 2022). Pigs immunized with DNase II-7 demonstrated a 45.7% decrease in the larvae muscle (Xu et al. 2021). While recombinant protein vaccines are becoming more common, the degree of immunoprotection is linked to the adjuvants, antigens, and delivery routes.

15. LIVE ATTENUATED VACCINES

Live attenuated vaccines lower the risk of *T. spiralis* infection while still stimulating an immune response. Mice immunized with radiation-attenuated larvae showed a 72.5 percent reduction in the muscles of larvae (Hafez et al. 2020). Live attenuated vaccines have significant defensive effectiveness because these methods closely mimic natural infection and provide a similar environment to that of *T. spiralis* infection. Though, their security is called into question owing to the probability of infection. The use of live attenuated vaccine, which are known for their strong protective immunity, is quickly being phased out (Santi et al. 2018).

16. SYNTHETIC PEPTIDE VACCINE

Various epitope peptides have the advantage of existence easier and faster to make than recombinant protein vaccines, and they may contain numerous protective epitopes. Mice who received an immunization of a synthetic peptide made up of forty amino acids and derived from the glycoprotein of *T. spiralis* saw a decrease of 64.3% in adult worms (Wait 2022). Mice vaccinated with a thirty-mer peptide antigen had 33.3% decrease in parasite female fertility. In recent years, vaccines targeting epitopes for bacterial, viral, and parasite infections have been rapidly produced. Epitope vaccines have several drawbacks, such as low immunogenicity and the requirement to be linked to a large transport protein (Parvizpour et al. 2020). To boost the immunogenicity of epitope vaccines, a new method comprising numerous antigenic peptides was created. Epitope-based vaccinations can be developed as chimeric vaccines, by producing various efficient epitopes. By using a chimera vaccination, it is possible to enhance the protection provided by epitope vaccines or prevent the immune system from being evaded by

parasites (Sanches et al.2021). Furthermore, T. spiral life cycle is complicated, resulting in a variety of antigens at distinct phases. *T. spiralis* infection can be effectively controlled with a multiepitope vaccination.

17. DNA VACCINES

DNA vaccines acquired popularity because of their potential to elicit a wide immune reaction and provide long-time immunity (Soleymani et al. 2022). In addition, DNA vaccines have been found to be more steady, economical, easy to produce, and harmless to distribute when linked to traditional protein vaccines. The fundamental drawback of DNA vaccines over protein vaccines is their low immunogenicity (Qin et al. 2021). The primary disadvantage of DNA vaccines beyond protein vaccines is their lack of immunogenicity. Recently, numerous DNA vaccines effective against *T. spiralis* infection have been identified in mouse models. According to a study conducted on mice, who were given a TsPmy DNA vaccine delivered through Salmonella, there was a reduction of 46.6% and 44.8% in ML and AD worm burdens (Wu et al. 2021). In mice treated with the pcDNA3.1(+)-Ts-NBLsp DNA vaccine, the worm burden of ML was reduced by 77.93% (Xu et al. 2020). Overall, DNA vaccines have lower immunogenicity than protein vaccinations due to low amounts of antigen expression. According to research, DNA positive protein immunization is an excellent technique for increasing protective effect and immune response. Mice vaccinated with Ts87 in a DNA-prime/protein-boost approach had 46.1% reduction in ML worm load. More approaches will be developed to improve the efficacy of DNA vaccinations as technology advances. The principal objective is to create a DNA vaccination that can be used safely in humans.

18. SYNTHETIC PEPTIDE VACCINES

Extensive investigation has been conducted to progress vaccines against *T. spiralis* infection, including recombinant proteins, DNA vaccines, and crude antigens. Currently, only a limited amount of research has been conducted on the effectiveness of peptide vaccines in suppressing *T. spiralis* infection. Multiepitope peptides have the advantage of being easier and faster to make than recombinant protein vaccines, and they may contain numerous protective epitopes (Gu et al. 2020). Selected a forty-mer synthesized peptide from T. spiral glycoprotein, mice treated with the peptide vaccine had 64.3% decrease in adult worms. Screened a forty-mer synthesized peptide from T. spiral glycoprotein, and mice treated with the peptide vaccine had 64.3% decrease in adult worms. (Gu et al. 2020). Female parasite fecundity was reduced by 33.3% in mice inoculated with a thirty-mer peptide antigen. Lately, vaccines targeting the infection caused by viruses, bacteria, and parasites have been produced rapidly using epitope technology. However, epitope vaccines have numerous drawbacks, including low immunogenicity and the essential to be coupled to a larger transporter protein. To boost the immune response of epitope vaccines, a new method comprising numerous antigenic peptides was created (Kazi et al. 2018). It is possible to create chimeric vaccines using epitope-based vaccines by combining multiple effective epitopes. As a result, a chimera vaccination could boost the epitope vaccine or avoid and protect parasite immune evasion. Furthermore, T. spiral life cycle is complex, resulting in a variety of antigens at distinct phases. *T. spiralis* infection can be effectively controlled with a multiepitope vaccination.

19. FACTORS OF VACCINE EFFECTIVENESS

Many reasons influence vaccine effectiveness, including antigen composition, transport routes, adjuvants, animal species, coinfection, inoculation doses, infective doses, and immunization strategy. *T. spiralis* has a multiple phases life cycle, which produces distinct antigens at each stage. Because the combination of antigens impacts vaccine effectiveness, identifying great antigens is critical for creating *T. spiralis* vaccines. During a trichinellosis infection, hosts release Th2-type cytokines (Gao et al. 2022). These cytokines are

responsible for increasing mast-cell proliferation and activation, which is necessary for removing the parasite from the intestine. It's important to note that different antigen candidates can induce different immune responses and provide varying levels of protection. Future research into the production of *T. spiralis* inoculations should concentrate on antigens that can provoke a Th2-type immune reaction. Choosing an appropriate adjuvant is critical in vaccine development. Adjuvants boost immune reactions elicited by parasite antigens and defend them from being, or removed, degraded and diluted by the host (Serradell et al. 2019). The use of Freund's adjuvant is being phased out due to its toxicity and particular damage to experimental animals (Serradell et al. 2023). Although few adjuvants outperform Freund's adjuvant in terms of antibody generation, numerous adjuvants can induce high antibody responses while causing less inflammation and tissue death. In recent decades, alternative adjuvants like Montanide ISA series adjuvants and Montanide IMS series adjuvants have been tested in mouse models to combat *T. spiralis* infection (Zhang et al. 2018). Coinfection may alter the host's immune response, reducing the efficacy of *T. spiralis* vaccinations. It needs to be seen if the immune reaction elicited by *T. spiralis* vaccinations may be inhibited or defused by infection with other organisms. In order to improve *T. spiralis* vaccines, it is important to consider the immune response generated by various infections. Animal models are well known for their use in vaccine development. Most investigations on vaccination protection have employed mouse representations rather than pig representations (Cai et al. 2022). Vaccine effectiveness, however, may differ based on animal type. Previous research from our group discovered that the immune reaction elicited by the same antigen differs across mice and pigs. To ensure the effectiveness of potential antigens in inducing immunity, it is necessary to validate the significant levels of immunity in swine models after testing in mouse models. Scientists have been working hard to develop and try out different methods for creating vaccines. Although mature immunization regimens have been developed in mice as models, they may not be accessible to pigs or people (Ali et al. 2022). Currently, there is no universally accepted approach for conducting studies using swine models. In order to develop effective *T. spiralis* vaccines in the future, it is crucial to carefully study the factors that affect their effectiveness.

20. CHALLENGES AND FUTURE PERSPECTIVE

Although there have been significant efforts and progress in searching for potential antigens and developing vaccines for *T. spiralis*, there are currently no effective vaccinations to prevent *T. spiralis* infection. Additional immunogenic antigens have been extracted and discovered due to the advancement of genomics, proteomics, and transcriptomics to generate efficient trichinellosis vaccines (Abbas et al. 2023). More and more ways are being used to improve vaccine effectiveness. DNA vaccines are becoming more popular due to their numerous benefits, including low cost and long-lasting protection. In terms of toxoplasmosis vaccinations, a DNA multicomponent vaccine reduced parasite cyst load by 80.22% (Zhang et al. 2018). The combination DNA vaccine could be a potential technique for increasing the efficacy of *T. spiralis* vaccinations. Furthermore, in mouse models, combined vaccination has been employed as a favorable method to boost the efficacy of *T. spiralis* vaccines. By combining the advantages of DNA and protein vaccines, the DNA plus protein vaccination technique can stimulate a strong immunological response and provide effective immune protection. VLP vaccines are a commonly used method as they have a tendency to induce strong immune reactions (Keshavarz et al.2019). This technique has been utilized for developing vaccines for toxoplasmosis and presents a new approach for producing vaccines for *T. spiralis*. Genetic engineering technologies have been used to create live-attenuated toxoplasmosis vaccines through the process of gene editing. The method has been used in the creation of toxoplasmosis vaccines and offers a fresh strategy for the production of *T. spiralis* vaccines. Since the discovery of genetic engineering technologies, gene editing has been used to produce live-attenuated toxoplasmosis vaccines (Zhang et al. 2022). Even though Toxoplasma gondii and *T. spiralis* have different physiological characteristics, the same method can be used to develop a vaccine for trichinellosis. T. spiral life cycle in

the host is complicated, involving a variety of host response modulation, antigens, and immune evasion. Because of these qualities, it is challenging to establish the best possible defense with a single *T. spiralis* antigen. With the advancement of genetics, new study thoughts are being offered for boosting the immunity rate of *T. spiralis* vaccines (Tang et al. 2022). Comprehensive studies on immune evasion and immunosuppression brought on by *T. spiralis* infection will aid in the development of more potent *T. spiralis* vaccines. The primary cause of human *T. spiralis* infection is the consumption of pork and pork-associated products. To present, *T. spiralis* vaccine research has been conducted in mouse models, and more study in pigs is needed. *T. spiralis* vaccines employing pig models provide financial and technical challenges, yet this is an important aspect in many vaccinology studies. Because the danger of *T. spiralis* infection in livestock is minimal under normal management conditions, little emphasis has been paid to the development of *T. spiralis* vaccinations. Regardless, *T. spiralis* vaccinations are a nonviolent technique that could help evade medication struggle. As a result, it is critical to instruct people on the significance and benefits of vaccination. Producing a vaccine for Trichinosis is still a major focus of research. To induce protective immunity against trichinellosis infection, scientists were testing numerous vaccine applicants, including recombinant proteins and DNA vaccines. An effective vaccination could help manage Trichinosis by preventing infection in animals and humans.

21. CONCLUSION

Trichinellosis is a serious parasite infection caused by *T. spiralis* that is predominantly transmitted to humans through the ingestion of contaminated meat. While the worldwide incidence of trichinellosis is low in comparison to other foodborne parasites, it remains a public health problem in some areas and can cause severe clinical symptoms if left untreated. Efforts to produce effective vaccinations against *T. spiralis* have shown promise, with many techniques being investigated, including synthetic peptides, DNA vaccines, and recombinant protein vaccines. However, difficulties in recognizing infected meat, as well as the intricacy of the parasite's life cycle, continue to obstruct efficient control. To effectively combat trichinellosis, researchers, public health administrators, and the food sector must work together. Improving surveillance, establishing stringent meat inspection processes, and boosting public knowledge about safe meat consumption habits are critical measures in avoiding and controlling trichinellosis epidemics. Research into new vaccine candidates and technology, as well as advances in diagnostic tools, will be critical in the continued fight against this viral infection. Finally, lowering the worldwide effect of trichinellosis on animal and human health will require a holistic approach that incorporates preventive measures, effective management techniques, and innovative immunization options.

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Evaluation of Therapeutic Efficacy of Nanoparticles Against Secondary Cystic Hydatidiosis

26

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ABSTRACT

Cystic echinococcosis (CE) is a widespread zoonotic parasitic disease caused by the hydatid cyst of Echinococcus granulosus. The World Health Organization classifies it as a neglected tropical disease, particularly affecting areas with free-roaming dogs scavenging on animal carcasses. In the Kurdistan region of Iraq, human CE is a significant public health concern, necessitating innovative treatments beyond traditional surgery. The reemergence of infection post-surgery is often attributed to the leakage of cystic fluid, emphasizing the need to address this issue during surgical interventions. Current chemotherapeutic options, such as benzimidazole derivatives, exhibit limitations, prompting research into alternative treatments. Flubendazole (FLBZ), a broad-spectrum anthelmintic, has shown promise in mouse models, but its hydrophobicity and low water solubility hinder its efficacy in humans. Nanomedicine offers a potential solution, with the use of biodegradable nanoparticles to enhance the solubility, absorption, and safety of FLBZ. This approach presents a novel strategy for treating E. granulosus infections, improving drug bioavailability and reducing adverse effects. Moreover, the application of nanoparticles in targeted drug delivery to hydatid cysts has gained attention. Nanoparticles, with their small size and high surface area, show promise in improving drug permeability and retention within cysts, thereby enhancing treatment outcomes and minimizing systemic side effects. Ongoing research explores the incorporation of nanoparticles, such as silver, gold, and zinc, to boost the efficacy of therapeutic compounds and address the challenges associated with hydatidosis treatment.

Keywords: Cystic echinococcosis, Echinococcus granulosus, nanomedicine, flubendazole, benzimidazole

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1. INTRODUCTION

A parasitic disease known as cystic echinococcosis (CE) is a widespread condition caused by the hydatid cyst known as Echinococcosis *granulosus*. Hydatid disease or hydatidosis are additional terms used to define it as a zoonotic illness (Kern et al. 2017). It was categorized by the World Health Organization as a neglected tropical illness (WHO 2010). The diseases is mostly associated to those areas or populations where free-roaming dogs are scavenging dead animal or disease due to animal carcass (Benyan et al. 2013).

The hydatid cyst of *Echinococcus granulosus* is the cause of the highly contagious parasitic disease known as cystic echinococcosis (CE). It is likewise portrayed as a zoonotic illness and considered a neglected tropical disease as well. In the Kurdistan region of Iraq, human CE is a significant public health concern, where residents of Arbil have a surgical incidence rate of 6.3 per 100,000 (Hassan et al. 2017). Although surgery is still the most common form of treatment, additional methods are crucial to the management of CE. One of the primary explanations that why the infection reemerges after the surgery is probably the leakage of the cystic fluid. Therefore, it is essential to remove the protoscoleces inside the cyst during surgery (Shi et al. 2016). Each scolicidal agents such as 20% saline, 3% hydrogen peroxide, 1.5% cetrimide-0.15% chlorhexidine (10% Savlon), 95% ethyl alcohol, 10% polyvinylpirrolidone-iodine (Betadine) act as a barrier in hydatid cyst surgery. In purely cystic hydatid liver disease, the risk of dissemination of the cyst contents can be avoided by injection of a potent scolicidal agent such as Savlon (Besim et al. 1998). Currently, cerebral hemorrhage, necrosis, and myelinolysis can occur from the commonly used scolicidal medicated hypertonic saline. However, this medication has major adverse effects because it can also produce hypernatremia, which can lead to these complications as well (Albi et al. 2002).

Benzimidazole was suggested by the WHO as a treatment for human infection. Instead of having parasiticidal effects, this medication is known to have parasitostatic ones. The ineffectiveness of benzimidazole as a therapy option may be due to its low water solubility and limited absorption. The lifetime and prognosis of CE patients are also negatively impacted by numerous serious side effects. These details emphasize the necessity for novel medications. As a result, numerous attempts have been undertaken by researchers to improve the substance's solubility, absorption, and accessibility (Siles-Lucas et al. 2018).

The clinical side effects of hydatid sore contamination are relying upon the site and aspect of the pimple. When the growths are slight but the sore size is expanding, the hydatid illness can be asymptomatic in its early stages. Impact from mechanical stresses on nearby organs, potential contamination, or anaphylactic shock after cyst rupture. The parasitic ailment known as hydatid disease, also known as cystic echinococcosis, is brought on by the formation of a fistula in a tapeworm. It can cause liver and other organs to develop blisters. (Gavara et al. 2015). The frequency is highest in areas with a high concentration of sheep farming, where free-roaming dogs at sick animals' organs. Due to the negative effects on people and their animals, it is also among the most serious helminthic infections in the nation with significant financial consequences. Nearby tissues could result in significant, even fatal medical signs (Hamad et al. 2022).

Cystic hydatidosis is caused by larval cestodes of the phylum Platyhelminthes (tapeworms). Their life cycle includes two hosts, one definitive and other is intermediate host People go about as unintentional transitional host. There are three stages of development in the life cycle: the eggs in the environment, the metacestode in the intermediate host, and the adult tapeworm in the final host. Metacestodes are ingested by the main host. The metacestodes grow into the tapeworm in the final host and release eggs into the environment. The intermediate host ingests the eggs that hatch into metacestodes, which move toward the liver, lungs, muscles and different organs of the moderate host (Gavara et al. 2015).

Cystic hydatidosis currently has two treatment options: chemotherapy and medical procedure (including mild laparoscopic, percutaneous waste consisting of cut and infusion. Additionally, the medical technique becomes illogical when lesions develop in various organs or in high-risk regions like the cerebrum and spinal tissues. Many efforts have been made to put up a successful immunization. However, there is currently no effective human vaccine against chronic hydatid disease (CHD) (Rokni 2009).

Benzimidazole derivatives like mebendazole and albendazole are the current chemotherapeutic experts used to treat hydatidosis. However, acne had a number of negative side effects, such as altered liver function, drowsiness loose stools, stomach pain, and migraines. Additionally, they were linked to baldness, thrombocytopenia, and severe leucopenia. Furthermore, because albendazole has been demonstrated to be teratogenic in animals, its usage is restricted (Yu et al. 2021).

The parasitology lab of the Veterinary Medication Staff received hydatid cyst from the livers and lungs of sheep. The outer layer of the hydatid growths was then washed with 70% ethyl alcohol. The lowest portion of the chambers became home to the protoscolices. The generated protoscolices were repeatedly washed with phosphate buffer saline after the supernatant was removed and then they were examined with 0.1% eosin. For further testing, the protoscolices that had more than 90% growth were selected (Norouzi et al. 2020).

Albendazole, fenbendazole, and other benzimidazoles are ineffectively water-solvent medications. The most popular strategy has been altered to serve as a suspension for oral organization, but regrettable GI absorption renders it poorly suited for root accessibility and reduces its resulting resistance to cystic echinococcosis (Küster et al. 2014). In an effort to address this problem, ricobendazole (also known as albendazole sulfoxide) was developed. It is the most potent anti-helminthic active metabolite of albendazole and has greater water solubility when compared to other potent benzimidazoles. Surfactants like polysorbate, bile salt, or cosolvents have been studied to increase the solubility of albendazole even though they are assimilation enhancers, but aggravate stomach-related mucosa. Additionally, it has been demonstrated that the solvency of albendazole was affected by the intense scattering with polyvinyl pyrolidone and poly-lactic corrosive nanoparticles (Küster et al. 2014).

In 94% of treated mice, albendazole (ABZ) sulfoxide was utilized to cure hydatid cysts. Due to the lengthy and expensive procedure involved in creating novel antagonists of parasite combinations, it is important to examine various bendazole subordinates and attempt to change the existing anti-helminthic in order to concentrate on their physicochemical and metabolic components effects (Garcia-Llamazares et al. 1998).

Flubendazole (FLBZ), a broad-spectrum anthelmintic, is a medication in the bendazole class. It is frequently employed to treat stomach nematodiasis in people, pets, and fowl. Additionally, recent research utilizing mouse models have shown FLBZ's efficacy against protoscoleces and hydatid sores under laboratory conditions and in mice (Gomes and Nagaraju 2001).

Under comparable trial circumstances, FLBZ has been shown to be more effective than ABZ against auxiliary mouse hydatid growths reference (Gomes and Nagaraju 2001). A prior investigation on sheep found minimal levels of FLBZ and its metabolite in plasma, but the protoscoleces in the animals' liver cysts definitely lost viability. In any event, FLBZ is unable to provide human CE with the usual sufficiency in such state of mind. FLBZ's poor action in humans is most likely explained by its hydrophobicity and low water solubility (Farhadi et al. 2018). Due to this, it has limited gastrointestinal absorptions, which causes low blood and hydatid cyst concentrations. In fact, the requirement for bigger doses and more frequent administration of the drug will result in more negative effects. In order to get over this limitation, it may be possible to dramatically boost FLBZ's absorption and systemic bioavailability, which would improve the drug's therapeutic benefits on human hydatid cysts (Farhadi et al. 2018).

In recent years, nanomedicine has enhanced both the effectiveness of treatments and the economic viability of widely used administration techniques. Particles of a submicron size (distance across 1m) are Nano-particles. In addition to increasing the properties of medications with poor water dissolvability, accessibility, drug kinetics and blood dissemination periods may also improve against chemotherapy for parasites. Improvements to polymeric nano-transporters have been made employing both biodegradable and non-biodegradable substances. A synthetic biological substance called methyl polyethylene glycol polycaprolactone (mPEG-PCL) is utilized to distribute hydrophobic medications. It is a diblock copolymer that is amphiphilic. Development a unique nano-formulation to improve the low water solubility of FLBZ and its absorption, effectiveness, and safety utilizing biodegradable nanoparticles (mPEG-PCL). Additionally, we looked into how well FLBZ-stacked mPEG-PCL nanoparticles worked to treat *E. granulosus* protoscoleces and sores (Farhadi et al. 2018). Later, the use of recently organized compounds, achieved by ligand-attaching NPs to modified surfaces, as well as electromagnetically created nanodrugs, could be useful for creating modified information as an alternative to the majority of currently available medications against these ignored parasitic hydatid disease (Khamesipour et al. 2021).

2. NANOPARTICLES

Nanoparticles have been explored for their expected remedial job in different illnesses, including parasitic diseases like hydatidosis. Their exceptional properties, for example, their little size and high surface region to-volume proportion, make them possibly appropriate for designated drug conveyance and upgraded drug bioavailability (Alanazi et al. 2010).

A few examinations have investigated the utilization of nanoparticles in conveying anthelmintic medications straightforwardly to the hydatid blisters to further develop drug viability and decrease foundational secondary effects. The nanoparticles might possibly upgrade drug porousness and maintenance inside the pimples, prompting further developed treatment results (Dutta et al. 2017).

The absolute huge advancements for illnesses following and treatment are nanotechnology and nanoparticles. Materials of nanoscale aspects (more modest than 100nm) are alluded to as "nanomaterials" and can be used to counterfeit normal nanoparticles (Roduner 2006).

Nanoparticles (NPs) have numerous uses in industries ranging from medicine to agriculture. Nanoparticles (silver, gold, zinc) are always being enhanced in the field of medicine for applications such as drug delivery, disease screening, and tissue design (Soares et al. 2018). As a result, nanotechnology has begun to play a crucial role in several fields, including catalysis, energy and climate, horticulture, optics, sensors, PCs, and a great deal more. One of the elements in these experiments that boosted the therapeutic compound's efficacy was the addition of nanoparticles (NPs) which increase the intra-cystic permeability of the curative substance. NPs are intensively explored nanostructures for new and better biomedical applications due to their size-related advantageous physicochemical features and biological usefulness, particularly their high antibacterial activity and non-toxic nature. The Iranian Nanomaterials Pioneers Co. supplied the small metal powder (99%, 20 nm-40 nm). The cleanest analytical grade metal oxide nanoparticles were used in this work (Soares et al. 2018).

3. CHARACTERISTICS OF NANOPARTICLES

Nanoparticles (NPs) have special qualities that can be employed in nanomedicine for the treatment and control of infections because of their small diameter (1-100 nm) as well as multiple structural forms. Due to issues like time-consumption, high toxic quality, inherent hazards, and various other flaws are

related to microbiology. There are a number of restrictions on the utilization of physical and chemical processes in nanoparticle synthesis. The term “green synthesis” refers to the union of nanoparticles derived from plants and it is recognized as a reliable, successful, and biologically appealing method widely applied in nanomedicine (Mühlebach 2018).

The microwave heating method has received a lot of attention for the mixing of nanoparticles because of a few features, such as uniform and precise warm diffusion, lower the time and energy needed to achieve amalgamation and enhancement of response time (Hamad et al. 2022).

4. PROPERTIES OF NANOPARTICLES

A few expected benefits of utilizing nanoparticles in treating cystic hydatidosis include:

5. DRUG CONVEYANCE UPGRADE

Nanoparticles can be intended to exemplify drugs to treat the illness. This embodiment can further develop drug dependability, dissolvability, and bioavailability, prompting more effective and designated drug conveyance to the pimples (Ahmad et al. 2003).

6. CONTROLLED DISCHARGE

Drug release can be controlled in nanoparticles, allowing for sustained drug release over time, which may be advantageous for long-term treatment (Begum et al. 2009).

7. DESIGNATED TREATMENT

Surface alterations of nanoparticles can empower them to focus on the cystic hydatid blisters, expanding the centralization of the medication at the site of contamination while diminishing foundational aftereffects explicitly.

8. IMAGING AND DIAGNOSTICS

Nanoparticles can be utilized for analytical purposes to help in the early identification of the diseases dynamics. A few examinations have researched the expected healing viability of nanoparticles against cystic hydatidosis:

A review distributed in the Diary of Medication Focusing in 2019 investigated the utilization of lipid-based nanoparticles for designated drug conveyance against Echinococcus granulosus protoscoleces. The researchers discovered that the nanoparticles improved albendazole's delivery, increasing its effectiveness against parasites (Iravani 2011).

In 2017, the Diary of Controlled Delivery distributed a concentrate on biodegradable polymeric nanoparticles stacked with albendazole for the treatment of hydatid growths. The outcomes showed that the nanoparticles had improved enemy of parasitic movement contrasted with the free medication. Further preclinical and clinical examinations are expected to completely assess the security and viability of nanoparticles as a remedial choice for cystic hydatidosis (Ahmad et al. 2003).

Before considering any novel treatments for this disease, it is essential to consult with medical professionals and stay up to date on the most recent research findings. The direction of medical services specialists for the most suitable and successful treatment of cystic hydatidosis is highly recommended (Begum et al. 2009).

9. ROLE OF SILVER NANOPARTICLES (AgNPs) TO CONTROL HYDATIDOSIS

AgNPs are suitable substitutes for other materials used in coatings, tissue scaffolds, and drug delivery. Other earlier investigations looked into the effectiveness of gold and silver nanoparticles from *Penicillium aculeatum* against *E. granulosus* protoscoleces. Biosynthesized AgNPs from various plant extracts, particularly *Eucalyptus globulus* extract, displayed outstanding efficacy against *E. granulosus*. Additionally, AgNPs could lessen the harmful effects of the preferred medication, albendazole (Hamad et al. 2022).

ABZ, the drug of choice to treat hydatid sore disease, has toxic effects that AgNPs may lessen. Necrosis, degeneration, steatosis, and increased serum hepatic proteins are just a few of the toxic effects of albendazole. Therefore, coating albendazole on AgNPs may be an effective way to increase ABZ's effectiveness against cystic echinococcosis (Hamad et al. 2022), they are readily available, have few adverse effects, and are inexpensive, nanoparticles are frequently evaluated and presented as alternatives by researchers. To avoid the secondary infection after hydatid cyst surgery, efficient scolicidal drugs are required. Although there have been numerous ways to produce nano sized particles. A century ago, the field of nano medicine as a modern science was recognized for the first time in the 1990s (Hamad et al. 2022).

Zizyphus spina-christi leaves were used for the green synthesis of AgNPs. AgNPs were administered orally to mice in dosages of 50 mg, 100 mg, 200 mg, and 300 mg/kg for a thorough assessment of momentary poisonousness, and assessments for dangerous signs were made constantly at 24, 48 hours and 14 days. According to tissue pathology analysis, the liver, kidneys, and digestive system of the mice displayed minor histological changes in compared to the control mice. The treated-contaminated mice showed a change in the liver hydatid sores' appearance from hyaline to smooth overcast as compared to the untreated infected mice (Hamad et al. 2022). AgNPs produced by biosynthesis have antagonistic to hydatic properties and are advised for use in the treatment of echinococcal sores. Due to its effects on both people and their livestock, it is also one of the most serious helminthic infections in the nation (Salih et al. 2020).

10. ROLE OF FERRIC OXIDE NANOPARTICLES (Fe2O3) TO CONTROL HYDATIDOSIS

Fe_2O_3 nanoparticle concentrations of 0.25, 0.5 and 1 mg/mL were added to the microtubes for the purpose of evaluating the drug action. A drop of protoscolex-rich sediment was also added. The tubes' fluids were gently combined. The test tubes were kept at 37 °C for 10, 30, and 60 minutes, respectively. The top phase was delicately removed at the end of each incubation period to prevent disrupting the hydatid cyst. The leftover, settling protoscolices were then delicately combined with 1 ml of 0.1% eosin dye. After incubating the fluid for 15 minutes, the top portion was discarded. In this way Fe_2O_3 nanoparticle composed in lab (Moazeni et al. 2017).

11. FERRIC OXIDE NANOPARTICLES ARE PREPARED

To evaluate the NPs' scolicidal properties, nanoparticle. In distilled sterile water, concentrations of 0.25%, and one milligram per milliliter were suspended (Shnawa et al. 2021).

12. ELECTRON MICROSCOPY FOR SCANNING

Three PBS washes were performed on electron microscopy for scanning *E. granulosus* protoscolices. Then, protoscolices were left to dry at room temperature. The protoscolices were then performed in

ethanol at increasing concentrations after drying. Finally, gold sputter coating was applied to processed samples before being analyzed (Rahimi et al. 2015).

Complex cystic echinococcosis cases are still typically treated with surgery. However, it has been linked to secondary spread or local recurrence. The inactivation of the protoscolices from hydatid cysts has been accomplished using a variety of chemical scolicidal treatments. Many of these scolicidal medications could have negative side effects, which would limit their use. For instance, among other drugs, Unfavorable side effects have been associated with 20% highly concentrated saline, 20% nitrate of silver, 0.5-1% cetrimide, ethanol, and 20 mg/mL albendazole medication sulfoxide (Hamad et al. 2022).

As an alternative to opening or removing cyst s, the eradication of protoscolices with scolicidal drugs has been suggested. This procedure is associated with high efficacy and little side effects. Additionally, patients who are not good clinical candidates can receive radiotherapy in conjunction with surgery (either before, during, or after).

Results showed that 20% nitrate of silver (20 min), 0.5–1% the chemical cetrimide (10 min), 20% hypertonic sodium chloride (15 min), a concentration of 95% ethyl alcohol (15 min), and 3% peroxide of hydrogen (15 min) all had scolicidal effects. Due to the use of nano-metal products, there is currently a higher emphasis on the need for efficient parasite management techniques. Because nanoparticles will probably pollute the environment, appropriate use rules and toxicity thresholds must be developed to lessen the impact on helpful bacteria, livestock, and food webs (Begum et al. 2009).

13. ROLE GOLD, SILVER, CHITOSAN AND OXIDIZED METAL AS A NANOPARTICLES

Numerous studies have shown that Ag, Au, chitosan, and oxidized metals have antiparasitic and suppressive actions on protoscolices. The concentration of selenium nanoparticles utilized ranged from 50 to 500 mg/mL over the course of 10 to 60 minutes, with a size range of roughly 80 to 220 nm. The findings showed that biogenic Se-NPs significantly reduce scoliosis at all concentrations, but particularly at 500 and 250 mg/mL after 10 and 20 min, respectively. Because Ag-NPs showed the most potent scolicidal effects, this study may lead to their usage in CE surgery (Torabi et al. 2018).

14. FORMATION OF GOLD NANOPARTICLES (AUNPS)

The gold nanoparticles were produced by the method of Turkevitch. 1ml of a 12.7 mM aqueous chloroauric corrosive (HAuCl4) mixture was added to 49 mL of distilled water. The mixture was heated while mixing until it started to bubble. The simmering mixture was then given 0.94 mL of a 38.8 M trisodium citrate solution after waiting for five minutes. After around two to three minutes, the blend's color turned reddish. The liquid was blended for 15 minutes, then cooled to the ambient temperature. The materials were spun down and repeatedly washed in distilled water to produce AuNPs (Çolak et al. 2019).

Au nanoparticles are commonly employed in research in medicine because they are thought to be safe, dependable, and biodegradable materials. According to a number of produced research, AuNPs didn't have any cytotoxic, genotoxic, identified ordered, or nearby side effects. In addition to being inactive and non-toxic, AuNPs also possess a special property known as the photo thermal effect. Circular AuNPs may transform the warm energy of the stored green laser light into warm energy through photo thermal impact (Çolak et al. 2019).

Warmth will disperse into the surrounding media, and by using AuNPs, localized warming can result in the destruction of warm cells. We take advantage of this phenomena when treating hepatic hydatid blisters

for protoscolices, and we degenrate these protoscolices by raising the temperature following localized heating by AuNPs. To put it another way, this idea promoted the employment of a laser to heat up AuNPs in a painful liquid, which eventually led to the passing of all the protoscolices. All quantities of gold nanoparticles significantly reduced the incidence of hydatid cyst scolices. The elimination of all protoscoleces in gold nanoparticles at a dose of 1 mg/mL took place in a period of sixty minutes (Napooni et al. 2019).

15. SCOLICIDAL ACTIVITY OF GREEN PRODUCED SILVER NPS

At various concentrations and exposure times, green-produced silver NPs have scolicidal action against protoscolices of Cystic Hydatid Disease (CHD). The results demonstrated that Ag-NPs exhibited significant scolicidal effects at all doses.?After 120 minutes of exposure, the doses of 0.1 and 0.15 mg/mL indicated mortality rates of 83% and 90%, respectively. Ag-NPs produced by biosynthesis had a 40% scolicidal activity at 0.025 mg/mL for 10 minutes. According to a report, because they are more affordable, safe, and non-toxic than the commonly utilized chemical materials for cystic hydatid disease (CHD) surgery, naturally occurring Ag-NPs may be considered as a potential scolicidal agent (Jalil et al. 2021).

Although this exposure time was too long for surgical procedures, Ag-NPs showed the highest scolicidal impact (80%) at 1 mg/mL dose after sixty minutes of contact. Since 10 minutes is the ideal amount of time for clinical conclusion, Ag-NPs have a 65.67% scolicidal impact in that time. The price of silver nanoparticles is identical to that of silver nitrate because they are only used in very small quantities. Metal oxide nanoparticles and Ag-NPs are impacted by the adsorption and penetration of the nanoparticles due to a change in the surface properties and a significant increase in the gap between the bands. The Ag-NPs have considerable scolicidal activity because they are smaller and more dispersed than silver nitrate (Hamad et al. 2022).

16. BIOGENIC SELENIUM NANOPARTICLES' PROTOSCOLICIDAL EFFECTS ON HYDATID CYST PROTOSCOLICES

Currently, many scolicidal agents which have serious drawbacks are used to inactivate cyst contents. Therefore, there is a critical need for surgeons to develop novel scolicidal medications with improved efficacies and fewer adverse effects. In this study, Bacillus sp. MSh-1, a recently identified marine bacterial strain, was used to create selenium nanoparticles that were tested for their ability to kill E. granulosus protoscoleces in vitro. We aseptically aspirated protoscolices from hydatid cyst-infected sheep livers. Se NPs with diameters ranging from 80 to 220 nm were used for 10 to 60 minutes at a variety of concentrations (50 to 500 g/ml). The vitality of protoscoleces was assessed using a 0.1% eosin stain (Mahmoudvand et al. 2014).

17. ALBENDAZOLE'S IMPACT ON HYDATID CYST PROTOSCOLECES WHEN USED ALONE AND WITH GREEN SYNTHESIZED ZINC NANOPARTICLES

The current study emphasizes green synthesis of the zinc nanoparticles using *Lavandula angustifolia* (Lamiaceae family), a plant with a wide range of therapeutic applications, including anti-inflammatory, anticancer, anti-oxidant, antiviral, and analgesic properties. Although other studies have proven that zinc nanoparticles that (ZnNPs) have a variety of natural and medicinal uses in microbial contamination, etc (Shakibaie et al. 2022).

17. BIOGENIC ZNNPS SETUP AND CHARACTERIZATION

The *L. angustifolia* components (ethereal parts) were extracted using the permeation process in 80% methanol for 72 hours at 21 °C. ZnNPs made using a technique that has been previously described. As mentioned earlier, the obtained ZnNPs were characterized using a spectrophotometer in the UV-visible range (JENWAY 6405), an X-ray diffractometer (Philips, PW1710), a filtering electron magnifying device (SEM, KYKY-EM3200), and Fourier change infrared spectroscopy analysis (FTIR, Shimadzu IR-470, Japan). Both by itself and in conjunction with albendazole, microwave therapy produces anti-parasitic effects on protoscoleces of hydatid sores (Norouzi et al. 2019).

18. ZNNPS CHARACTERIZATION

Suppl 1 had a description of the Zn NPs amalgamation. The largest assimilation was found to occur between 230 and 330 nm, according to a horrifying UV-vis analysis. ZnNPs produced by green synthesis have a globular form and a few masses of different sizes. The majority of the ZnNPs were between 50 and 60 nm in size, while their size ranged from 30 to 80 nm (Kohansal et al. 2017).

The microwave heating technique has received a lot of attention for the amalgamation of nanoparticles among the many methods for the amalgamation of nanomaterials because it possesses a few elements, such as indistinguishable and specific intensity dispersing, speeding up response and thereby lowering the significant investment expected to achieve union (Norouzi et al. 2019).

The description of the Zn NPs amalgamation as a vital component was introduced in Zinc (Zn), although it is fundamentally unclear how many different substances and macromolecules work and function in cell growth and the synthesis of DNA, RNA, and proteins. Green mixed ZnNPs ranged in size from 30 to 80 nm; the majority of these nanoparticles were between 50 and 60 nm in size. The greatest scolicidal effect of ZnNPs was observed in vitro at a concentration of 200 ng/ml, where it eliminated 81.6% of protoscolices. However, after 10 minutes of exposure, the protoscolices were completely eliminated by the combination of these nanoparticles with ALZ, especially at a concentration of 200 g/ml. However, in comparison to in vitro measurement (Norouzi et al. 2019).

North of 2000 cases of hydatid disease have been treated with benzimidazoles and monitored for up to a year in various studies. According to the results of these tests, the blisters disappeared in 10–30% of the patients; no morphological changes were seen in 20–30% of the cysts; and in 50–70% of the patients, a considerable regression in cyst size was seen. In general, factors like the size and age of the sores, the thickness of the host's implied connective tissue, calcification, growth complexity with multiple compartments or girl blisters, the ability of the medication to penetrate the pimple wall, and the ingenuity of a sufficient amount of the medication or its dynamic metabolite at the parasite area affect response to treatment (Shakibaie et al. 2022).

19. ZNNPS'S IN VITRO PROTOSCOLICIDAL IMPACTS

Effects of ALZ alone and in combination with ZnNPs on hydatid blister protoscoleces. The observed results showed strong scolicidal effects of ZnNPs compared to the control group (p 0.001), especially when combined with ALZ. ZnNPs showed the highest recorded scolicidal impact, eliminating 81.6% of protoscolices at a concentration of 200 g/ml. The combination of these nanoparticles with ALZ, particularly at a dosage of 200 g/ml, completely eradicated the protoscoleces after 10 minutes of presentation (Shakibaie et al. 2022).

20. AFFECT ON PROTOSCOLECES FROM EX VIVO

Injecting ZnNPs into hydatid blisters significantly increased the mortality of protoscoleces, especially when combined with ALZ. In any case, compared to in vitro testing, the studied medications took longer to display their scolicidal effects. ZnNPs at a concentration of 200 g/ml killed roughly 68.3% of protoscoleces after being present for 60 minutes; but, after 20 minutes, ZnNPs at the same concentration placed close to an ALZ killed 100% of protoscoleces (Shakibaie et al. 2022).

21. THE INITIALIZATION OF OPOPTOSIS

Caspase-3 enzyme activity was used to determine whether apoptosis had occurred in hydatid sore protoscoleces. After treating protoscoleces with various concentrations of ZnNPs for two days in a row, the concentration of released NA-p was determined in order to evaluate the changes in caspase-3 protein mobility. The outcomes demonstrated that at concentrations of 50, 100, and 200 g/ml, ZnNPs enhanced the caspase-3 protein by 13.4%, 27.3%, and 34.8%, respectively (Norouzi et al. 2019).
Unfortunately, the majority of these scolicidal drugs that are injected into sores have adverse effects that limit their use, such as sores that bleed excessively and unfavorable effects on living tissue, like liver cell corruption. Several investigations on the protoscolicidal and antihydatidcyst properties of different nanoparticles, such as silver nanoparticles, have been conducted at concentrations ranging from 1 to 4000 g/mL, however, their findings have been variable and occasionally conflicting. The union approach of the nanoparticles, the efficacy measurement strategy, and the application strategy may have an effect on the viability of the nanoparticles (Shakibaie et al. 2022).

22. GOLD NANOPARTICLES (AU-NPs)

For extraction, water soluble and dry plant powder were mixed in a ratio of 1:10, and the resulting mixtures were then shaken for one hour at 180 rpm. At that time, they were exposed for 40 minutes at 60°C to an ultrasonic shower (Shrewd Clean). The extracted materials were concentrated at 40 degrees Celsius using a vacuum rotary evaporator. The salvage was maintained at a cool temperature in a drab container until testing (Napooni et al. 2019).

23. UV-VISIBLE ANALYSIS

UV-vis spectroscopy was used to track the progression of the Au-NCs synthesis reaction at a wavelength of 350–680 nm. For this purpose, a sample was diluted with deionized water at a ratio of 1:10 and the absorption spectra were measured using a UV-VISIBLE spectrophotometer from LSI-Alpha (Çolak et al. 2019).

24. FILTERING ELECTRON MAGNIFYING INSTRUMENT INVESTIGATION

24.1. THE MORPHOLOGY OF GREEN SYNTHESIZED AU-NCS

Using a scanning electron microscope (SEM) with a 15 kV, 10x, and 1 nm resolution, the morphology of greenly produced Au-NCs was assessed. X-ray Diffraction (XRD) Analysis Green produced Au-NCs were subjected to XRD crystallography inside the channel run of a common point (10 to 80°C). By measuring

the X-ray source of a copper light with a wavelength of X bars in = 1.52 A0 by an XRD device display 2000 APD, XRD diffraction was selected (Çolak et al. 2019).

25. IN VITRO PROTOSCOLICIDAL EFFECTS FOR IN-VITRO PROTOSCOLICIDAL

Take into account that in test tubes, protoscoleces (1 mL) were freely revealed to 1 mL Au-NCs at 100–400 g/mL. The tube filling was gently combined and then exposed to 37°C for 10, 20, 30, and 60 minutes. After each period of production, the supernatant was collected, and 0.05 mL of eosin recolor (0.1%) was added to the mixture. Even if the common sense and dead parasites independently rose up without color and pink, the mortality rate of the treated protoscoleces was observed beneath a light amplification device. Independently, the dissolve silver nitrate and regular saline were linked as the positive and negative medications (Napooni et al. 2019).

26. EX VIVO PROTOSCOLICIDAL IMPACTS OF AU-NCS

To consider the ex vivo properties of Au-NPs on protoscoleces, about half of the substance of the hydatid cyst were arranged of, and after that Au-NCs at 100–400 mix and examine under SEM (Napooni et al. 2019).

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Zoonotic Parasitic Disease Control Strategies: Phytotherapy

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ABSTRACT

Zoonotic diseases have significant challenges to the global health by presenting a continuous threat to the animal and human populations. Ectoparasites and endoparasites are also responsible for transmission of zoonotic diseases. Ectoparasites like ticks, fleas, mosquitoes are responsible for transmission of Lyme disease, babesiosis, plague, malaria, dengue, zika and West Nile Viruses. Some arthropods like sandflies which transmit leishmania in humans and animals. Endoparasites like tapeworms are responsible of echinococcosis and cysticercosis and some soil transmitted roundworms and hookworms also cause toxocariasis and cutaneous larva migrans. Trichinella spiralis is the roundworm of pig and its spread by consumption of undercooked meat of pork and it's commonly known as pork worm. Some of protozoan water borne parasites like giardia and cryptosporidium are also responsible for gastrointestinal illnesses. Toxoplasma gondii oocyst shed in cat feaces and it's dangerous for pregnant women's and most of time it causes abortion in females. Many of these parasites develop resistance due to excessive use of synthetic acaricides like pyrethroids, macrocyclic lactones, organophosphate and carbamates. So its alternative is herbal or medicinal plants like garlic, neem, cloves and wormwood extract which contain bioactive compounds that can kill or inhibit parasites. Certain medicinal plants also offer nutrients to animal and boost the immune system along with antiparasitic properties. Phytotherapy along with conventional medicine reduce side effect and enhance the efficacy of treatment. In this book chapter we will focus on zoonotic parasitic control strategies through herbal or medicinal plants.

Key words: Phytotherapy, Ectoparasites, Endoparasites, Acaricides

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1. INTRODUCTION

Zoonotic diseases have led to the production of significant challenges to global health by presenting a continuous threat to animal and human populations. The main causes of infection of transmission of zoonotic diseases include endoparasites and ectoparasites (Abdel-galil and Aboelhadid 2021). Endoparasites are microorganisms that live and multiply within the host, while those that cause externally infest the host are called ectoparasites. Zoonotic endoparasitic diseases, such as toxoplasmosis, cryptosporidiosis, and echinococcosis, are caused by protozoa and helminths that can affect animal and human health (Abo-EL-Sooud 2018).

The parasitic life cycles are complex depend on hosts and vectors, and exhibit different modes of transmission. Life cycles of these parasites are significantly affected by human activities (urbanization, deforestation, and climate change) which change their emerging and spreading pattern (Akhtar et al. 2012). In comparison, vectors such as ticks, mosquitoes, and fleas, are mainly involved in the causation and transmission of zoonotic ectoparasitic diseases. Lyme disease, one of the well-known zoonotic diseases, is caused by the bacterium *Borrelia burgdorferi* and transmitted by ticks. It affects the integumentary, skeletal, cardiac, and nervous systems, resulting in a multi-systemic disorder (Akhtar et al. 2012).

Fleas cause the transmission of the bacterium *Yersinia pestis* which results in the bubonic plague. Furthermore, global-health-threatening diseases such as malaria and West Nile Fever are caused by mosquitoes transmitting the plasmodium parasite and West Nile Virus (Al-Zanbagi 2009). Phytotherapeutics (herbal or traditional medicines) are herbs and plants that possess medicinal properties and are used to treat disease conditions in animals and humans. Throughout history, phytotherapeutics has been used to treat morbidities and infections, manage pain, and treat and control both endoparasites and exoparasites (Al-Zanbagi 2011). Using plants as therapy dates back to old times when ancient civilizations utilized their nature-related knowledge to cure pathological conditions. Phytotherapeutics have been regularly used in traditional healing practices by several cultures (Al-Zanbagi and Zelai 2008).

In recent years, there has been an increase in the concerns related to drug resistance, side effects of synthetic therapeutics, and the recognition of the medicinal potential of plants natural compounds, which resulted in a resurgence of interest in the use of phytotherapeutics.

Extensive use of synthetic anti-parasitic drugs has made the parasites resistant to them (Andreotti et al. 2013).

Several medicinal herbs possess exceptional anti-microbial and cytotoxic activities, and their use is beneficial in controlling both ectoparasites and endoparasites.

Biologically active compounds of these medicinal plants exhibit their anti-parasitic activity by targeting the parasite genome (DNA), damaging the cellular integrity, and interrupting the nervous system of the parasites (Annan-Prah et al. 2012).

Many plant extracts and their secondary metabolites show excellent anti-protozoal activity by hampering the growth of Plasmodium, Trypanosoma, Leishmania, Trichomonas, and intestinal helminths. Considering the extensive use of traditional therapeutics, the World Health Organization (WHO) has recognized the role of phytotherapeutics in the Alma-Ata Declaration 1978 of Health-for-All (Arab et al. 2006).

2. HISTORY OF PHYTOTHERAPY

Phytotherapy is an ancient healing practice that involves using plants and their extracts to treat various health conditions. This brief history of phytotherapy explores the origins and development of this traditional healing approach (Attisso 1979). The roots of phytotherapy can be traced back to prehistoric times when early humans relied on their knowledge of the natural world to identify plants with medicinal properties. Archaeological evidence and ancient writings from civilizations like Egypt, Mesopotamia, China, and India reveal the use of herbal remedies in their healing practices (Awais et al. 2011).

The classical Greek and Roman periods were instrumental in shaping phytotherapy as a formal medical discipline. Renowned figures such as Hippocrates and Dioscorides extensively documented the medicinal uses of plants, laying the groundwork for subsequent generations (Bauri et al. 2015). During the medieval and Renaissance eras, monasteries played a pivotal role in preserving and advancing herbal knowledge. The Age of Exploration further enriched phytotherapy with the discovery and exchange of medicinal plants from various regions across the globe (Beigh and Ganai 2017).

In modern times, the field of phytotherapy observed significant developments due to advancements in chemistry and pharmacology. While the rise of modern pharmaceuticals gained prominence, herbal medicine continued to be valued in traditional healing practices worldwide (Benoit-Vical et al. 2000). Today, phytotherapy remains an essential component of traditional medicine in many cultures and has found its place within complementary and alternative medicine (CAM) in Western societies. The integration of ancient herbal wisdom with modern scientific validation continues to drive its relevance and recognition in promoting health and well-being (Brown et al. 1998).

3. MODES OF ACTION OF PHYTOTHERAPEUTICS

The active compounds present in plants responsible for their medicinal properties are known as phytochemicals. These bioactive substances interact with the body's physiological processes to exert therapeutic effects (Casida 1980). Modes of action of phytotherapeutics in treating and controlling parasites include *1. Anthelmintic Properties*: Many phytotherapeutic compounds possess anthelmintic activity, meaning they can kill or expel parasitic worms (helminths) residing in the host's gastrointestinal tract or other organs. *2. Insecticidal and Acaricidal Properties:* Some phytochemicals act as natural insecticides and acaricides, effectively eliminating ectoparasites such as fleas, ticks, mites, and lice (Choi et al. 2008).

3. Immunomodulatory Effects: Certain phytotherapeutics can modulate the host's immune response, bolstering its defense mechanisms against parasites. *4. Antiprotozoal effects:* Phytochemicals can disrupt the membrane integrity of protozoa, interfere with their energy metabolism, and inhibit their ability to invade host cells. *5. Repellent Action:* Some plant extracts act as repellents, deterring parasites from infesting the host in the first place (Christenhusz and Byng 2016). Table 1 and 2 highlights the mode of action of antiparasitic plants against ectoparasites and endoparasites.

4. PHYTOTHERAPEUTICS FOR ENDOPARASITES

There are several herbal plants with properties to act as antiparasitic treatment, they include a) Wormwood (*A. absinthium*): it contains the compound artemisinin, which exhibits potent antiparasitic properties against various endoparasites (Gefu et al. 2000). It is particularly effective against intestinal worms such as roundworms and hookworms. It has a long history of use in traditional medicine for various purposes, including anti-parasitic medicine. b) Black Walnut (*J. nigra*) is a tree native to North America, and its various parts, including the hulls, leaves, and bark, have been traditionally used for medicinal purposes (George et al. 2008). It is believed to possess antiparasitic properties, particularly

against intestinal parasites. Its anthelmintic effects are due to the presence of certain active compounds, such as juglone, tannins, and flavonoids. c) Garlic (*A. sativum*) contains several bioactive compounds that contribute to its antiparasitic properties and are as follows: i) Allicin: a sulfur-containing compound that is formed when garlic is crushed or chopped and is one of the most potent and biologically active compounds and has a proven activity against a wide range of parasites, including protozoans and helminths ii) Diallyl Disulfide (DADS): another sulfur-containing compound of garlic exhibiting antiparasitic activity against intestinal parasites (protozoa and helminths) (Gouda et al. 2014) iii) Ajoene: a sulfur-containing compound, shown to have antiparasitic effects, particularly against the malaria parasite (*Plasmodium spp*.) and certain skin parasites like scabies mites (*Sarcoptes scabiei*). iv) S-Allyl Cysteine (SAC): a bioactive and water-soluble compound found in garlic, contributes to the overall antiparasitic properties of garlic (Hadimani and Gupta 2011). v) Sulfur compounds: and their collective presence contributes to the herb's overall antimicrobial and antiparasitic effects d) Papaya (*C. papaya*) contains i) Papain, a proteolytic enzyme found in the latex or milky sap of unripe papaya fruit and is known for its digestive properties and antiparasitic activity against intestinal parasites (Hammond et al. 1997). This enzyme helps break down the protective outer layer of parasites, making them more susceptible to the body's immune response and other treatments ii) Carpaine, an alkaloid found in papaya leaves has demonstrated antiparasitic properties iii) Flavonoids (quercetin and kaempferol) act as antimicrobial and antiparasitic iv) Tannins are polyphenolic compounds and contribute to the fruit's antimicrobial and antiparasitic effects v) Alkaloids exhibit antiparasitic properties vi) Cysteine Proteinases: contribute to antiparasitic activity (Hördegen et al. 2003).

Table 1: Mode of action of antiparasitic plant against ectoparasites**.**

No.	Plant	Constituents	Mode of Action	References
1	Neem (*Azadirachta indica*)	Azadirachtin, Nimbidin, Nimbin	Disruption of Reproduction and Growth	(Chungsamarnyart and Jansawan 2001)
		Neem oil	Disruption of feeding and digestion	(Cordeiro et al. 2005)
		Limonoids	Cell membrane damage	(Costa et al. 2006)
		Gedunin	Immune system modulation	(de Almeida et al. 2012)
		Neem oil	antimicrobial activity,	(Diaz Lira et al. 2005)
2	Eucalyptus (*Eucalyptus globulus*)	Eucalyptol 1,8-Cineole	Interfere with cell membrane integrity	(Ekanem et al. 2004)
		Limonene	Disrupt the growth and survival of various parasites	(Ekanem and Andi Brisibe 2010)
		Alpha-Pinene and Beta-Pinene	Interfere with cell membrane integrity	(Costa et al. 2006)
		Terpinen-4-ol	Disturb the growth	(Fajimi and Taiwo 2005)
3	Lavender (*Lavandula angustifolia*)	Linalool	Disrupt the cellular activities	(Fajimi et al. 2003)
		Linalyl acetate	Impairs the digestive function	(Fajimi et al. 2002)
		Camphors	Growth disruption	(Fernandes et al. 2008)

5. PHYTOTHERAPEUTICS FOR ECTOPARASITES

a) Neem (*A. indica*): It contains different bioactive compounds that contribute to its antiparasitic properties. The different parts of the neem tree, including the leaves, seeds, bark, and oil, contain these compounds such as: i) Azadirachtin: a primary bioactive compound found in neem seeds and acts as a potent insecticide and antiparasitic agent interferes with the development and growth of various insect larvae (mosquito larvae and agricultural pests) ii) Nimbin and Nimbidin: possess antifungal, antibacterial, and antiparasitic properties, and are effective against various parasites and pathogens (Hoste et al. 2005) iii) Gedunin: a limonoid compound shown to have antimalarial activity iv) Salannin: another limonoid that exhibits antiparasitic activity against various pests and parasites v) Quercetin: a flavonoid

that has demonstrated antimicrobial and antiparasitic properties (Hounzangbe-Adote et al. 2005) vi) Beta-Sitosterol: a phytosterol found in neem leaves and seeds, possesses antiparasitic effects against certain parasites vii) Azadirone and Azadiradione: These compounds are found in neem oil and have insecticidal and acaricidal (killing mites) properties viii) Neem Volatile Oil: This oil contains various volatile compounds contributing to its antiparasitic effects (Jang et al. 2007).

Table 2: Mode of action of anti-parasitic plant against Endo-parasites

No.	Plant	Constituents	Mode of Action	References
1	Wormwood (*Artemisia absinthium*)	Artemisinin	• Formation of free radicals • Disruption of membrane structure • Heme accumulation leads to toxicity • Inhibition of parasitic growth and development	(Costa et al. 2006)
2	Black Walnut (*Juglans nigra*)	Juglone	Disruption of parasites physiology and metabolism	(de Almeida et al. 2012)
		Tannins	Precipitation and inactivation of parasite cell proteins	(Diaz Lira et al. 2005)
		High ORAC value	Neutralizes free radicals. Immunomodulation.	(Ekanem et al. 2004).
3	Garlic (*Allium sativum*)	Allicin	• Interfering with the structure and function of parasitic cellular components • Disruption of the integrity of the cell membranes and metabolism	(Ekanem and Andi Brisibe 2010)
		Allyl Cysteine (SAC)	• Immunomodulatory properties. • Antioxidant activity.	(Costa et al. 2006)
4	Papaya (*Carica papaya*)	Chymopapain proteolytic enzymes	Degrade parasite proteins	(Ekanem and Andi Brisibe 2010)
		Immune modulation		(Costa et al. 2006)
		Carpaine	Antioxidant activity	(Fajimi and Taiwo 2005)
		Flavonoids	Antimicrobial properties	(Ekanem and Andi Brisibe 2010)

b) Eucalyptus (*E. globulus*) oil contains cineole, which acts as a natural insect repellent and can be used to control ectoparasites. Eucalyptus is a fast-growing evergreen tree native to Australia, but it is now cultivated in many parts of the world for its medicinal and aromatic properties (Jansawan et al. 1993). The essential oil extracted from it is particularly well-known for its antiparasitic and antimicrobial effects. The primary constituents of Eucalyptus essential oil are: i) Eucalyptol (1,8-Cineole): it is the major active compound typically comprising 60-80% of the oil. It is responsible for the characteristic aroma and many of the medicinal, antimicrobial, and antiparasitic properties ii) Alpha-Pinene and Beta-Pinene: These are monoterpenes and contribute to the oil's antimicrobial activity and can also help deter certain parasites iii) Limonene and Terpinen-4-ol: both are monoterpene with antiparasitic properties iv) Terpinen-4-ol and Alpha-Terpineol: these are alcohol that exhibits strong antimicrobial, antifungal, insecticidal, and antiparasitic properties v) Phenolic compounds: such as catechins and flavonoids possess antimicrobial and antiparasitic effects (Kaaya et al. 1995).

c) Lavender *(L. angustifolia),* a popular aromatic herb known for its calming and soothing properties. While lavender is primarily valued for its use in aromatherapy and relaxation, it also possesses certain bioactive compounds that may exhibit antiparasitic properties against ticks and fleas (Kavitha et al. 2012). However, it's essential to understand that lavender's antiparasitic effects are relatively mild compared to other herbs specifically known for their antiparasitic activity. The constituents of lavender that may contribute to its antiparasitic properties include: i) Linalool: its significant amount is present in the oil and is known for its pleasant floral scent with demonstrated parasitic properties (Khan et al. 2008) ii) Linalyl Acetate and Camphor: which may contribute to the overall antiparasitic effects of

lavender. Lavender may have some potential for supporting the body's natural defense against parasites due to its mild antimicrobial properties (Kiss et al. 2012).
d) Citronella (*Cymbopogon nardus*): Citronella oil is a well-known natural mosquito repellent that can be useful for controlling blood-sucking ectoparasites. The essential oil of citronella is composed of several constituents, and while some of them have shown antimicrobial activity, their direct antiparasitic effects against internal parasites have not been extensively studied (Kostadinovic et al. 2012). The main constituents of citronella essential oil include: i) Citronellal: a major component of the oil, responsible for its lemon-like scent. It exhibits insect-repelling properties and has some antimicrobial activity against bacteria and fungi ii) Geraniol and Citronellol: these have antimicrobial activity and are known for their insect-repelling properties iii) Geranyl Acetate: present in the oil and contributes to its aromatic profile (Lans et al. 2007).

6. INDIRECT METHOD TO COMBAT THE PARASITES

The use of Condensed Tannins (CT) affects the helminths in 2 ways:

6.1. INDIRECT EFFECT

The indirect effect includes feeding the animals with forages rich in tannin amount, resulting in CT release and formation of abomasum-degraded CT-Protein complex (Lee et al. 2008). This complex helps in combating protein loss caused by helminth infestation and supports more protein release to overcome parasite-generated losses.

6.2. DIRECT EFFECT

Direct effect includes the formation of CT-chillates with surface proteins of the parasite body, impairing the normal functioning of the vital organs of parasites (locomotory, digestive, and reproductive organs) (Macarenco et al. 2001). Table 3 enlists the plants used against ruminant endoparasites. Table 4 shows the medicinal plants used for the treatment of various parasitic infections.

7. MEDICINAL PLANTS USED FOR THE TREATMENT OF ARTHROPOD INFESTATION

In a study, tobacco leaves and steam extracts were shown to be completely efficacious against lice and kept repelled the parasite for 56 days in African goats. Neem skin cream showed excellent antiparasitic activity when mixed with shampoo foams. Table 5 shows the medicinal plants used against arthropod infection.

8. FUTURE PERSPECTIVES AND UPCOMING DIRECTIONS

There is an increase in interest in the role of phytotherapeutics in controlling zoonotic parasitic diseases in the field of both traditional and modern medicine (Sandoval-Castro et al. 2012). The use of Phyto-medicines acts as a potential alternative approach to traditional antiparasitic drugs, and suggests several future perspectives and upcoming directions in this field:

8.1. PHYTOCHEMICAL RESEARCH

The objective of the ongoing research is to identify and isolate the active biological compounds of the plants that show anti-parasitic activity.

Table 3: Plants used for ruminants endoparasites

Animals	Scientific names of plants	English names of plants	Used parts	Parasite Types	References
Sheep	*Achellia millefolium L.*	Yarrow	Whole, Extract	GIT Nematodes	(Madzimure et al. 2011)
	Alnus glutinosa L.	Alder	Shoots	Trematodes	(Mandeel and Taha 2005).
	Artemisia absinthium L.	Wormwood	Aerial parts, Extract, Whole, Leaves	Roundworms including, *Toxocara vitulorum*, *Haemonchus contortus* and *Trichostrongylus colubriformis* Tapeworms, *Eimeria spp.*	(Matovu and Olila 2007)
	Artemisia campestris L.	Field wormwood	Leaves, Extract	Effective against roundworms especially *H. contortus*	(Michels et al. 2011)
	Artemisia maritime L.	Sea wormwood		Nematodes	(Min and Hart 2003)
	Artemisia vulgaris L.	Mugwort	Leaves, extract	It is effective against roundworms, especially *T. colubriformis*	(Min et al. 2005)
	Betula pubescens Ehrh.	Downy birch	Leaves, Bark	Nematodes, Trematodes & Cestodes	(Molan et al. 2009)
	Calluna vulgaris L.	Hill/Heater	Leaves, Bark	Flukes (Trematodes)	(Molan et al. 2000)
	Cichorium intybus L.	Chicory	Whole	It's effective against Gastrointestinal tract roundworms & lungworm infections	(Mothana et al. 2014)
	Dryopteris filix-mas L.	Male Fern	Roots	Roundworms including, *Trichostrongylus colubriformis* It is also effective against *Fasciola spp.* and *Dicrocoelium spp*. of class trematodes	(Mudi and Bukar 2011)
	Humulus lupulus L.	Hop	Whole, Roots	It is effective against helminths, especially tapeworms and flukes	(Mwangi et al. 1995)
	Juniperus communis L.	Juniper	Bark, Roots	It is effective against Trematodes, especially liver flukes	(Madzimure et al. 2011)
	Lepidium sativum L.	Garden cres	Whole, Seeds	Helminths especially trematodes	(Mandeel and Taha 2005)
	Nigella sativa L.	Garden fennel	Seeds, Extract	It's effective against gastrointestinal tract roundworms & tapeworms	(Matovu and Olila 2007)
	Pastinaca sativa L.	Wild parsnip	Aerial parts	Endoparasites	(Michels et al. 2011)
Sheep	*Pyrus communis L.*	Pear	Berries	Roundworms	(Min et al. 2005)
	Salix spp.	Willow	Bark, Leaves	It is effective against helminths, especially tapeworms & flukes	(Molan et al. 2009)
	Symphori-carpos albus L.	Snowberry	Leaves	Cestodes	(Molan et al. 2000)
	Tanacetum vulgare L.	Tansy	Aerial parts, Whole, Leaves, Seeds	Roundworms including, *Trichostrongylus colubriformis* Trematodes and Cestodes	(Mothana et al. 2014)
	Urtica dioica L.	Common nettle	Whole, Seeds	It is effective against helminths, especially flukes	(de Almeida et al. 2012)
	Valeriana officinalis	Common valerian	Roots	Roundworms including *T. colubriformis*	(Diaz Lira et al. 2005)

Goat	*Artemisia absinthium L.*	Wormwood	Aerial parts, Extract, Whole, Leaves	Roundworms including, *T. colubriformis, H. contortus, T. vitulorum.* Tapeworms *Eimeria protozoal spp*.	(Ekanem et al. 2004)
	Cichorium intybus L.	Chicory	Whole	It is effective against gastrointestinal roundworms and lungworms	(Ekanem and Andi Brisibe 2010)
	Artemisia campestris L.	Field wormwood	Leaves, Extract	Roundworms (*H. contortus*)	(Costa et al. 2006)
	Dryopteris filix-mas L.	Male-fern	Roots	Roundworms (*T. colubriformis*) Flukes (*Fasciola Spp.* and *Dicrocoelium spp.*)	(Naidoo et al. 2008)
	Juniperus communis L.	Juniper	Berries, Roots	It is good against flukes and is effective against liver flukes.	(Ndumu et al. 1999)
	Nigella sativa L.	Garden fennel	Extract, Seeds	Gastrointestinal tract (GIT) Roundworms, Tapeworm	(Niezen et al. 2002)
	Pastinaca sativa L.	Wild parsnip	Aerial parts	Endoparasites	(Nweze and Obiwulu 2009)
	Symphori-carpos albus L.	Snoeberry	Leaves	Cestodes	(Nwosu et al. 2011)
Cow	*Artemisia absinthium L*	Wormwood	Aerial parts, Extract, Whole, Leaves	Roundworms (*T. colubriformis, T. vitulorum and H. contortus*) Tapeworm, *Eimeria spp.*	(Nwude and Ibrahim 1980)
	Acorus calamus L.	Sweet-flag	Roots	It is effective against helminths.	(Orengo et al. 2012)
	Artemisia vulgaris L.	Mugwort	Leaves, Extract	Nematodes, *T. colubriformis*	(Paolini et al. 2004)
	Cichorium intybus	Chicory	Whole	GIT Nematodes, Lungworm,	(Papazahariadou et al. 2010)
	Dryopteris filix-mass L.	Male fern	Roots	Roundworms, *T. colubriformis*. Flukes, (*Dicrocoelium spp. Fasciola Spp.*)	(Patel et al. 2009)
	Iris Pseudocorus L.	Yellow iris	Roots	Helminths	(Ekanem et al. 2004)
	Juniperus communis L.	Juniper	Berries, Roots	It is effective against trematodes especially (liver fluke)	(Ekanem and Andi Brisibe 2010)
	Lotus corniculatus L.	Bird`s-foot-trefoil		Roundworms, (*Ostertagia ostertagi* and *Cooperia oncophora*) Lungworm (*Dictyocalus eckerti*)	(Costa et al. 2006)
	Pastinaca ssativa L.	Wild parsnip pear	Aerial parts	Endoparasites	(Ekanem and Andi Brisibe 2010)
	Quercus Robur L.	Pedunculate oak	Nuts	Helminths	(Costa et al. 2006)
	Salix spp	Willow	Bark, Leaves	It is effective against helminths, especially tapeworms and flukes	(Fajimi and Taiwo 2005)
	Senecio Vulgaris L.	Groundsel	Leaves	Cestodes	(Ekanem and Andi Brisibe 2010)
	Symphori- carpus albus	Snowberry	Leaves	Endoparasites	(Costa et al. 2006)

An in-depth understanding of the bioactive compounds of medicinal plants' is now possible by using advanced phytochemical analysis techniques (Mass spectrometry and Nuclear magnetic resonance) (Sathiyamoorthy et al. 1999).

8.2. MECHANISMS OF ACTION

Phytotherapeutics can be effectively used if their anti-parasitic modes of action are well understood. The understanding of the parasites-phytocompounds interaction at the molecular level can reveal more of plants antiparasitic characteristics.

8.3. SYNERGY AND COMBINATION THERAPY

Studies are being conducted to find what different combinations of plant extracts possess synergism that improve their antiparasitic activity (Smith-Schalkwijk 1999). Using phytotherapeutics in combination with conventional drugs may also result in enhancement of the therapeutic results and reduction in the development of parasitic resistance (Su and Mulla 1999).

8.4. CLINICAL TRIALS AND VALIDATION

While the use of several medicinal herbs in antiparasitic therapy has been documented by old civilization knowledge however extensive clinical studies are required for the validation of their efficacy and safety. More randomized controlled trials (RCTs) are being conducted to establish the evidence-based use of phytotherapeutics against zoonotic parasites.

8.5. FORMULATION DEVELOPMENT

Developing standardized and stable formulations of phytotherapeutics is crucial for their widespread use. This includes creating extracts, capsules, or topical formulations with consistent levels of active compounds to ensure reproducible outcomes (Tariq and Tantry 2012).

8.6. BIOAVAILABILITY AND PHARMACOKINETICS

Understanding the bioavailability and pharmacokinetic properties of plant compounds is crucial for optimizing dosing regimens and ensuring that therapeutic levels are achieved in the body.

8.7. PLANT BIOTECHNOLOGY

Advancements in plant biotechnology, such as genetic engineering and recombinant DNA technology, may facilitate the production of high-yield, standardized, and genetically modified plants with enhanced antiparasitic properties and targeted molecular drug delivery.

8.8. ETHNOPHARMACOLOGICAL STUDIES

Collaborations between traditional healers, Eastern medicine doctors, and medical scientists can lead to the discovery of novel plant-based medicines. This will improve our understanding of traditional medicine effectiveness, safety, bioavailability, and applications.

8.9. ONE-HEALTH APPROACH

Adopting a "One-Health" approach, which recognizes the interdisciplinary approach of human, animal, and environmental health, can help address zoonotic diseases more effectively. Phytotherapeutics may play a role in the transmission of zoonotic diseases to humans.

Table 4: Medicinal plants used for the treatment of various parasitic infections including *Toxoplasma gondii*

Plant Name	Parts Used	Extraction Method	Biological effect	References
Vernonia colorata	Stems and leaves	Air-dried, powdered, and ethanolic extract	It has the anti-toxoplasmic activity	(Pereira and Famadas 2006)
Zingiber officinale	Stems and Leaves	Air-dried, powdered, and ethanolic extract	It has the anti-toxoplasmic activity	(Pirali-Kheirabadi and da Silva 2010)
Sophora flavescens	Stems and Leaves	Air-dried, powdered, and ethanolic extract	It has the anti-toxoplasmic activity	(Poyares et al. 2005)
Torilis japonica	Stems and Leaves	Air-dried, powdered, and ethanolic extract	It has the anti-toxoplasmic activity	(Fajimi and Taiwo A 2005)
Euricoma longifolia	Roots	Air-dried, powdered, and methanolic extract	It has the anti-toxoplasmic activity	(Ekanem and Andi Brisibe 2010)
Callotropis procera	Leaf	Air-dried, grounded and ethanolic soak	It has an anti-malarial effect.	(Russo et al. 2009)
Pulicaria crispa	Leaf	Air-dried, powdered and methanolic extract	It has the anti-malarial and anti-cancer activity	(Niezen et al. 2002)
Euphorbia retusa	Leaf and stem	Dried at room temperature, powdered and methanolic extract	It has the Anti-bacterial activity	(Nweze and Obiwulu 2009)
Rumex spinose	Leaf	Air-dried chloroformic and methanolic extract	It has the anti-fungal (*Candida albicans*, *Alternaria alternate*, *Saccharomyces cerevicsiae*)	(Refahy 2011)
Ocradenus baccatus	Leaves and flower	Air-dried powdered and methanolic extract	It has anti-malarial, anti-leishmanial, anti-trypanosomal, and hypocholesterolemic effect	(Regassa 2000)
Lycium shwii	Leaves	Oven-dried grounded and methanolic extract	Hypoglycemic Anti-plasmodial and anti-trypanosomal effect	(Refahy 2011)
Curcuma longa	Stem and leaf	Air-dried water and ethanolic extract	It has the anti toxoplasmic activity	(Poyares et al. 2005)

Table 5: Medicinal plants used for the treatment of arthropods infestation

Plant	Part used	Active compound	Efficacy	Reference
Stemona collinsae	Root	Extract	*In vitro* and *in vivo* against *B. microplus* (Mortality of Nymph & Adult)	(Zeineldin et al. 2018)
Aganonerion polymorphum	Leaves and stem	Crude ethanolic extract	Mortality of *Boophilus microplus*	(Zaman et al. 2012)
Calotropis gigantean	Leaf and stem	Crude ethanolic extract	*Mortality of B. miroplus*	(Niezen et al. 2002)
Margaritaria discoidea	Leaf & stem (Bark is more acaricidal)	Hexane extract	Nymph mortality of *Rhipicephalus appendiculatus*	(Nweze and Obiwulu 2009)
Osimum suave	Aerial parts	Oil extracted by steam distillation	Larvae mortality of *R. appendiculatus*	(Youn et al. 2003)
Pimenta dioica	Leaf	Hexane extract / Essential oil	Mortality and inhibit oviposition in *B. microplus*	(Wink 2012)
Azadirachta indica	Seed	Oil extract	In vitro acaricidal against *Amblyoma variegatum* (Larvae mortality)	(Youn and Noh 2001)

Tamarindus indicus	Mature fruit	Aqueous & (10% Ethanol extract)	Mortality of *B. microplus (Engorged females)*	(Wichtl 2004)
Euphorbia obovalifolia	Aerial parts	Crude extract	Inhibitory effect on all stages of *Rhippicephalus decoloratus*	(Viegi et al. 2003)
Dahlstedtia pentaphyla	Root	Ethanol extract (terpenoids)	In vivo spray on bovines Adulticide against *B. microplus*	(Waller et al. 2001)
Copaifera reticulate	Stem & Leaf	Extract (Terpenoids)	Larvicidal against *B. microplus*	(Urban et al. 2008)
Tephrosia vogelii	Leaf	Methanol aqueous and other extracts	In vitro efficacy against various genra of *Ixodid ticks* Cidal (Nymph & Adult)	(Turolla and Nascimento 2006)
Hypericum polyanthimum	Aerial parts	Crude methanolic extracts	In vitro Larvicidal (100% @ high concern.) against *B. microplus*	(Uchegbu et al. 2011)
Magonia pubescens	Stem, Bark	Crude Ethanolic extracts	Larvicidal against *R. sanguineus*	(Trojan-Rodrigues et al. 2012)
Calea serrata	Leaf & stem	Hexane extract Precocene	Inhibit *B. microplus* egg hatching	(Thamsborg et al. 2001)
Aloe ferox & Ptaeroxylon oblicum	Fresh leaves (crushed)	Overnight soaked in water	High degree control of *B. microplus*)	(Tipu et al. 2002)
Pelargonitum Roseum & Ecucalyptus globulis	Aerial parts	Essential oil	Adulticidal effect on *B. annulatus*	(Singh et al. 2011)
Lavendula augustifolia	Leaf	Essential oil	Acaricidal effect on *B. microplus*	(Seely et al. 2008)
Tetradenia riperia	Leaf	Essential oil	In vivo & in vitro adulticide against *B. microplus*	(Schmahl et al. 2010)
Lippia javanica	Leaf	Aqueous extract (Phenolic glycosides, flavonoids)	In vivo Adulticid against *B. microplus*	(Chungsamarnyart and Jansawan 2001)
Nicotiana tabacum	Leaf	Essential oil & (precocene II) isolated from it	In vitro toxic to larvae of *B. microplus*	(de Almeida et al. 2012)
Calatropis procera	Flower	Aqueous extract	Effective against all developmental stages (Dose 7 time-dependent response) of *B. microplus*	(Fernandes et al. 2008)
Trachyspermum ammi	Seed	Essential oil	Reduced average weight of ticks, no of ticks & Larval viability reduced	(Tipu et al. 2002)
Strychnos spinosa & Solanum incanum	Fruit	Extracts	Cattle pen trial *in-vitro* & *In-vivo* with amitraz as reference control and effective on all stages. The efficacy of *S. incanum* fruit extract higher	(Singh et al. 2011)
Cymbopogon martini	Leaf	Essential oil & Precocene II isolate from it	Toxic to larvae of *B. microplus*	(Seely et al. 2008)

8.10. REGULATORY CONSIDERATIONS

Establishing appropriate regulations and quality control for phytotherapeutics is vital to ensure their safety, efficacy, storage, and applications.

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Coproantigen as a Tool for Monitoring Echinococcus Granulosus Infection in Definitive Host

28

Teroj A Mohammed

ABSTRACT

This chapter delves into the utilization of Coproantigen as a diagnostic tool for *Echinococcus granulosus* infection in definitive hosts, primarily focusing on canine echinococcosis. *Echinococcus granulosus*, the causative agent of cystic echinococcosis, poses a significant public health challenge due to its zoonotic nature. The traditional diagnostic methods face limitations in sensitivity and specificity, highlighting the need for more efficient techniques. The chapter extensively explores the morphology, life cycle, and transmission dynamics of E. granulosus, emphasizing its diverse impact on both definitive and intermediate hosts. Significant attention is devoted to the Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test, a groundbreaking advancement in the field of veterinary parasitology. This test exhibits high specificity and sensitivity, enabling early detection of infections in dogs, even before the presence of eggs in faeces. The chapter evaluates the advantages, limitations, and practical applications of the Coproantigen test, including its role in active surveillance, monitoring treatment efficacy, and implementation in endemic regions. The comprehensive analysis underscores the necessity of incorporating the Coproantigen test into broader echinococcosis control programs. Combining this diagnostic tool with other methods and enhancing its accessibility in resource-limited settings are crucial steps towards effective management of the disease. This chapter contributes significantly to our understanding of canine echinococcosis and presents viable strategies for mitigating its zoonotic threat to public health.

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1. INTRODUCTION

Echinococcosis, caused by the dwarf dog tapeworm, is a highly endemic and significant parasitic zoonotic disease worldwide (Borji et al. 2013; Ananda et al. 2015). Its historical impact on human health is long-standing, with references to the disease found in ancient texts of Judaism, such as the Talmud, indicating the recognition of echinococcosis by Jewish people since ancient times (OIE 2022).

The scientific understanding of echinococcosis began to emerge in the 17th century when it was discovered that hydatid cysts found in infected humans were of animal origin. In 1766, Pierre Simon Pallas correctly predicted that the hydatid cysts found in humans were actually larval stages of tapeworms (Howorth 1945). Further progress in understanding the disease occurred in the 18th and 19th centuries. In 1782, the cyst and head of the tapeworm were described by Goeze, and in 1786, *Echinococcus (E.) granulosus* was accurately described by Batsch (Tappe et al. 2008). During the mid-19th century, Carl von Siebold conducted experiments that confirmed *E. granulosus* was causing the hydatid cysts in dogs. In the following decades, more details about *E. granulosus* and its life cycle, as well as its role in causing the disease, were elucidated (Howorth 1945).

1.1 TAXONOMY OF *ECHINOCOCCUS GRANULOSUS*

The taxonomy of the genus Echinococcus has undergone various reviews over the years, with earlier classifications based on morphological and biological observations of natural and experimental infections (Kumaratilake and Thompson 1982; Thompson 2017). The current arrangement of the genus Echinococcus within the family Taeniidae is shown in Table 1.

Within the genus Echinococcus, there are nine recognized species that affect humans and animals, as shown in Table 2. Among these, *E. granulosus sensu lato* is a complex of ten genotypes (G1-G10) that includes four distinct species*: E. granulosus sensu stricto* (G1-G3), *E. equinus* (G4*), E. ortleppi* (G5), and *E. canadensis* (G6-G10). Each species exhibits unique morphological, epidemiological, and transmission dynamics characteristic (Torgerson et al. 2006; Nakao et al. 2007; Hüttner et al. 2008; OIE 2022).

Advancements in genetic studies have confirmed the high intra-specific variation within Echinococcus species, leading to the recognition of the *E. granulosus* sensu lato complex with its ten genotypes. These genotypes differ in various aspects, shaping the epidemiology and transmission patterns of the disease (OIE 2022).

While significant progress has been made in understanding echinococcosis, challenges remain in the effective monitoring and control of the disease. Its complex life cycle, varying genotypes, and different transmission dynamics call for robust diagnostic methods, like the Coproantigen ELISA test, to detect and monitor infections in dogs accurately. Addressing the limitations of diagnostic tools and incorporating comprehensive surveillance and control strategies are crucial to combat this zoonotic disease effectively and protect both human and animal populations from its impact (Borji et al. 2013; Ananda et al. 2015).

1.2 MORPHOLOGY OF *ECHINOCOCCUS GRANULOSUS*

The morphology of *E. granulosus* involves distinct life stages, each with unique characteristics.

1.2.1 EGG

The eggs of *E. granulosus* are spherical or ovoid in shape and measure about 24-40 µm in diameter (Fig. 1). They consist of two layers; an outer thin wall or shell, and an inner embryophore with radial striations. The embryophore contains a developing oncosphere, which is the first larval stage of the parasite. The oncosphere has three pairs of hooklets that are used for penetration into the host's tissues (Soulsby 1982; Zhang et al. 2021; OIE 2022; Teroj 2022).

Echinococcus eggs are very resistant to environmental conditions and can remain infective for up to a year in soil (Lahmar et al. 2007).They can also survive in water and damp sand for several weeks at specific temperatures. However, they are sensitive to direct sunlight, dry heat, freezing, and chemical disinfectants (OIE 2022). The eggs are passed in the feces of the definitive host (usually dogs or other canines) and are ingested by intermediate hosts (such as sheep, cattle, or humans). The peak period of egg production by the adult tapeworm occurs 40 to 80 days after infection (Soulsby 1982).

Table 1: Taxonomy of the genus Echinococcus

Phylum	Class	Subclass	Order	Family
Platyhelminths	Cestoda	Eucestoda	Cyclophyllidea	Taeniid

Table 2: Classification of the genus Echinococcus

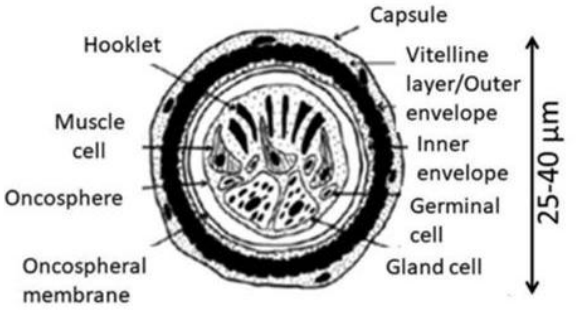
Genus	Note
E. granulosus sensu stricto (G1-G3)	This is the most common and widespread species that causes cystic echinococcosis in humans and animals. It mainly infects dogs as definitive hosts and sheep, goats, cattle, camels, and pigs as intermediate hosts. It has a cosmopolitan distribution and is endemic in many regions of Africa, Asia, Europe, Oceania, and South America.
E. equinus (G4)	This species mainly infects horses as intermediate hosts and dogs as definitive hosts. It has a restricted geographic distribution and is mainly found in Europe, Africa, and Asia. It causes cystic echinococcosis in humans but at a lower frequency than *E. granulosus sensu stricto.*
E. ortleppi (G5)	This species mainly infects cattle as intermediate hosts and dogs as definitive hosts. It has a worldwide distribution but is more prevalent in Africa and South America. It causes cystic echinococcosis in humans but at a lower frequency than *E. granulosus sensu stricto* (Torgerson et al. 2006; Davidson et al. 2012).
E. canadensis (G6-G10)	This species comprises five genotypes that infect different intermediate hosts such as camels, cervids, pigs, rodents, and lagomorphs. It has a worldwide distribution but is more prevalent in North America, Asia, and Africa. It causes cystic echinococcosis in humans but at a lower frequency than *E. granulosus sensu stricto*.
E. multiloculari s	This is the most pathogenic species that causes alveolar echinococcosis in humans and animals. It mainly infects foxes as definitive hosts and rodents as intermediate hosts. It has a circumpolar distribution and is endemic in many regions of Asia, Europe, North America, and Alaska.
E. oligarthrus	This species mainly infects felids as definitive hosts and rodents as intermediate hosts. It has a neotropical distribution and is mainly found in Central and South America. It causes polycystic echinococcosis in humans but at a very low frequency.
E. vogeli	This species mainly infects bush dogs as definitive hosts and rodents as intermediate hosts. It has a neotropical distribution and is mainly found in Central and South America. It causes polycystic echinococcosis in humans but at a very low frequency.
E. shiquicus	This species mainly infects Tibetan foxes as definitive hosts and plateau pikas as intermediate hosts. It has a restricted geographic distribution and is only found in the Tibetan plateau of China. It causes alveolar echinococcosis in humans but at a very low frequency.
E. felidis	This species mainly infects lions as definitive hosts and antelopes as intermediate hosts. It has a restricted geographic distribution and is only found in the Serengeti National Park of Tanzania. It causes cystic echinococcosis in humans but at a very low frequency.

1.2.2 ADULT STAGE

The adult stage of *E. granulosus* is a tapeworm that resides in the small intestine of the definitive host. It is typically less than a centimeter long (2-7 mm) and consists of 3-5 proglottids (Fig. 2), occasionally up to 6 proglottids (Soulsby 1982; Muller et al. 2002). Each proglottid has a single genital opening on the lateral margin that alternates irregularly from side to side. The first two proglottids are immature, While the penultimate proglottid is sexually mature and contains male and female organs. The last proglottid is

gravid (full of eggs) and constitutes about half of the total length of the tapeworm (Constantine et al. 1993; Muller et al. 2002; Zhang et al. 2021; Teroj 2022).

The scolex (head) of *E. granulosus* is characteristic of taeniids. It has a rostellum (a protrusion) with 28-40 small hooks (22-34 µm) and large hooks (30-40 µm) in length (Fig. 3). It also has four muscular suckers that help to attach to the intestinal mucosa of the host. These features are essential for the survival and reproduction of the tapeworm in its adult stage. The scolex can also be used for identification purposes (Shield 1969; Reissenweber et al. 1975; Constantine et al. 1993; Teroj 2022).



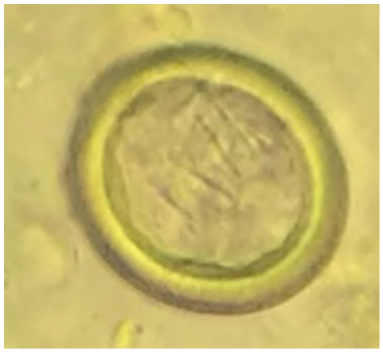


Fig. 1: Morphological structure of *E. granulosus* egg (Zhang et al. 2021 and Teroj 2022)

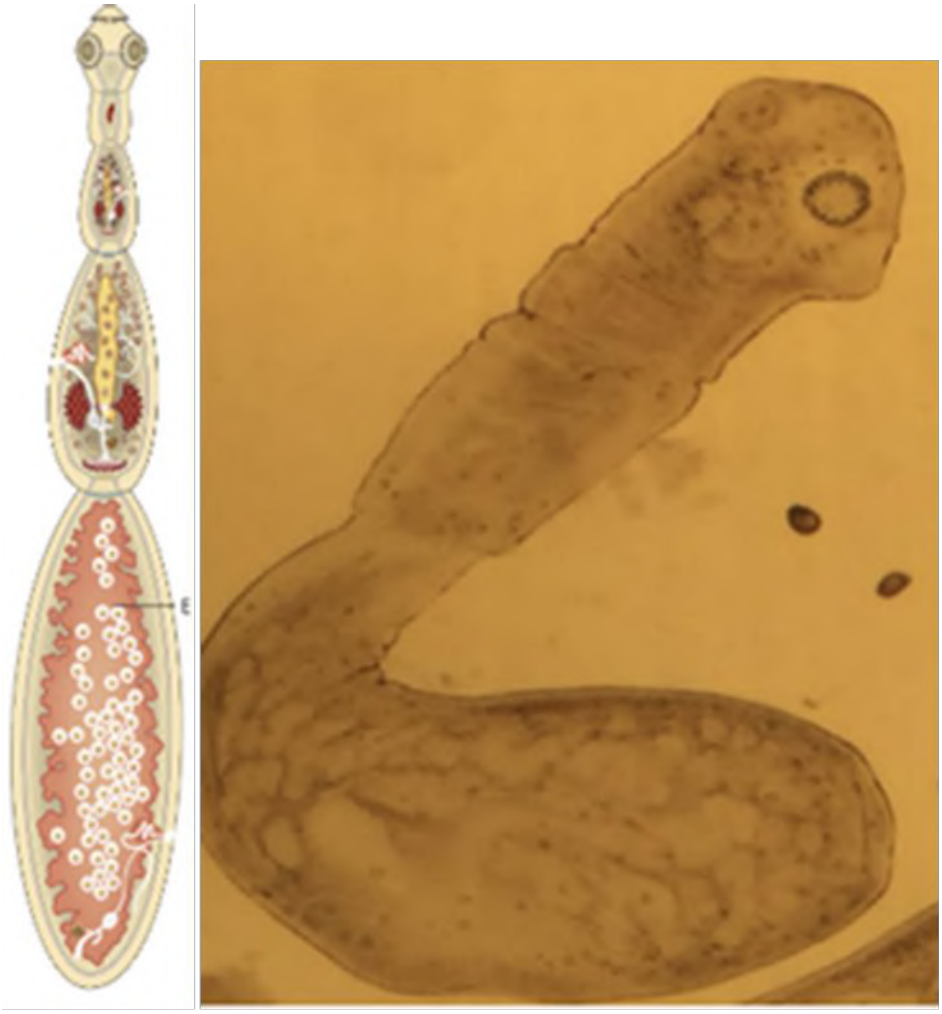


Fig. 1: Morphological structure of adult *E. granulosus* (Zhang et al. 2021 and Teroj 2022)

1.2.3 HYDATID CYST

The hydatid cyst is the larval stage of *E. granulosus* and can develop in various organs of the intermediate host, especially the liver and lungs. It has a complex structure composed of several distinct components. The outermost component is the laminated membrane, which forms a non-nucleated hyaline cuticle of about 1 mm thickness. It is produced by the germinal layer, which lines the inside of the cyst and overlays the laminated layer. The germinal layer is attached to the laminated layer by finger-like processes that extend into it (Fig. 4). This layer is permeable to water (PUMP 1963; Richards et al. 1983; Ingold et al. 1998; Brunetti et al. 2010).

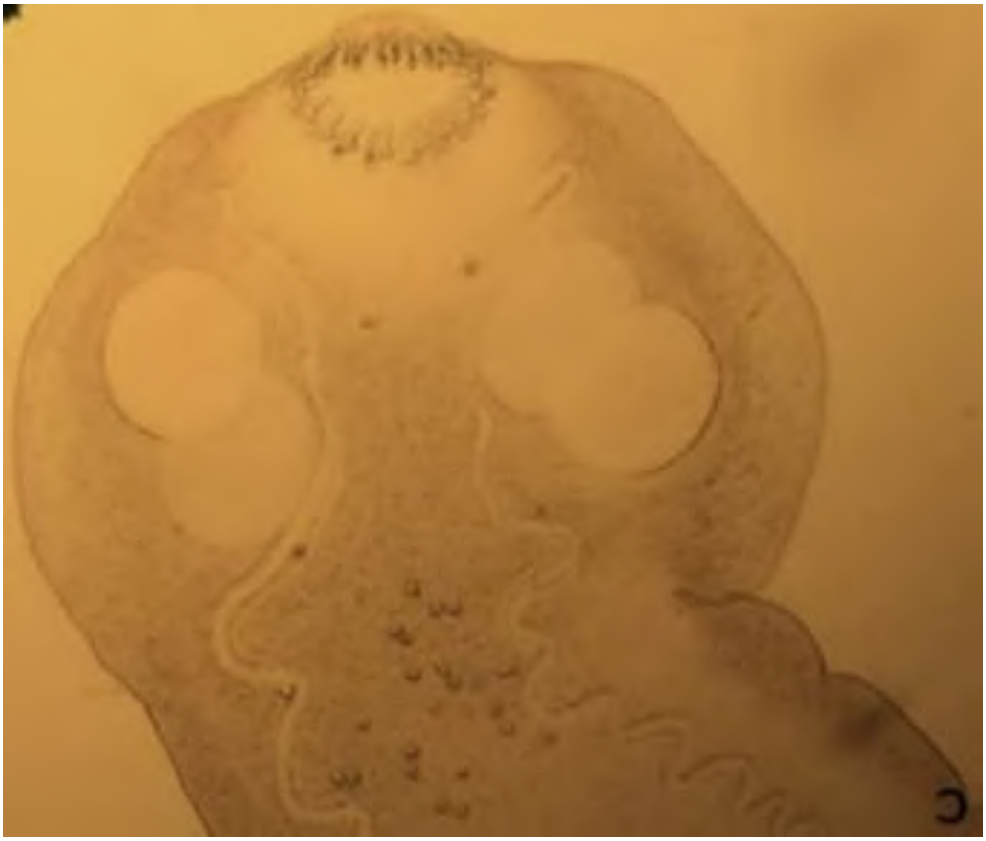


Fig. 2: Morphological structure of *E. granulosus* scolex (Teroj 2022)

The cyst contains a fluid called hydatid fluid, which is typically colorless or slightly yellowish. It has a specific gravity of 1.012 and a pH ranging from 6.7 to 7.2. The hydatid fluid comprises various components such as albumin, creatinine, lecithin, urea, a small amount of glucose, sodium chloride, sodium, calcium, and enzymes (Kassis and Tanner 1976).

Another essential component of the cyst is the brood capsule, which has a highly vacuolated wall with nuclei present at irregular intervals. Within the brood capsule, debris from degenerated protoscolices can be observed. Surrounding the entire cyst is a fibrous tissue capsule (Gottstein 1992). In some cases, the cysts may be sterile and fail to produce brood capsules (acephalocyst), while in others, the brood capsules may not generate scolices (Paul and Stefaniak 1997).

The protoscolices are another critical element of the cyst. They consist of scolices with a rostellum and suckers that can be either invaginated or evaginated. Microscopically, they appear grain-like, and hydatid

sand refers to the granular material containing free protoscolices, daughter cysts, hooks, and calcareous bodies (Mehlhorn 2008).

The hydatid cyst is a significant stage in the life cycle of *E. granulosus* and can cause cystic echinococcosis in humans and animals. The complex components of the hydatid cyst highlight the remarkable adaptability of this parasite within its hosts, making it a challenging and significant public health concern (Zeibig 1997; Budke et al. 2013).

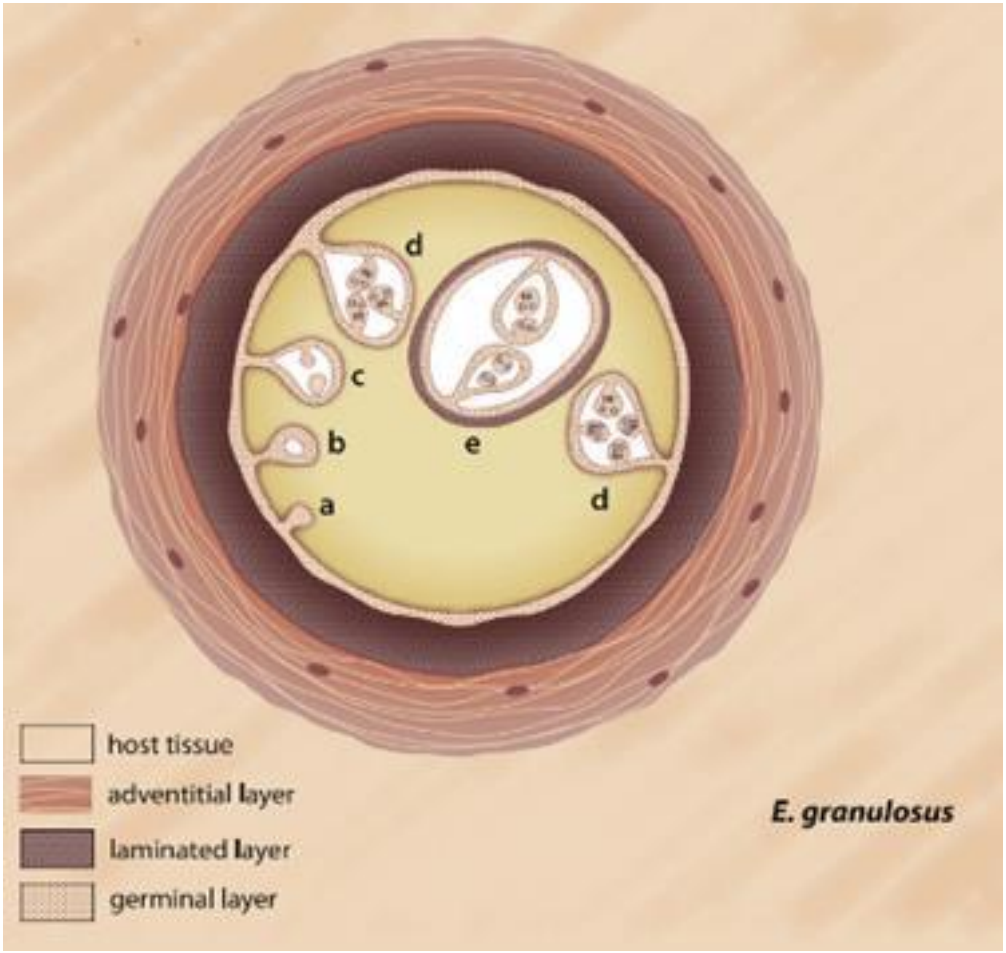


Fig. 3: Morphological structure of *E. granulosus* hydatid cyst (Brunetti et al. 2010)

1.3 LIFE CYCLE OF *ECHINOCOCCUS GRANULOSUS*

The life cycle of the genus Echinococcus can be divided into two main kinds based on the hosts involved: the domestic or pastoral life cycle and the natural or sylvatic life cycle. Each kind involves different definitive and intermediate hosts, highlighting the adaptability and complexity of the parasite's life cycle (Sing 2015; Bowman 2020).

1.3.1 DOMESTIC OR PASTORAL LIFE CYCLE

In the domestic or pastoral life cycle, dogs serve as the definitive host, where the adult tapeworms reside in their small intestine. The infection in dogs occurs when they ingest uncooked offal, such as the liver, lungs, or other organs from intermediate hosts, which containing fertile metacestodes. Domestic farm

animals, including sheep, goats, and cattle act as the intermediate hosts in this cycle. These intermediate hosts become infected by ingesting the eggs of Echinococcus through contaminated food, water, or vegetation (Morar and Feldman 2003).

Once inside the dog's small intestine, the eggs hatch, releasing the oncosphere. The oncosphere penetrates the intestinal wall and is passively carried by the bloodstream to various organs, where it develops into the metacestode stage, forming hydatid cysts. The hydatid cysts primarily develop in the liver and lungs of intermediate hosts but can also affect other organs (Morar and Feldman 2003) (Fig. 5).

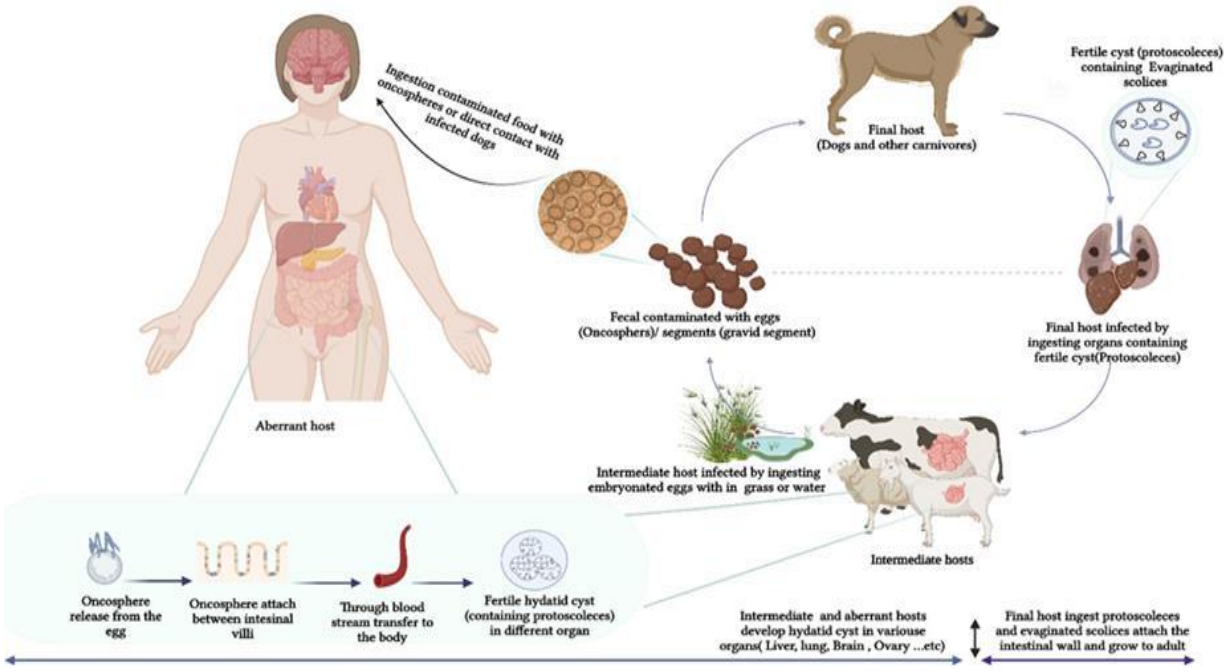


Fig. 4: *E. granulosus* life cycle.

In this cycle, the dog serves as the definitive host, shedding infectious eggs through proglottids in its feces. If dogs roam freely in areas where livestock graze, they can contaminate the environment with these eggs, leading to the infection of intermediate hosts and perpetuating the life cycle (Craig et al. 1995).

1.3.2 NATURAL OR SYLVATIC LIFE CYCLE

In the natural or sylvatic life cycle, the life cycle primarily involves wildlife. Wolves and certain species of cervids (e.g., moose, caribou, and reindeer) act as the definitive hosts in this cycle. The definitive hosts become infected by consuming the organs or tissues of intermediate hosts harboring metacestodes. Similar to the domestic cycle, the intermediate hosts become infected by ingesting the eggs of Echinococcus from contaminated food, water, or vegetation (Almulhim and John 2023).

The life cycle in wildlife settings is more complex and involves interactions among different animal species. Wild carnivores, such as wolves, play a crucial role as definitive hosts, shedding infectious eggs into the environment through their faeces. The contamination of the environment with these eggs can lead to the infection of intermediate hosts, which, in turn, can be consumed by the definitive hosts, completing the life cycle (Craig et al. 1995). Additionally, hunters who handle and consume the meat of infected wildlife

may accidentally become hosts of the parasite if proper food hygiene practices are not followed (Morar and Feldman 2003).
The natural or sylvatic life cycle is often self-sustaining within wildlife populations, and human involvement as accidental hosts is relatively less common compared to the domestic cycle (Morar and Feldman 2003). Both life cycles are summarized in the Table 3.

2. *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS

Canine echinococcosis, caused by the parasitic tapeworm *E. granulosus*, poses a significant zoonotic threat. Domestic dogs (Canis familiaris) serve as the primary host for this parasite.

Table 3: *E. granulosus* life cycles

Life Cycle	Definitive Host	Intermediate Host	Transmission	Prevention
Domestic or Pastoral	Dog	Sheep, goats, cattle	Ingesting uncooked offal from intermediate hosts, releasing oncospheres that develop into hydatid cysts in the liver and lungs of intermediate hosts.	Deworming dogs, improving food hygiene practices, and vaccination of intermediate hosts.
Natural or Sylvatic	Wolves, cervids	Small mammals, livestock	Ingesting the organs or tissues of intermediate hosts harboring metacestodes, which develop into hydatid cysts in the liver and lungs of intermediate hosts.	Education, improved food hygiene practices, and vaccination of intermediate hosts.

Dogs are particularly susceptible to the intestinal form of echinococcosis, making them crucial in the transmission cycle of this parasitic infection. Remarkably, *E. granulosus* infections in dogs do not manifest as disease, even in cases of heavy infestations (Grosso et al. 2012). However, in young dogs heavily burdened with the parasite, a potbellied appearance may be observed, and there is a risk of small intestine obstruction (Soulsby 1982).
The intricate interaction between Echinococcus parasites and their canine hosts occurs within the small intestine. The tapeworms adeptly penetrate between the intestinal villi, make their way into the crypts of Lieberkühn. There they firmly attach themselves using suckers and rostellar hooks to the epithelial lining. Despite this intimate relationship between the parasites and their host's gut, the infection usually does not cause significant harm. Nevertheless, some minor changes may occur, including slight cellular infiltration of the intestinal mucosa and localized flattening of epithelial cells. Additionally, the presence of the parasites stimulates an increase in mucus production in the small intestine (OIE 2022).

3. DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS

As Echinococcus tapeworms reside in the intestines of dogs, where they release excretory/secretory products from their scolex region into the surrounding environment. This process can trigger the production of circulating antibodies in the dog's immune system. These antibodies are believed to play a role in the host's defense against the parasites and may have implications in the diagnostic process (OIE 2022).
Diagnosing *E. granulosus* infection in definitive hosts, such as dogs, can be challenging due to the morphological similarities among the eggs of various Echinococcus species and Taenia parasites. Additionally, the characteristic small segments of Echinococcus tapeworms may be absent from the dog's feces or easily overlooked during routine examinations. To overcome these difficulties, veterinarians and parasitologists employ various diagnostic methods for both living and deceased dogs (OIE 2022).

3.1 DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN LIVING DOGS

In living dogs, fecal examinations using flotation or sedimentation techniques are commonly employed to detect parasite eggs (Jenkins et al. 2023). However, due to intermittent egg shedding, these tests may yield false-negative results. As an alternative, coproantigen tests offer higher sensitivity and specificity by detecting specific antigens produced by adult Echinococcus worms. Additionally, serological tests, such as ELISA and Western blot, play a crucial role in detecting specific antibodies produced by the dog's immune system in response to the parasitic infection. These serological assays are particularly useful for identifying chronic infections or when eggs are not present in the faeces (Chamekh et al. 1992; Paduraru et al. 2023). These coproantigen tests significantly improve the accuracy of diagnosis (Abbasi et al. 2003).

3.2 DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN DEAD DOGS

For the systematic analysis of canine intestinal content, commence by making a sterile incision in the dog's abdomen to carefully access the intestine (El-Shehabi et al. 2000). With precision, tie off both the pyloric and anal ends of the intestine. Immediately collect the intestinal contents in sterile containers, ensuring no contamination. Given the sensitivity of the samples, they should be stored in an icebox to maintain integrity and be transported to the lab within a maximum of three hours. Following the collection, for ethical and environmental safety, incinerate the canine remains on-site. Upon arrival in the lab, segment the intestine into four equal portions. Subsequently, longitudinally slice open each segment and immerse it in a 0.15 M phosphate buffer saline solution with a pH of 7.2 for exactly five minutes. Using a sterilized spatula, scrape the mucosal lining gently, depositing the content into clean glass dishes. Let the samples settle in 1,000 ml conical Nalgene graduates. After multiple rinses with the phosphate buffer solution, inspect the aliquots meticulously under a dissecting microscope for detailed analysis (El-Shehabi et al. 1999).

4. TRADITIONAL METHODS FOR DIAGNOSING *ECHINOCOCCUS GRANULOSUS* INFECTION

The diagnosis of *E. granulosus* in the definitive host, such as domestic dogs, can be challenging due to the morphological similarities between *E. granulosus* eggs to those of *E. multilocularis*, and various Taenia species. Moreover, egg excretion is irregular, making it difficult to reliably identify them in fecal samples microscopically. However, there are specific techniques available to aid in the detection process (El-Shehabi et al. 1999).

4.1 DETECTION OF EGGS AND PROGLOTTIDS

Faecal samples can be examined using flotation techniques, wherein the eggs are concentrated by suspending the feces in a liquid with a higher specific gravity. Additionally, the perineal skin of dogs can be examined using clear adhesive tape, which is pressed against the skin, transferred to a microscopic slide, and examined. Despite these methods being helpful, distinguishing between the eggs of different parasites can still be a challenge (Varcasia et al. 2004; Benito et al. 2006; OIE 2022).

Apart from eggs, proglottids of *E. granulosus* can also be detected in faecal samples. Proglottids are tapeworm segments that are spontaneously discharged by dogs and can often be found on the surface of samples. Examining these segments can aid in correctly diagnosing the presence of *E. granulosus* in the definitive host (Ajlouni et al. 1984; Craig et al. 1995).

4.2 ARECOLINE HYDROBROMIDE PURGATION

The method of arecoline purging is recognized as a reliable technique for the detection of *E. granulosus* infection in canine populations, as documented by Benito et al. (2006). The central component, arecoline hydrobromide, is a parasympathomimetic drug that predominantly affects the small intestine's smooth muscle, resulting in tapeworm paralysis. For effective outcomes, it is critical to administer the drug, available in both tablet and liquid forms, either orally or, in some instances, per rectum. Based on extensive research by Craig et al. (1995), Dakkak et al. (2017), and OIE (2022), the suggested dose spans from 1.75 mg/kg to 3.5 mg/kg of body weight, demonstrating effectiveness in a majority of dogs. Post-administration, the drug induces purgation, facilitating the tapeworms' expulsion via faeces. It's imperative to promptly collect this purged material in leak-proof bags and to preserve it by saturating in either a 10% formalin or 85% ethanol solution, as recommended by Benito et al. (2006). Following preservation, the samples are ready for a comprehensive examination to determine the degree of *E. granulosus* infection.

4.3 IMMUNODIAGNOSTIC METHODS

Immunodiagnostic methods for *Echinococcus spp.* in the final host has significantly advanced over the years. Early attempts at immune-diagnosis were made in the early 20th century, and several immunological and molecular methods have since been applied to the diagnosis of intestinal stages of both *Echinococcus spp.* and *Taenia spp.* (Gasser et al. 1988; JC et al. 1992; Deplazes et al. 1992; Benito et al. 2006).
Among the major methods developed and assessed are serological tests and molecular techniques. Serological tests, such as enzyme-linked immunosorbent assay (ELISA) and Western blot (Gasser et al. 1988; Craig et al. 1995; Kouguchi et al. 2010), rely on detecting specific antibodies produced by the host's immune system in response to the presence of *Echinococcus* antigens (Al-Khalidi and Barriga 1986). The choice of diagnostic method depends on a number of factors, including the stage of infection, the availability of resources, and the clinical presentation of the patient. In general, serological tests are a good first-line option, as they are relatively inexpensive and easy to perform (Benito et al. 2006).

5. COPROANTIGEN TESTS

The development of immunodiagnostic methods has significantly advanced the detection of canine echinococcosis. Among these methods, the Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test stands out as a powerful tool for diagnosing this parasitic infection (Allan and Craig 1989; JC et al. 1992; Deplazes et al. 1992; Deplazes et al. 1994).
To perform the Coproantigen ELISA test, researchers have generated polyclonal antibodies against somatic or excretory/secretory antigens of adult *E. granulosus* (Deplazes et al.,1992; Deplazes et al. 1994; Allan and Craig 2006). This test can detect coproantigens highly specific to the genus *Echinococcus* in dogs as early as 5-10 days after infection, even in cases where eggs are not yet present in the faeces (Deplazes et al. 1992).
One significant advantage of detecting specific antigen(s) in faecal samples from definitive hosts is its high probability of correlation with current infection, as it directly indicates the presence of the parasite in the intestine. This method has been successfully utilized in various countries to diagnose canine echinococcosis (Varcasia et al. 2004; OIE 2022).
The Coproantigen ELISA test represents a valuable tool in canine echinococcosis surveillance and control programs, enabling early and accurate diagnosis of infected dogs. By promptly identifying infected animals, health authorities can implement appropriate measures to prevent the spread of the parasite to

humans and other susceptible hosts, ultimately contributing to public health and the reduction of zoonotic risks associated with this disease. As research in immunodiagnosis continues to progress, it is likely that even more sensitive and specific methods will be developed to enhance our ability to combat this significant zoonotic threat effectively (WHO 2012).

5.1 VALIDATION AND SENSITIVITY OF COPROANTIGEN TESTS

5.1. SPECIFICITY

The detection of *E. granulosus* coproantigens using the Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test has been shown to be highly specific (Deplazes et al. 1992; Allan and Craig 2006). The test, which employs antibodies raised against *Echinococcus spp.* antigens, has proven its capability to specifically detect infected faeces from dogs harboring *E. granulosus* parasites while yielding negative results in hosts infected with other types of parasites, including *Taenia* species. The specificity of this test has been reported to reach an impressive 98% and higher (Allan and Craig 2006).

5.2. SENSITIVITY

The sensitivity of the coproantigen test for *E. granulosus* detection is influenced by the worm burden within the definitive host's intestine. In cases where faecal samples are obtained from animals harboring only a few worms, the sensitivity of the coproantigen test may be lower. However, as the worm burden increases, so does the sensitivity of the test. For instance, the sensitivity of coproantigen testing reaches 100% when the worm burden exceeds 1000 worms, and it remains high at 93.3% when the worm burden ranges between 200 and 1000 worms. The test's sensitivity is still satisfactory, ranging between 85% and 93.1%, even when the worm burden is in the range of 50 to 100 worms (Allan and Craig 2006).

5.3. ADVANTAGES

The Coproantigen ELISA test offers several advantages as enlisted in Table 4 that make it a valuable tool in the diagnosis of canine echinococcosis (Craig et al. 1995; Allan and Craig 2006).

5.4 APPLICATION OF COPROANTIGEN TESTS FOR MONITORING INFECTION IN DOGS

The Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test is an essential tool for monitoring and controlling Echinococcosis in the final host (dogs). This highly specific and sensitive diagnostic method allows for the early and accurate detection of *E. granulosus* infections in the definitive hosts, primarily domestic dogs. By employing this diagnostic tool in a systematic manner, public health authorities and veterinarians can implement effective measures to reduce the prevalence of the parasite and its transmission to humans and other susceptible hosts (Allan and Craig 2006).
The Coproantigen ELISA test can be used for a variety of purposes, including:

5.4.1. ACTIVE SURVEILLANCE OF *E. GRANULOSUS* INFECTIONS IN DOG POPULATIONS

Veterinarians and public health officials can conduct regular testing campaigns, especially in areas known to have high prevalence rates of the parasite. By testing faecal samples obtained either directly from the

rectum or from the ground, authorities can identify infected dogs early in the course of the disease, even before the shedding of eggs. This early detection allows for prompt treatment and isolation of infected animals, minimizing the risk of transmission to humans and other animals (Allan and Craig 2006; WHO 2002).

5.5.2. MONITORING THE EFFECTIVENESS OF CONTROL MEASURES AND TREATMENT INTERVENTIONS

Following the administration of anti-parasitic drugs to infected dogs, veterinarians can use the test to assess treatment efficacy. The coproantigen level in faecal samples typically decreases within a few days after successful treatment. By monitoring this decline, veterinarians can ensure that infected dogs are effectively treated and pose a reduced risk of transmission (Allan and Craig 2006).

Table 4: Copro-antigen advantages

Advantage	Notes
Sample Collection Flexibility	Faecal samples can be obtained either directly from the rectum or from the ground where dogs defecate. Some studies have even demonstrated that dry faeces can be used for testing.
Ease of Sample Storage	Faecal samples can be tested on the same day of collection or stored in a refrigerator or deep-frozen at -20°C until use.
High Genus-Specificity	The coproantigen test exhibits a high level of genus-specificity and accurately identify the presence of *Echinococcus spp.* without cross-reacting with antigens from other parasites. This specificity has been reported to exceed 95%.
Early Detection	The coproantigen can be detected in faeces even before the shedding of eggs by the adult worms. Additionally, the level of antigen does not depend on the number of eggs present, making it a reliable indicator of infection.
Environmental Stability	The coproantigen remains stable in environmental conditions for several months, and it retains its detectability even across a range of temperatures from -80°C to 35°C. This stability allows for the safe storage and transportation of faecal samples for testing purposes.
Monitoring Treatment Efficacy	The coproantigen level in faecal samples decreases within 1 to 5 days after treatment, providing a means to monitor the efficacy of therapeutic interventions.

5.5.3. SURVEILLANCE OF DOGS IN REGIONS WHERE *E. GRANULOSUS* IS ENDEMIC

By conducting regular testing of canine populations in these areas, health authorities can identify high-risk zones and target control efforts more effectively. This information aids in developing localized prevention and control programs, including public awareness campaigns and interventions targeting specific risk factors (Craig et al. 1995; Varcasia et al. 2004).

5.5.4. MASS SCREENING OF DOGS IN COMMUNITIES WHERE THERE IS A HIGH LIKELIHOOD OF HUMAN EXPOSURE TO THE PARASITE

In such settings, identifying and treating infected dogs can play a crucial role in breaking the transmission cycle and reducing the incidence of human echinococcosis (Ahmad and Nizami 1998; De et al. 2010).

5.6 LIMITATIONS AND CHALLENGES

The Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test, despite its numerous advantages and valuable applications for monitoring and controlling *E. granulosus* in dogs, faces certain limitations and challenges that need to be addressed for its optimal use in combating this zoonotic disease. One of

the main challenges is the variation in sensitivity depending on the worm burden within the definitive host's intestine. The test's sensitivity is generally higher when the worm burden is high, but it decreases when the number of worms is low, potentially leading to false-negative results in dogs with low parasite loads. Cross-reactivity with closely related parasites, such as other *Taenia* species, also poses a risk of false-positive results in regions where multiple taeniid parasites coexist (Siavashi and Motamedi 2006). Additionally, the timing of detection can be critical as the presence of antigen in faecal samples may decline within a few days after effective treatment, leading to false-negative results shortly after treatment. Proper sample collection, transportation, and storage are crucial to obtaining accurate results, which can be logistically challenging, especially in free-roaming or uncooperative animals. Furthermore, the test's implementation requires specialized laboratory equipment and trained personnel, which can increase the overall cost of testing and may limit its accessibility in regions with limited resources. To overcome these limitations, complementary diagnostic methods, such as serological tests, imaging techniques, and coproscopy (egg detection in faecal samples), should be used in combination with the Coproantigen ELISA test to improve overall accuracy. By addressing these challenges and employing a comprehensive approach to diagnostics, veterinary and public health efforts can better combat *E. granulosus*, protect human health, and reduce transmission in dog populations. Efforts should be made to improve the accessibility and affordability of the test in regions with high disease burden, ultimately contributing to the successful control of this zoonotic disease (Craig et al. 1995; Guarnera et al. 2000; WHO 2002; Allan and Craig 2006).

6. CONCLUSION

Coproantigen ELISA test offers a valuable tool for monitoring and controlling Echinococcosis in dogs, the definitive host. Its high specificity and sensitivity allow for early detection of infections, enabling effective control measures to be implemented. However, limitations such as reduced sensitivity with low worm burden and cross-reactivity with other parasites necessitate a comprehensive approach. Combining the ELISA test with other diagnostic methods, improving accessibility in resource-limited settings, and addressing logistical challenges are key to maximizing its effectiveness. By overcoming these hurdles and employing a multifaceted approach, veterinarians and public health officials can effectively combat Echinococcosis, protect human health, and safeguard dog populations.

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Zoonoses Associated with Geohelminthiases

29

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ABSTRACT

This chapter presents a general and updated overview of the different zoonoses associated with soil-transmitted helminth infections. The topics presented in the chapter are: Angiostrongyliasis; Ascariasis; Capillariasis; Lagochilascariasis; Mammomonogamiasis; Strongyloidiasis; Cutaneous larva migrans. In this context, zoonotic diseases are a major public health problem because animals are the source of human pathogens. The emergence and re-emergence of zoonotic diseases have been attributed to a number of anthropogenic and natural factors, including vector biology, urbanization, climate change, animal migration and trade, travel and tourism, among others. Animals are the origin of a large number of infectious diseases that affect humans. About 60% of newly discovered human infections are zoonotic. Because of this, it is important to consider the areas where we live and the risks of latent diseases in the regions. In addition, it is extremely important to give importance to common wound accidents, because it is better to prevent silent diseases that can be hidden for years through good food and wound asepsis and go to the doctor.

Key words: zoonotic diseases, parasite, helminths, one health, sustainability.

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1. INTRODUCTION

1.1. ANGIOSTRONGYLIASIS

Angiostrongylus (*A.*) *cantonensis* and *A. costaricensis* are the two main roundworm species that cause angiostrongyliasis, also referred as rat lungworm disease. *A. costaricensis* is linked to ingesting raw vegetables contaminated with the parasite's larvae (Rojas et al. 2021). It is the primary cause of angiostrongyliasis and is primarily associated with eating contaminated raw or undercooked snails or slugs, which act as intermediate hosts (Cowie 2017).

A. cantonensis reproduces as adult worms in the pulmonary arteries of rats, where its eggs hatch into larvae in the lungs and are subsequently expelled in the feces. The larvae grow to the infectious stage within the snails after they have consumed the excrement. Rats that eat infected snails continue the cycle by allowing the larvae to enter the brain through circulatory system. (Ringelmann and Heym 1991). Third-stage larvae can inadvertently enter human bodies through the consumption of raw or undercooked infected snails or contaminated product. The larvae migrate to the brain, where they can cause severe, lifelong neurological damage and eosinophilic meningitis. Mild headaches, skin sensitivity, more severe motor disturbances, digestive problems, coma, and even death are among the symptoms. The diagnosis is difficult and depends on a number of factors, including imaging, serological testing, presence in parasite-prone areas, food consumption history, and diagnosis. Treatment is not definitive, and management involves pain relief, spinal taps to decrease intracranial pressure, and corticosteroids to reduce inflammation. Anthelmintics may be used but remain controversial due to the immune reaction to dying worms (Kaplan et al. 2020).

1.1. DISCOVERY AND DISTRIBUTION IN SNAILS

A. cantonensis, first discovered in 1935 in China, was later confirmed to be connected to the disease in Hawaii in 1961. Since then, it has become a global concern, with cases reported in various regions, including the continental USA, where it has been found in snails and rats, notably in Florida and other states. Worldwide, nearly 3,000 cases have been reported, with higher numbers in regions like Thailand, Taiwan, China, and Pacific islands of Southeast Asia, Australia, the Caribbean, and both North and South America (Cowie 2017). Using *Achatina fulica* and *Pomacea canaliculata* as intermediate hosts and *Rattus norvegicus* and *R. rattus* as natural definitive hosts in different regions of the world, this parasite infects a variety of snail species (Hu et al. 2018).

2. ASCARIASIS

Zoonoses are an important public health concern because animals are the source of more than 60% of human pathogens. The emergence and reemergence of zoonotic diseases have been attributed to a number of anthropogenic and natural factors, including vector biology, urbanization, climate change, animal migration and trade, travel and tourism, and others. Animals are the source of a large number of infectious diseases that affect humans. Around 60% of newly discovered human infections are zoonotic, meaning that over 70% of these pathogens originate from wildlife species (Rahman et al. 2020).

Geohelmintiasis, also called soil-transmitted helminthiasis (STH), is a common neglected tropical illness primarily affecting human intestines and caused by parasitic worms. It is a serious public health issue, especially in areas with limited resources and poor hygiene standards. "Geohelmintiasis" refers to the intimate relationship that exists between the spread of these helminths and the infiltration of soil by their infectious stages (WHO 2023).

Beyond causing digestive problems, geohelmintiasis has other negative effects as well. Prolonged and severe infections can cause anemia, stunted growth and development, reduced school attendance and performance, malnourishment, and cognitive decline. These outcomes can prolong a cycle of poverty and lower economic productivity in the impacted communities (WHO 2023).

Humans and pigs are infected with parasitic nematodes called *Ascaris lumbricoides* and *A. suum*, respectively. With 1.2 billion infections worldwide, *A. lumbricoides*, better known as the human roundworm, is one of the most common parasites. The Americas, China, East Asia, and sub-Saharan Africa are the main regions where infections are found. Ascariasis, the related illness brought on by an *A. lumbricoides* infection, accounts for an estimated 10.5 million disability-adjusted life years and adds significantly to the disease burden. Approximately 122 million cases of morbidity with serious health consequences occur each year. Ascariasis is still considered a neglected tropical disease in spite of its effects (Schindler-Piontek et al. 2022). Conversely, *A. suum* is a common parasitic nematode that infects pigs and its high prevalence rates have been found in pig populations. Few swine herds are totally free of *A. suum* infection, although the prevalence of infection varies based on geographic location and farm management techniques. Pigs with porcine ascariasis have poorer health and performance, which lowers feed-to-gain ratios and causes liver condemnation, both of which have a major negative financial impact (Dold and Holland 2011).

Taxonomically speaking, there is little genetic difference between the *Ascaris* species that infect humans and pigs—1.3% in the first internal transcribed spacer (ITS-1) and 3-4% in the mitochondrial genome (mtDNA) sequence, respectively. The two species are morphologically identical. Strong affinities between the two species are indicated by their close phylogenetic relationship (Easton et al. 2020). A recent study on experimental cross-transmission have shown that pigs can contract *A. lumbricoides* infections and vice versa (Tee et al. 2022). Human infections with *A. lumbricoides* were found to contain worms that originated in pigs in non-endemic areas, indicating that pigs may be a possible source of infection for human populations. But molecular epidemiological research in *Ascaris*-endemic areas has revealed little to no cross-infection between host species, indicating constrained gene flow amongst genotypes. Since *A. lumbricoides* and *A. suum* have a major impact on human and pig health, it is imperative to understand their life cycle and characteristics in order to develop effective control and prevention strategies. The feco-oral route is one of the ways that *Ascaris* species transmit disease throughout their life cycle. When consumed, infectious eggs hatch in the small intestine and the larvae move through the mucosa to the proximal colon and caecum. They then shed their L2 cuticle as they pass through the portal blood and arrive at the liver. The larvae then move to the lungs, where they pass through the alveolar space before being ingested and going back into the small intestine. In the small intestine, *A. suum* larvae undergo another molt to become L4 stage larvae. By day 24 after infection, the larvae have reached sexual maturity and have undergone another molt to become L5 stage larvae (Schindler-Piontek et al. 2022).

In both pigs and humans, the hepato-tracheal migration takes place 10–14 days after egg ingestion. Although most adult worms are eliminated from the intestines by the 23rd week of infection in pigs, they can stay there for up to a year. Adult worms are 15–25 cm in length for males and 20–35 cm for females. An estimated 200,000 eggs can be produced daily by female worms, though this number varies depending on the worm load. Embryonated eggs can survive in the soil for up to 15 years before being expelled in feces. The larvae go through two molts within the egg during embryonation (Schindler-Piontek et al. 2022).

As one of the most widespread parasitic infections worldwide, ascariasis is expected to infect 807 million people by 2020. Searching PubMed, Embase, Web of Science, and Google Scholar for studies published between 2010 and 2021 that reported the prevalence of *Ascaris* infection in humans, a systematic review and meta-analysis was developed to estimate the global prevalence of *Ascaris* infection in humans (Holland et al. 2022). The authors identified 1,060 studies, of which 184 met the inclusion criteria, and

included studies that reported the prevalence of *Ascaris* infection in humans, whether they were cross-sectional, case-control, or cohort studies. *Ascaris* infection was pooled at a prevalence of 20.2% (95% CI: 18.9-21.5%). Africa had the highest prevalence (33.2%), followed by Latin America (17.7%) and Asia (23.4%). North America (2.9%) and Europe (9.7%) had lower prevalence rates. The most recent estimates of the worldwide prevalence of *Ascaris* infection are given by this study. According to the findings, ascaris infection poses a serious threat to public health, especially in low- and middle-income nations. The geographic distribution and risk factors for giardiasis, amebiasis, and ascariasis in Mexican children were examined by Zavala et al. (2020). The authors create a database of the incidence of these infections in children between the ages of five and nine using publicly available data from Mexico's thirty-two states. Additionally, they gathered information on socioeconomic and environmental variables that might be connected to the prevalence of these infections. While amebiasis was more common in the central states of Mexico, ascariasis was more common in the southern states. The nationwide distribution of giardiasis incidence was more uniform. The authors also discovered a correlation between high rainfall, a low socioeconomic status, and limited access to toilets and piped water. The results of this study indicate that the risk of parasitic infections in children from Mexico varies significantly based on geography and socioeconomic status.

A. lumbricoides has been linked to a number of pathologies in humans, which can result in a range of health issues and complications. Intestinal obstruction, malnutrition, pneumonia, hepatobiliary complications, appendicitis, and allergic reactions are among the illnesses and ailments linked to ascariasis (Dold and Holland 2011).

It is crucial to remember that not everyone who has ascaris will have symptoms or problems. Numerous variables, including the quantity of worms present, the length of the infection, and the person's immune system, can affect the severity of the infection and related illnesses. The prevention and control of ascariasis and its associated complications depend heavily on good hygiene practices, sanitation, and deworming programs. Medications to remove the worms from the body and reduce symptoms are usually used to treat ascariasis (Barbosa et al. 2017).

Here are some ascariasis treatments that are frequently used:

2.1. ANTIHELMINTHIC MEDICATIONS

The main treatment for ascariasis consists of medications created expressly to destroy and eradicate parasitic worms. For ascariasis, the most commonly prescribed anthelminthic drugs are albendazole, mebendazole and ivermectin (Conterno et al. 2020).

2.2. SYMPTOMATIC TREATMENT

Symptoms of ascariasis include nausea, diarrhea, and abdominal pain. These symptoms can be lessened with over-the-counter drugs like antiemetics for nausea and antispasmodics for abdominal pain. Probiotics (Pryshliak et al. 2022) and certain plants (Aschale et al. 2022; Tan et al. 2023) have both been used as treatments to lessen symptoms and reinfection.

2.3. HYGIENE AND SANITATION

To stop the infection from spreading to others and from re-infecting oneself, proper hygiene and sanitation practices are crucial. This entails keeping living spaces clean, washing hands frequently—especially before meals and after using the restroom—and thoroughly cleaning fruits and vegetables before consumption (WHO 2023).

It is crucial to remember that the length and dosage of treatment can be changed based on the patient's age, the severity of their infection, and other variables. For a precise ascariasis diagnosis and suitable treatment regimen, speaking with a medical expert is advised.

Even though deworming programs have been put in place, effective elimination still requires an understanding of transmission dynamics, including zoonotic transmission, and the development of focused treatment plans. Diverse population genetic studies have revealed evidence of human-pig zoonotic transmission. There has been evidence of interbreeding between *A. lumbricoides* and *A. suum*, and genetic variations between the parasites that infect humans and pigs point to a complicated worldwide population expansion (Easton et al. 2020). This emphasizes the necessity of a one-health approach and shows how important it is to take into account both human and pig parasites in the control of human ascariasis. The significance of comprehending and managing the spread of human ascariasis is underscored by the development of a reference-quality *Ascaris* genome and additional genetic analyses that corroborate the interconnectedness of *A. lumbricoides* and *A. suum* populations (Wang 2021).

3. CAPILLARIASIS

Nematodes of the genus *Capillaria* are the source of the zoonotic infection known as capillariasis. Even though the *Capillaria* genus contains 300 species, only a small number of them, including *Capillaria (C.) phillipinensis* and *C. hepatica*, are responsible for most human infections. Due to eating raw or undercooked fish, *C. phillipinensis* causes intestinal capillariasis (Mahendra and Gutama 2024).

3.1. LIFE CYCLE

Capillaria species normally go through several stages in their life cycle, beginning with the adult worm living inside its host's body and laying eggs there. Depending on the species and the site of the infection, these eggs are then expelled from the host through their urine or feces. The eggs develop in the external environment and, after a few weeks, become infectious larvae. The infectious larvae can then enter the host by consuming tainted food or drink or by coming into direct touch with contaminated objects (Harvey et al. 2023; Mahendra and Gutama 2023).

Additionally, depending on the species, the larvae can grow on intermediate hosts. Some *Capillaria* species use specific insects or other invertebrates as intermediate hosts, allowing the larvae to mature before infecting the final host. The life cycle may manifest as either direct or indirect, contingent upon the particular species and the engagement of intermediary hosts (Fig. 1) (Harvey et al. 2023).

3.2. HOST RANGE AND SPECIFICITY

Capillaria species exhibit a broad spectrum of prospective hosts, with distinct species having adapted to infect various categories of animal hosts. Several *Capillaria* species, such as those targeting rodents, demonstrate a predilection for specific host types, while others may parasitize birds, fish, or reptiles. Each species may display either a specific host preference or a more extensive capacity to infect diverse animal species. Some *Capillaria* species are host-specific, whereas others have the capacity to infect several hosts and transmit disease to humans through zoonotic transmission (Santos et al. 2001).

3.3. PATHOGENESIS AND CLINICAL PRESENTATION

Depending on the species involved, the organs affected, and the severity of the infection, the pathogenesis of *Capillaria* infections can differ dramatically. *Capillaria* species infections can be asymptomatic, mild, or severe, resulting in a range of clinical manifestations in varied hosts (Santos et al. 2001).

The most prevalent type of infection in mammals is intestinal capillariasis, which mostly affects the small intestine. Animals with the infection may exhibit signs like diarrhea, weight loss, anorexia, and gastrointestinal pain. Intestinal damage, perforation, and inflammation brought on by severe infections can affect gut health. Depending on the species involved and the organs affected, *Capillaria* infections in birds might present as respiratory or gastrointestinal issues. Coughing, sneezing, nasal discharge, and respiratory distress can all be symptoms of respiratory capillariasis in birds. In contrast, gastrointestinal capillariasis may result in weight loss, diarrhea, and malabsorption. *Capillaria* infections frequently cause inflammation, edema, and epithelial damage in the stomach of fish. The growth rates of infected fish may be slower, their ability to absorb nutrients may be affected, and they may be more vulnerable to other infections (Dubey et al. 2018).

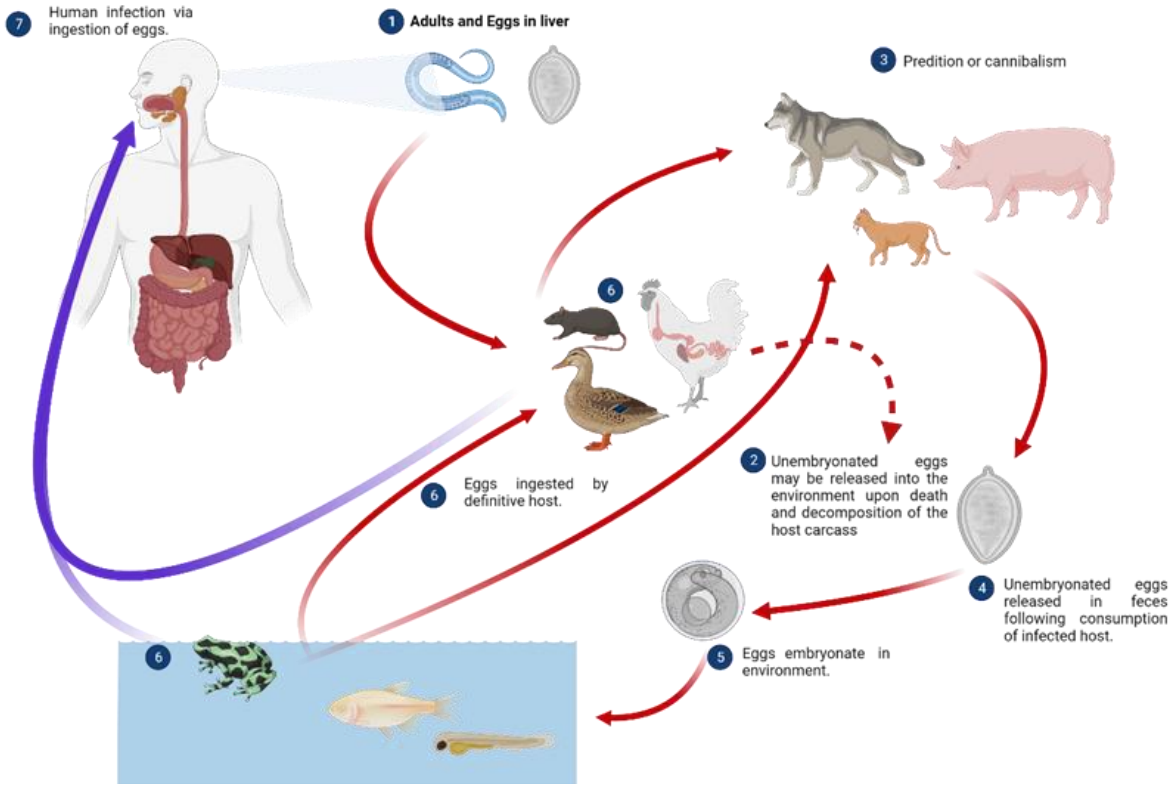

Fig. 1: The life cycle of *Gnathostoma* species. (Source: Centers for Disease Control and Prevention, www.dpd.cdc.gov/dpdx/gnathostomiasis/index.html) 1) Mature nematodes aggregate into a neoplastic mass within the gastrointestinal tract of animals acting as their exclusive hosts, with the subsequent expulsion of their eggs in fecal matter. Within the hepatic parenchyma of the host, adult worms are profoundly entrenched, where they deposit numerous eggs within the surrounding parenchymal tissue. 2,3) The eggs ensnared within the host's hepatic parenchyma persist therein until the expiration of the host organism, or more probable, until it undergoes predation or scavenging. 4) Predatory or scavenging organisms ingest embryonated eggs, thereby facilitating a proficient mechanism for the dissemination of eggs into the surrounding environment. This pathway stands as the predominant environmental route of transmission. 5) Environmental egg development necessitates exposure to air and humid soil for infectious maturation. 6) The continuum persists upon the ingestion of embryonated eggs by a suitable mammalian host. 7) Infectious eggs undergo hatching in the intestinal milieu, liberating larvae at the first developmental stage. Subsequently, these larvae traverse the intestinal wall and migrate through the portal vein, reaching the hepatic parenchyma within a span of 3-4 days. Created with BioRender.com, modified of www.cdc.gov/parasites/

3.4. ZOONOTIC POTENTIAL

Some *Capillaria* species possess zoonotic potential, indicating their capability to infect humans and induce diseases. An exemple is *C. philippinensis*, which instigates intestinal capillariasis in humans, predominantly in Southeast Asia. The transmission of this species occurs through the ingestion of raw or insufficiently cooked fish harboring the infective larvae. In human hosts, intestinal capillariasis can manifest as chronic diarrhea, abdominal pain, malabsorption, and weight loss. Another instance is *Capillaria hepatica*, a parasitic affliction caused by the nematode *C. hepatica*. This parasite predominantly targets the liver in various mammals, encompassing humans, rodents, and certain other animal species. (Frean 2020).

3.5. TRANSMISSION

Rats and mice are the most common rodent hosts for *C. hepatica.* When people unintentionally consume food or water that has parasite eggs in it, they become infected. The eggs, which contaminate the environment, are excreted by infected rodents in their waste. The eggs hatch once they are inside the human body, and the larvae move to the liver where they mature and become adult worms (Santos et al. 2001).

3.6. CONTROL AND PREVENTION

A multifaceted strategy is required to prevent and treat *Capillaria* infections. Maintaining clean and hygienic environments can help to lower the risk of infection in animal husbandry situations. To control *Capillaria* infections in domesticated animals, proper cleanliness, waste management, and routine deworming of animals are crucial. To prevent infections in fish farming, maintaining the quality of water sources is essential. Fish can be identified and isolated if they show symptoms of infection, which will stop the parasite from spreading throughout the population. Public health awareness efforts are essential in informing people about the dangers of consuming raw or undercooked fish as well as other possible infection sources in zoonotic cases involving *Capillaria* species. Additionally, implementing food safety regulations and proper cooking practices can minimize the risk of transmission to humans (Dubey et al. 2018).

4. LAGOCHILASCARIASIS

It is known as a parasitic disease and reports in humans are rare. The helminth responsible for this disease in humans is *Lagochilascaris (L.) minor* (Barbosa et al. 2017; Barreto et al. 2018). Human Lagochilascariasis can also be found in cats and dogs. This disease is considered an emerging zoonosis in America distributed from Mexico to Argentina and the Caribbean islands (Barrera-Pérez et al. 2012; Christello Trindade et al. 2019).

It is a silent and chronic disease, and over the course of years the symptoms may be hidden. The clinical picture of the disease presents itself until the parasite invades the subcutaneous tissues, central nervous system and bone tissues (Solano-Barquero et al. 2022).

L. minor is mainly reported in wild felines, with rare reports in domestic felines. Due to the rarity of disease, the life cycle has not yet been clearly reported (Lanfredi et al. 1998) (Fig. 2).

Although lagochilarscariasis is an uncommon parasitic disease in humans, people must be cautious on probable outbreaks due to the climate change (Quintana de Moura et al. 2012; Solano-Barquero et al. 2022).

5. MAMMOMONOGAMIASIS

Mammomonogamus (a helminth) infection in humans (also known as syngamosis) is quite rare (Castaño et al. 2006; Alves de Almeida et al. 2018). It is produced by the nematode *M. laryngeus*, which parasitize the respiratory tract of some animals like cattle (Echeverry et al. 2011). Infected humans present symptoms like other diseases with respiratory manifestations (Bentivi Pulcherio et al. 2013). One of the main symptoms of the disease is a persistent cough, sometimes causing coughing up blood (Angheben et al. 2009). These symptoms are caused by the parasite settling in the respiratory tract (Nosanchuk et al. 1995; Agosu et al. 2021) (Fig. 3).

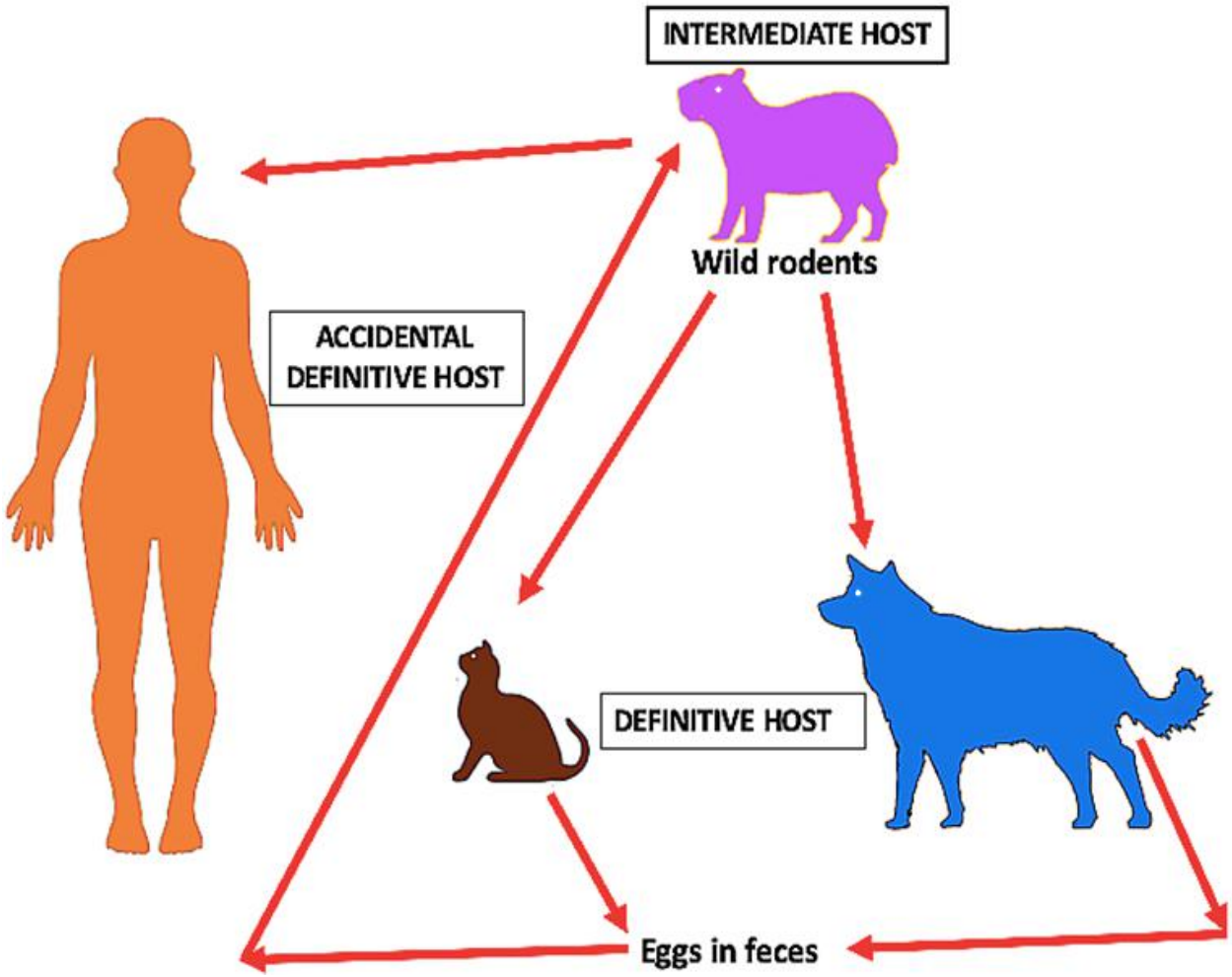

Fig. 2: Probable life cycle of *Lagochilascaris minor*. (Figure made by Carlos Ramón Bautista-Garfias).

The general life cycle of *M. laryngeus* is as follows: humans or cattle ingest Infective eggs or larvae in contaminated food, or water. Then, larvae penetrate intestinal walls into mesenteric veins and migrate to lungs where it develops into adult worms. Adult worms move to tracheolaryngeal region and there attach to mucosal walls. In this region, male and female worms form Y shape, and sexual reproduction occurs. After this, eggs produced and are coughed up and can be reswallowed. Eggs not swallowed are expelled in feces (Fig. 3) (Echeverry et al. 2011).

6. STRONGYLOIDIASIS (*STRONGYLUS* SPP.)

The causative agent of strongyloidiasis is a geohelminth *Strongyloides (S.) stercoralis*. This is a parasite inhabiting the small intestine of the host. The name of this parasite was accepted by Bavay (1876) and was reported by the physician when he observed French soldiers returning from Vietnam with diarrhea and larvae in the feces (Grove 1990).

The life cycles of each species vary in some respects. In the case of *S. stercoralis* the females located in the intestine deposit several eggs in the intestinal epithelium which cause them to develop into larvae and emerge as larvae in the feces (Grove 1980). Due to this variable for the detection of parasite in feces, it is recommended to carry out a wider sampling and on different days (Fernandez-Chavarria 2011).

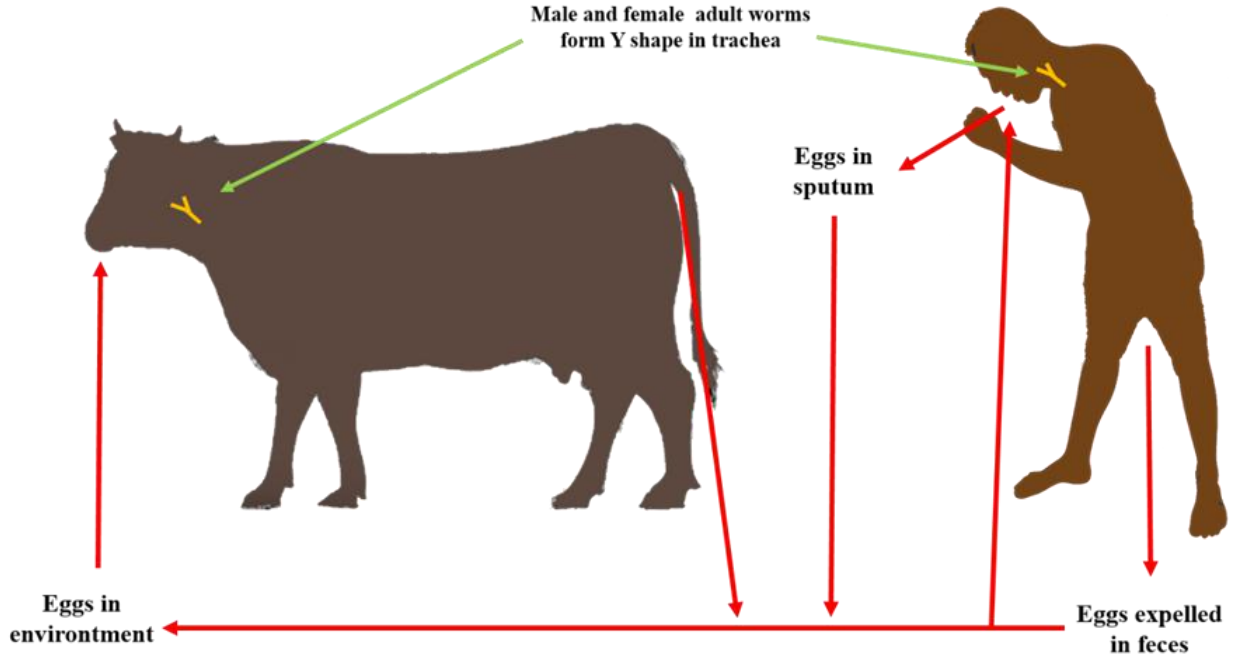

Fig. 3: Life cycle of *Mammomonogamus laryngeus*. (Figure made by Carlos Ramón Bautista-Garfias).

6.1. LIFE CYCLE

In general, the Strongylidae family presents a direct cycle with two phases: exogenous and endogenous. The adult parasites are lodged in the large intestine and the eggs produced by the females are excreted in the feces. Subsequently, the L1 larva hatches from the egg and grows into the infective larva (L3). Infection occurs passively after ingestion of L3 (with a sheath) found in the pasture. There are other types of Strongyloides, but the most common type infecting humans is *S. stercoralis* (Catalogue of Life 2023).

6.2. CLINICAL PICTURE

Once infected, the person starts showing signs and symptoms including intradermal migration of Strongyloides (Larva currens), itchy feet, wheezing, coughing, fever, diarrhea, nausea/vomiting and weight loss. These symptoms may present differently depending on the stage of the disease (Farthing et al. 2018).

6.3. DIAGNOSIS

Tests used for the diagnosis of *S. stercoralis* include the diagnostic techniques by coproparasitological methods, culture of feces on agar plates, the direct method, the modified Ritchie method and the Baerman method. Of these methods, greater sensitivity has been observed in the stool culture tests on agar plates and the Baerman technique (Figueroa 2002; Farthing et al. 2018).

7. CUTANEOUS LARVA MIGRANS

Cutaneous larva migrans (CLM), colloquially known as "creeping eruption" or "sandworm disease," represents a parasitic cutaneous infection induced by the migration of larval stages of certain hookworm species within the epidermal and upper dermal layers. Regions characterized by tropical and subtropical climates, particularly those marked by deficient sanitation and a substantial population of stray dogs and cats, are predisposed to this malady. Individuals inadvertently assume the role of hosts upon exposure to environments contaminated with infectious larvae (Hochedez and Caumes 2007).
Cutaneous Larva Migrans (CLM) stands as the most prevalent dermatological affliction of tropical origin associated with travel. This dermatosis stems from the subcutaneous migration of Ancylostoma larvae. The nomenclature associated with this skin ailment, including "creeping eruption," "creeping verminous dermatitis," "sand worm eruption," and "plumber's itch," contributes to its perplexity. The designation "hookworm-related cutaneous larva migrans" (HrCLM) has been employed to characterize this disorder (Hochedez and Caumes 2007). Specific hookworm species, during their larval stage, precipitate CLM by traversing the epidermis and upper dermal layers, manifesting as a dermatological malady. Principal offenders include larvae of *Ancylostoma (A.) braziliense* and *A. caninum*, predominantly affecting dogs and cats. Additional, albeit less phylogenetically related parasites encompass *Uncinaria (U.) stenocephala*, *Ancylostoma tubaeforme*, *Gnathostoma spinigerum*, certain strains of *Strongyloides stercoralis*, bovine parasites (*Bunostomum phlebotomum*), murine parasites (*Strongyloides myopotami*), and parasites found in wild canids (*S. procyonis*). *Toxocara canis* species are responsible for the onset of ocular and visceral larva migrans (Otamendi et al. 2011). Through the feces of sick animals, these worms discharge eggs into the environment. The larvae become infectious when the eggs hatch in warm, damp soil or sand. The infectious larvae can enter the skin of people who encounter these polluted places and cause CLM (Hochedez and Caumes 2007).

7.1. ETIOLOGICAL AGENT

The major species causing HrCLM are *A. braziliense*, *A. caninum*, or *U. stenocephala*, which are distinguished by slightly elevated and erythematous tracks. They measure roughly as one centimeter. One or more, linear or, more frequently, serpiginous, ramified, and interwoven, may exist. The width of tracks varies from 2 to 4 mm, and the length can vary greatly, sometimes up to many centimeters. Pruritus frequently occurs along with tracks. The larvae puncture the corneal layer of the epidermis when people meet soil contaminated by animal waste. Because humans are incidental hosts that disrupt normal larval development, CLM is self-limiting. However, the creeping eruption could persist for a few months. (Feldmeier et al. 2006).

7.2. EPIDEMIOLOGY

In impoverished communities in tropical and subtropical areas of developing nations, CLM is common, especially in areas with inadequate sanitation and a high population of stray dogs and cats. There

have also been reports of autochthonous cases or small outbreaks in temperate countries such as the United States, Germany, France, Great Britain, and New Zealand (Feldmeier et al. 2006). It usually affects visitors and travelers who engage in activities such as walking barefoot on beaches that involve contact with contaminated sand or dirt. The majority of clinical data on this parasitic skin illness comes from observations made by travelers returning from the tropics. Only one study had examined clinical characteristics in individuals living in areas where the disease was endemic (Heukelbach et al. 2004).

7.3. LIFE CYCLE AND PATHOGENESIS

The parasitic hookworm goes through various phases in its life cycle, the first of which is the larvae. Due to their inability to complete their life cycle in humans, these larvae migrate beneath the skin of the host, causing CLM (Freedman et al. 2006).

The small intestine of infected dogs and cats is home to adult hookworms, which cling to the intestinal wall and feed on blood. The eggs laid by the female hookworms are expelled in the feces of the host and number in the millions each day. Through their feces, afflicted animals pass the eggs of *A. braziliense* and *A. caninum* from their bodies. These eggs are extremely tolerant of their surroundings and can remain in the soil for several weeks to months. The hookworm eggs hatch and first-stage larvae (L_1) emerge from the eggs in warm and damp habitats like sandy beaches or soil (Hochedez and Caumes 2007). When people come into contact with sand or dirt that has been polluted with the infectious larvae (L_3), they become unintentional hosts of CLM. The larvae can actively pierce the skin, particularly in places where the skin is thin or injured. Once the infectious larvae have entered the epidermis, they cannot finish their life cycle in people. Instead, they move through the epidermis and higher dermal layers of the skin, resulting in the cutaneous larva migrans rash, which is characterized by a serpiginous or winding rash. The larvae are unable to develop further inside the skin. Instead, they move, producing the recognizable rash and excruciating itching.

Over time, the body's immune response may lead to the elimination of the larvae, and the rash resolves without specific treatment. However, the duration of the infection and resolution of symptoms can vary from weeks to months (Jourdan et al. 2018). As the larvae die or are eliminated by the immune system, the symptoms subside, and the skin gradually heals (Fig. 4). It is essential to note that humans are accidental hosts for these hookworm larvae, and they cannot complete their full life cycle in the human body. The larvae cannot mature into adult worms or reproduce within humans. The infection is generally self-limiting, and the larvae are eventually eliminated by the immune response (Hochedez and Caumes 2007; Jourdan et al. 2018).

7.4. DIAGNOSIS

Clinical features are the primary basis for the diagnosis of CLM. A medical professional will examine the unique appearance of the skin rash, which typically presents as a raised, reddish and elevated lesion that is intensely itchy (Leung et al. 2017). The healthcare professional will work to distinguish CLM from other skin disorders, such as scabies, allergic responses, and other parasite infections, that may have comparable clinical presentations. The form and spread of the rash should be carefully examined to help to differentiate CLM from other skin conditions (Hochedez and Caumes 2007).

7.5. TREATMENT

Treatment for CLM aims to eliminate the hookworm larvae causing the infection, accelerate healing, and alleviate symptoms. CLM typically resolves on its own within weeks to months, as the rash often

spontaneously disappears when the larvae either die or are eliminated by the immune system. However, if the rash does not improve or if there is extreme itching or discomfort, medical treatment might be recommended. The use of anthelmintic treatments, which are medicines that can kill parasitic worms, is the main therapy strategy for CLM. Hookworms can be treated with albendazole, an oral drug, along with other parasitic infections (Freedman et al. 2006).

8. TRICHIURIASIS (*TRICHIURIS SUIS*)

Trichocephalosis is caused by *Trichuris* spp or trichocephalus (*T. trichiura*, *T. vulpis* and *T. suis*), a nematode that is in the large intestine and mainly affects children (Carrada-Bravo 2004).
Trichuris is transmitted to humans in different ways including the infection by *Trichuris vulpis* and *Trichuris suis* from dogs and pigs (Nejsum et al. 2020).

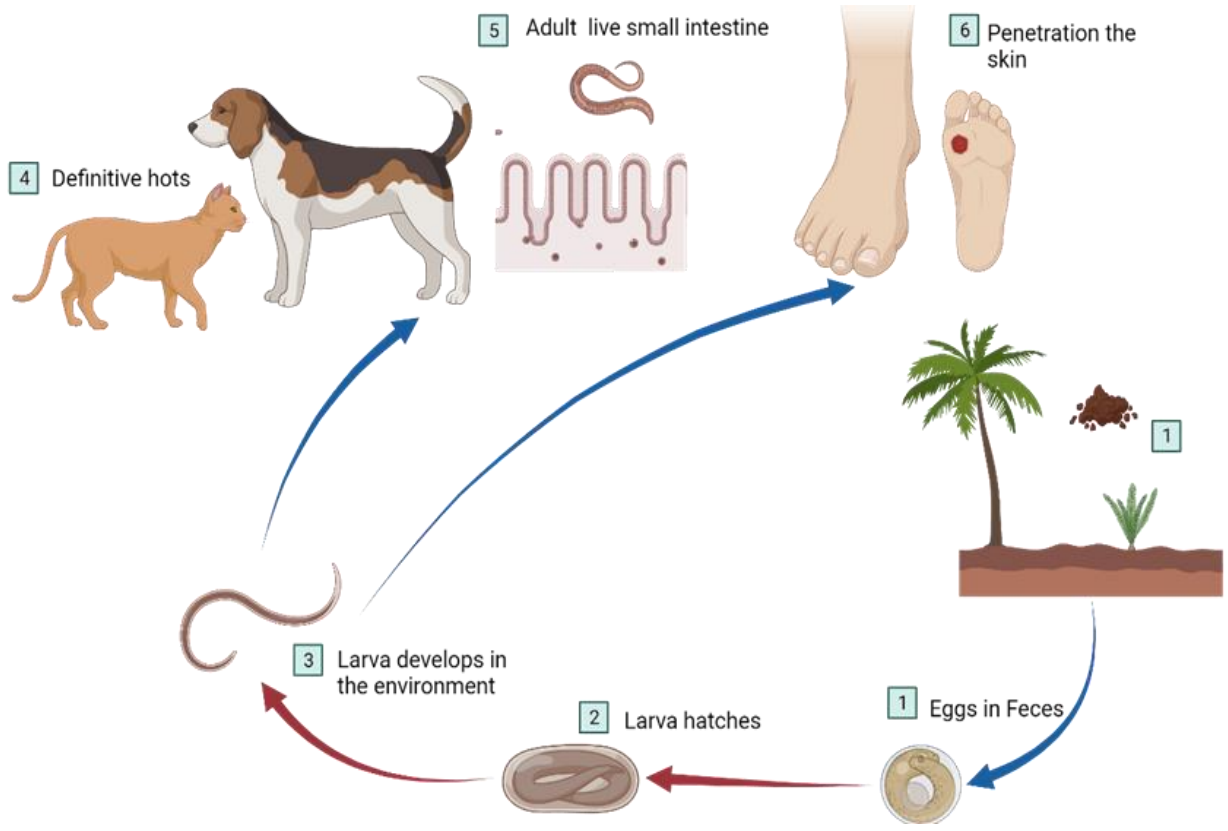

Fig. 4: Cycle in the final host resembling the cycle in the human species quite a bit. Created with BioRender.com, modified of https://www.cdc.gov/dpdx/hookworm/index.html

When it comes to *T. trichiura*, females are found in the mucosa and lay eggs daily. These eggs hatch in feces and the surrounding environment, and only a small percentage of them are able to embryonate until the first larval stage develops in the egg under the right conditions. These eggs contain infectious larvae that, upon ingestion by a new host, settle in the small intestine before moving on to the large intestine, where they eventually mature into adults (Carrado-Bravo 2004; Nejsum et al. 2020). Zoonotic species, such as *Trichuris* spp., can spread through improper treatment of animal feces and environmental

contamination. For *T. vulpis* in dogs, the prepatent period lasts 70–90 days, but for *T. suis*, it lasts in 41–45 days.

8.1. CLINICAL PICTURE

Symptoms include inflammation, growth retardation in children, oedema, hemorrhages, anemia, abdominal pain, and occasional diarrhea, and in case of massive infection, appendicitis may occur. However, the absence of symptoms has been observed in several hosts (Bundy 1994).

8.2. DIAGNOSIS

Recently, coproparasitological tests such as flotation technique, Baerman technique and others are of great help to identify *Trichuris* spp. in asymptomatic hosts (Carrada-Bravo 2004). Eggs observed under the microscope have a peculiar lime-shaped form as shown in Fig. 5.

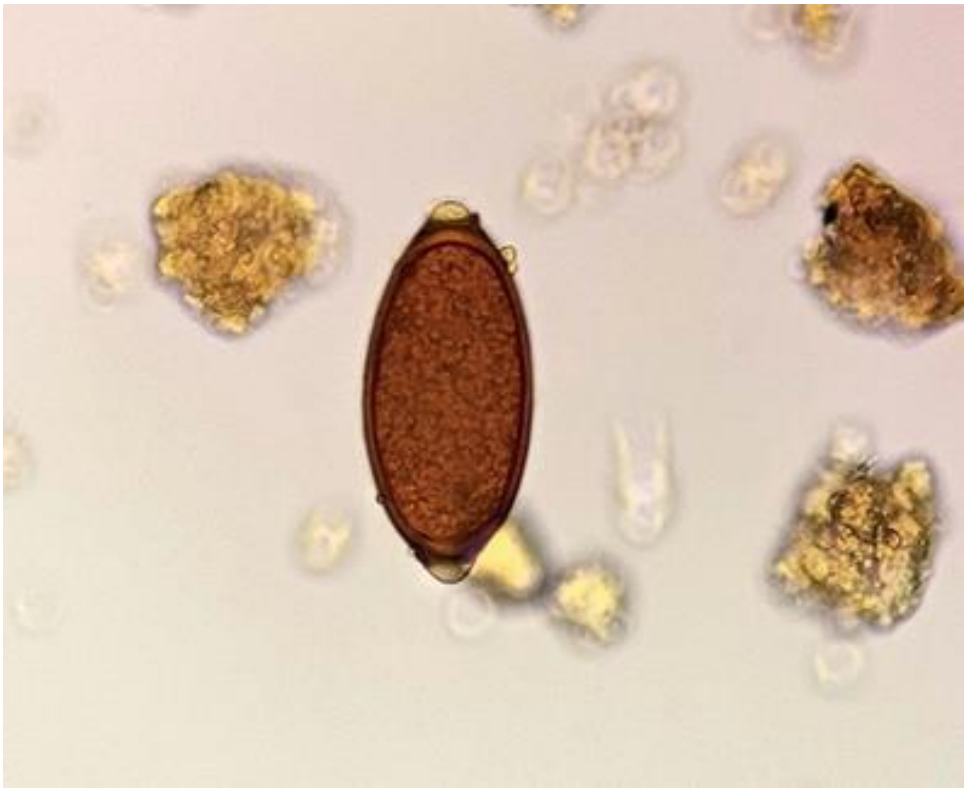

Fig. 5: Egg of *Trichuris trichiura* (Taken from Cauich-Echeverria and Franco-Zetina 2021).

9. CONCLUSION

Zoonotic diseases are a major public health problem because animals are the source of human pathogens. The emergence and re-emergence of zoonotic diseases have been attributed to a number of anthropogenic and natural factors, including vector biology, urbanization, climate change, animal migration and trade, travel and tourism, among others. Animals are the origin of a large number of infectious diseases that affect humans. About 60% of newly discovered human infections are zoonotic. Because of this, it is important to consider the areas where we live and the risks of latent diseases in the regions. In addition, it is extremely important to give importance to common wound accidents, because it

is better to prevent silent diseases that can be hidden for years through good food and wound asepsis and go to the doctor.

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Ornamental Fishes and Zoonotic Problems to Animals and Public Health

30

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ABSTRACT

The ornamental fish trade and aquarium hobby have grown exponentially, aided by the globalization of commerce and the ease of transport. However, the movement of ornamental fish also poses threats to ecosystems, animal health, and public health. Ornamental fishes may serve as carriers or vectors of potentially zoonotic organisms, including viral, bacterial, fungal and parasitic pathogens. Mycobacteriosis, caused by Mycobacterium spp. and exophiala infections are frequent among imported tropical freshwater fishes. Clinical signs vary from chronic wasting, skin lesions and hemorrhages to non-apparent infections, thus facilitating disease transmission to other fishes or humans. Free-living pathogens are also dispersed to new habitats through aquarium release and contaminated water disposal. Protozoa like Cryptocaryon or Amyloodinium can produce disease both in marine fishes and humans, although unusual. Trematodes inhabiting freshwater and estuarine fishes utilize other animals as intermediate/paratenic hosts and are capable of infecting humans if infected fish are consumed raw or improperly cooked. However, the greatest public health risks from the ornamental fish trade likely come from exposing immunocompromised aquarists and fish-handlers to opportunistic bacterial, fungal, and mycobacterial infections. Thus, education regarding proper hygiene practices should accompany the promotion and sale of ornamental fishes. Additional research is needed to better characterize pathogens associated with the ornamental fish trade and implement biosecurity measures to prevent introduction of invasive organisms. This book chapter reviews the diversity of potential zoonotic organisms identified among imported ornamental fishes, pathological findings, transmission routes to humans, as well as diagnosis, treatment and preventive guidelines.

Keywords: Ornamental fishes, Zoonotic infections, Pathological findings, Transmission routes

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1. INTRODUCTION

1.1. ORNAMENTAL FISHES AND ZOONOTIC PROBLEMS

Ornamental fishkeeping is a popular hobby worldwide, with millions of enthusiasts cherishing the vibrant colors and diverse shapes of these aquatic pets. From stunning freshwater species like guppies and angelfish to mesmerizing marine fish like clownfish and tangs, the allure of ornamental fishes knows no bounds. However, beyond their beauty lies a lesser-known aspect of this hobby: the potential zoonotic problems associated with keeping and handling these captivating creatures (Hossain and Heo 2021).

Zoonotic diseases, also known as zoonoses are infectious diseases that can be transmitted from animals to humans and vice versa. While ornamental fishes may not be the first animals that come to mind when discussing zoonotic concerns, they can indeed harbor a range of pathogens that pose health risks to both their owners and others in contact with them. Though the likelihood of contracting a zoonotic disease from ornamental fish is relatively low, it is essential to be aware of the potential risks to ensure responsible and safe fishkeeping practices (Weir et al. 2012).

Table 1: Zoonotic Diseases of Ornamental Fishes

Disease name	Etiology	Mode of transmission
Fish Tuberculosis (Mycobacteriosis	Mycobacterium spp. (bacterium)	Contaminated water
Aeromoniasis	Aeromonas spp	Drinking contaminated water and foods
Cryptococcosis	fungus Cryptococcus	Through inhalation of spores
Salmonellosis	*Salmonella typhimurium*	Fecal oral route
Erysipeloid	*Erysipelothrix rhusiopathiae*	Direct contact with infected animals
Henneguya and Myxobolus Infections	*Henneguya ictaluri*	Injesting raw or undercooked infected fish
Fish Handler's Disease	*Erysipelothrix rhusiopathiae*	Direct contact with infected fish
Spring Viremia of Carp	*Rhabdovirus carpio*	Infected fish eggs
Lymphocystis Disease	Lymphocystivirus (virus)	Mechanical injury, stress
Gyrodactylus and Dactylogyrus Infections	Flukes (Monogenean Parasites)	Translocation of live fish

This introduction seeks to shed light on the intersection of ornamental fishes and zoonotic problems, highlighting the types of zoonotic diseases associated with these fish, the modes of transmission, and the significance of adopting preventive measures. As the ornamental fish trade continues to grow, with new species being introduced and distributed globally, understanding the zoonotic risks becomes paramount for safeguarding public health and the well-being of aquatic ecosystems (Millington et al. 2022).

Ornamental fishes are beloved pets that enhance the beauty and tranquility of home aquariums and public displays. However, it is crucial to be aware of potential zoonotic problems associated with these fishes, as certain diseases can be transmitted between humans and fish (Passantino et al. 2008).

1.2. IMPORTANT ZOONOTIC DISEASES

1.2.1. FISH TUBERCULOSIS (MYCOBACTERIOSIS) - ETIOLOGY AND PATHOGENESIS

Fish tuberculosis is caused by *Mycobacterium marinum*, a bacterium that can infect both humans and fishes (Jacobs et al. 2009). In fish, the bacteria primarily invade the digestive tract, skin and internal

organs, leading to chronic granulomatous inflammation. In humans, the bacteria can enter the body through skin abrasions or ingestion, causing localized infections. Direct contact with infected fish, contaminated water or environmental surfaces can transmit the bacteria. In humans, transmission occurs through skin contact with infected fish, fish tanks or aquarium water (El Amrani et al. 2010).

1.2.2. CLINICAL FINDINGS

Fish may exhibit symptoms such as emaciation, lethargy, skin ulcers and fin rot. Internal organs may show granulomas and caseous necrosis (Ferguson 2007; El Amrani et al. 2010). In humans, symptoms include skin lesions, typically on hands or arms which may appear as nodules, ulcers or abscesses (Petrini 2006; Hashish et al. 2018). Systemic infections are rare but may cause joint pain, fever and swollen lymph nodes. In fish, post mortem examination may reveal granulomatous lesions in the internal organs, liver, spleen and kidneys. In humans, skin biopsies may show caseating granulomas.
Fish tuberculosis is more prevalent in certain species, such as angelfish, gouramis and discus fish. People with compromised immune systems such as those with HIV/AIDS are at higher risk of developing severe infections.

1.2.3. TREATMENT AND CONTROL

Treatment involves isolating infected fish and providing appropriate antibiotic therapy. In humans, antimicrobial therapy is necessary for severe infections. To prevent transmission, practice good hygiene, thoroughly clean aquarium equipment and avoid exposure to contaminated water or infected fish.

1.3. AEROMONIASIS

1.3.1. ETIOLOGY AND PATHOGENESIS

Aeromoniasis is a bacterial infection caused by Aeromonas species commonly found in ornamental fishes (Hossain and Heo 2021). The bacteria invade fish tissues, causing systemic infection. In humans, the bacteria can enter the body through open wounds or ingestion (Batra et al. 2016). Direct contact with infected fish, contaminated water or equipment can transmit the bacteria. In humans, transmission occurs through exposure to infected fish or contaminated water.

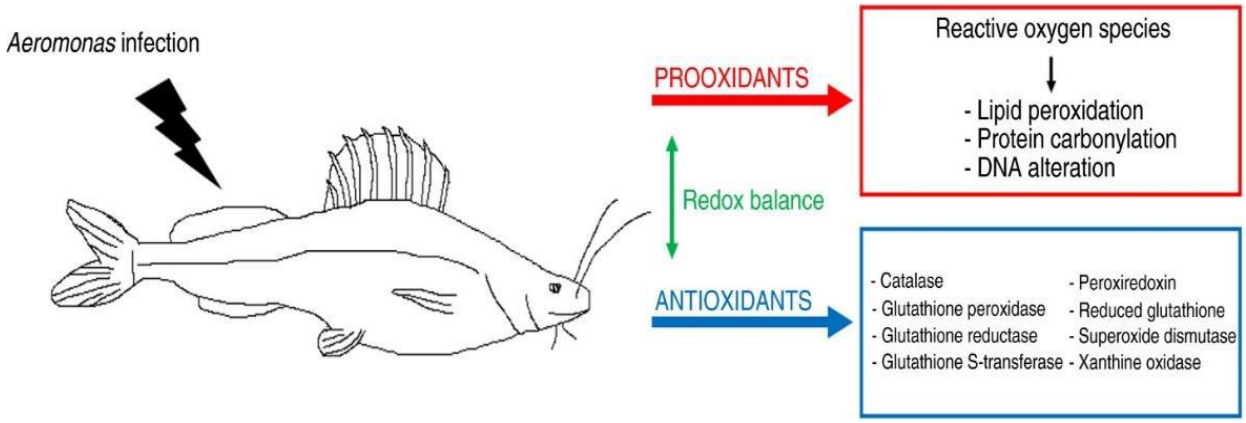

Fig. 2: Aeromoniasis infection (Applied microbiology internation-Wiley).

1.3.2. CLINICAL FINDINGS

Fish may exhibit symptoms such as fin rot, skin ulcers, hemorrhages and abdominal distension. Internal organs may show congestion and hemorrhages. In humans, symptoms include diarrhea, abdominal pain, wound infections and cellulitis (Alhazmi 2015). Systemic infections can occur in immunocompromised individuals.

Aeromoniasis can affect a wide range of fish species. Human infections are often associated with fish handling, fish cleaning or exposure to contaminated water. In fish, post mortem examination may reveal hemorrhagic enteritis, congestion and necrotic lesions in the liver and spleen. In humans, tissue samples may show necrotizing fasciitis or cellulitis.

1.3.3. TREATMENT AND CONTROL

Antibiotics are commonly used to treat infected fish. In humans, appropriate antimicrobial.

1.4. CRYPTOCOCCOSIS

1.4.1. ETIOLOGY AND PATHOGENESIS

Cryptococcosis is a fungal infection caused by *Cryptococcus neoformans* and *Cryptococcus gattii* (Kwon-Chung et al. 2017; D'souza et al. 2011). Fish can become infected by ingesting fungal spores and humans can acquire the infection through inhalation of fungal particles. Direct contact with contaminated water or exposure to infected fish can transmit the fungi to humans. Inhalation of aerosolized fungal particles from fish tanks or handling infected fish can also lead to transmission (Datta et al. 2009; Harris et al. 2012; May et al. 2016).

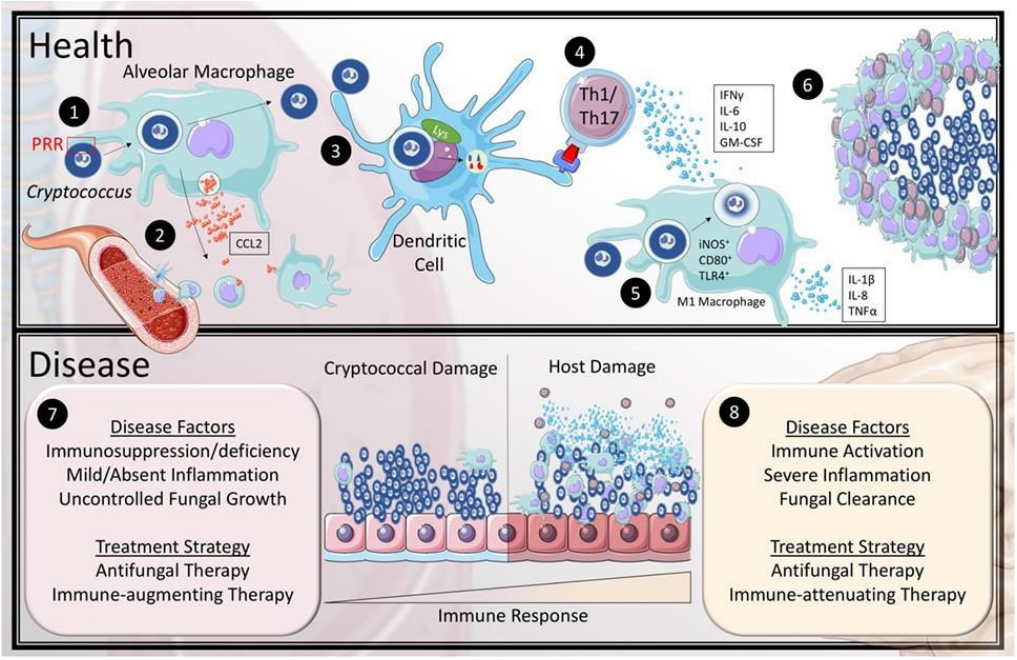

Fig. 3: Etiology of Cryptococcosis (Crossen et al. 2022).

1.4.2. CLINICAL FINDINGS

Fish may show respiratory distress, lethargy, skin ulcers and abnormal swimming behavior. Internal organs, particularly the brain and lungs may exhibit granulomas and necrosis. In humans, cryptococcosis primarily affects the respiratory system, causing pneumonia-like symptoms such as cough, shortness of breath and chest pain. In severe cases, it can spread to other organs, including the central nervous system, leading to meningitis (Damiani et al. 2020).

Cryptococcosis is more commonly associated with tropical and subtropical regions. People with weakened immune systems such as those with HIV/AIDS or organ transplant recipients are at higher risk of developing severe infections. In fish, post mortem examination may reveal granulomatous lesions in the brain, kidneys and other organs. In humans, tissue samples may show granulomatous inflammation and encapsulated yeast-like organisms.

1.4.3. TREATMENT AND CONTROL

Antifungal medications, such as fluconazole or amphotericin B is used for both fish and human infections. Reducing exposure to fungal spores through proper hygiene and minimizing aerosolization in fish tanks are essential preventive measures (Acheson 2020).

1.5. SALMONELLOSIS

1.5.1. PATHOGENESIS

Salmonellosis is a bacterial infection caused by Salmonella species (Bibi et al. 2015). Fish can become carriers of the bacteria through contaminated water or food sources. In humans, infection occurs through ingestion of contaminated fish or their products. Direct contact with infected fish or exposure to contaminated water or equipment can transmit the bacteria. Consuming undercooked or raw infected fish can also lead to human infection (Antunes et al. 2018; Gazal et al. 2018; Akinjogunla et al. 2011; Dib et al. 2018).

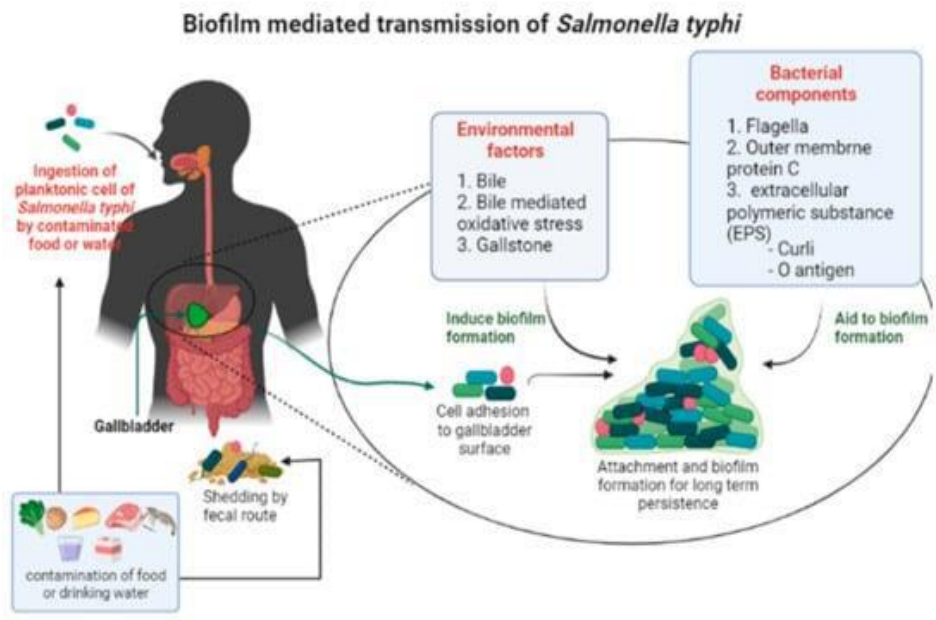

Fig. 4: Salmonella mode of transmission (Jahan et al. 2022).

1.5.2. CLINICAL FINDINGS

Fish may exhibit signs of systemic infection, including lethargy, loss of appetite, skin lesions, and hemorrhages in the internal organs (Mawa et al. 2021). In humans, salmonellosis presents as gastrointestinal symptoms such as diarrhea, abdominal pain, nausea and fever. Severe cases can lead to bloodstream infections and complications.

Salmonellosis is a widespread zoonotic disease associated with various animal species, including fish. Proper food handling and preparation practices are essential to prevent transmission. In fish, post mortem examination may reveal necrotic foci in the liver, spleen and intestines. In humans, stool cultures can confirm the presence of Salmonella.

1.5.3. TREATMENT AND CONTROL

Antibiotics may be used to treat infected fish but prevention is crucial. Proper hygiene practices, safe food handling and thorough cooking of fish are essential to prevent salmonellosis in humans (Bakhiet and Zaroug 2020).

1.6. ERYSIPELOID

1.6.1. ETIOLOGY AND PATHOGENESIS

Erysipeloid is a bacterial infection caused by *Erysipelothrix rhusiopathiae* (Rostamian et al. 2022). Fish can carry the bacteria asymptomatically and humans can contract the infection through direct contact with infected fish or their contaminated environments. Contact with contaminated fish or exposure to water or surfaces contaminated with the bacteria can transmit the infection to humans (Coutinho et al. 2012; Mavrot et al. 2020). Penetrating injuries or cuts on the skin increase the risk of infection.

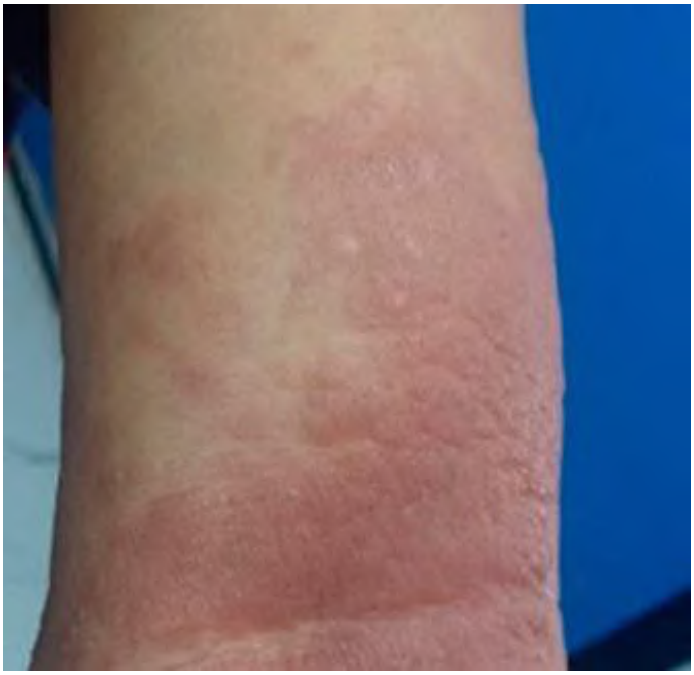

Fig. 5: Erysipeloid infection (Parajuli et al. 2021).

1.6.2. CLINICAL FINDINGS

Fish may not exhibit significant clinical signs but carriers can shed the bacteria into the surrounding water. In humans, erysipeloid usually affects the skin, causing a localized, painful and red rash that may resemble cellulitis. In some cases, joint pain, swelling and fever can also occur (Xu et al. 2019).
Erysipeloid is more commonly associated with handling raw seafood, including infected fish. Occupational groups such as fishmongers, aquarium workers and seafood processors are at higher risk of infection (Cruz and Martin-Ezquerra 2023). No specific post mortem lesions are typically observed in fish as the infection is usually asymptomatic.

1.6.3. TREATMENT AND CONTROL

Antibiotics such as penicillin or erythromycin are commonly used to treat erysipeloid in humans. Preventive measures include using gloves when handling fish, maintaining good hygiene practices and promptly cleaning and treating any wounds (Rostamian et al. 2022).

1.7. HENNEGUYA AND MYXOBOLUS INFECTIONS

1.7.1. ETIOLOGY AND PATHOGENESIS

Henneguya and myxobolus are parasites belonging to the group of myxozoans (Okamura et al. 2018). They infect the muscle tissues of fish and can potentially cause infections in humans. Contact with infected fish or exposure to contaminated water can transmit the parasites to humans. Ingesting raw or undercooked infected fish can also lead to human infection.

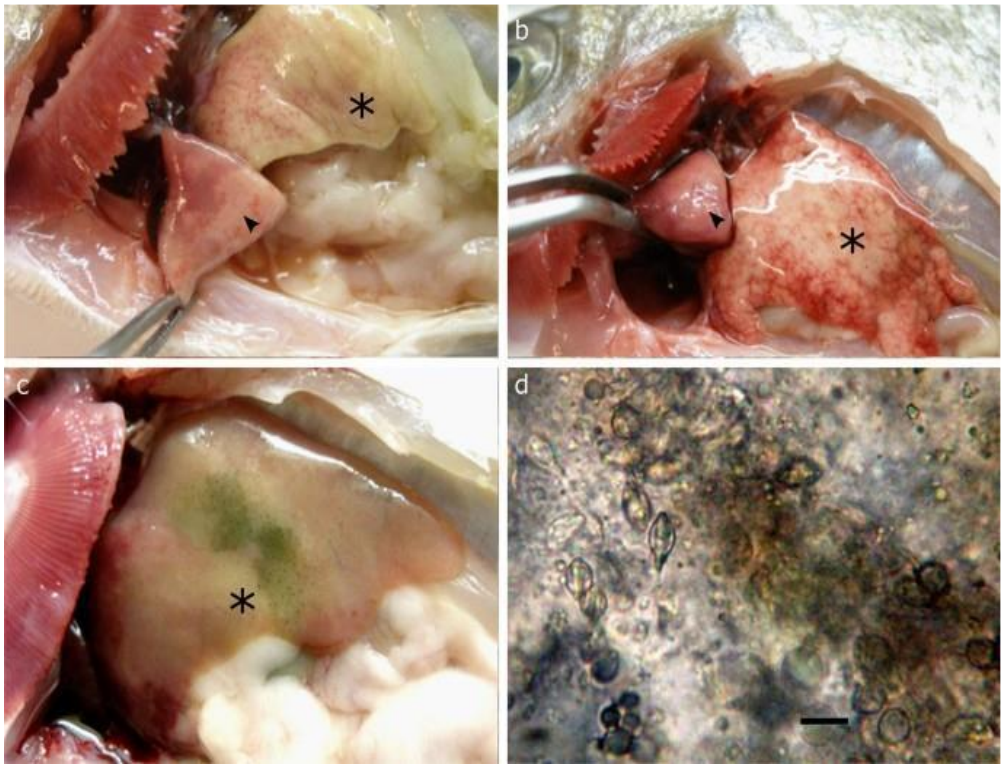

Fig. 6: Infected hearts (arrowheads) with noticeable myocardium degeneration. Haemorrhagic and degenerated livers (asterisks) (a and b) with bile imbibition (c). d) Mature spores of Henneguya aegea n. sp. in fresh liver tissue squash under light microscopy (×400). Scale-bar: 10µm (Katharios et al. 2020).

1.7.2. CLINICAL FINDINGS

Fish infected with henneguya and myxobolus may exhibit no apparent clinical signs but the parasites can cause cysts or plasmodia in the muscle tissues (Müller et al. 2023). In humans, infection by henneguya and myxobolus species is rare and symptoms are not well-documented. However, potential manifestations may include localized pain, inflammation and swelling at the site of contact. The risk of human infection is relatively low but individuals who consume raw or undercooked fish, particularly from endemic regions may be more susceptible. In fish, post mortem examination may reveal cysts or plasmodia within the muscle tissues.

1.7.3. TREATMENT AND CONTROL

There is no specific treatment for henneguya and myxobolus infections in humans. Prevention involves proper cooking of fish to kill any potential parasites and avoiding contact with contaminated water or infected fish (Lebanan and Mohilal 2021).

1.8. FISH HANDLER'S DISEASE

1.8.1. ETIOLOGY AND PATHOGENESIS

Fish handler's disease also known as Fish Tank Granuloma or Fish Poisoning Dermatitis, is caused by various bacteria including *Mycobacterium marinum*, *Vibrio vulnificus* and *Streptococcus sp*. These bacteria can enter the human body through cuts, wounds or breaks in the skin when handling infected fish or contaminated water (Sobuj et al. 2022). Direct contact with infected fish or exposure to contaminated water can transmit the bacteria to humans. Fish handler's disease is commonly associated with cuts or abrasions on the hands or arms.

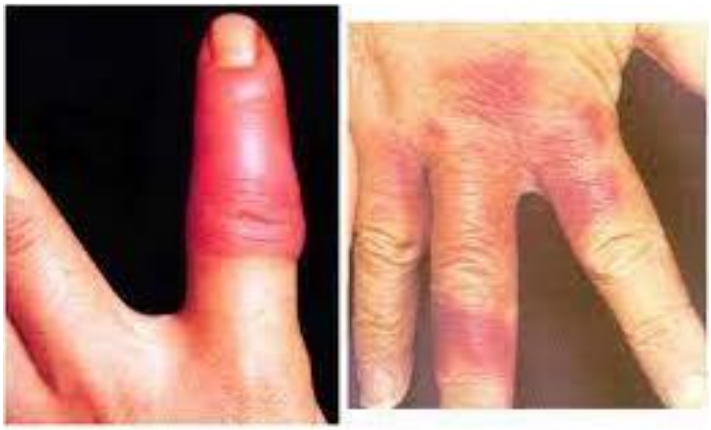

Fig. 7: Fish handler pathogenicity (Sobuj et al. 2022)

1.8.2. CLINICAL FINDINGS

Fish may not show any apparent signs of infection but they can carry the bacteria asymptomatically. In humans, fish handler's disease presents as skin infections characterized by redness, swelling and the formation of painful nodules or ulcers. Systemic symptoms such as fever and swollen lymph nodes can also occur in severe cases (Bezabih 2022). Fish handlers, aquarium workers and individuals involved in fish cleaning or processing are at a higher risk of contracting this disease. It is more common in warm-water fish species. No specific post mortem lesions are typically observed in fish.

1.8.3. TREATMENT AND CONTROL

Antibiotic therapy, typically with tetracycline-based antibiotics or fluoroquinolones is commonly used to treat fish handler's disease in humans. Preventive measures include using protective gloves when handling fish, promptly cleaning and treating any wounds, and practicing good hygiene (Pate et al. 2019).

1.9. SPRING VIREMIA OF CARP

1.9.1. ETIOLOGY AND PATHOGENESIS

Spring Viremia of Carp (SVC) is a viral disease that primarily affects carp species including koi and goldfish. The disease is caused by the *Rhabdovirus carpio* which can infect fish and potentially pose a risk to humans (Jia et al. 2020). Direct contact with infected fish, exposure to contaminated water or handling contaminated equipment can transmit the virus to humans. Ingesting infected raw fish or fish products may also lead to human infection.

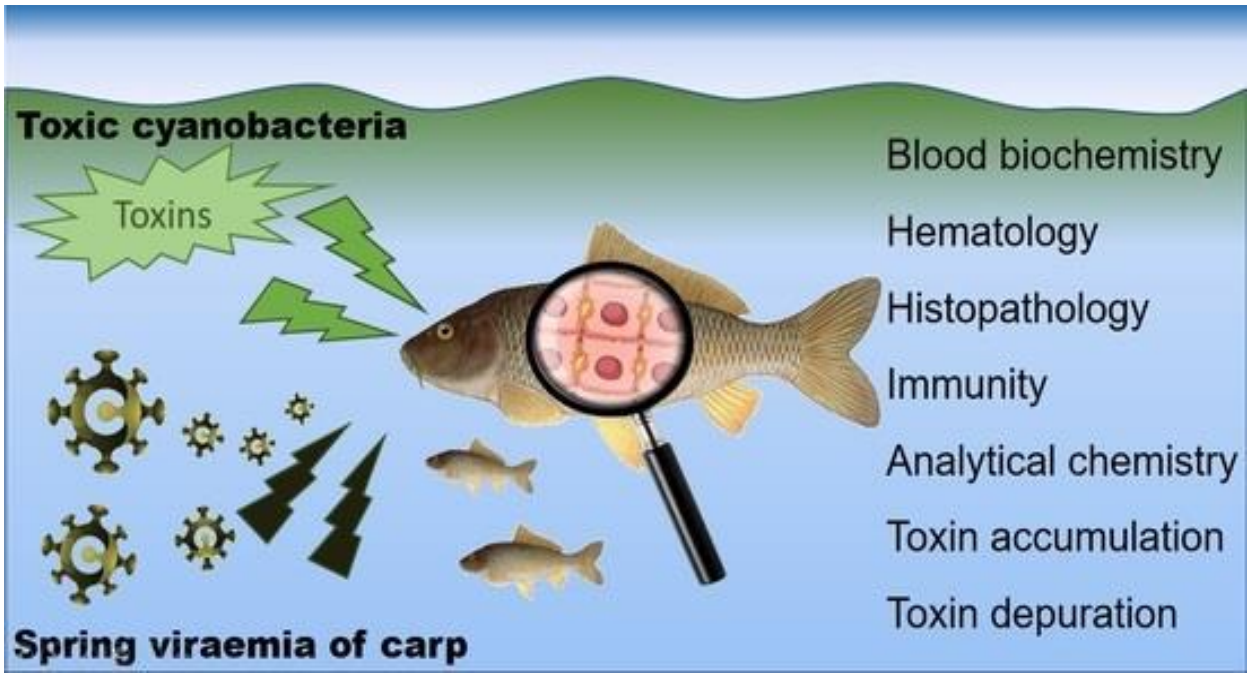

Fig. 8: changes in biochemistry due to SVC (Palikova et al. 2021)

1.9.2. CLINICAL FINDINGS

Fish infected with SVC may show a range of clinical signs, including lethargy, hemorrhages, swollen abdomen and gill abnormalities. Mortality rates can vary depending on the severity of the infection (Zhang et al. 2019). SVC is not well-documented in human cases, and symptoms in humans are generally unknown. The risk of SVC transmission to humans is low. However, individuals who handle infected fish or consume raw or undercooked infected fish may be at a higher risk (Clouthier et al. 2021). In fish, post mortem examination may reveal hemorrhages, congestion and abnormalities in the internal organs particularly the liver and spleen (Liang et al. 2022).

1.9.3. TREATMENT AND CONTROL

There is no specific treatment for SVC in humans. Preventive measures include proper cooking of fish to ensure virus inactivation and avoiding contact with infected fish or contaminated water (Xie et al. 2022).

1.10. LYMPHOCYSTIS DISEASE

1.10.1. ETIOLOGY AND PATHOGENESIS

Lymphocystis disease is a viral infection caused by the Lymphocystivirus which affects a variety of fish species. The virus causes the formation of characteristic white or pinkish nodules or growths on the skin and fins of infected fish (Borrego et al. 2017). Direct contact with infected fish or exposure to contaminated water can transmit the virus to humans. Although, it is believed that the virus can cause localized skin infections in humans who come into contact with infected fish (Benkaroun et al. 2022).

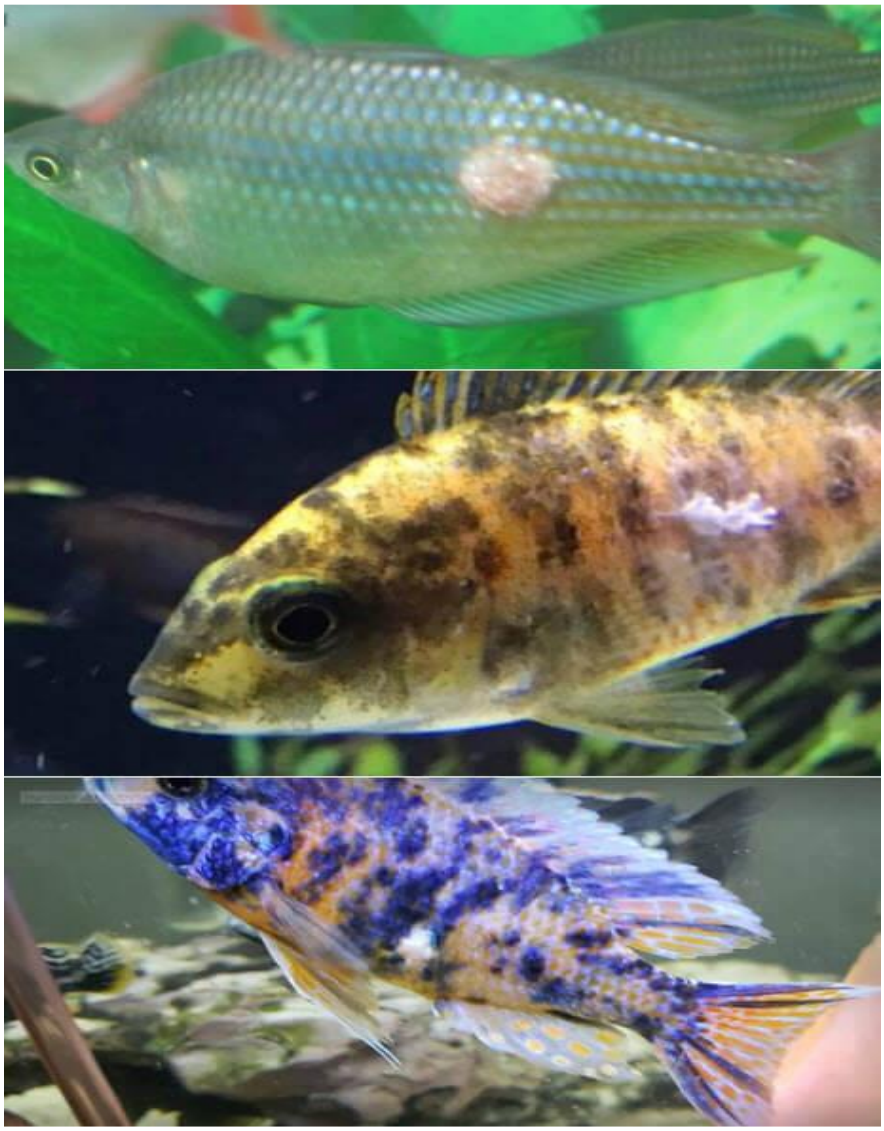

Fig. 9: Lymphocystis Disease (https://aquariumscience.org/)

1.10.2. CLINICAL FINDINGS

Infected fish develop nodules or growths on the skin, fins and occasionally the gills. These growths can vary in size and number and may cause secondary infections (Harikrishnan et al. 2010). In humans the risk of infection is low but if transmission occurs, it can result in localized skin infections characterized by redness, swelling and the formation of small raised nodules. Lymphocystis disease is commonly observed in both wild and captive fish populations. The transmission of the virus to humans is rare and usually occurs through direct contact with infected fish or contaminated water (Nurliyana et al. 2023). In fish, post mortem examination may reveal the presence of characteristic nodules or growths on the skin, fins or gills.

1.10.3. TREATMENT AND CONTROL

There is no specific treatment for Lymphocystis disease in fish. Prevention involves quarantining new fish arrivals, maintaining good water quality and avoiding overcrowding. In humans, treatment is typically supportive, focusing on wound care and prevention of secondary infections (Volpatti and Ciulli 2022).

1.11. GYRODACTYLUS AND DACTYLOGYRUS INFECTIONS

1.11.1. ETIOLOGY AND PATHOGENESIS

Gyrodactylus and dactylogyrus are ectoparasitic flatworms that infect the gills, skin and fins of fish. While these parasites primarily affect fish, there is a theoretical risk of human infection through direct contact with infected fish or contaminated water (Rastiannasab et al. 2016). Contact with infected fish or exposure to contaminated water can potentially transmit the parasites to humans. Ingesting raw or undercooked infected fish may also lead to human infection (Wahab et al. 2021).

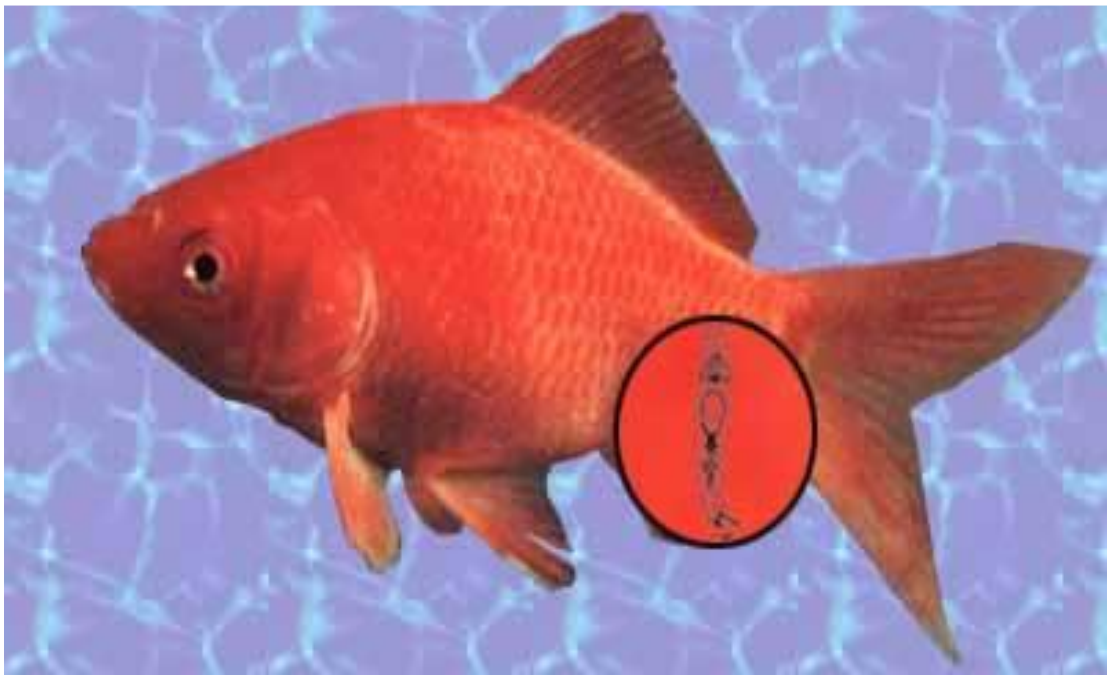

Fig. 10: Gyrodactylus and Dactylogyrus infection (dactylogyrus.htm).

1.11.2. CLINICAL FINDINGS

Infected fish may display clinical signs such as increased mucus production, respiratory distress, flashing behavior and skin or fin damage. Human infections with gyrodactylus and dactylogyrus species are extremely rare and specific clinical findings in humans are not well-documented. The risk of human infection is considered low. However, individuals who handle infected fish or consume raw or undercooked infected fish may be at a higher risk. In fish, post mortem examination may reveal the presence of parasites attached to the gills, skin or fins (Iqbal and Haroon 2014).

1.11.3. TREATMENT AND CONTROL

Treatment for gyrodactylus and dactylogyrus infections primarily focuses on fish, using various antiparasitic medications. Preventive measures include proper hygiene, ensuring fish are sourced from reputable suppliers, use of turmeric extract and cooking fish thoroughly before consumption (Jayasundara and Hettiarachchi 2012).

2. RISK FACTORS AND MODES OF TRANSMISSION OF ZOONOTIC PROBLEMS IN ORNAMENTAL FISHES

Aquarium fish, usually referred to as ornamental fish are common pets all around the world. These stunning aquatic species can occasionally harbour zoonotic infections that are hazardous to human health (Ziarati et al. 2022). Risk factors and mode of transmission of zoonotic diseases in ornamental fished are described below.

2.1. RISK FACTORS

2.1.1. HIGH POPULATION DENSITY

The high fish population density in pet shops and ornamental fish farms can promote the spread of zoonotic diseases. It is more likely for infectious conditions to occur when fish are in close contact with one another (Haenen et al. 2020).

2.1.2. STRESS AND POOR HUSBANDRY

Ornamental fish that are in stressed condition or unhealthy are more susceptible to infections, especially zoonotic diseases. Poor husbandry techniques, including poor diet, overcrowding, and poor water quality, can weaken these fish immune systems and increase their risk to pathogenic diseases.

2.1.3. INTRODUCTION OF NEW FISH

Zoonotic infections can enter the environment when new fish are added to an aquarium or when ornamental fish are introduced that were collected in the wild. The danger of infection can be high since new fish may carry the disease without showing symptoms, especially if the precautions and standard methods are not followed (Moratal et al. 2020).

2.1.4. CONTAMINATED WATER SOURCES

Contaminated water used in aquariums can serve as a resivior for zoonotic pathogens. Untreated tap water, polluted gravel or substrate, and live feed organisms added to the aquarium can all be sources of contamination. (Rahman et al. 2020).

2.1.5. LACK OF AWARENESS AND EDUCATION

It is possible that many aquarium owners are unaware of the risks of ornamental fish spreading infectious diseases. Zoonotic infections may spread more readily if people are not well informed about disease transmission and preventative techniques.

3. MODES OF TRANSMISSION

3.1. DIRECT CONTACT

Direct contact with infected ornamental fish is a common mode of transmission for zoonotic diseases. Handling infected fish or coming into contact with contaminated water can lead to the transfer of pathogens to humans (Gauthier 2015).

3.2. WATERBORNE TRANSMISSION

Infected fish can release zoonotic viruses into the water, which can then contaminate the aquarium water. These bacteria may infect humans when they accidentally take in water, have open wounds, or inhale contaminated water droplets during fish handling

3.3. CONTACT WITH FOMITES

Nets, aquarium accessories, and other inanimate objects may act as fomites and contain zoonotic infections. These objects may be used as means of disease transmission between fish and people if they are not properly sanitized.

3.4. INGESTION OF CONTAMINATED FOOD

Feeding raw or uncooked ornamental fish, seafood which carrying the pathogens of a certain disease can lead various human diseases if proper food safety measures are no adopted.

3.5. AEROSOL TRANSMISSION

In the aquarium water, specific zoonotic diseases might be aerosolized, especially during water changes or maintenance procedures. Humans who breathe in these airborne particles may get respiratory disorders.

4. PREVENTIVE MEASURE

4.1. VACCINATION PROTOCOLS

Before adding new fish to an aquarium with existing species, it is important to follow the right vaccination cure in order to handle any possible zoonotic disease carriers.

4.2. HYGIENE PRACTICES

Zoonotic infections can be prevented by routine hand washing and the use of personal protective equipment, such as gloves, while managing aquarium water or fish.

4.3. WATER QUALITY MANAGEMENT

The occurrence of zoonotic pathogens in the aquarium environment can be reduced by maintaining adequate water quality by suitable filtration, frequent water changes and effective disinfection techniques (Cardoso et al. 2019).

4.4. EDUCATION AND AWARENESS

In order to encourage ethical fishkeeping practises and protect the general public's health, aquarium owners must be informed about zoonotic hazards and preventative actions.
It is essential to comprehend the danger variables and means of transmission of zoonotic issues in ornamental fishes in order to stop diseases that affect humans. The possible zoonotic hazards associated with ornamental fishes can be successfully reduced by raising awareness to people, upholding basic cleanliness, and educating aquarium owners.

4.5. BIOSECURITY

- Emphasize the importance of biosecurity in reducing the pathogens of ornamental fish.
- Providing proper guideline for vaccination protocols, best sanitization methods, maintaining the vaccination processes, spreading the infected fish and appropriate water quality management techniques.
- Discuss the benefits of vaccination with people in preventing the zoonotic diseases in ornamental fish populations (Mocho et al. 2022).

4.6. PUBLIC AWARENESS CONCERNING ETHICAL PET OWNERSHIP

- Describe the significance of informing the public about the dangers of zoonotic infections caused by ornamental fish.
- Talk about good pet-ownership habits, such as managing and discarding fish waste properly.
- Emphasize the need of getting veterinarian help when problems with an ornamental fish's health arise.

4.7. REGULATION AND IMPORTATION

- Discuss the need of imposing restrictions on the purchase and import of ornamental fish in order to stop the spread of zoonotic illnesses from foreign sources.

Address the role of local and international governments in observing and preventing the spread of disease.

4.8. ONE HEALTH APPROACH

- Describe the "One Health" philosophy and how it applies to the control of infectious diseases in ornamental fish.
- Draw attention to the cooperation of veterinary, environmental, and human health specialists in disease surveillance and control (Lee et al. 2022).

4.9. CASE STUDIES AND SUCCESS STORIES

- Provide examples of successful disease management and control programmes for populations of

ornamental fish.

- Give instances of nations or areas that have successfully lowered zoonotic hazards in the trade of ornamental fish (Mecha and Gonzales-Plasus 2023).
- Reiterate how important it is to treat and control disease in ornamental fish in order to reduce the spread of diseases that are zoonotic.

Emphasise the need of promoting ethical pet ownership, promoting public awareness, and working with other nations to protect both human and animal health.

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Zoonotic Parasitic Infestations in Fish and their Impact on Public Health and Aquatic Ecosystems

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ABSTRACT

Parasites represent a major threat to wild and farmed fish stocks, resulting in significant economic losses in fisheries and aquaculture globally. In addition, some fish parasites have a zoonotic potential, posing risks to human health through consumption of infected raw or undercooked fish. These primarily include helminth parasites, such as nematodes, cestodes, trematodes and acanthocephalans. Anisakid nematodes are among the most common fish-borne zoonotic parasites, causing human infection via consumption of third stage larvae in raw or undercooked fish hosts. An additional nematode, Capillaria philippinensis, may also be transmitted by ingesting infected fish, although freshwater fish are the typical second intermediate hosts. Some major groups of cestodes, including diphyllobothriid tapeworms, use fish as secondary intermediate hosts and can infect humans that eat plerocercoid larvae in raw or poorly cooked fish. Digenean trematodes also utilize fish as secondary intermediate hosts, with some species occasionally infecting human definitive hosts if metacercariae in raw or pickled fish are ingested. While clinical manifestations vary widely, they may include allergic reactions, gastrointestinal disturbances, malnutrition, anemia or larva migrans syndromes. Preventive measures lie in public education regarding risks related to consumption of raw or undercooked fish. Additional research should aim to elucidate fish-zoonotic parasite transmission pathways, geographic distributions, genetic resistance and diversity, improve diagnostic techniques, and develop evidence-based guidelines for control and eradication. This book chapter reviews major groups of parasitic zoonosis related to fish hosts, focusing on life cycles, epidemiology, pathologic findings in fish and humans as well as diagnosis, treatment and prevention.

Keywords: Parasitic disease, Zoonotic infections, Pathological findings, Transmission routes.

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1. INTRODUCTION

Fish are an integral part of the human diet and play a crucial role in global food security. However, consumption of fish also increases the risk of parasitic zoonoses (Shamsi 2019). Parasitic zoonosis refers to diseases caused by parasitic organisms that can spread from animals to humans and cause serious risks to public health. Fish can act as intermediate or definitive hosts for a wide range of parasites, making them potential vectors for these zoonotic pathogens (Lõhmus and Bjorklund 2015).

With the growing global demand for seafood and the expansion of aquaculture practices, parasitic zoonotic diseases in fish have gained increased attention (Shamsi 2020). As more people rely on fish as their primary protein source, it is imperative to understand the transmission, pathogenesis, and prevention of these parasitic infections. This chapter provides an overview of parasitic zoonoses that infect fish, their potential impact on human health, and the importance of effective control measures to mitigate the risks.

1.1. FISH AS INTERMEDIATE HOSTS FOR PARASITES:

Fish serve as intermediate hosts for a diverse range of parasites belonging to different taxonomic groups. These parasites typically complete part of their life cycle within fish, undergoing developmental stages that enable them to infect their definitive hosts, which may include mammals, birds or other aquatic organisms. As intermediate hosts, fish can harbor parasitic larvae, cysts, or eggs that, when ingested by humans, can develop into mature parasites and cause zoonotic infections (Gabagambi et al. 2019).

1.2. COMMON PARASITIC ZOONOTIC DISEASES IN FISH: NUMEROUS PARASITES HAVE BEEN IDENTIFIED AS POTENTIAL

Zoonotic agents transmitted through fish consumption. Among these, certain helminths (worms) and protozoans stand out as significant contributors to parasitic zoonosis. For instance, tapeworms of the genus diphyllobothrium and the liver fluke species Clonorchis sinensis are notorious examples of zoonotic helminths commonly associated with fish consumption (Cong and Elsheikha 2021).

1.3. TRANSMISSION ROUTES TO HUMANS

The transmission of parasitic zoonosis from fish to humans can occur through various routes. The most common method of disease transmission is eating undercooked, raw or poorly prepared fish

containing live parasites (Shamsi and Sheorey 2018). Additionally, direct contact with infected fish or contaminated water during recreational activities or occupational exposure in aquaculture and fishing industries can also pose risks of transmission.

1.4. CLINICAL MANIFESTATIONS IN HUMANS

Humans that get parasitic zoonotic infections can have a variety of clinical signs from minor gastrointestinal issues to serious systemic illnesses. The parasite type, the quantity of infectious stages taken in and the immunological health of the infected person are all common factors in determining the severity of an infection. Some parasitic zoonosis may remain a symptomatic for long period therefore there is necessary to make an accurate diagnosis and timely treatment for cure (Bao et al. 2019).

1.5. IMPACT ON PUBLIC HEALTH AND AQUATIC ECOSYSTEMS

Parasitic zoonotic diseases in fish can have substantial implications for both human health and the balance of aquatic ecosystems. Human infections can lead to increased healthcare costs, reduced productivity, and in severe cases, life-threatening conditions. Moreover, heavy parasite problems in fish populations can affect their growth, reproduction and survival, potentially disrupting aquatic food chains and ecological stability (Buchmann 2022).

1.6. FISH-BORNE TREMATODE INFECTIONS

Various other trematode parasites, such as heterophyidae and echinostomatidae which can be transmitted to humans through infected fish consumption (Caffara et al. 2020) . These parasites can cause gastrointestinal symptoms and other health issues.
Parasitic zoonosis in fish refers to the transmission of parasitic infections from fish to humans, leading to potential health risks. The most common method of disease transmission is zoonotic disorders which can naturally spread from animals to people. Fish can harbours various parasitic organisms and when humans come into contact with infected fish or consume raw or undercooked fish, they may become infected with the parasites. These parasites can cause a variety of health problems in humans and some can be severe or even life-threatening, particularly in individuals with weakened immune systems. Here are certain parasitic diseases listed in table and explained below:

Sr. No.	Disease	Etiology	Fish organ affected
1	Anisakiasis	*Anisakiasis spp.*	Digestive tract
2	Echinococcosis	Tapeworm species	Eggs of fish
3	Clonorchis	*Clonorchis sinensis*	Liver and bile ducts
4	Gnathostomiasis	*Gnathostoma spp.*	Skin, muscle and eyes
5	Diphyllobothriasis	*Diphyllobothrium spp.*	Digestive tract
6	Heterophyiasis	*Heterophyes spp.*	Digestive tract

2. PARASITIC DISEASES

2.1. ANISAKIASIS

The most common parasitic disease of marine fish and squids is anisakiasis, caused by the nematode *Anisakis simplex*. The disease is known to be zoonotic which means it can be transmitted from fish to

humans (Eiras et al. 2018). Anisakiasis is caused by the *Anisakis simplex* nematode which can infect marine fish and squid.

2.1.1. OUTBREAK

Anisakiasis outbreaks have been documented in a number of places, notably in Japan and other nations where raw fish eating is popular. Between 2018 and 2019, The center of disease control and prevention (CDC) documented 19737 cases of anisakiasis (Sugiyama et al. 2022). Molecular identification of larvae revealed 88.4% patients get infected with species *Anisakis simplex.* This zoonotic disease is most common in countries consuming large quantity of seafood (Sugiyama 2010; World Health Organization 2022).

Anisakis simplex is a parasitic nematode that can infect a variety of marine fish and squid. The nematode's life cycle involves three hosts: a marine mammal, a crustacean, and a fish or squid. Humans can become infected when they eat raw or undercooked fish or squid that contain Anisakis larvae.

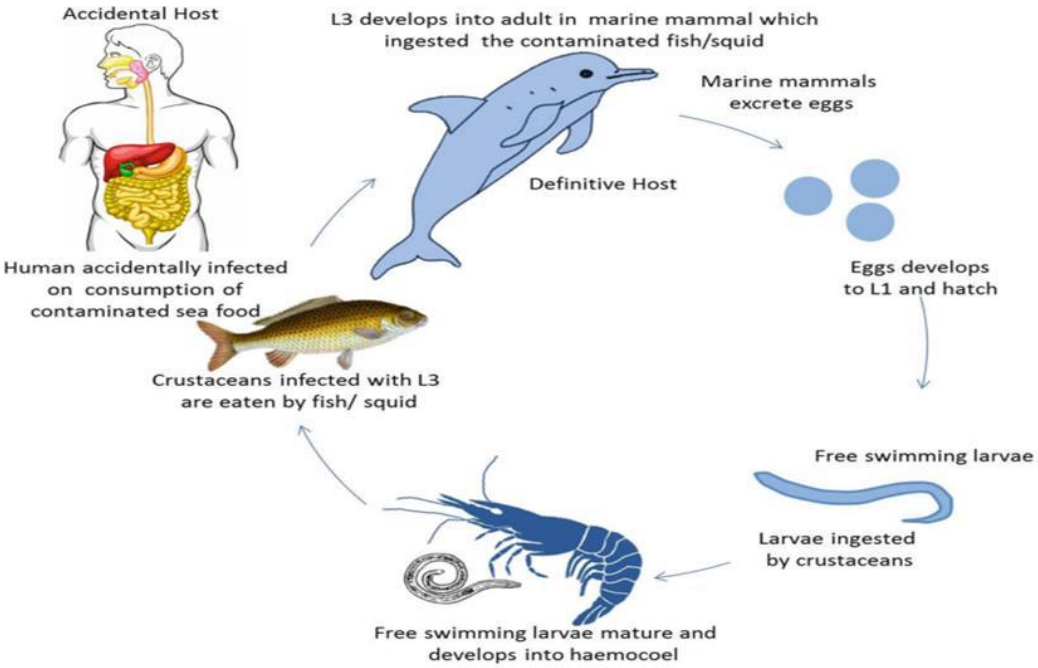

Fig. 1: Life cycle of Anisakis simplex (Tak 2022).

2.1.2. PATHOGENESIS

The most common method of disease transmission is through Anisakis larvae that can enter the digestive tract wall of humans who ingest raw or undercooked fish or squid that has been containing the larvae, leading to inflammation and tissue damage. This may result in symptoms including nausea, vomiting, diarrhea and abdominal discomfort. (Audicana and Kennedy 2008).

The larvae can also be transmitted through contaminated water or by handling fish or squid with bare hands (Mattiucci and Nascetti 2008). The most common cause of anisakiasis in Japan is caused by *A. simplex s.s*. nematodes but in Europe and South Korea *A. pegreffii* is the main culprit. *A. pegreffii* nematodes are frequently removed along with fish viscera when making sushi and sashimi, this may help to explain why there are fewer cases of *A. pegreffii* anisakiasis in Japan. *A. simplex s.s* nematodes penetrate the muscles of different fish species more readily than *A. pegreffii*. Additionally, fish habitat

can support the distinction between South Korea and Japan more popular anisakid nematode species (Suzuki et al. 2021).

Symptoms of anisakiasis typically occur within a few hours after eating infected fish or squid and can include abdominal pain, nausea, vomiting, and diarrhea. In serious situations, the infection may result in problems such as intestinal blockage and allergic reactions. Postmortem lesions associated with anisakiasis may include inflammation and tissue damage in the digestive tract, particularly in the stomach and small intestine.

2.1.3. TREATMENT AND CONTROL

Anisakiasis can be treated with endoscopy to remove the larvae from the digestive tract. In severe cases, surgery may be necessary to remove the larvae and repair any tissue damage. Prevention of anisakiasis involves avoiding eating undercooked fish or squid, especially if it has been discovered that it contains Anisakis larvae. Additionally, the larvae can be killed and the risk of infection is reduced by freezing fish at -20°C for at least 24 hours. (Sakanari and Mckerrow 1989).

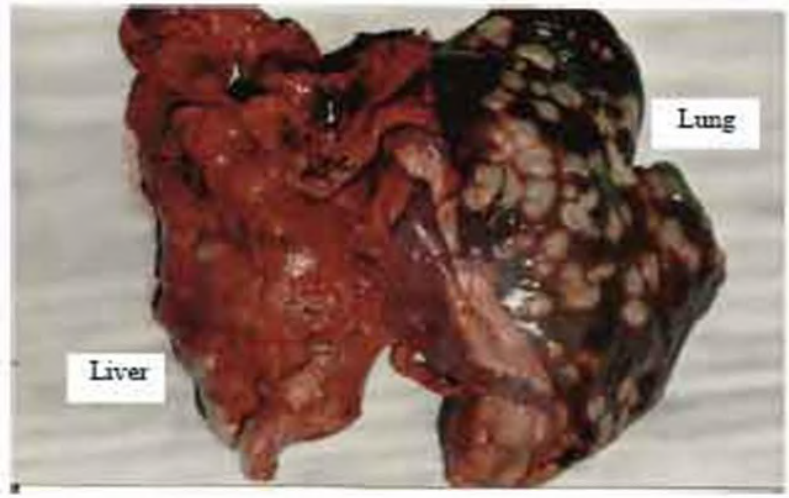

Fig. 2: Echinococcosi effect on liver and lungs (Abuseir, 2021)

2.2. ECHINOCOCCOSIS

2.2.1. ETIOPATHOLOGY

The larval stages of cestodes (tapeworms) from the genus Echinococcus are primarily transmit echinococcosis, also known as hydatidosis or hydatid sickness. Cystic echinococcosis is the most common type and is caused by Echinococcus granulosus (sensu lato). Alveolar echinococcosis is caused by a different species, E. multilocularis, which is becoming more widespread. "Neotropical echinococcosis" is linked to two solely New World species, E. vogeli and E. oligarthrus; although E. vogeli produces the exceedingly rarely seen unicystic type, E. oligarthrus causes the polycystic form (Hijjawi et al. 2018).

Ingested eggs from animal faeces hatch in the stomach and release oncospheres, which are juvenile forms of the parasite encased in an embryonic envelope. These eggs may be found on the fur of dogs or other animals. Through the intestinal wall, oncospheres enter the body and travel through the bloodstream before settling in the liver, these also settle in the lungs or less frequently the brain, the

bone or other organs. *E. granulosus* oncospheres change into cysts in tissue and then these cysts progressively develop (typically over several years) to become hydatid cysts which are enormous, fluid-filled unilocular tumours. Within these cysts, brood capsules with countless tiny infectious protocolizes develop. Millions of protocolizes as well as > 1 L of highly antigenic hydatid fluid may be present in large cysts. Sometimes, daughter cysts develop inside or outside of main cysts. A liver cyst that ruptures or leaks might cause an infection to spread to the peritoneum (Thompson 2020).

Hand-to-mouth transmission of live tapeworm eggs from pet waste causes human illness. The parasite eggs, which may survive in the environment for weeks are spread by infected tapeworm animals, dog faeces that contaminate the nearby environment. Infectious protoscoleces originate in cysts that the larvae generate after penetrating the intestinal mucosa, entering the portal system and reaching multiple organs. It is found all across the world but is most common in northern Europe, Asia and North America. Normal hosts for the adult tapeworm include dogs, coyotes and foxes. By using contaminated food and water, people can contract the parasite larval stages. Hydatids in the liver can cause stomach discomfort, nauseousness and vomiting. Chest discomfort, shortness of breath and a persistent cough are clinical indicators that of the lungs infection. The presence and amount of pressure on the surrounding tissues of the hydatid cysts will determine any additional symptoms. Anorexia, weight loss and weakness are non-specific symptoms.

2.2. CONTROL AND PREVENTION

It is controlled by limiting the spread of the parasite. Limiting the locations where dogs are permitted and forbidding animals from ingesting meat contaminated with cysts are two prevention methods. Prevent dogs from eating contaminated dead bodies.

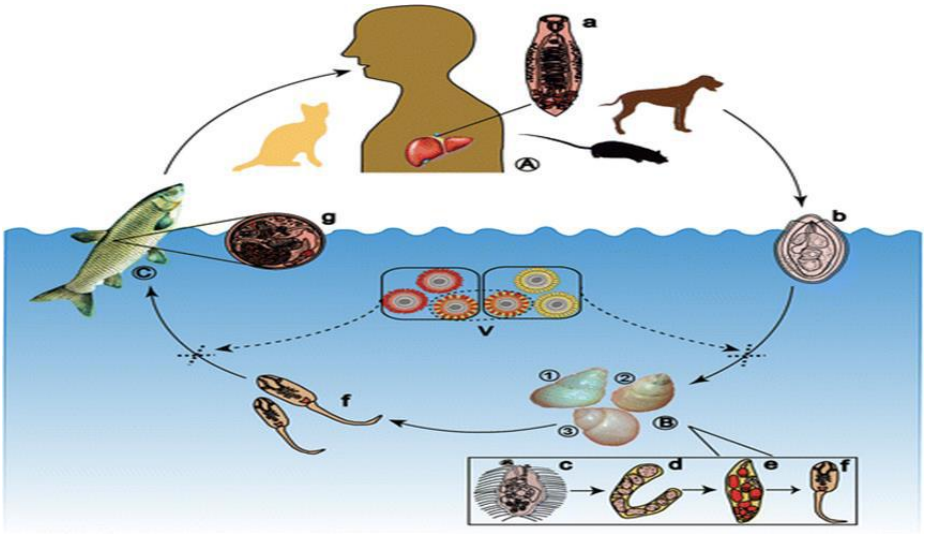

Fig. 3: Clonorchis mode of transmission (Tang et al. 2016).

2.3. CLONORCHIS

2.3.1. ETIOLOGY

The trematode known as *Clonorchis sinensis* which is carried by fish and causes a disease called clonorchiasis. It is also known as the Chinese liver fluke. Most cases of clonorchiasis are brought on by eating fish that has been infected with *C. sinensis* in endemic areas of the world such as East Asia.

2.3.2. PATHOGENESIS

Most infected people are thought to have a benign tumor in the early stages of infection (fewer than 100 flukes) and they rarely ever show symptoms. Acute cholangitis symptoms such as jaundice, right upper quadrant stomach discomfort, nausea, vomiting, anorexia, malaria and fever, can appear in patients with very high parasite loads (>20 000). There are various symptoms involving the liver and biliary system that can be brought on by chronic clonorchiasis. Gallstones, especially calcium carbonate stones are more likely to develop as a result of cholelithiasis which is commonly seen and probably connected to *C. sinensis*. Because of the hyperplastic alterations caused by *C. sinensis*, the gallbladder wall contracts frequently which causes bilirubinate and mucin to precipitate on the parasite eggs (Chamadol et al. 2019).

Clonorchis is primarily transferred when raw or un-cooked contaminated seafood is consumed. Tapeworms can survive in freshwater environments for several years, and can infect a variety of fish species. Most of the Eastern hemispheres of the world, particularly East Asia and portions of Russia are home to *C. sinensis*. It used to be common in Japan as well, but with the mechanization of agriculture after World War II, it has largely disappeared.

Light trematode infections are frequently asymptomatic; symptoms usually appear in people who have had an infection for a longer time or who have a higher worm stress. Heavier infections might result in eosinophilia, hepatomegaly, epigastric pain, fever, chills, and epigastric discomfort during the acute phase. Later, diarrhea might happen. Usually, symptoms continue for two to four weeks. In severe infections, chronic cholangitis can proceed to liver parenchymal atrophy and portal fibrosis. If the biliary tree is blocked by a number of flukes, jaundice may develop.

2.3.3. TREATMENT AND PREVENTION

Praziquantel is the preferred medication for the treatment of clonorchiasis. The World Health Organisation (WHO) advises either 40mg/kg in a single treatment or 25 mg/kg orally three times daily for two to three days to achieve cure rates above 90%. Prevention of Clonorchis involves proper cooking of fish to kill any tapeworm larvae that may be present. Freezing fish at -20°C for at least 24 hours can also kill the tapeworm larvae. Additionally, good sanitation practices and proper disposal of fecal matter can help prevent the spread of infection (Choi et al. 2010).

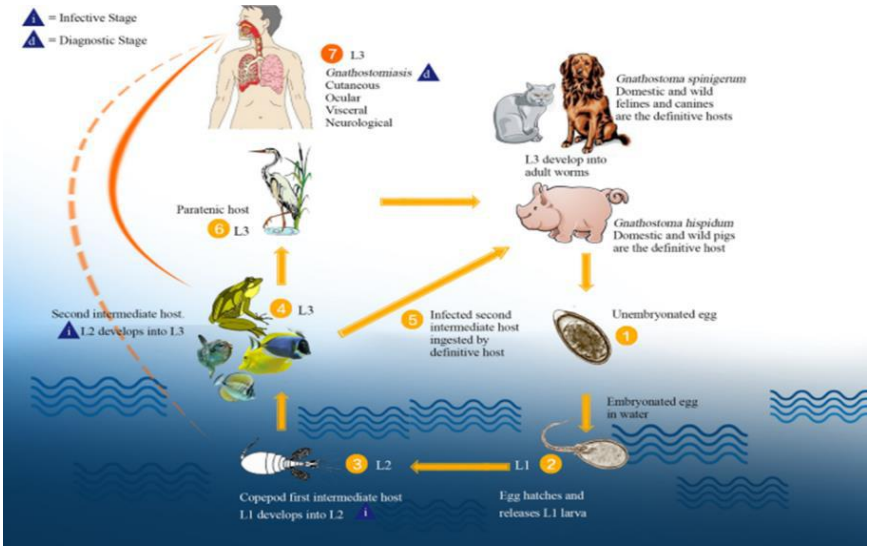

Fig. 4: Infective and diagnostic stages of Gnathostomiasi (Liu et al. 2020).

2.4. GNATHOSTOMIASIS

2.4.1. ETIOLOGY

A zoonotic disease spread by food is gnathostomiasis. its etiological agent is third stage larvae of *Gnathostoma spp.* Human gnathostomiasis is a condition that is often documented in underdeveloped nations as well as in non-endemic regions of advanced countries. The rising intake of reptiles, uncooked freshwater fish, frogs, snakes and poultry that carry L3 larvae is mostly to blame for an increase in human gnathostomiasis cases (Liu et al. 2020).

Eosinophilia and migrating bumps are typical signs of infection. In severe situations, L3 can infiltrate internal organs and tissues including the eyes, spinal cord and brain which can result in blindness, a coma, and even death. It can also cause neurological disorders like paralysis.

The diagnosis can be done on the base of clinical signs Elevated blood eosinophil Living in or traveling to endemic region. Eating undercooked or raw fish or poultry.

2.4.2. TREATMENT

There is no any specific effective treatment for gnathostomiasis. Larvae can be removed surgically and it is the only effective treatment. Surgical removal can only be done in superficial migration. For visceral migration rubrical removal is impracticable, in this case we can give drugs like praziquantel, metronidazole, thiabendazole and quinine. But they do not have obvious efficacy.

2. 5. DIPHYLLOBOTHRIASIS

2.5.1. ETIOLOGY

It is a parasitic disease caused by the tapeworms Diphyllobothrium latum and Diphyllobothrium nihonkaiense. These tapeworms infect a variety of fish, including salmon, trout, and perch, and can be transmitted to humans who consume raw or undercooked infected fish (Richardson et al. 2012). Consumption of contaminated fish results in diphyllobothriasis. The complex life cycle of the tapeworms involves a variety of hosts, including fish and mammals. The adult tapeworms may reach lengths of up to 10 metres and lay up to one million eggs every day which are excreted in the feces of infected host (Kuchta et al. 2017).

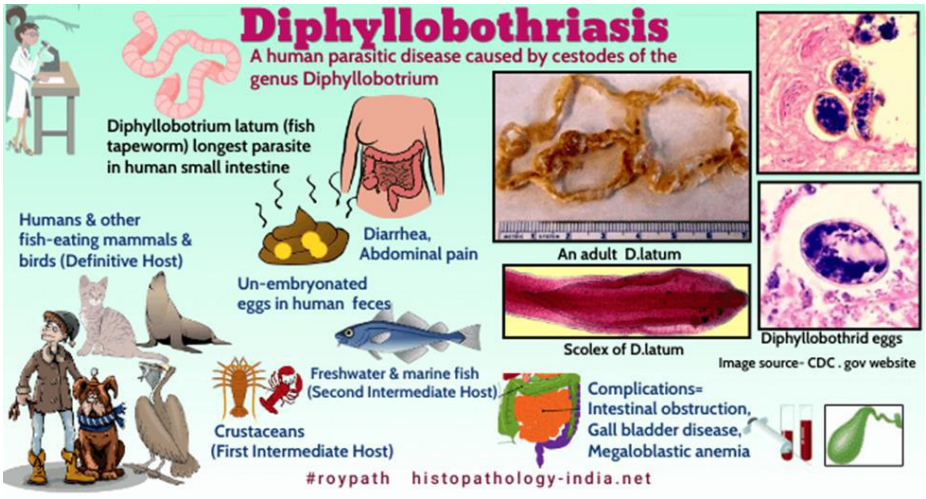

Fig. 5: Diphyllobothriasis life cycle (Dr Sampurna Roy MD).

When humans consume raw or undercooked infected fish, the tapeworm larvae travelled to the small intestine where they attach themselves to the intestinal walls to develop into adult form. These tapeworms can cause a variety of gastrointestinal symptoms, including abdominal pain, diarrhea, and weight loss. In severe cases, they can also lead to vitamin B12 deficiency and anemia (Kuchta et al. 2014).

2.5.2. MODES OF TRANSMISSION

Diphyllobothriasis is primarily transmitted through the intake of raw or under-cooked infected fish. These tapeworms can survive in freshwater environments for several years, and can infect a variety of fish species (Torgerson et al. 2011). Diphyllobothriasis is found world widely however, it is more commonly seen in regions where raw or undercooked fish is a common food source. Outbreaks of diphyllobothriasis have been reported in several countries, including Japan, Russia, and Chile (Ikuno et al. 2018). The clinical signs of diphyllobothriasis can include abdominal pain, diarrhea, and weight loss. In severe cases, it can also lead to vitamin B12 deficiency and anemia. Postmortem examination of fish infected with Diphyllobothrium spp. May show gross and microscopic lesions in the gastrointestinal tract, including inflammation and hemorrhage in the intestine.

2.5.3. TREATMENT AND CONTROL

Diphyllobothriasis can be treated with anthelmintic drugs, such as praziquantel and niclosamide. These drugs work by killing the tapeworms in the intestine, allowing them to be passed out of the body in the feces. Prevention of diphyllobothriasis involves proper cooking of fish to kill any tapeworm larvae that may be present. Freezing the fish for 24 hours at -20°C can kill the larvae of tapeworm. Additionally, good sanitation practices and proper disposal of fecal matter can help prevent the spread of infection.

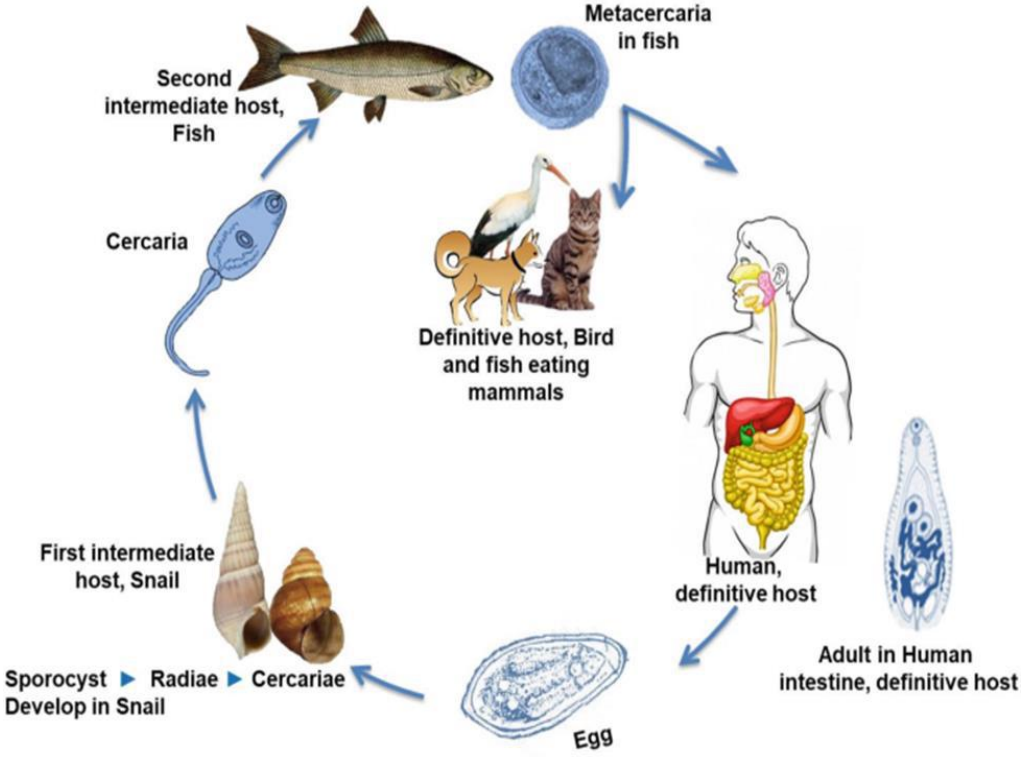

Fig. 6: Life cycle of heterophyiasis (Mahanta 2022).

2.6. HETEROPHYIASIS

- Heterophyiasis is a parasitic infection caused by the trematode worm *Heterophyes heterophyes.*
- The parasite is mostly found in the Middle East, North Africa and the Far East in specific freshwater fish (Hadyait et al. 2018).
- Humans contract the infection by ingesting undercooked or raw infected fish that contain the parasite's larvae (Shamsan and Al-Jobory 2018).
- In the small intestine, the larvae grow into adult worms that cause symptoms such as diarrhea, indigestion, nausea and vomiting (Iqbal and Ashraf 2017).
- The infection may occasionally result in inflammation of pancreas or gallbladder.
- Heterophyiasis is typically treated with the anti-parasitic drug praziquantel.
- Prevention involves avoiding raw or undercooked fish, properly cooking fish, and properly disposing of fish waste.
- Risk factors for heterophyiasis include living in or traveling to areas where the parasite is prevalent and consuming raw or undercooked fish.
- Diagnosis is typically made through a stool sample analysis to identify the presence of the parasite's eggs.
- While heterophyiasis is generally not life-threatening, it can cause significant discomfort and complications if left untreated.

3. PREVENTION OF PARASITIC ZOONOSIS

In fish, the main concern is making sure the fish is fully cooked before eating. The parasites are eliminated from fish when cooked at temperatures that are high enough to kill them. Informing the public about the adverse effects of eating raw or undercooked fish and the need of following the proper guidelines for food safety is also important (Bibi et al. 2015).

It is important to identify and treat parasite zoonosis in fish to reduce hazards to human health and encourage safe fish-eating practices. It is essential to routinely check fish populations for parasites and put appropriate control measures in action for protecting public health and maintaining food safety standards.

Diverse parasites that may spread from fish to humans are the source of parasitic zoonotic diseases in fish. It is critical to understand the mechanisms influencing their transmission as well as the steps to avoid and control these infections because these parasites can complete their life cycles in both fish and humans. The specific requirements are listed below:

3.1. PARASITIC DIVERSITY IN FISH

Protozoa, helminths (worms) and crustaceans are parasites that live within fish. Some of these parasites may infect people and spread disease because they are zoonotic (Poulin 2014). Freshwater fish liver flukes are thought to be present in 45 million humans and 680 million more are thought to be at risk of infection (Saijuntha et al. 2021).

3.2. COMPLEX LIFE CYCLES

Numerous zoonotic parasites have complex life cycles that include numerous hosts. Fish operate as the parasitic intermediate hosts while humans or other mammals become their final hosts, where it matures, reproduces and cause disease (Urdeş and Hangan 2013). In order to stop transmission to people, it is vital to fully understand their life cycles.

3.3. ROUTES OF TRANSMISSION

Fish-borne parasitic infectious diseases can spread to people in a number of ways, by the raw fish, unhygienic conditions and due to improper handling of fish (contaminated water) (Chaisiri et al. 2019). Fish farmers, fisherman, owners all can affected due to water contamination and fish infections due to parasites. Swimmers, divers, and water sport participants come into direct contact with water bodies which contain parasitic zoonotic infections.

3.4. TEMPERATURE FACTOR

Water temperature is one of the ecological components that affects the transmission of parasitic zoonotic infections in fish. Parasite growth and infectiousness are frequently temperature-dependent processes (Wongsaroj et al. 2014). Some parasites life cycles may be accelerated by warmer waters. Stressed fish are more susceptible to disease when the water is contaminated with organic matter or chemicals the incidence of zoonotic parasites can be impacted by the presence of intermediate hosts and vectors in aquatic habitats.

3.5. FEEDING HABITS AND FISH SPECIES

The sensitivity of certain parasites to different fish species may differ. Furthermore, a fish exposure to parasites in the environment can be influenced by its consuming food preferences, such as carnivorous or omnivorous diets.

3.6. CONSUMPTION PATTERNS AND HUMAN BEHAVIOR

Human behavior like food preparation and preferences can significantly impact parasitic zoonotic infections. awareness of the risks and follow management rules make you enjoy a better life, free from pathogens (Broglia and Kapel 2011).

3.7. FISH HEALTH

Fish with a damaged immune system or those under stress are vulnerable to parasite infestations. The incidence of parasites in fish populations can be decreased in aquaculture by proper management and health monitoring (Kuton et al. 2019).

3.8. DIAGNOSTICS AND SURVEILLANCE

Finding parasitic zoonotic infections in fish requires effective diagnostic and monitoring techniques. Regular inspections can spot sick fish and prevent them from getting into the food supply.

3.9. PRECAUTIONS

There are several ways to reduce the danger of parasite-related zoonotic diseases in fish and people: a. Proper Cooking: Killing parasites and ensuring safe eating require cooking fish at the right temperatures. b. Freezing: Freezing fish at the right temperature and for a proper duration of time can help eliminate certain parasites. Implement quarantine and health certification procedures for fish farms and

aquaculture facilities. d. Public Education: Inform the general population about the dangers of ingesting raw or undercooked fish as well as the value of good cleanliness (Ziarati et al. 2022).

Fish parasitic zoonotic diseases represent a serious risk to human health and must be controlled and eradicated using a multidisciplinary strategy. Designing efficient measures to protect both fish populations and people who consume them requires an understanding of the mechanisms controlling the transmission, prevalence, and life cycles of these parasites (Cong and Elsheikha 2021).

4. CONTROLLING AND PREVENTING PARASITIC ZOONOTIC IN FISH

Fish parasitic zoonotic diseases are a serious hazard to the health of aquatic animals as well as human populations. Effective preventative measures and control tactics are crucial for preserving public health and the integrity of aquatic ecosystems. Drawing from reputable sources and scientific literature, this section provides an overview of the main preventative strategies and management techniques for parasitic disease outbreaks in fish.

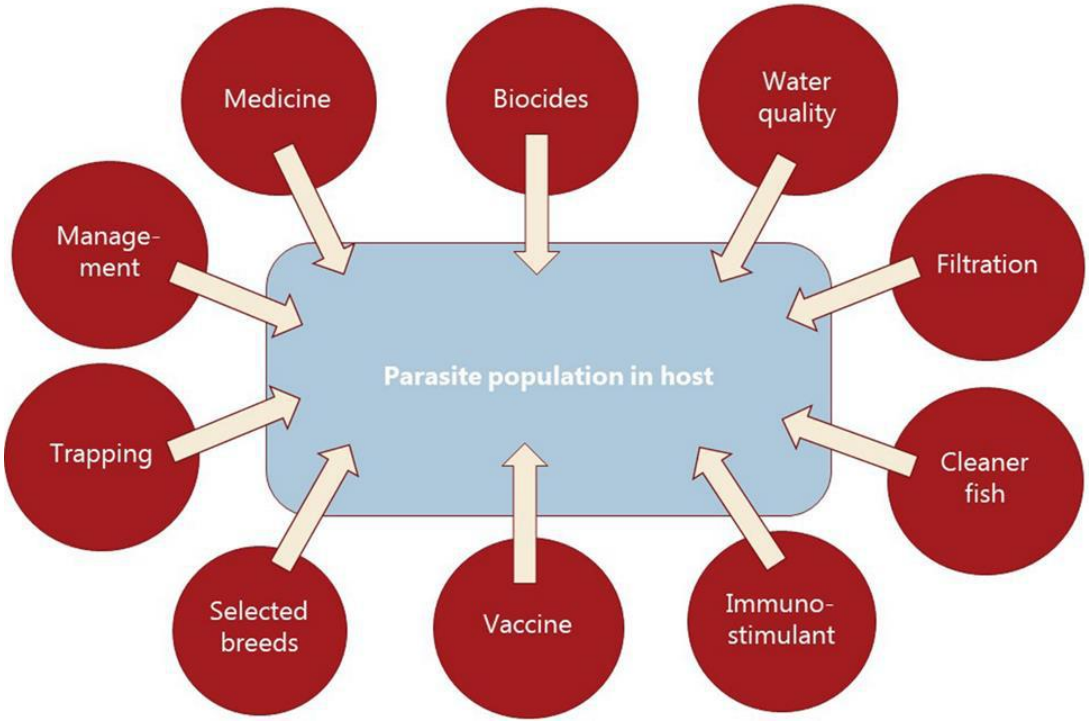

Fig. 7: Control of parasitic diseases in aquaculture (Buchmann, 2022).

4.1. QUARANTINE PROTOCOLS AND BIOSECURITY

- Strict biosecurity controls may be put in place in fish farms and other aquaculture facilities to assist limit the introduction and spread of parasitic infections.
- Before introducing freshly purchased fish stocks to existing populations, quarantine them to evaluate their health state and make sure they are parasite-free.
- Keep a close eye on and check fish shipments often to look for and remove any potential parasite disease carriers (Williams et al. 2022).

4.2. MANAGING QUALITY OF WATER

- Keeping the water at its optimum level is essential to lowering fish stress, which might lower their susceptibility to parasite conditions.
- To ensure a healthy aquatic environment, regularly check water parameters including temperature, pH, ammonia and dissolved oxygen levels.

4.3. PROPER NUTRITION MANAGEMENT

- Giving fish a healthy and nutritious diet helps boost their immune systems and increase their resistance to parasite infection.
- Maintain the proper stocking densities and check for nutritional deficiencies in fish to reduce stress.

4.4. SANITATION

- Follow strict hygiene procedures while handling fish and performing aquaculture activities to avoid cross-contamination.
- Conduct routine cleaning and disinfection of buildings, tanks, and machinery to avoid the accumulation and spread of parasitic germs.

4.5. IPM, OR INTEGRATED PEST MANAGEMENT

Instead of only chemical treatments using an IPM approach can help reduce parasite zoonotic infections. Manage parasite populations by using biological or natural predators and lessen the usage of chemical controls (Falkenberg et al. 2022).

4.6. DISEASE MONITORING AND DIAGNOSIS

- Conduct routine disease monitoring and health inspections on fish populations to look for early indications of parasite diseases.
- Use reliable diagnostic techniques and seek expert help as necessary since accurate diagnosis is essential for effective treatment of an infection (Bardhan 2022).

4.7. USE OF ANTIPARASITIC DRUGS

- When parasitic infections are confirmed use the proper antiparasitic drugs in accordance with the recommended doses and treatment procedures.
- When parasitic infections are confirmed Select the most efficient and secure treatment methods in cooperation with fish health professionals and veterinarians.

4.8. REDUCING CROSS-CONTAMINATION

- Use distinct equipment and nets for each group to avoid cross-contamination between various fish populations.
- To prevent the spread of parasites, isolate sick fish right away from healthy fish to minimize the risk of infection (Williams et al. 2020).

4.9. ENVIRONMENTAL MANAGEMENT

- Evaluate how aquaculture practices affect the environment and natural ecosystems.
- Reducing water pollution and managing waste properly can help to lower the danger of parasite outbreaks.

4.10. PUBLIC HEALTH AWARENESS

- Educate fish farm owners, workers and consumers about the risks associated with fish farming, consuming and trading fish infected with zoonotic parasites

Encourage safe food handling techniques such as appropriate cooking and freezing techniques to destroy parasites.

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Zoonotic Infertility Due to Toxoplasma Gondii

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ABSTRACT

Toxoplasmosis is the infection of *Toxoplasma gondii.* It is an apicomplexan parasite that infects both animals and humans, worldwide. Its definitive hosts are felines. So, pet cats are the main toxoplasma reservoirs affecting the human population directly. Toxoplasma, resides in ovaries and hence leads to reduced reproductive potential, infertility, abortion or congenitally diseased offspring. Although famous for causing infertility in females, toxoplasma is also suspected to affect the male reproductive system. Besides causing infertility in humans, toxoplasma also affects the economics of the livestock industry. The main issues caused by toxoplasmosis are abortion, damage to the reproductive system and reduction in value of the breeding stock. In addition to the effect on reproductive health, toxoplasma is also suspected to greatly affect the brain and neurologic functions of the individuals with congenital infection. Conclusively, toxoplasmosis is a problematic disease that requires utmost care for control and prevention. Preventive measures including education of people about transmission routes and reservoirs should be implemented to control its spread effectively. Hygiene protocols should be developed and eradication measures should be taken to the reservoirs and transmission sources of toxoplasma.

Keyword: Toxoplasmosis, Feline, *Toxoplasma gondii*, Ovaries, Infertility.

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1. INTRODUCTION

An obligate apicomplexan parasite is *Toxoplasma gondii* famous for infecting both humans and animals all across the globe (Tonouhewa et al. 2017). The final hosts of this parasite are the cat family or felids. However, the latest research has shown the success of its development in mice when placed under certain conditions of inhibition by enzymatic action and modification of diet (Attias et al. 2020). The intermediate hosts of *T. gondii* include several types of birds along with terrestrial and aquatic mammals (Attias et al. 2020).

Toxoplasmosis is a disease of cosmopolitan factors found in humans and several types of mammals. Its etiologic agent is an opportunistic protozoan known as *T. gondii* mainly transmitted through oral route, acquired congenitally and by blood exchange. According to an estimate it has infected around 33% of the world's population (Tenter et al. 2000; Dubey and Jones 2008; Jones and Dubey 2010). Mostly in adult hosts it will not cause any serious issues. On the other hand, it can produce reduced mental functionality and loss of vision in children with congenital infection. It can also produce severe diseases in patients with disorders leading to compromised immunity. The latest research indicates that *T. gondii* infection is often discovered in association with the condition of abdominal hernia (Alvarado-Esquivel and Estrada-Martínez 2011). The plan of action followed by *T. gondii* for infecting hosts and transmitting the disease is multifaceted. Its life cycle pathway involves three stages of development of the parasite (tachyzoite, bradyzoite, and sporozoite) (Attias et al. 2020). The intermediate hosts of *T. gondii* include humans too. Humans can get infected by *T. gondii* through several transmission routes including

(i) Consumption of water contaminated with infective oocysts.

(ii) Consumption of vegetables and fruits grown in contaminated water.

(iii) Consumption of undercooked or raw meat infected with bradyzoites or tachyzoites of *T. gondii* (Dubey et al. 2009a).

(iv) Unsafe blood transfusion.

(v) Unsafe transplantation of organs contaminated with cysts or tachyzoites.

(vi) Congenital infection transmitted from the mother to the fetus via the placental route.

2. LIFE CYCLE

The definitive hosts of this parasite are the feline family that acquire the infection by carnivorism or ingesting the sporulated oocysts. However, ingestion of raw, non-pasteurized milk or milk products can also rarely serve as a potential source of *T. gondii* infection (Chiari and Neves 1984; Stelzer et al. 2019; Attias et al. 2020). Mussels and Oysters can act as host reservoirs for the infective stage of oocysts. These oocysts can later on produce infection after being ingested by other animals (Lindsay et al. 2004; Coupe et al. 2019; Monteiro et al. 2019; Attias et al. 2020). Being final hosts felids are responsible for harbouring the maturation stage of parasites that reside in their intestines. Once matured these parasites expel a large number of infective oocysts into the intestine. These oocysts are then passed out to the environment along with faeces. This release of infective oocyst can last from three to 18 days post-infection of the feline (Montazeri et al. 2020).

Progression in life cycle of *T. gondii* necessitates presence of both intermediate hosts and definitive hosts. Only if both hosts are available then life cycle of *T. gondii* (Fig. 1) will reach its completion after going through the asexual and the sexual phases of replication. The sexual portio of its life cycle progresses only in the intestines of the felids that are its definitive hosts. The infection in the intermediate hosts which includes the warm-blooded animals begins after they ingest oocysts contaminated foods or drinks. These are the same infective oocyst that were once released by a feline along with faeces (Montoya and Liesenfeld 2004). In the early few days of infection the actue phase

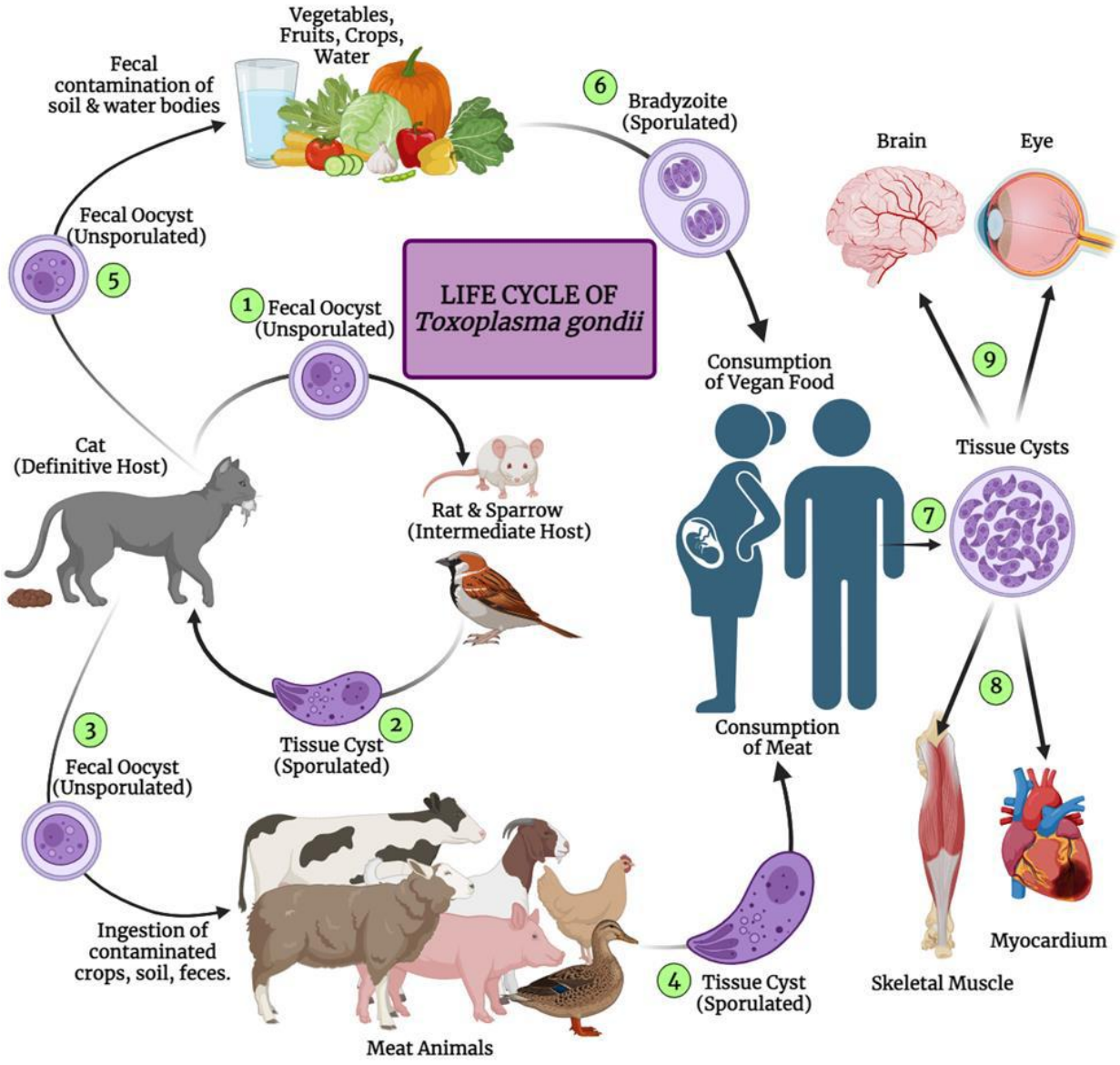

Fig. 1: Life Cycle of *Toxoplasma gondii*.

occurs marking rapid replication of the tachyzoites leading to an immense increase in their numbers The tachyzoites then mature into bradyzoites with the passage of time. Later on it begins to form tissue cysts that parasitize the cells of host. The toxoplasmosis infection may become lethal if it gains entry into an immuno-compromised patient, this situation will be aggregated by the reverse formation of tachyzoites from bradyzoites. Besides definitive hosts the intermediate hosts are also responsible for transmission of toxoplasmosis as they release tachyzoites and tissue cysts. Transmission pathways (Fig. 2) of toxoplasmosis include congenital, peroral and blood transfusion (Dubey 2010). The initial cases of human toxoplasmosis were reported in Jiangxi Province of China during the year 1964 (Xie 1964). Since the reports of first epidemic in China became public several human cases were also reported. This shifted the focus of researchers leading to the first epidemic survey launched for surveillance of toxoplasmosis.

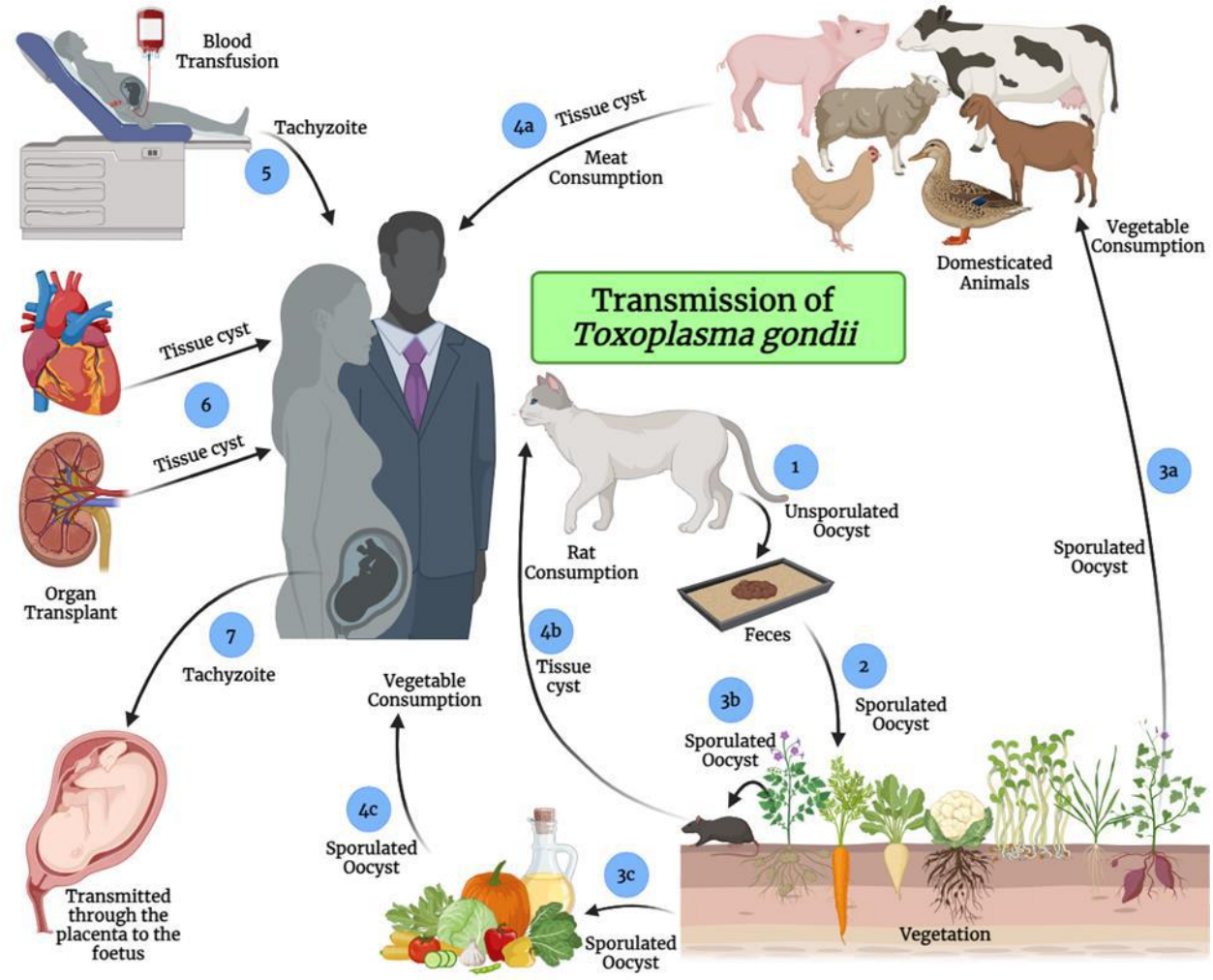

Fig. 2: Zoonotic transmission of *Toxoplasma gondii* via different routes.

This survey was initiated and performed during the year of 1978 in Guangxi Province of China (Chen et al. 2005). Despite these efforts the toxoplasmosis cases reported from China were barely recognized by the clinicians of the west as most of the information published from China was in Chinese and a small part of information was presented in English. The increasing incidence rate of *T. gondii* infections and a rise in the number of clinical cases was observed mostly (Xia et al. 2001; Li et al. 2002; Quan 2006) patients with impaired immune response systems. Similar trends were in patients that were congenital carriers of toxoplasmosis and psychosis. These reasons are enough to draw the attention of researchers to identify toxoplasmosis as a serious threat to the global public.

3. EPIDEMIOLOGY

Toxoplasmosis infection in animals or humans is caused by toxoplasma parasite that is prevalent worldwide. The rate of infection is variable as it depends on the geographical region of infection and the climate conditions of that area (Tonouhewa et al. 2017). Other factors affecting the prevalence of

toxoplasma infection include gender, age, geographic characteristics and contact with animals (Stelzer et al. 2019). Toxoplasma infections are often marked by inconsistent degrees of clinical symptoms that appear during infection. The state of clinical signs is dependent upon inoculum size and virulence of the parasitic strain that infected the host. Another dividing factor in this infection is the level of effectiveness of the host's immune response system (Mose et al. 2020).

4. PATHOLOGY

Toxoplasmosis has often been reported as a disease of the reproductive system. So, it mainly presents its infection symptoms by disturbing the normal reproductive parameters of the hosts. This disturbance appears as a negative impact on the reproductive functionality female host (Abdoli et al. 2012). Toxoplasmosis induces a programmed cell death cascade in the fundamental reproductive cells also known as the spermatogonial cells, through direct or indirect interference (Saki et al. 2020). This cell death cascade results in the reduction of sperm quality in humans as the number of main production cells is reduced (Zhou et al. 2003). Furthermore, reduced sperm quality also affects the fertility of male rats, when infected experimentally, by reducing it significantly (Terpsidis et al. 2009; Saki et al. 2020). Many researchers have reported a notable connection between the seropositivity of *T. gondii* and the occurrence of abortion in small ruminants. These reports originated from certain districts in the central region of central Ethiopia (Gebremedhin et al. 2013). In small ruminants like sheep, the toxoplasm infection may result in early embryonic death. After early embryonic death, the fetus may be disposed of by the body through resorption, fetal death, abortion, mummification, and stillbirth (Edwards and Dubey 2013). Such losses in the small ruminant industry ultimately lead to severe economic losses and affect the overall financial performance of the livestock industry (Tonouhewa et al. 2017; Etter et al. 2019).

The impact of *T. gondii* infections appears on the economics of the sheep rearing industry and other livestock business operations when the increased number of abortions leads to an increase in lambing/kidding interval of the livestock animals. Similarly, death and culling of infected animals lead to a reduction in milk production. Additionally, the presence of reproductive disease reduces the market value of the breeding stock. All these factors when combined together in the presence of endemic toxoplasmosis, lead to major economic losses for the livestock industry (Gebremedhin et al. 2013).

The stage of infection severity in the case of toxoplasmosis depends on the phase of gestation in which the ewe gets infected with the toxoplasma. Infection with *T. gondii* at the preliminary stage of gestation often leads to lethal consequences (Dubey et al. 2009b; Gebremedhin et al. 2013). In hosts with competent immune systems, the toxoplasma infection may persist asymptomatically. On the other hand, in the case of human hosts with impaired immune response systems such as in cases of disease like AIDS, the infection may turn into an ugly situation with dire effects and may lead to some serious problems (Frimpong et al. 2017; Etter et al. 2019). In the manner when this infection happens in women going through pregnancy it leads to the production of a fetus with congenital toxoplasmosis. A fetus that is congenitally affected by this infection faces increased severity of the disease along with elevated risks that are dependent on the time of maternal infection and are often accompanied by developmental malformation of the fetus, abortion, or reduction in quality of life for the child if it survives (Frimpong et al. 2017; Etter et al. 2019; Mose et al. 2020).

While toxoplasma is an agent with zoonotic disease transmission abilities it can be still controlled and prevented in both animals and humans all across the globe. These efforts to control toxoplasmosis are hindered in sub-Saharan regions of Africa due to several types of factors. The hampering factors include a lack of resources that in turn leads to high levels of poverty, the absence of proper diagnostic capabilities necessary for disease identification, limited abilities of disease surveillance bodies to operate

in that region, and poor practices regarding veterinary care (Hammond-Aryee et al. 2014). Having a proper implementation system in place for assurance of good veterinary practices is insanely crucial for the control of toxoplasmosis because its main reservoir is animals from which it can be transmitted to humans through the faecal-oral route and ingestion of undercooked or raw food or meat contaminated with Toxoplasma. These are the main transmission routes concerned with the transmission of infection in humans (Mose et al. 2020). All this pertains to the fact that effective control of toxoplasma infection necessitates raising enough awareness of good veterinary practices among veterinarians and relevant staff that they start practising proper protocols for personal safety and hygiene, improve their cooking and eating habits, monitor their intake routines and make corrections where needed, and lastly, they should emphasise accurate diagnosis of the disease so that factual disease reports can be presented to assess actual conditions of toxoplasmosis transmission and biological burden (Ramírez et al. 2017).

Correct diagnosis is very important for control and prevention of toxoplasmosis and it involves a few direct methods. The two main methods used for toxoplasma identification include molecular biology-based molecular techniques and immunology-based immunodiagnostic methods. The methods of direct diagnosis for toxoplasma infection involve the extraction of parasitic agents or bioassay, cell culture, and histology. The methods of immunodiagnostic include the immunofluorescent assay (IFA), Sabin–Feldman dye test (SFT), modified agglutination test (MAT), hemagglutination assay, enzyme-linked immunosorbent assay (ELISA), avidity, western blot, recombinant antigens, immunocytochemistry, and immunohistochemistry. The techniques of molecular diagnosis include Polymerase Chain Reaction (PCR), PCR-restriction fragment length polymorphisms (PCR-RFLP), real-time PCR, high-resolution melting (HRM) and loop-mediated isothermal amplification (LAMP) (Ramírez et al. 2017). Infection of *T. gondii* leads to initiation of IgM appearance in the host. The rise of IgM is followed by the emergence of IgA and IgE antibodies once an interval of about two weeks has passed since infection (Montoya 2002; Daka et al. 2015). The number of IgG antibodies begins spiking in the host after approximately four months have passed since the start of the infection. This elevated amount of IgGs then persists throughout the lifetime of the host (Daka et al. 2015).

Toxoplasma infection in a host individual with a competent immune response system resolves without any treatment (Muhie and Keskes 2014). On the other hand, the host individuals having an impaired immune system require dosing of antibiotics like clindamycin, sulfonamides, spiramycin, and pyrimethamine to be used for treatment of the infection (EFSA 2007; Vogel et al. 2010). The combination of drugs like sulfadiazine and pyrimethamine is suitable and effective for use in the treatment of newborns, infants, and pregnant women. Similarly, an antibiotic spiramycin has been proven effective for use in pregnant women to prevent congenital transmission of toxoplasma from an infected host mother to a healthy unborn fetus. It has been proven effective in preventing the infection but not for the treatment of latent infections as antibiotics do not have the capability to the bradyzoites in sufficient concentrations (Overton and Bennet 2010; Daka et al. 2015).

5. INFERTILITY IN WOMEN

In women, it has been experienced that there occurs no association between toxoplasmosis and sterility. However, some experiments have explained that *T. gondii* causes reproductive problems in mice species, and the main cause behind this problem was hypogonadotropic hypogonadism which is secondary to hypothalamic dysfunction, and during estrous cycling cessation, different histopathological changes were observed accompanied by impaired folliculogenesis and decrease in the development of corpus luteum (Stahl et al. 1994; Antonios et al. 2000). Spontaneous abortion, hydatidiform mole, stillborn, sterility and teras are the outcomes that are observed because of *T. gondii* exposure in pregnant women. Women

who have historically poor obstetric outcomes have seroprevalence of 14.2% (Zhang and Wang 2006) to 33.9% (Gong et al. 2006) which is much greater than the normal pregnancies that happen in China. A survey of 68 cases of oviducal sterility because of *T. gondii* showed a prevalence of 44.1%, which is too higher than that observed during normal pregnancies of women (3.3%) (Wei et al. 2005), clearly suggesting that oviducal sterility can develop because of *T. gondii* infections. Male sterility has also been linked to *T. gondii* infection. According to recent zoopery investigations, *T. gondii*-infected male rats exhibited a considerable rise in sperm abnormalities along with a significant drop in reproductive indices such as sperm motility and concentration (Terpsidis et al. 2009). When mice are experimentally infected with *T. gondii*, then similar results are obtained (Yang et al. 2005). (Zhou et al. 2002) discovered that toxoplasma transmission in sterile human spouses was higher than in fertile ones. This finding may have something to do with the increased levels of anti-sperm antibodies in toxoplasma-infected couples. In a recent study, 16% of 100 sterile males with *T. gondii* infection tested positive for IgM, and 13% tested positive for CAg, both of which are significantly higher than the percentage of healthy men (Qi et al. 2005). In Luoyang,Henan province, when seroprevalence of male sterility because of *T. gondii* was investigated, it was 19.8% (Yue et al. 2006), to 22.8% in Yan'an, Shaanxi province (Hui et al. 2003), again higher than that of detected in healthy men. The investigations and relevant studies in China suggest that *T. gondii* infection can be a cause of male sterility (Lu 1998).

Preventing direct contact with infection sources, such as cats, contaminated settings, eating raw or undercooked meat, maintaining good personal hygiene, and washing hands, is the main strategy for preventing toxoplasmosis (Daka et al. 2015). The other strategy for disease control is to eliminate mechanical vectors responsible for transmission, such as flies, cockroaches, and rats in the surrounding environment (Muhie and Keskes 2014).

6. TOXOPLASMOSIS INFECTION AND BEHAVIOURAL CHANGES IN ANIMAL

Latent infection of *T. gondii* can affect the performance of the central nervous system especially in a young growing body. This phenomenon backs the claims of neurologic changes occurring in toxoplasmosis in animals. This evidence can be also used to support the reports of behavioural changes appearing in animal models experimentally infected with latent toxoplasmosis. Observation of the toxoplasma-infected rodents served as strong evidence of reduced learning, poor memory task achievements and poor performance although to the researcher's surprise, no cysts were seen in the hippocampi of infected animals. On the other hand, several cysts were discovered throughout the brain. These occurrences suggest that the alteration in neurophysiology serves as the basis for anomalous behaviour shown by the infected animals (Daniels et al. 2015). Several researchers have reported observations of similar changes in the behaviour of the infected mice and cats (Joanne 2007). The changes were interpreted by reporting researchers as a manifestation of the invading parasite's neurotrophic manipulative function performed for the completion of its life cycle. The altered behaviour of rodents makes them more prone to be preyed upon by cats making this a cascade fall to the definitive hosts of the parasite. Recent research has now revealed that initiating treatment against *T. gondii* can resolve behavioural alterations bringing it back to normal. These reports provided the researchers with new avenues for managing cases of human schizophrenia (Zhu et al. 2003; Joanne 2007).

7. DETECTION OF *T. GONDII* OOCYSTS IN FAECAL SAMPLES

The discoveries unveiled in this study were unique from reports of other authors (Dabritz et al. 2007; Bizhga 2017). In the region of Bangladesh, the number of well-organized animal farms is limited and cats

can be seen wandering everywhere in the areas where study was being conducted. The wandering of stray cats meant the contamination of the grass or pasture. So a strategy was developed to keep the livestock like cattle, sheep and goats confined within the enclosure or pasture for grazing. This strategy reduced the risk of infection that they could get from the environment contaminated with sporulated oocysts of *T. gondii* being shed by cats through faeces. This reduces the chances of people getting an infection after ingestion of raw or undercooked meat of these animals. Additionally, it is rare because of the food habits of Bangladeshi people who cook everything well before consumption. On the other hand, people may get infected by consuming unwashed fruits and vegetables as they might be contaminated due to the environment of the kitchen garden where they were present along with sporulated oocysts. People can get infected accidentally while they are working in situations to come in contact with infective oocysts like gardens and fields etc. During warmer seasons (March to June) toxoplasmosis displays a higher prevalence. Contamination Soil by *T. gondii* oocysts is not a stagnant event as it can differ depending on the presence of excreta of a feline with oocysts in a faeces environment with climate conditions such as moisture and temperature (Dubey 2004; Casartelli-Alves et al. 2015). Various reports have verified a connection between outbreaks of toxoplasma infection and contamination of water bodies toxoplasma oocysts (Cook et al. 2000; Dubey 2004; Karanis et al. 2013; Krueger et al 2014). Once the soil becomes contaminated with oocysts, the oocysts may reach the water body source by wind or rain and then water becomes contaminated. Adult cats were affected more than young (<4 months old) by this protozoa. It is expected that with increasing age, the exposure of cats to *T. gondii* infection also increases (Miro et al. 2004; Alvarado et al. 2012). This may be due to feeding cats with raw meat and infrequent cleaning of the litter box (Besne Merida et al. 2008). Young cats might get infection via congenital transmission from infected dams or from milk.

All of the studied cats were free-roaming but with limited access to households and none had a specific owner. In general street cats are more prone to *T. gondii* infection compared to household cats because street cats could acquire the infection through catching rodents, birds, reptiles and raw food scraps (Sah et al. 2019).

8. CONCLUSION

Toxoplasma gondii is an apicomplexan and obligate parasite. Its life cycle is divided into several hosts including warm blooded mammals as intermediate and felids as the definitive hosts. It resides in intestines of feline hosts and matures into infective oocysts that can be then expelled through feces. It can spread among hosts through oral route as by consumption of contaminated edibles, blood exchange and congenitally. Although toxoplasma infection is unable to cause much damage in normal adult hosts. In adult hosts it persists asymptomatically. On the other hand, its congenital transmission in the fetus can produce some serious symptoms in the host, especially one related to abnormal neurological behavior. These anomalies may include reduced brain performance, poor memory and inability to achieve tasks. It affects memory despite having cysts in hippocampi.

Toxoplasmosis also affects the farmers economically besides its disease impact. The losses occur in the form of abortions especially in sheep and loss of breeding stock value due to reduced fertility and gestation disorders. Toxoplasma affects fetuses because it survives in ovaries and has high chances of transmission to the fetus during pregnancy leading to early embryonic death which can further aggravate into abortion, mummification or maceration. It can be easily controlled for saving fertility of women by teaching them proper hygienic measures, eliminating hosts like pests and flies, preventing contact of pet cats with toxoplasma.

Additionally, it can also affect the fertility of males. In contrast to normal young adults it severely affects the adults with impaired immune system as in case of acquired immunodeficiency syndrome (AIDS) or such similar diseases. Its prevalence increases in summer as compared to winter. Toxoplasmosis, although problematic can be easily controlled by educating people to cook food properly before consumption, stay away from unsafe blood transfusion and follow proper treatment protocols for preventing its transmission.

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Toxoplasmosis

33

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ABSTRACT

Toxoplasmosis, caused by the globally prevalent parasite Toxoplasma gondii, features a complex life cycle involving both sexual and asexual reproduction, utilizing cats as definitive hosts. The parasite's genetic diversity, diverse transmission routes, and potential impact on human reproductive function underscore the need for further research to elucidate its epidemiological significance and the relative importance of different transmission pathways. In 1908, Nicolle and Manceaux's discovery of T. gondii in a Tunisian gundi was pivotal for toxoplasmosis research, shaping the nomenclature based on its coccidian-like structure. Toxoplasmosis imposes significant economic losses on the food industry, leading to costly recalls and an annual financial impact of 7.7 billion USD in the United States. The disease, primarily transmitted through T. gondii oocysts or tissue cysts, poses varied clinical risks, with asymptomatic or mild symptoms in some, but severe manifestations in high-risk populations, underscoring the importance of preventive measures and timely medical intervention. Toxoplasmosis, challenging to diagnose due to diverse clinical presentations, is managed through crucial laboratory tests like serology, PCR, and immunohistochemistry, with ongoing research emphasizing improved diagnostics and treatment outcomes. Treatment strategies vary, with spontaneous resolution in healthy individuals and a combination therapy involving pyrimethamine, sulfadiazine, and leucovorin/folinic acid for severe cases, highlighting preventive measures, including proper hygiene and public health education, to reduce T. gondii transmission and infection risks. Ongoing research targets enhanced diagnostics, vaccine development, novel therapeutics, and deeper insights into T. gondii biology, aiming to alleviate the disease burden and inform effective control measures for both human and animal populations.

Keywords: Toxoplasma gondii, Epidemiological significance.

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1. INTRODUCTION

Toxoplasmosis is a common parasitic disease affecting animals and humans worldwide. Toxoplasma gondii (T. gondii) is a protozoan parasite causing toxoplasmosis (Mehnaz et al. 2019). It's an obligatory intracellular organism, belongs to the phylum Apicomplexa (Besteiro 2014). T. gondii is genetically diverse with 15 distinct genotypes, differing in virulence, tissue tropism, and distribution. (Gibson et al. 2011). The life cycle of T. gondii is complex, with both sexual and asexual reproduction. It requires two host species: the definitive host, usually a cat, and the intermediate host, which can be various warm-blooded animals, including humans (Zúquete et al. 2022). Inside the definitive host (typically a cat), T. gondii undergoes sexual reproduction, resulting in the production of oocysts excreted in the cat's feces. These oocysts can survive in the environment for extended periods, ranging from months to years. Intermediate hosts can be infected by consuming contaminated food or water containing these oocysts (Maier et al. 2019). After entering an intermediate host, T. gondii multiplies asexually and forms cysts in organs like the brain, eyes, and muscles. Infection can occur in humans and other warm-blooded animals by consuming undercooked or raw meat with tissue cysts from infected animals. Ingesting oocysts from contaminated food, water, or soil can also cause T. gondii infection (Hill and Dubey 2018).

T. gondii, is a versatile protozoan that can infect various host species and has evolved multiple transmission routes within and between them (Kagira et al. 2020). When T. gondii is initially contracted during pregnancy, the parasite can be vertically transmitted to the fetus through the placenta, carried by tachyzoites (Agarwal et al. 2022). T. gondii can spread from one animal to another in three different ways during its life cycle. First, animals can get infected by swallowing infectious oocysts found in the environment. Second, they can become infected by eating the meat or organs of other animals containing tissue cysts or tachyzoites (AM and Health 2017). Furthermore, transmission can also occur when tachyzoites are present in blood products, tissue transplants, or unpasteurized milk (Iano et al. 2019). The relative importance of these routes in the epidemiology of the disease is currently unknown (Farahani et al. 2020). T.gondii. In intensive farm management areas a notable decrease in the occurrence of T. gondii was observed in animals raised for meat production (Kagira et al. 2020).

Toxoplasmosis is known for its capability to infect a wide variety of animal species, including birds, rodents, livestock, and even marine mammals (El Fadaly et al. 2022). Research has shown that T. gondii infection can have an impact not only on female reproduction but also on male reproductive function, causing impairment (Xu et al. 2022). Clinical studies have reported a notably high prevalence of toxoplasmosis among infertile men (Mishra et al. 2022). Additionally, there are indications of venereal transmission of T. gondii (Hlaváčová et al. 2023). T.gondii. It can also affect various hormones, potentially leading to insufficient male reproductive function (Dalimi and Abdoli 2013).

2. HISTORY

In 1908, Nicolle and Manceaux described *T. gondii* in Tunis, identifying it in the gundi's tissues. Nicolle named the infectious organism *T. gondii* based on its coccidian-like structure, marking a crucial milestone in the study of toxoplasmosis (Cox 2002). In 1939, Wolf, Cowen, and Paige established *T.*

gondii as a causative agent of human disease. Additionally, its medical significance became evident when it was found in the tissues of a congenitally infected infant (Keohane et al. 2020). In 1948, a major breakthrough came with the Sabin-Feldman dye test, a specific antibody test for *T. gondii* It helped identify the parasite as widespread among warm-blooded hosts worldwide. Similarly, in 1957, *T. gondii* significance was acknowledged in the veterinary community due to its involvement in sheep abortion outbreaks. These discoveries were pivotal in understanding the importance of *T. gondii* (Ferguson 2022). Around the same period, Splendore identified the presence of *T. gondii* in the tissues of a rabbit in Brazil (Dubey 2020).

However, it was not until 1970 that scientists fully elucidated the life cycle of the parasite. During this period, they discovered that felids, including cats, act as the definitive host for *T. gondii* (Iano et al. 2019). Recent research found *T. gondii* infections in marine wildlife, like sea otters, indicating contamination from land-washed oocysts. This raises concerns about the environmental impact and parasite transmission (Bahia-Oliveira et al. 2019).

3. ECONOMIC IMPACT

Toxoplasmosis creates a substantial economic burden on healthcare systems due to high costs for diagnosis, treatment, and complication management. Additionally, the disease impacts the food industry's economy, as *T. gondii*contaminated meat products can lead to costly recalls, financial losses, and reduced revenue for businesses involved in their production and distribution (Basso et al. 2019). Economic loss of 7.7 billion USD annually has been recorded in united states of America (Kruszon-Moran et al. 2001).

3. LIFE CYCLE

Until 1970, only the asexual stages of *T. gondii*, including tachyzoites, trophozoites, bradyzoites and cystozoites, were documented (Wu et al. 2021). The documentation of the sexual cycle of *T. gondii* and the discovery of its environmentally resistant stage, the oocyst stage, were first reported in 1970 (Francia et al. 2020). After ingestion, Toxoplasma parasite multiplies rapidly as tachyzoites during the acute phase, then establishes in various organs. *T. gondii* has three infectious stages: tachyzoites, bradyzoites, and sporozoites (Lüder and Rahman 2017). Humans and animals mainly get infection by consuming bradyzoite or oocyst stage of the cycle. After ingestion, bradyzoites and sporozoites transform into tachyzoites in the body's tissues. Interconversion between tachyzoites and bradyzoites is critical, as bradyzoites are more resistant to drugs, and reactivation into tachyzoites causes severe toxoplasmosis in AIDS patients (Ashander et al. 2021). Following infection with any infective stage, tachyzoites multiply in various cells and eventually encyst in tissues, especially the brain (Hill and Dubey 2018). Tissue cysts can persist within the host for extended periods, possibly throughout their life span. One hypothesis proposes that these cysts may occasionally rupture, releasing bradyzoites. In immunocompetent hosts, the immune system is believed to effectively eliminate these released bradyzoites (Blanchard et al. 2020). Immunosuppressed individuals may experience multiplication and spread of bradyzoites released from tissue cysts to other organs. The exact mechanism of toxoplasmosis reactivation remains unclear. It is uncertain if bradyzoites from older cysts can directly form new cysts or if they first need transition through the tachyzoite stage (Guegan et al. 2019). Bradyzoites are more resistant to chemotherapy than tachyzoites, making their fate in host tissues clinically important. Different criteria are used to distinguish between tachyzoites and bradyzoites (Wang et al. 2022). Tachyzoites have a centrally located nucleus and few or no PAS-positive granules, mainly seen during the acute infection phase. Bradyzoites have a terminally located nucleus,

numerous PAS-positive granules, and are enclosed in a resilient cyst wall, mostly found during the chronic infection phase. However, the transitional stages and reverse transformation between tachyzoites and bradyzoites lack clearly defined structural characteristics or antigenic properties (Dubey 2020).
Felines, acting as definitive hosts, can get infected by carnivorous feeding (consuming mammals and birds) or ingesting sporulated oocysts. Oocysts can remain infectious in mussels. Although rare, the consumption of unpasteurized milk or milk products can be a potential source of transmission (Teixeira et al. 2020). After consuming Toxoplasma oocysts excreted by cats, the parasite forms tissue cysts that persist within the body for extended periods, often throughout the host's life. These cysts are commonly found in tissues like the heart, skeletal muscles and central nervous system. If an animal infected with Toxoplasma is eaten, viable parasites present in the tissues can transmit the infection to a new host (Hill and Dubey 2018) (Fig. 1).

4.TRANSMISSION

Toxoplasmosis is mainly a zoonotic disease, meaning it can be transmitted between animals and humans. The primary mode of transmission for *T. gondii*, the parasite causing toxoplasmosis, is through ingesting the parasite's oocysts or tissue cysts (Kagira et al. 2020). Oocysts are shed in the feces of cats infected with

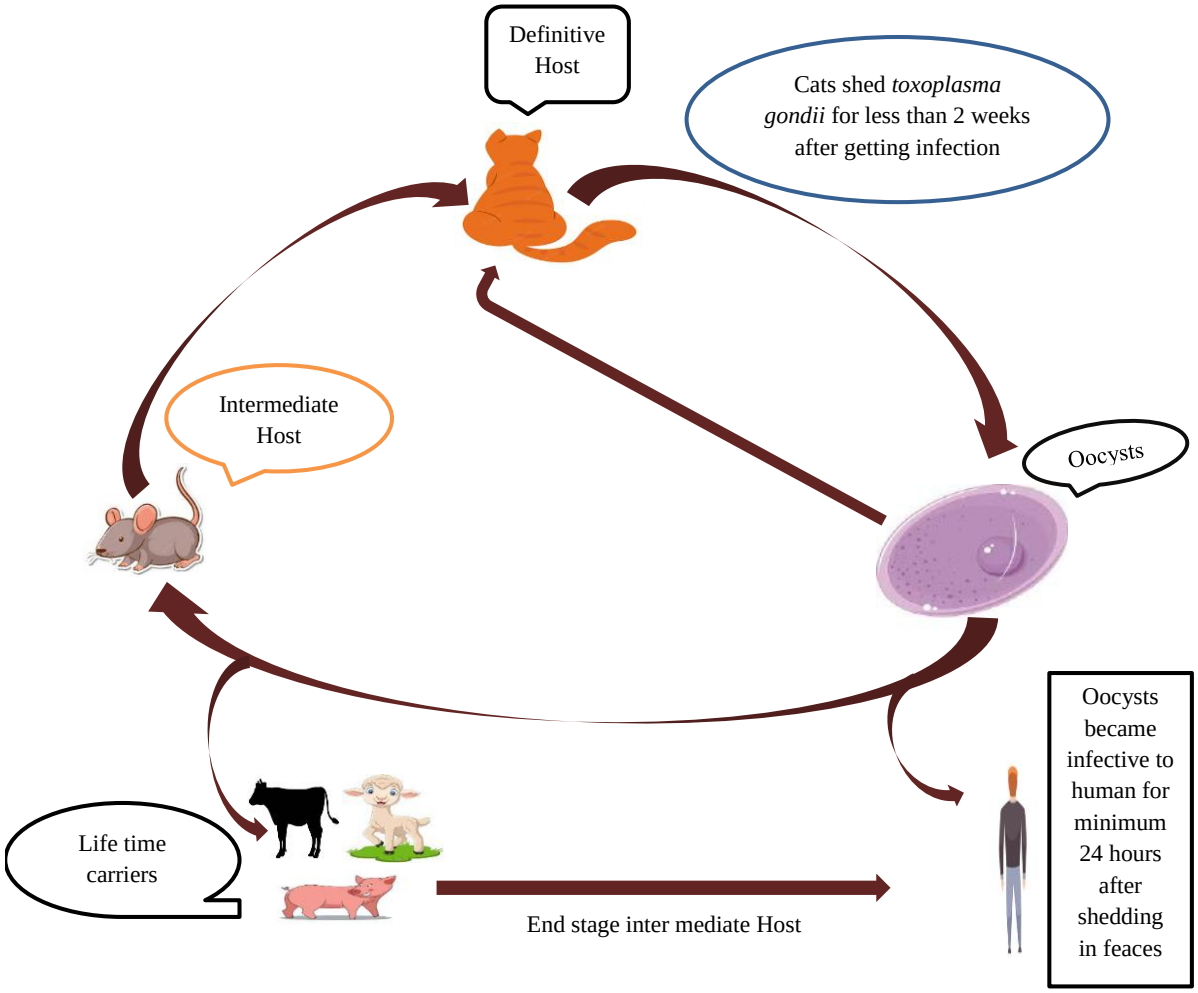

Fig. 1: Life cycle and one health.

T.gondii, the parasite's definitive host. These oocysts can persist in the environment for months to years, depending on factors like temperature and humidity (Bahia-Oliveira et al. 2019). Humans can contract *T.gondii* infection by accidentally ingesting oocysts found in contaminated soil, water, or food. Vegetables grown in oocyst-contaminated soil or meat contaminated during processing can be sources of infection (Chaudhry et al. 2022). Intermediate hosts, including rodents, livestock, and marine mammals, can acquire *T. gondii* infection by ingesting oocysts from contaminated soil or water, or by consuming tissue cysts in infected meat (Graziosi et al. 2023). Humans can get *T. gondii* infection by eating undercooked or raw meat from infected animals. Consuming unpasteurized dairy products like milk and cheese from infected animals can also lead to its transmission. However, transmission through organ transplantation or blood transfusion from an infected donor is relatively rare (Graziosi et al. 2023). Congenital transmission of *T. gondii* can occur when a pregnant woman gets infected with the parasite during pregnancy. The parasite can cross the placenta and infect the developing fetus, leading to severe complications like miscarriage, stillbirth, or birth defects (Graziosi et al. 2023).

5.CLINICAL PRESENTATION IN HUMANS

Clinical signs of toxoplasmosis in humans vary based on age, immune status, and parasite transmission route. With a healthy immune system, the infection may go unnoticed or cause mild flu-like symptoms like fever, headache, and muscle aches that usually resolve in a few weeks (Al-Malki 2021). In 2017, the Wisconsin Department of Health Services, Division of Public Health, investigated a febrile illness outbreak among retreat attendees who consumed intentionally undercooked, locally sourced venison. The investigation was prompted by a physician's report, and preliminary testing suggested a potential link to toxoplasmosis (Elbadawi et al. 2021).*T. gondii* infection is primarily influenced by the individual's immune status. In immunocompetent individuals, infections are usually asymptomatic, with lifelong latent infection (Fong et al. 2021). While most healthy people with *T. gondii* infection show no symptoms, a few may experience fever, malaise, and swollen lymph nodes.

Severe or life-threatening illnesses can rarely occur from infections caused by virulent parasite strains (Aga et al. 2020). Around 2% of healthy individuals may experience retinochoroiditis, inflammation of the retina and choroid. *T. gondii* can cause ocular disease congenitally or after birth, leading to symptoms like acute retinochoroiditis with pain, photophobia, tearing, and vision loss. Recurrent episodes can follow the acute phase (Choi et al. 2018). Immunocompromised individuals, like those with HIV/AIDS or organ transplantation, may face severe and life-threatening *T. gondii* infection. The parasite can cause encephalitis (brain inflammation), leading to neurological symptoms like seizures, confusion, and behavioral changes (Marra et al. 2020).

Congenital *T. gondii* infection can result in diverse manifestations in infants, such as hydrocephalus, microcephaly, cerebral calcifications, retinochoroiditis, blindness, epilepsy, motor retardation, and anemia (Khan and Khan 2018). Emerging evidence associates *T. gondii* infection with neuropsychiatric disorders. Studies show higher *T. gondii* antibodies prevalence in some neuropsychiatric patients, but more research is needed to understand the association's nature and mechanisms (Liesenfeld et al. 2011). Toxoplasmosis in pregnant women can cause congenital infection with severe consequences like fetal death, stillbirth, or long-term neurological and developmental complications in infants. Pregnant women should take preventive measures and seek prompt medical care if they suspect exposure to *T. gondii* (Dehority et al. 2020).

6. DIAGNOSIS

The severity of congenital toxoplasmosis depends on the timing of infection during pregnancy, with early infections associated with more significant complications. Pregnant women infected with *T. gondii* may

not show symptoms but can still transmit the infection to their fetuses, resulting in severe consequences for the developing baby (Rostami et al. 2018). Diagnosing toxoplasmosis can be challenging due to its varied clinical presentation. The wide range of possible symptoms and some cases having no symptoms or self-limited manifestations contribute to the complexity (Guarnera et al. 2022). Laboratory tests are vital for confirming toxoplasmosis. Serologic testing detects antibodies to *T. gondii*, while PCR testing detects the parasite's DNA, aiding in accurate diagnosis and confirmation (Ozgonul and Besirli 2016). Toxoplasmosis shows diverse clinical presentations, from asymptomatic to severe and life-threatening. Accurate diagnosis and timely treatment are crucial to minimize complications and improve patient outcomes (Gajurel et al. 2018).

Diagnosis of toxoplasmosis can be challenging due to nonspecific symptoms and potential delays in onset after initial infection. Various laboratory tests, including serological techniques, are available for diagnosing toxoplasmosis (L'ollivier et al. 2019). Extensivly serological techniques are used to diagnose toxoplasmosis, considering diverse clinical and immunologic characteristics. Ongoing efforts aim to further improve these methods (Schlüter et al. 2014). Recent progress in serological diagnosis of toxoplasmosis includes identifying novel immunogenic proteins and innovative antigen production. Incorporating recombinant antigens and multiepitope chimeric peptides has notably enhanced serological diagnostic methods (Ahmadpour et al. 2020)

Serotyping offers a less invasive alternative to genotyping, characterizing different parasite lineages. It has become the preferred method for toxoplasmosis diagnosis due to widespread usage (Pitt 2018). Serological tests measure *T. gondii* antibodies in the bloodstream, mainly IgG and IgM. IgG antibodies indicate a past infection (Mohtasebi et al. 2020), IgM antibodies are found in acute infections, while IgG antibodies indicate chronic infections. Both IgG and IgM antibodies together suggest an acute infection (Fricker-Hidalgo et al. 2016).

The PCR test is used to detect *T. gondii* DNA in bodily fluids like blood, cerebrospinal fluid, and amniotic fluid. It is important for diagnosing acute infections and monitoring treatment effectiveness (Karanis et al. 2018). Researchers have developed a DNA-based assay to identify *T. gondii* using PCR. The PCR technique amplifies a segment of the parasite's DNA, specifically the P30 gene. Following gel electrophoresis, the amplified DNA can be detected directly on the gel or via Southern hybridization using either radioactive or non-radioactive DNA probes (Hemphill et al. 2022). The assay has successfully detected *T. gondii* DNA in various isolates, even when combined with human or mouse DNA. When combined with clinical data, CT scans, and serology, PCR assay is expected to enhance toxoplasmosis diagnosis in immunosuppressed, immunocompromised patients, and fetal tissues (Wang et al. 2015).

The Immunohistochemistry (IHC) test is commonly used to identify *T. gondii* in tissue samples, especially when there is a suspicion of congenital toxoplasmosis, as the parasite may be present in the placenta or fetal tissue (Harrison 2000; Nurcahyo et al. 2017). To confirm diagnosis and assess parasite dissemination in different tissues, Immunohistochemistry (IHC) was conducted for *T. gondii* on formalin-fixed, paraffin-embedded (FFPE) tissues (Bauer et al. 2021).

Cultivating *T. gondii* in a lab is a specific test, but it is not commonly used for toxoplasmosis diagnosis due to slow parasite growth and the need for specialized facilities (Brenier-Pinchart et al. 2021). MRI and CT scans can be used alongside lab tests to identify *T. gondii* in the brain or other organs, serving as additional diagnostic tools for infection detection (Karanis et al. 2018).

7. TREATMENT

The treatment approach for toxoplasmosis is determined by factors such as infection severity and the individual's health condition. Treatment is adapted to ensure effective management (Al-Malki 2021). In

healthy individuals, treatment may not be necessary as the infection can be cured spontaneously. However, for weakened immune system patients, pregnant women, a combination therapy of pyrimethamine, sulfadiazine, and leucovorin or folinic acid is the typical first-line treatment to combat the infection and manage symptoms (Goodwin et al. 2017).

Pyrimethamine, a folate antagonist, is used to treat active toxoplasmosis in combination with medications like sulfadiazine or clindamycin. It effectively inhibits *T. gondii* proliferation by targeting the folate metabolic pathway in a synergistic action with sulfadiazine (Aspöck 2000). Sulfadiazine, a sulfa drug, is used in combination with pyrimethamine to treat active toxoplasmosis. It inhibits the growth of *T. gondii* and is commonly used as an additive drug in the treatment of ocular toxoplasmosis (Verbraak et al. 2002). Clindamycin, an antibiotic, is combined with pyrimethamine to treat active toxoplasmosis. It inhibits the growth of *T. gondii*. Spiramycin, an antibiotic, is used to treat toxoplasmosis in pregnant women. It prevents the replication of *T. gondii* in the placenta and fetus. Spiramycin crosses the placenta, diffusing into cord blood and amniotic fluid, concentrating in placental tissue. Pharmacokinetics have been documented during the second and third trimesters. Administering spiramycin during pregnancy significantly reduces the risk of fetal infection.

8.PREVENTION AND CONTROL

Preventive measures include avoid eating undercooked meat, practicing good hygiene, and preventing contact with cat feces or contaminated soil. Routine screening for toxoplasmosis in pregnant women is recommended to reduce the risk of congenital toxoplasmosis. Preventive measures are vital in controlling toxoplasmosis and minimizing health complications (Longcore et al. 2019).

T. gondii can be found in raw or undercooked meat, particularly in pork, lamb, and venison. To lower the infection risk, cook meat to a safe temperature (at least 160°F for ground meat and 145°F for whole cuts) (Kijlstra and Jongert 2009). Fruits and vegetables can be contaminated with *T. gondii* from soil or water. It's crucial to wash them thoroughly before consumption (Jones and Dubey 2012), so washing hands with soap and water after handling cat litter, gardening, or touching soil is essential (Jones et al. 2010). Pregnant women and immunocompromised individuals should avoid cleaning cat litter boxes or handling cat feces. If unavoidable, wearing gloves and thorough handwashing afterward is recommended (Kravetz and Federman 2005). Cat owners should keep their pets indoors and avoid feeding them raw meat to reduce infection risks. Humans can get infected through contaminated soil, water, undercooked meat. Direct cat contact is not a primary risk due to short oocyst shedding. Cats become infected by consuming infected prey. Keeping cats indoors reduces transmission to humans.

To reduce *T. gondii* transmission, practice proper hand washing and hygiene. Cook meat thoroughly, wash fruits and vegetables, and avoid cat feces, especially during pregnancy. Pregnant women and immunocompromised individuals should avoid handling cat litter boxes and delegate the task to others (van Gils et al. 2018). In addition to personal and environmental hygiene measures, public health education and awareness campaigns play a vital role in increasing knowledge and understanding of toxoplasmosis and its prevention. Together, these efforts aid in the prevention and control of toxoplasmosis, reducing the risk of infection (Ferreira et al. 2019).

9.FUTURE DIRECTIONS FOR RESEARCH AND CONTROL

Toxoplasmosis ongoing research on control measures are essential for reducing the burden of disease and improving outcomes for individuals affected by toxoplasmosis (Singla et al. 2012).

Future directions for research and control of toxoplasmosis include:

9.1. ENHANCED DIAGNOSTIC METHODS AND SCREENING

Improvements in diagnostic testing, such as the development of more accurate serological tests, can enable earlier detection and intervention. Regular screening, particularly for pregnant women, can aid in identifying cases for timely treatment (Longcore et al. 2019).

9.2. VACCINE DEVELOPMENT

The development of effective vaccines against toxoplasmosis can significantly impact disease control. Ongoing research is focused on live attenuated, subunit, and DNA-based vaccines, necessitating further evaluation of their safety, efficacy, and optimal immunization strategies (Hamilton et al. 2019). Toxovax, a live-attenuated vaccine based on the tachyzoites of *T. gondii* S48 strain, is currently the only commercially available toxoplasmosis vaccine (Chu and Quan 2021).

9.3. NOVEL THERAPEUTIC APPROACHES

New and effective therapies are needed for treating toxoplasmosis, especially in immunocompromised individuals. Research is exploring potential drug targets, such as enzymes involved in the parasite's cell wall synthesis, to develop improved therapeutics with enhanced efficacy and reduced toxicity (Dupouy-Camet et al. 2019).

9.4. ADVANCING PARASITE BIOLOGY

Enhanced knowledge of *T. gondii* biology contributes to the development of new diagnostic tools, therapeutic targets, and vaccines. Technological advancements like genomics and proteomics offer insights into the parasite's pathogenesis and aid in identifying potential drug targets (Zhao and Ewald 2020). In summary, ongoing research and development efforts are vital in reducing the impact of toxoplasmosis and improving outcomes. Advancements in diagnostics, vaccines, therapeutics, and parasite biology helps in the development of targeted control measures and overall disease management.

10. CONCLUSION

Toxoplasmosis, caused by the parasite Toxoplasma gondii, affects both people and animals worldwide. This parasite has a complicated life cycle that involves cats and other animals. People can get infected by eating contaminated food or undercooked meat. Recent research suggests a possible connection between *T. gondii* and male reproductive problems. Scientists are studying the parasite's genes and biology to develop drugs and vaccines. It's important to understand how it spreads and its impact to manage toxoplasmosis, which costs a lot for healthcare and the food industry. *T. gondii* has different infection stages, leading to long-lasting infections in both people and animals. Cats release tough oocysts into the environment, which can contaminate various sources. The parasite spreads through ingesting oocysts or tissue cysts. While healthy individuals often show no symptoms, it can harm those with weak immune systems or newborns. Diagnosis is tricky, requiring special tests, and treatment involves specific medications. Preventive steps include cooking food thoroughly, good hand hygiene, and avoiding cat feces. Collaborative efforts are essential for effective control, and future research aims to improve diagnosis, vaccines, treatments, and our understanding of the parasite's biology to reduce the impact of toxoplasmosis on both humans and animals.

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Diagnostic Tools for Zoonotic Infections

34

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ABSTRACT

The recent growth of infectious diseases stemming from zoonotic origins has placed a significant global burden on both morbidity and mortality. Additionally, it has generated substantial economic challenges. The complexity and dynamism of the resurgence and epidemiology of zoonoses are shaped by diverse factors which can be broadly classified into human-related, pathogen-related and climate related parameters. The diagnosis of animal diseases serves a dual role as both the origin and solution to various ailments. It is instrumental in managing and preventing diseases, playing a crucial part in overall disease control. Therefore, the imperative for the development of veterinary diagnostic tools becomes apparent, ensuring comprehensive animal welfare and effective monitoring of disease spread. Here we discussed various molecular and non-molecular diagnostic methods for zoonotic infections. These diverse approaches include viral metagenomics, clinical recognition, standard labortary assessments, and labortary tests. By examining recent advancements in diagnostic methodologies, this chapter aims to underscore the importance of ongoing research and innovation in the field of zoonotic disease diagnostics for enhanced public health preparedness and intervention strategies.

Keywords: Disease management, Diagnostic, Zoonotic, Viral metagenomics, Clinical tests, PCR, DNA fingerprinting, DNA based composition, Radioimunoassay.

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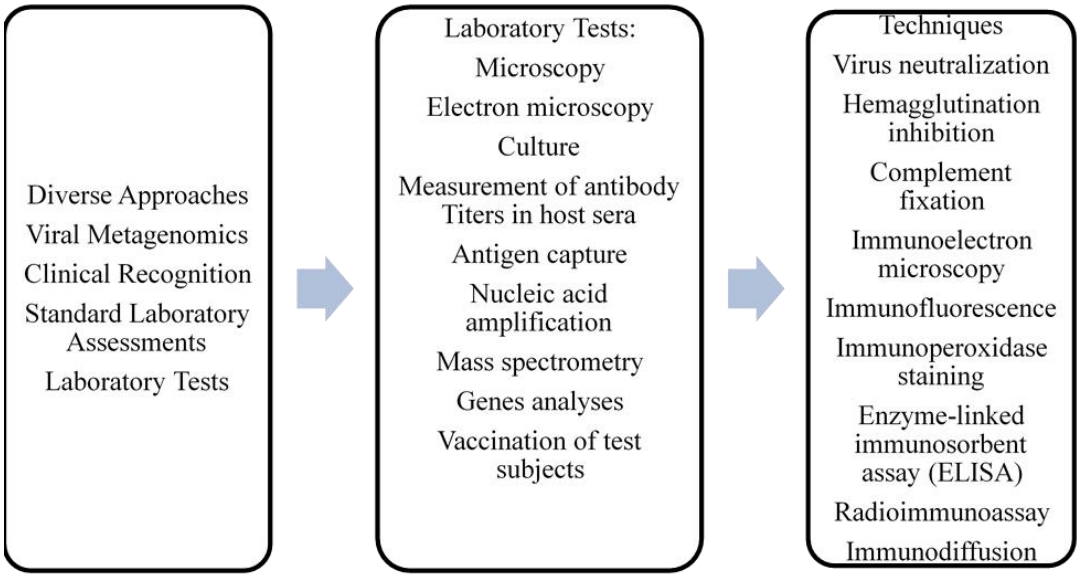
1. INTRODUCTION

Some unidentified and mysterious illnesses with no proper treatment have been observed all over the globe (He et al. 2020; Smolarz et al. 2021; Shafaati and Zandi 2022). Table 1 shows rapid diagnosis for molecular and non-molecular zoonotic infections.

Furthermore, such diseases are unique to specific regions of the world and have spread to new ones (Malki et al. 2020). Therefore, we need to be able to spot emerging diseases and determine its cause (Connolly et al. 2021).

Table 1: Rapid diagnosis for molecular and non-molecular zoonotic infections.

Molecular diagnostic methods	Non-molecular diagnostic methods
• Polymerase chain reaction (PCR) • Hybridization • Metagenomics • Gene probing • DNA Fingerprinting • rRNA sequencing • DNA Base composition • Electron Microscope • Mass Spectrometry • Restriction Endonuclease and Oligonucleotide Fingerprinting	• Cultures • Beta-Glucan Assay • CAGTA Assay • Histopathology • Glutomannan assay • Serological Tests (Antigen tests, Antibody tests) • Optical microscope



2. DIVERSE APPROACHES

The technique that incorporates simultaneous and diverse approaches is the most promising for evaluating novel diseases (Basith et al. 2020).

2.1. VIRAL METAGENOMICS

A culture-independent method called viral metagenomics is used to examine all viral genetic populations in biological material (Sommers et al. 2021). This methodology becomes a powerful tool for identifying

new and emerging viruses, considering that animals remain a reservoir for the virus that can cause zoonosis. Less than 1% of bacterial hosts have been cultivated, so it is difficult to identify and measure the dynamics of the viral population in the environment (Zhang et al. 2022). On the whole, these restrictions can be overcome by metagenomics investigation of uncultured viral communities, which can also offer insights into the makeup and organization of environmental viral communities.

2.2. CLINICAL RECOGNITION

An immediate individual or group recognizes a disorder that does not share traits with any other recognized condition (Shah et al. 2020). It could be an illness with a recently identified or previously documented ailment with an atypical presentation. As a result, it is advantageous to alert hospital infectious disease specialists, emergency room physicians, and other combatants to the potential for a novel disease or one that has not previously been observed in the region (Sami et al. 2021).

2.3. STANDARD LABORATORY ASSESSMENTS

It could be an illness with a recently identified or previously documented ailment with an atypical presentation. Therefore, it is advantageous to alert hospitals for novel disease specialists, emergency room physicians, and other combatants to the potential for a novel disease or one not previously observed in the region to encourage such recognition.

2.4. EPIDEMIOLOGY STUDIES

The epidemiological field is a scientific, systematic, and analytical study of the frequency, distribution, and origins of medical symptoms and events in particular groups (such as communities, schools, cities, regions, nations, and the world) (Luca et al. 2022).
The investigations may shed light on the new condition's distribution, the race, age, and sex of vulnerable people, the immune response of those who are resilient and those who are potential animal vectors or recipients, the lubricating period, and the mode of transmission (Belbasis and Bellou 2018).

2.5. LABORATORY TESTS

All conceivable laboratory tests need to be performed when an indication of a new and significant disease is indicated. These exams comprise of:

- Microscopy
- Electron microscopy
- Culture
- Measurement of antibodies serum
- Antigen capture assays
- Nucleic acid amplifications
- Mass spectrometer
- Genes Measurements
- Vaccination of test subjects

2.6. MICROSCOPY

A microscope can be used for a variety of things, depending on its type. While some are utilized in instructional settings, others are appropriate for biological purposes (DeVree et al. 2021).

Light microscopy can be performed quickly, but precision depends on the equipment's quality and the microscopist's competence. The ability of doctors to employ microscopy for diagnosis outside of an accredited laboratory is frequently constrained by regulations. To differentiate between invasive illness and surface colonization—a distinction that is difficult to make using culture methods-microscopic inspection of tissue may be necessary (Richert-Poggeler et al. 2019).

3. MICROSCOPY USING ELECTRONS AND IMMUNOELECTRON MICROSCOPY

These techniques are best suited for quickly detecting viral isolates from cell cultures and actual specimens (Madanayake et al. 2023). Electron microscopy only allows for family-level identification, whereas immunoelectron microscopy with a suitable, particular antibody may allow for the creation of finer differences (Gulati et al. 2019).

4. CULTURE

Culture is the development of microorganisms located in a nutrient-rich either liquid or solid medium; more organisms make identification easier. Antimicrobial susceptibility testing is also made more accessible by culture (Namdari et al. 2019).

5. IMMUNOLOGIC TESTS

The antigens are used to search for pathogen-specific antibodies in the patient's samples. Detection antibody for a pathogen's antigen in the patient's samples. Although there are different protocols for handling specimens, they should normally be chilled or frozen if testing needs to be postponed to avoid bacterial contamination proliferation (Normann et al. 2020). There are numerous techniques used or put forth for the cultivation-independent characterization of infectious pathogens (Gilboa et al. 2020; Preena et al. 2020). Through the use of molecular pathogen discovery techniques, new pathogens connected to both acute and chronic illnesses have been discovered during the past ten years (Belizario et al. 2021).

6. LIMITATIONS

6.1. THE PATHOGEN'S HOST RANGE

A pathogen once exclusive to dogs, swine, or cats now threatens human life (Tomori and Oluwayelu 2023).

6.2. MODIFICATION OF TISSUE TROPISM

A virus that in the past only occasionally caused moderate enteritis now frequently causes severe myocarditis and severe encephalitis (Shieh 2022).

6.3. IMMUNE EVASION

Large DNA viruses with many genes that help them escape or manipulate the host's immunity include orthopox viruses (Verdonck et al. 2022). These genes could undergo mutations that alter the virus's host range. Mutations can cause viruses of all sizes, including RNA viruses, to switch species. Additionally, some

RNA fragments are capable of recombining to create new variations that are immune-evading for both humans and the animals that serve as their reservoirs (Ma et al. 2019).

6.4. ENVIRONMENTAL EFFECTS

Human beings' activities include constructing routes across the bush and moving animals or vectors (Mishra et al. 2021).

6.5. ZOONOSES WITHOUT A VECTOR

Pathologists can now identify the root cause of dying more quickly than ever before and frequently when it would have been impossible in the past, using techniques like polymerase chain reaction (PCR) testing, in situ hybridization, and immunohistochemistry (Sledzinskaet al. 2021).

Table 2: Main categories of tests used to determine an accurate diagnosis of a viral outbreak in an animal

Taxonomy	Techniques	Examples	References
Family (genus)	• Complement Fixation • Electron Microscopy • Cytopathology	GBV-A, a virus from the family Flaviviridae, is found in wild monkeys, whereas GBV-B, from the genus Hepacivirus, is found in infected tamarins. Pegivirus is the name of the fourth genus in the Flaviviridae GBV-D and GBV-C family observed in bats, rodents, horses, and pigs	(Koonin et al. 2021)
Species (types)	Neutralization	Brown bats' Eptesicus fuscus and Myotis lucifugus were examined for the presence of the rabies virus and antibodies that can neutralize it.	(Gold et al. 2020)
Subtype	Kinetic neutralization Monoclonal antibody serology	Tembusu virus (TMUV), a new flavivirus that is developing in ducks, was neutralized by an antibody response in 2010. Influenza-neutralizing antibodies A virus and the effectiveness of their preventative measures in mice	(Lv et al. 2019)
Variant	• Restriction Endonuclease • Oligonucleotide fingerprint • Nucleic acid hybridization	Dromedaries with MERS-CoV Infection variant detection are detected and in pregnant white-tailed deer, SARS-CoV-2 and its alpha form were found and also in Norway rats. Endonuclease fragments were used to find the *bla*CTX-M-15 and *bla*CTX-M-1 (CTX-M ESBL gene variants) in Escherichia coli isolates and animal faeces	(Lado et al. 2021; Cool et al. 2022; Higgins et al. 2023)
Mutant (including by point mutation)	Nucleic acid sequencing	Asiatic lions in India were infected with the SARS-CoV-II Delta mutation	(Karikalan et al. 2022)

7. VIRAL DIAGNOSIS

There are three main categories of tests used to determine an accurate diagnosis of a viral outbreak in an animal:

1. Characterization and isolation of the causative virus.
2. Measurement and detection of antibodies.
3. Direct demonstration of viral nucleic acids, viral antigens, virions, or in tissues, secretions and excretions.

There are numerous interesting diagnostic tests employing nanomaterials that are focused on the chemicals that cause animal diseases, but many of them have not yet reached a balance between specificity, sensitivity, cost-effectiveness, and repeatability. (Ramakrishnan et al. 2021). Table 2 shows main categories of tests used to determine an accurate diagnosis of a viral outbreak.

7.1. SPECIMEN PREPARATION FOR INOCULATION

Once the material has arrived at the laboratory, it should be handled and immunized as soon as feasible. If delays of more than a day are anticipated, the specimen may be frozen. Swabs are treated by rotating them in the vessel of transport and applying significant pressure to the container's side to release the substance (MacDonald et al. 2022). The excrement is dispersed using a vortex mixer. Tissue samples are meticulously cut with scissors and homogenized in a glass or mechanical homogenizer.

Table 3: The main serological methods in diagnostic virology.

Techniques of choice	Working Principle	Examples	References
Virus Neutralization	Antibody prevents cytopathology, protects animals, or decreases plaques by neutralizing the virion's infectivity.	Antibodies against the West Nile virus were discovered in wild birds from Southern Spain. Virus-neutralizing antibodies and related variables against rabies in the immunized domestic dogs of Kathmandu Valley, Nepal.	(Ferraguti et al. 2016) (Rimal et al. 2020)
Hemagglutination Inhibition	Antibody suppresses the hemagglutination of viruses.	In Kerman, Iran's southeast, dogs were used in an experiment to test for the avian H_9N_2 influenza virus's ability to hemagglutinate.	(Saberi et al. 2019)
Complement Fixation	Complement is bound by the antigen-antibody complex, rendering it effective to lyse hemolysis-sensitive sheep red blood cells.	Complement fixation test for detection of specific antibodies against bovine brucellosis in chosen peasant association in Guto Gida district, East Wollega Zone, Oromia Ethiopia	(Tosisa et al. 2020).
Immunoelectron Microscopy	Electron microscopy can reveal virions that have aggregated into antibodies.	After a single dose of immunization, immunoelectron microscopy-based immunogenicity of a recombinant VSV-vectored SARS-CoV vaccine generated strong protection in rhesus monkeys.	(Shan et al. 2022)
Immunofluorescence	The fluorescent antibody binds to subcellular antigens and fluoresces under ultraviolet light microscopy.	To investigate the seroprevalence of *Bartonella henselae* in Egyptian cats and people, an immunofluorescent test was performed	(Sayed et al. 2022)
Immunoperoxidase Staining	The intracellular antigen is recognized by the peroxidase-labeled antibody, and upon the addition of substrate, colored precipitate results.	*Brucella melitensis* in experimentally infected foetuses is diagnosed by immuno-peroxidase staining in the liver of the foetus.	(Mazlan et al. 2021)
ELISA (Enzyme-linked immunosorbent assay)	The reaction causes the substrate to change colour when an antibody or antigen that has been enzyme-labeled binds to it.	To find antibodies against *Toxoplasma gondii* in a cohort of hunting dogs, researchers used an ELISA with the modified agglutination test (MAT)	(Bellatreche et al. 2022)
Radioimmunoassay	An antigen or antibody that has been radiolabeled binds to it, such as when it is connected to the solid phase.	The ELISA test was developed based on radioimmunoassay to test samples from potentially infected animals.	(Broussard 2020)
Immunodiffusion	In a gel, soluble antigens and antibodies precipitate in clear lines.	Agar gel immunodiffusion assay for the diagnosis of canine brucellosis using Brucella ovis antigen	(Bolotin et al. 2022)

Membrane filters with an average pore diameter of 0.45m filter out contaminating microorganisms before inoculation. Then mycoplasmas can pass through these kinds of filters. The suspension needs to be refiltered using 0.22m filters to get rid of mycoplasmas after the virus has been successfully isolated and grown to a significant titer. Faeces and tissue homogenates should be diluted by at least a factor of 10 before being centrifuged at 1000g for 15 minutes to produce a filterable supernate. The virus concentration is believed to be very low, so a high dosage of antibiotics may be chosen for filtration (Diaz-Linan et al. 2021).

7.2. UTILIZING SEROLOGY TO IDENTIFY VIRAL ISOLATES

The main serological methods used to identify various viruses have been enlisted in Table 3.

7.3. COMPLEMENT FIXATION

Indirect diagnostic tests with great sensitivity like the complement fixation test (CFT) have a lot of false-positive outcomes (Elschner et al. 2021).
Inactivated serum samples are diluted twice in Complement fixation test (CFT) buffer and combined with CFT antigen and complement hemolytic units. Sera, complement, and antigen are combined in the plates and left to interact overnight at 4°C. This allows the complement to be fixed.
The plates are then incubated for 45 minutes at 37°C with a 2% suspension, then centrifuged for 5 minutes at 600g, and then the process is repeated. Samples exhibiting 25-75% hemolysis are regarded as suspicious, samples showing 100% hemolysis in a dilution are classified as negative. The classification of all suspicious test results is affirmative (Elschner et al. 2019).
Complement fixation can be utilized to classify an isolate and perform initial screening on it. Essentially a slightly streamlined version of the complement fixation of complement fixation test, the immune-adherence hemagglutination test is currently employed more commonly to identify antibodies than antigens (Coombs 2012).

7.4. HEMAGGLUTINATION AND INHIBITION OF HEMAGGLUTINATION

Numerous viral families' virions attach to red blood cells to hemagglutinate them. Hemagglutination is suppressed if antibodies and viruses are combined before the inclusion of red blood cells (Cho et al. 2022). Dissociating the virions using detergents can boost the hemagglutination level of some viruses, such as the canine distemper virus (Suarez et al. 2020). Antisera may need to be prepared to get rid of hemagglutination inhibitors that aren't specific. Test for determining the titer of antibodies to viral hemagglutinin by inhibiting hemagglutination. In titers, dilutions are stated as reciprocals (Spackman and Sitaras 2020).

8. METHODOLOGY

1. Periodate is applied to the sera, which is subsequently subjected to heat at 56°C for a half-hour to inactivate non-specific hemagglutination inhibitors.
2. After that, the sera are diluted in microtiter wells. The appropriate viral strain is then injected with hemagglutinating units into each well. Each well receives 0.05 ml of red blood cells following a 30-minute room temperature incubation period (Chivukula et al. 2021; Zhang et al. 2022).
3. Hemagglutination has been prevented where there is enough antibody to coat the virions, and as a result, at the bottom of the well, the erythrocytes condense to form a button (Saegerman et al. 2021).

Erythrocytes get agglutinated by the virus when there is insufficient antibody present. For example, the horse lacked any antibodies that could have blocked the hemagglutinin-inducing effects of this particular influenza virus strain. The first dose of the vaccine resulted in some antibody production, and the second dose induced a booster response (Drozdz et al. 2020).

8.1. NEUTRALIZATION OF VIRUS

A unique antibody may neutralize a virus's infectiousness through several different processes. To get rid of general viral inhibitors. The serum first needs to be inactivated by being heated at 56°C for 30 minutes (Cuevas et al. 2022). Suitable cell cultures are infected with serum-virus combinations. Afterward, they are allowed to develop until the virus-only controls show cytopathic effects by reducing a virus's ability to infect, an antibody shields cells against viral annihilation (Amanat et al. 2020).

8.2. RESTRICTION ENDONUCLEASE AND OLIGONUCLEOTIDE FINGERPRINTING

For the majority of routine diagnostic purposes, even to the level just mentioned, it is often not necessary to isolate antigenically. However, there are circumstances in which going further to identify differences between variants or subtypes within a particular serotype could provide viral epidemiological data. Similarly, viral DNA can be isolated from infected cells or virions, and the fragments can then be separated using agarose gel electrophoresis after being cut with properly chosen restriction endonucleases (Guo et al. 2022).

Ethidium bromide per silver staining is required to obtain restriction endonuclease fragment patterns, which are frequently referred to as fingerprints. All dsDNA virus families have found a use for methodology, particularly referred to as fingerprints. All dsDNA virus families have found use for the methodology, particularly in epidemiology research but also in pathogenesis research. These methods' resolution allows us to distinguish different isolates of the same viral species, even if they did not all come from the same epizootic, depending on the viral family. Minor amounts of genetic drift, which are typically not reflected in serological differences, can occasionally be detected using this technique (Laudermilch and Chandran 2021).

8.3. ELECTRON MICROSCOPY FOR THE DIRECT DETECTION OF VIRIONS

Electron microscopes are used for quick viral diagnosis. Negative staining techniques and understanding the concentration of virions are crucial for accuracy. Technology in the medical field is constantly advancing. This method can diagnose viral skin disease using vesicular fluid, scrapings, or scabs. It can also be used for accurate diagnosis in cell culture (Hopfer et al. 2021).

8.4. IMMUNOELECTRON MICROSCOPY

It is a technique that uses immune serum to increase the susceptibility of electron microscopic techniques. After mixing the sample with the antibody for an overnight period, the sample is typically cleared by low-speed centrifugation.

The immune complexes are subsequently centrifuged at a low speed to form pellets, which are then negatively stained. The antibody used could be a mixture of these antibodies, serum from an old animal that is hyperimmune to many viruses, or type-specific monoclonal or polyclonal antibodies (Zhang et al. 2022).

8.5. DIRECT IDENTIFICATION OF VIRAL ANTIGENS

These techniques rely on the direct interaction of viral particles, or antigens, with specific antibodies that have been prelabeled in some way to quickly identify the interaction, in situ in tissues, excretions, or secretions. The labeling techniques used—are immunoperoxidase staining and radioimmunoassay.
The labeling methods are divided into four categories: radioimmunoassay, immunoperoxidase staining, immunofluorescence, and ELISA. Viral antigens can also be identified using two proven serological techniques, precipitation and complement fixation (Bassani-Sternberg et al. 2016).

8.6. IMMUNOFLUORESCENCE

Immunofluorescence is a method of unique significance in the quick identification of viral infections due to its specificity, speed, relative simplicity, and sensitivity (Zhang et al. 2022).
The classic instance of immunofluorescence is the diagnosis of rabies, for which immunofluorescence has been recognized as diagnostic for more than 20 years (Chiebao et al. 2019).

9. STAINING WITH IMMUNOPEROXIDASE

An alternate approach involves using an enzyme-labeled antibody to locate and detect viral antigens in infected cells. The process results in a pore-durable, non-fading, and anatomically clearer preparation than immunofluorescence, and it uses less expensive equipment. Similar steps and ideas apply to immunofluorescence (Burrell et al. 2017).
The conjugated antibody, bound to the antigen through a direct or indirect procedure, is detected by adding a substrate suited for the specific enzyme. In the case of peroxidase, this is H_2O_2 coupled with a benzidine derivative. Endogenous peroxidase, which is found in many tissue cells, especially leukocytes, causes false positive results, which is a drawback of the method. By using a diligent approach and proper controls, this issue can be avoided (Arshad et al. 2022).

9.1. RADIOIMMUNOASSAY

A radioactive element, most frequently 125I, serves as the label in radioimmunoassay. The technique is incredibly sensitive, allowing the detection of viral antigens at low concentrations ranging from 10–12M. There are numerous radioimmunoassay techniques available. The principles are the same for both direct and indirect approaches as for immunofluorescent staining.
In the most basic configuration, a sample that might contain a virus or viral antigen is allowed to attach to the bound antibody, washed, and then an antiviral antibody is measured in a gamma counter after additional washing (Burrell et al. 2017).
A more popular approach is indirect radio-immunoassays, which include a second layer of 125I-labelled anti-IgG as an indicator antibody in place of the detection antibody. Different animal species must be used to generate the antiviral antibodies that make up the capture and detection antibodies (Inoue et al. 2010).

9.2. DIRECT ISOLATION OF LEPTOSPIRES FROM CLINICAL SAMPLES

A common method for identifying Leptospira directly from clinical samples is dark-field microscopy (DFM). Early detection of leptospires in bacterial infections is crucial. The direct fluorescent antibody (DFM) is

highly sensitive for detecting leptospires in both blood and CSF, with a sensitivity rate of 64.7%. DFM is a good way to diagnose the early stages of an illness, with high sensitivity in identifying leptospires in blood and CSF. A skilled specialist is needed and 100 fields on each slide must be studied to deem it negative. As the illness progresses, DFM's sensitivity may decrease.

9.3. LEPTOSPIRES' CULTURE

A Bunsen burner will be used to aseptically add blood samples dropwise to the semi-solid medium that has been prepared. Within 24 hours, the sample-containing medium will be switched over to the liquid media. Every day for three months, each tube will be examined for the development of leptospires, and after every 2-3 weeks, they will be routinely switched to a new medium. The media developed by Stuart, Korthoff, and Fletcher is also utilized for culture. Although cultivation is the most common method of detection, its application for routine isolation is debatable because it requires a longer period for development, with a gestation period of 6–20 hours (Hornsby et al. 2020).

9.4. SEROLOGICAL METHODS

The serum of people who have contracted Leptospira can be tested using a variety of serological assays for antibodies against the parasite. We can detect IgG and IgM antibodies with MAT and ELISA- based test kits, and a wide variety of commercial fast diagnostic card tests that are already on the market (Trozzi et al. 2023).

9.5. MOLECULAR TECHNIQUES

Molecular methods can quickly and accurately diagnose infections, including leptospires, in medical or environmental samples (Girones et al. 2010).

9.6. POLYMERASE CHAIN REACTION (PCR)

Due to its specificity, sensitivity, and capacity to identify even the smallest amounts of nucleic acid particles. PCR is the best way to identify infections. Quantitative PCR is better than MAT or culture for quick and reliable results (Wood et al. 2019). This finding has important implications for medical and epidemiological analyses of this worldwide neglected disease. Leptospira is also detected using a variety of PCR techniques, including nested PCR, randomly primed PCR, etc. The drawbacks of PCR are similar to those of other diagnostic techniques and include complex lab requirements, degradation, false-positive results, initial validation, etc. These problems necessitate highly competent and trained personnel as well as a significant financial commitment (Hoorfar et al. 2004).

9.7. QUANTITATIVE PCR

PCR techniques detect Leptospira, but have limitations such as specialized equipment, sample degradation, and false positives. qPCR (real-time PCR) is preferred for accurate results in a short time by monitoring the DNA amplification rate. To evaluate disease severity, bacterial DNA amount and density must be assessed, often through targeting the LipL32 gene. qPCR is a reliable method for the early detection of Leptospirosis but is Challenging to implement in low-resource labs due to the required skilled personnel and expensive equipment (Ruijter et al. 2021).

9.8. IMMUNOCAPTURE-POLYMERASE CHAIN REACTION

New techniques have been developed to quickly identify leptospires from clinical samples, including the immunocapture-polymerase chain reaction methods created in 2018 using ELISA and PCR. A combination of molecular and serological methods is important for an accurate diagnosis of leptospires. The IC-PCR technique is a powerful tool that rapidly identifies leptospires and provides crucial information about specific serovars or serogroups present. This study shows it to be more accurate than traditional PCR methods (Jian et al. 2020).

9.9. OTHER MOLECULAR METHODS

Other molecular methods such as RAPD (random amplified polymorphic DNA), nucleic acid probe techniques, DNA-DNA hybridization, REA (restriction enzyme analysis), fingerprinting, PFGE (pulsed-field gel electrophoresis), etc., are available in highly developed laboratory settings. They support research into and comprehension of genetic diversity and genomic diversity profiles.
Several diagnostic techniques are available to detect and diagnose Leptospirosis, including ELA (IgM-Enzyme Immunoassay), MCAT (microcapsule agglutination test, LEPTO Dipstick, macroscopic SAT, IHA (indirect haemagglutination assay), and LEPTO Dri-dot. However, these rapid tests are less sensitive and specific than IgM rapid and immunochromatography techniques, despite the availability of several serological rapid tests.

CONCLUSION

Our ability to diagnose the cause of the disease is greatly improved by following all the stages consistently and sequentially. This method not only increases the scientific knowledge base but also helps zoo employees conduct retrospective studies and aids other researchers with the new database. It also enhances the ability of the zoological community to recognize disease trends and enables researchers to identify diseases that pose a threat to captive and wild animals.

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