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One Health Triad

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ONE HEALTH TRIAD
Volume 3

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PREFACE

The well-being of humans and animals is pretty much interdependent. It's impossible to ensure human health, food security and food safety, and welfare without considering animal and environmental health.

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, human and environmental health. This book provides the core concepts of One Health approach with a critical focus on the key challenges i.e., zoonotic diseases and environmental degradation. The objective is to cover epidemiological interactions of various infectious diseases and their environmental and 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, human and environmental 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

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Mosquito-Borne Dengue Fever-An Update

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INTRODUCTION

Globally, dengue fever (DF) is a highly endemic contagious disease and has a significant socioeconomic and health impact on many tropical and subtropical regions. Pakistan is one of the most affected countries for the past two decades with the first outbreak reported in 1994 (Nasir et al. 2022). This mosquito-borne viral infection characterized by nausea, headache, weakness, severe muscular and joint pain, lymphadenitis, and skin rashes. Swollen palms and soles, gingivitis, and intense eye pain are only a few symptoms of dengue fever. Dengue fever has the potential to worsen and develops into a more severe form named dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) (Gan et al. 2021; Rajeen and Mayurathan 2022).

Four serotypes of dengue have distinct epidemiological patterns and they can co-circulate within an area and many countries are hyper-endemic to these serotypes. Dengue has huge impact on human health and the world economies. According to an estimate, 390 million people are affected by dengue virus infections (95% credible interval 284–528 million) with over 25,000 deaths/year globally, of which 96 million (67–136 million) manifest clinically. According to WHO, the number of dengue cases increased over 8 times since 2000 from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Moreover, reported deaths augmented from 960 to 4032 within this period, affecting mostly younger age group (Stica et al. 2022; WHO 2022).

Geographic Distribution

The epidemiology of vector-borne diseases is directly influenced by climate change. Scientists agree that dengue viruses first infected monkeys in Africa or Southeast Asia between 100 and 800 years ago before transmission to humans. However, the spread of viruses was greatly due to the global transfer of Aedes mosquitoes that occurred as a result of World War II. Dengue fever (DF) is thewidest spread vector-borne disease worldwide, with the highest disease burden (Kulkarni et al. 2022). The region of Southeast Asia experience recurrent and cyclical epidemics of dengue throughout the year. Geographical location, time and demography also indicate the prevalence of dengue fever. Presently, the clinical worth of deceptive dengue infections remains undetermined, but it is supposed that deceptive dengue plays a vital role in the transmission of dengue in the absence of an epidemic (Gan et al. 2021).

Etiology

The dengue virus is a single strand RNA genome of ~11 kb, and translated into a single poly-protein. It belongs to the flavivirus genus and *Flaviviridae* family. The genome RNA encodes 3 structural protein molecules (Capsid, pre-membrane, Envelope) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The 4 strains of closely related serotypes named DEN-1, DEN-2, DEN-3, and DEN-4 are reported that vary in antigenicity (Kothai and Arul 2020). There are several different types of flaviviruses, including the tick-borne encephalitis virus (TBEV), the Japanese encephalitis virus (JEV), and West Nile virus. DENV, Yellow Fever Virus (YFV), and Zika Virus (ZIKV) are transmitted by arthropods or arboviruses (Higuera and Ramírez 2019).

During DENV replication, virion binds itself with the surface molecules of cells and receptors; still this binding has not been fully identified. Then virus is internalized through receptor mediated endocytosis. Glycoproteins on the virus surface involves in the fusion of viral membrane and cellular membrane at low pH of endosomes. This situation enables the virion to disassemble and release its RNA into the host cell cytoplasm. After that viral RNA is translated into polyprotein with the help of cellular and viral enzymes (proteases). Hence, non-structural proteins of dengue virus are accountable for viral RNA replication (Chan 2021).

The core reason of dengue fever infection is an infected *Aedes* (*A.*) *aegypti* mosquito bite, and in addition to this, vertical transmission may also be acquired accidentally, especially from pregnant women via placenta, blood products (infected), organ transplantation, and also due to needle stick injury (Kothai and Arul 2020).

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Pathophysiology

There are still many unknown facts regarding DENV pathogenesis and the host immune response. Dengue fever is an acute serious condition characterized by high-grade fever with frontal headache, myalgia, as well as nausea, vomiting, and rash that affects adults and older children. The main symptoms of the disease include leukopenia, thrombocytopenia with hemorrhagic tendencies, capillary leak syndrome, bleeding in the nose, gastrointestinal tract, and gums (Kathiriya et al. 2020; Kalimuddin et al. 2021). The viral envelope glycoprotein presents in the virus aids in attachment to host cells. Infected cells, such as monocytes, are a primary target of cytokines that drive innate immune responses to DENV via three mechanisms. (a) During localized infection of the skin, the dengue virus triggers degranulation of mast cells and releases inflammatory mediators such as proteases, leukotrienes, and histamine which promote edema at the injection site and increased vascular permeability. (b) During systemic infection, viremia occurs due to elevated levels of mast cell products in serum and the release of TNF, leukotrienes, and vascular epithelium growth factors (VEGF) that enhance vascular leakage from plasma. (c) During secondary infection, antibodies mediated enhanced (ADE) enhanced MC degranulation through crosslinking of FERC. Studies have shown that MCs are activated by endogenous products that lead to the degranulation of mast cells and mosquito saliva co-injected with arboviruses (Imad et al. 2020; Sugianto 2021).

Transmission

All four serotypes of DENV are transmitted to humans by a single bite of infected female mosquitoe, mainly the *A. aegypti* mosquito, and the infected person's blood results in viremia in an early illness that lasts for 2 to 12 days. Approximately 8 to 10 days later, the virus is released into the mosquito's saliva and transmitted to other tissues, including the salivary glands. When it bites another person, the mosquito's saliva spreads the infection to that person (Fig. 1). The mosquito is unaffected by the virus in any way (Gwee et al. 2021). It has also been documented that vertical transmission (from mother to child) of DENV is a considerable risk for adverse pregnancy (Chawla et al. 2014). The various reported cases of DENV infection through different routes has been mentioned in Table 1.

The Virus

Aedes mosquitoes especially *A. aegypti* are primary vector of the dengue virus. The typical range for these mosquitoes is round about 35° N and 35° S while altitude is approximately 3300 ft. They frequently bite in the morning and evening. This virus primarily affects humans but can also elicit primates from another genus. DENV is a positive single strand RNA genome constituting four unique serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). The genome encodes 3 underlying proteins (capsid [C], pre-membrane [prM], and envelope [E] and seven nonstructural proteins [NS]) by viral proteases and the host (Huang et al. 2014). Within every serotype, particular genotype or heredities have been recognized and exhibiting the most hereditary fluctuations in dengue serotypes. Nonetheless, determination keeps on being a prevailing topic in the development of dengue virus. Secondary dengue diseases are frequently connected with European genotype like DENV-2 and DENV-3 (Roy and Bhattacharjee 2021).

The Vectors

Individual serotypes of dengue virus are transmissible through a bite of contaminated female Aedes mosquitoes to people, particularly *A. aegypti.* It generally found in north of 1000 m because of low temperature. The undeveloped stages of *A. aegypti* are found around stagnant water that is closely linked to human dwellings (Tedjou et al. 2019). Research proposes that most of the females may spend their whole life in or around human dwellings where grown-ups arise. *A. albopictus, A. polynesiensis* and a few kinds of *A. scutellaris* are accredited the incidents of the dengue (Ononamadu et al. 2021). Every one of these genera has a particular natural, social, and topographical distribution. *A. albopictus* has taken many years to spread from Asia to Africa, America, and Europe, because their eggs can stay adapted for a long time, without any trace of water (Kothai and Arul 2020).

The Host

After incubation period of 4-10 days, contamination due to any of the four viruses serotype can cause a large numbers of diseases, mostly asymptomatic or subclinical (Krishnamoorthy et al. 2022). Primary infection is responsible for long term defensive behavior of a body's immune system against serotype infections. From 2 to 3 months after primary infection, without any long term cross-defensive resistance, people experiencing contamination are sheltered from clinical illness with a particular serotype (Wei Xiang et al. 2022). In the course of primary infection in infants and secondary infection, antibody-dependent enhancement (ADE) of the infection has been assumed as a mechanism of action to define the severity of dengue (WHO 2022). According to this model, cross-reactive and non-neutralizing antibodies are composed that bind with epitopes present on the surface of heterologous infective virus during primary infection and facilitate the entry of the virus in the Fc-bearing cells. Viral load increases with expanding infected cells and activate the host immune response like mediators which results in the capillary leakage. During secondary infection, memory T cells (cross-reactive) are triggered and further proliferate to release cytokines and correlate the

Table 1: Reported healthcare-associated transmission of dengue virus

Virus	Routes of transmission	Comment	References
Dengue	Blood transfusion	Donated blood, from which RBC's transfused recipients; fever and myalgia developed after 3 days of transfusion and was detected with DENV-2.	(Perera et al. 2020)
	Bone marrow transplant	A bone marrow donation caused the death of a 6-year-old Puerto Rican who infected with DENV-4.	(Bhat et al. 2018)
	Needle-stick	Several medical professionals became infected after needle-stick injuries during care of returned travelers diagnosed with dengue.	(Grobusch et al. 2020)
	Renal transplant	Dengue hemorrhagic fever developed in renal transplant recipients.	(Delfino and Mazzali 2022)
	Mucocutaneous	A medical professional became infected with DENV-3 after being splattered in face by blood from a febrile traveler return from Peru diagnosed with dengue.	(Ullah et al. 2019)

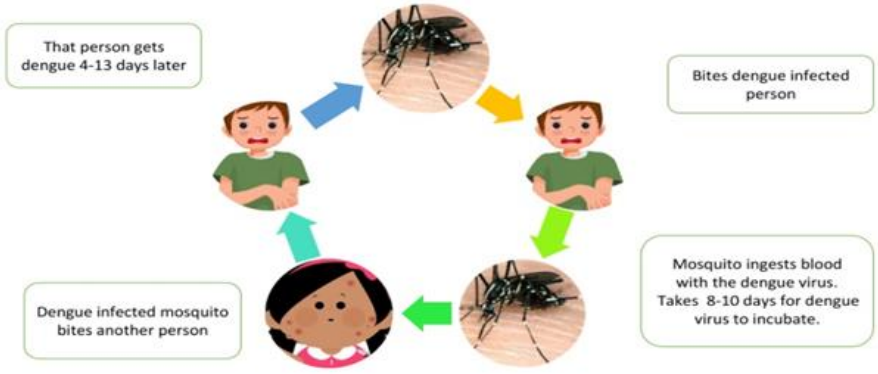

Fig. 1: Transmission of dengue fever

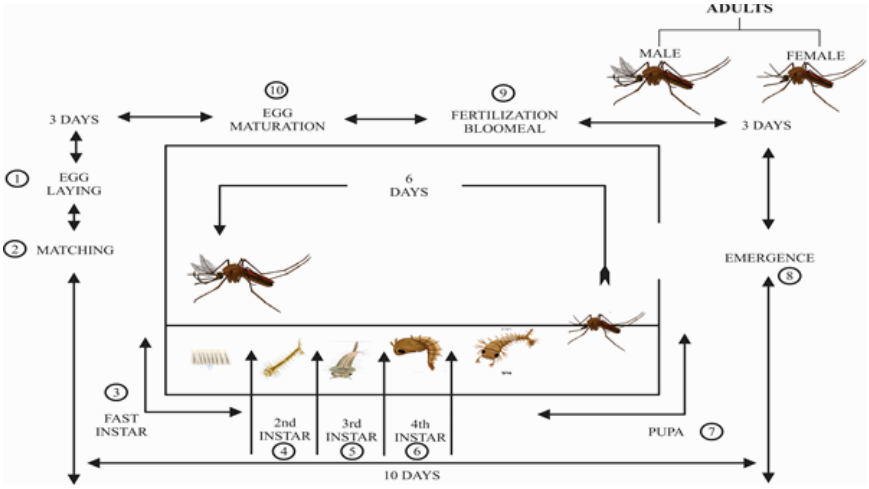

Fig. 2: Life cycle of *A. aegypti*: Female *A. aegypti* lays eggs on the inner walls of artificial containers. As the containers fill with water, mosquito larvae hatch from the eggs. The larva metamorphose into pupa after four larval stages which are named as four instars

severity of the disease. Research studies show that dysfunction of endothelial cells can mediate plasma leakage and can also be linked with the augmentation of infected T cells, monocytes, monokines, cytokines, complement system and generation of mediators (Uno and Ross 2018).

Life Cycle of *Aedes aegypti*

A. aegypti is a primary vector of viruses that cause dengue fever. It is geologically distributed in tropical and subtropical areas and utilizes an abundance of artificial containers for breeding (Tedjou et al. 2019). *A. aegypti* is a polymorphic type of arthropods that undergoes complete metamorphosis. An adult's life span ranges from 2-4 weeks depending on the environmental temperature and climate. During entire life, a female member lays ova about 4-5 times (Fig. 2). There are three polytypic forms of *A. aegypti* that have been found including (a) sylvan type which is a rural form that reproduces in forests, especially in tree holes, (b) domestic in urban habitats, and (c) peridomestic form that breeds in ecologically modified areas (Calma and Medina 2020).

Manifestations

Three clinical forms have been found such as dengue fever, dengue shock syndrome and dengue hemorrhagic fever in individuals infected with dengue (Kothai and Arul 2020).

Most dengue virus infections are not symptomatic which means that when a patient with fever has only mild symptoms, DENV is not yet recognized as the infection's primary cause. With the influenza-like dengue fever and dengue shock syndrom, each of three clinical presentations has a different level of symptom severity. In many cases, dengue virus infections may sometimes be fatal or life-threatening and develop to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) while mild febrile DF is often not lethal (Umakanth and Suganthan 2020).

Typically, symptoms start to manifest within 3-10 days during the incubation period. The clinical manifestations for DHF and DSS range in intensity from minor symptoms to severe life-threatening symptoms. Due to the ambiguous clinical presentation and lack of knowledge on the pathophysiology and molecular pathways underlying the disease, predicting the transition from mild symptoms to severe DHF/DSS is still challenging (Kothai and Arul 2020). According to the WHO, febrile episodes that are about 40°C for 2-7 days are characteristic of DF and are frequently accompanied by rash, nausea, vomiting, and headache. The severity of the preceding symptoms may increase after 3 to 7 days, along with the appearance of new symptoms such as abdominal pain, nasal bleeding, insomnia, and restlessness. Leukocyte counts are often increased and hepatic aminotransferase activity is mildly enhanced in instances of mild dengue fever, according to laboratory testing. If no therapeutic measures are adopted when these symptoms first appear, the disease will proceed to a severe form (DHF/DSS). Clinical interventions at this stage and ongoing monitoring are required, especially in the endemic area, to stop vascular leakage (Ahmad et al. 2020).

Any of the four identified DENV 1-4 serotypes causes severe dengue infection. Individuals having a history of dengue infection with a heterogeneous serotype are more likely to develop DHF/DSS. Severe DHF/DSS may affect 5–10% of the patients, and if left untreated, it can be fatal. Significant bleeding especially from the digestive system is another feature in addition to thrombocytopenia (50,000/mm^3), which may affect up to 50% of DHF cases. Remarkably, the quantity of platelets in the blood and the incidence of DHF are negatively correlated. Further, the precise mechanism responsible for this correlation is still being investigated. Vascular fragility is a result of decreased platelet numbers, loss of function and other factors and it may increase the possibility of bleeding and plasma leaks (Umakanth and Suganthan 2020). The DENV induces thrombocytopenia by direct contacting with megakaryocytes and platelets which in turn inhibit or activate platelet counts. Deep shock, also known as dengue shock syndrome, can be brought on by hypotension and systolic pressure that persist. DSS that lasts a long time can increase the risk of developing further issues such excessive bleeding, diffuse intravascular coagulopathy (DIC), respiratory arrest, multi-organ failure, and rarely meningitis and encephalopathy that results in death (Madi et al. 2014).

Along with the normal symptoms, dengue can also have an impact on a number of other bodily functions like dengue encephalopathy is earlier considered to be exclusively linked with dengue hemorrhagic fever and dengue shock syndrome (Trivedi and Chakravarty 2022). The Guillian Barre Syndrome (GBS) and transverse myelitis are two more neurological diseases that resemble with the dengue. The course of dengue infection is further divided in to three phases such as febrile, serious and recovery as mentioned in Table 2 (Kothai and Arul 2020).

Diagnosis

The possibility of a prompt and accurate diagnosis is occasionally exacerbated by the fact that the manifestations of DF are identical to several other diseases such as typhoid fever or malaria. Diagnosis of dengue initiates with a clinical sign of febrile phases of illness, dengue patients often have fever accompanied by nausea, body pain, maculopapular rashes, bleeding nose, and gums (WHO 2022).

In order to effectively combat the disease, it is crucial to make an early and accurate diagnosis of dengue infection in the laboratory. According to estimates, up to 50% of dengue cases could go undiagnosed. This is particularly for those who reside in or travel to locations where tropical infectious diseases are widespread, the signs and symptoms of dengue differ vary greatly from those of other viral infections. Avoiding severe instances and reducing the financial burden of the illness until an availability of anti-viral vaccine, is crucial for diagnosis in early and accurate manner (Kothai and Arul 2020).

The major advance laboratory tools used for detecting dengue infection involve; (a) nucleic acid amplification tests (NAAT) to identify the specific virus serotype; (b) genomic sequences and viral isolation from mosquito cell lines and (c) ELISA to detect antigen and antibodies (Huang et al. 2014). For early detection of dengue infection, two screening methods; direct and indirect approaches have been used. The former is used for detection of NS1 antigens and viral RNA from patient's blood infected with viremia in case of acute febrile phase. The latter is used in post febrile phase where IgG and IgM antibodies are detected by Mac-ELISA. The rapid and reliable method used for diagnosis of dengue virus is the extraction of RNA from blood, serum, tissues, saliva, and urine and perform reverse transcriptase PCR (RT-PCR) (District 2019).

For the first time, the neutralizing antibodies measured by neutralizing test was developed by Russell named as Plaque Reduction neutralizing tests (PRNT). The neutralizing antibodies inhibit dengue virus infection and offer greater specificity in separating DENV- specific antibodies from those that are cross reactive *flavivirus* antibodies. Since PRNT requires a lot of labor, takes a long time and has a low throughput, it is not frequently employed in dengue diagnosis

Table 2: Phases of dengue infection

Phases	Symptoms	Duration
Febrile	High grade fever, headache, vomiting, rash	2-7 days
Serious	Organ dysfunction, fever, severe bleeding from GIT, DSS and DHF	1-2 days
Recovery	Serious pruritus, bradycardia, maculopapular rash,	2-3 days

Table 3: Laboratory diagnostics for dengue with specimen and sensitivity.

Diagnostic methods	Technique	Specimen	Sensitivity
Antibody detection	IgM detection	Serum, plasma, whole blood	61.5-100
	IgG detection		46.3-99
	Rapid IgM detection		20.5-97.7
Antigen detection	Viral antigen detection (NS1)	Serum, plasma	54.2-93.4
Antigen-antibody combined detection	NS1 and IgM	Serum, whole blood	89.9-92.9
	NS1 and IgG/IgM		93.0
Viral detection	Virus isolation (cell culture)	Whole blood, serum, tissues	40.5
	Viral RNA RT-PCR		58.9-100
	Viral RNA (NASBA) RT-PCR		98.5

Table 4: Natural sources activity against *A. aegypti*

Plant	Common Name	Part Used	Reference
Boesenbergia rotunda	Temukunci	Roots used to make paste	(Akram et al. 2021)
Kaempferiaparviflora	Thai ginseng	Leaves and stem	(Balaji et al. 2022)
Carica papaya	Papaw	Leaves	(Teh et al. 2022)
Solanumvillosum	Red nightshade	Berry	(Siam et al. 2022)
Combretumcollinum	Weeping bushwillow	Shoots	(Schultz et al. 2021)
Azadirectaindica	Neem	Leaves	(Dwivedi et al. 2021)
Citrus limetta	Sweet lemon	Peel extract	(Bailão et al. 2022)
Acalyphaalnifolia	Copper leaf	Leaf	(Subbiah 2021)
Delonixelata	White gulmohur	Leaf	(Suresh et al. 2020)

even though it is still the assay for immunity studies that is most frequently utilized (Lima et al. 2022). In order to get around the limitations of PRNT, newer tests have been created such as the ELISA-based spot and microneutralization test, the fluorescent antibody cell sorter that based on dentritic cell specific intercellular adhesion of molecule-3-grab-bing Non-integrin expressor DC assay. Immune fluorescence test, capture ELISA and hemagglutination assays are used for the diagnosis of DENV infection in early stage by using hematological and biochemical indicators (Limkittikul et al. 2022). Laboratory diagnostics for dengue with specimen and sensitivity has been mentioned in Table 3 (Lima et al. 2021; Alidjinou et al. 2022).

Treatment of Dengue Fever

Currently, to cure the dengue fever no specific treatment is available. Typically, the fluid replacement along with the use of analgesics and proper rest is satisfactory. Acetaminophen can be used for the treatment of fever. The use of drugs like corticosteroids, aspirin, and NSAIDs should be evaded (Kellstein and Fernandes 2019). Research studies have been carried out by Novartis Institute for Tropical Diseases (NITD), Singapore to find out the inhibitors of target proteins of dengue virus to decrease the load of virus in active infection. The acute form of dengue fever necessitates fluid therapy and treatment of hemorrhage. The patients with dengue shock should be admitted to an intensive care unit. Ringer lactate which is an isotonic solution could be used in patients who are deficient in intravascular volume (Yokokawa 2020).

A hemostatic drug such as carbazochrome sodium sulfonate (AC-17) (due to capillary stabilizing activity) reduces the high permeability of blood vessels. This vascular hyper-permeability may be induced by vasoactive components via an agonist induced inhibition of phosphoinositide hydrolysis. Fluid therapy is used in the critical phase. There is inadequate evidence regarding the quantity and fluid selection. Fluids which could be used to increase the volume are 5% albumin, normal saline, plasma or plasma substitutes, ringer lactate and 5% glucose diluted in ratio 1:2 or 1:1 in normal saline (Hasan et al. 2016).

The fluid therapy is based on the principles comprising oral as well as intravenous fluid intake depending upon the condition of the patient. The purpose of this fluid therapy is to prevent hypovolemia. However, the excessive fluid therapy is prohibited. Crystalloids like 0.9% saline are recommended as first line I/V fluids (Kajimoto and Kitajima 2020). The intake of I/V fluids in patients can be increased gradually to minimize the risks. The use of acetaminophen prevents the use of NSAIDs such as acetylsalicylic acid and ibuprofen because of their increased risk of bleeding. The patients with reduced hematocrit should be transfused with blood (van Bergen et al. 2022).

The drugs obtained from natural sources have larvicidal and mosquitocidal activity against *A. aegypti*. The important natural cures are mentioned in Table 4.

Prevention and Control

In December 2015, Sanofi Pasteur was licensed to develop the first dengue vaccine Dengvaxia® (CYD-TDV) which is now approved by regulatory authorities in almost 20 countries. Additional analysis was performed in November 2017 to find out the serostatus at the time of vaccination release. The results of the study showed that the group of volunteers (without prior dengue virus infection) who participated in the trial study were deduced to be seronegative at their first vaccination and had a great risk of severe dengue and hospitalization in comparison to unvaccinated participants. Therefore, use of CYD-TDV vaccine is allowed for 9-45 years old people with laboratory established prior dengue virus infection (WHO 2022). The risk of dengue infection is increased in seronegative vaccinated individuals because they are exposed to natural dengue infection for the first time as the live-attenuated Dengvaxia® triggers an initial immune response to dengue. Strategic Advisory Group of Experts from World Health Organization (WHO) updated its recommendations from April 2018 assuming that pre-vaccination screening method must be recommended for nations contemplating CYD-TDV immunization, in which only people who are seropositive for dengue can be immunized. In 2019, Food and Drug Administration also approved Dengvaxia® as dengue vaccine (Biswal et al. 2022).

Currently, avoiding the bite of vector mosquito is the only way to avert dengue virus. This could be done by avoiding traveling to the areas where dengue is endemic. Mosquito netting is also used but its use is not much beneficial because *Aedes* bites during daytime. The mosquito indoor sprays can also be used for elimination of mosquito (Wang et al. 2020). Recently, non-chemical techniques have been categorized as "biopesticides," which simply refers to eradicating the pathogen with substances derived from living creatures. To find a powerful agent, it is necessary to investigate biological control agents such as diverse predators and parasites, i.e., viruses, fungus, bacteria, etc. The use of different viruses and predators as biological mosquito control agents has been documented. Wolbachia is an intriguing prospective new dengue biocontrol method against Wolbachia infection uses inherited endosymbiotic bacteria to make mosquito populations resistant to arboviruses and exhibit low significance against vector (Ritchie 2018).

Conclusion

Dengue fever is a rising public health issue throughout the world. For disease prevention, all dengue-endemic countries require more effective surveillance systems. A vaccination is urgently needed to reduce dengue fever-related morbidity and mortality. Several medicinal plants have been identified that have significantly inhibited response towards dengue but still effective and proper treatment needs to show positive and therapeutic outcomes. In addition, distinct serotypes in dengue endemic can be managed with the help of respective vaccine.

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2

Tick-Borne Encephalitis - A Threat to Life

AUTHORS DETAIL

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INTRODUCTION

Tick borne encephalitis is a serious arbo-viral zoonotic infection in human affecting their Central Nervous System (CNS) and commonly found in Asia and Europe (Ruzek et al. 2019). The virus is transmitted by Ixodes ticks spp. and taxonomically belongs to the family Flaviviridae and genus Flavivirus (Simmonds et al. 2017). Transmission of virus typically occurs during the infestation of tick hence, the incidence of TBE is linked with expansion of these ecto-parasites (Salat and Ruzek 2020). Additionally, it is also transmitted through ingestion of TBEV-infected milk and milk products. Sheep, goat, horses, dogs, rodents and other animals is its reservoir host and human are dead end host (Buczek et al. 2022). The most serious form of TBE virus is inflammation of brain and spinal cord known as encephalomyelitis (Gritsun et al. 2003).

It was earliest narrated in Austria and detached in Russia, in the years 1931 and 1937 respectively (Valarcher et al. 2015). TEBV is an enveloped, spherical, positive sense, RNA (single stranded) virus and roughly 50 nm in width. It is appeared in three distinct forms viz. mild, moderate and severe. This viral genome is encoding one polypropylene that split into 3 structural (C, M, E) and seven non-systemic proteins. Its nucleo-capsid is comprised of viral nucleic acid and capsid protein C, which is enveloped by a lipid protein consisting of protein M and E (Füzik et al. 2018). The principal part of viral exterior surface is Protein E and take part as virus-neutralising antibodies while post infection (Heinz and Stiasny 2012).

TBE is transmitted both transtadially and transovarially between their developmental stages. Ticks have long life cycle and TEBV have ability to survive throughout their developmental stage yet, its cycle is affected by certain factors as microclimate, host factor and environmental changes. In the winter season, some tick's activity become limited. Furthermore, ticks mostly active in plantation weathers with sufficient amount of moisture and increased temperature. During moulting, it's size contract with discharge of water and toughness of skin and until the upcoming spring, ticks develop itself for cold season (Wondim et al. 2022).

Etiology

Tick-borne encephalitis (TBE) is a serious infectious disease that affects the central nervous system (CNS) of animals and humans (Ruzek et al. 2019). About 10,000 to 15,000 cases are reported in Europe and Asia annually (Bogovic and Strle 2015). TBE virus (TBEV) is the causative agent of the disease, that represents arboviruses, including viruses, which are transmitted by blood-sucking arthropods. Phylogenetical character of the virus relates it to the Flaviviridae family and genus Flavivirus (Simmonds et al. 2017). TBEV includes 3 sub-types namely:

1) The European subtype that is transmitted by *Ixodes* (*I.*) *ricinus* ticks
2) The Far eastern subtype that is transmitted significantly by *I. persulcatus* and
3) The Siberian subtype that is transmitted by *I. persulcatus.*

The viral genome is a single-stranded RNA genome that encodes one polyprotein and split into three structural viz. C, M and E and seven non-structural proteins. The nucleocapsid of the virus consists of the viral nucleic acid and capsid protein C. The nucleocapsid is enveloped by a lipid membrane containing two proteins i.e., M and E (Füzik et al. 2018). Protein E is the main surface antigen, which allows the host cells to mediate infection by binding with the surface receptors (Heinz 1986).

Epidemiology

This virus is endemic in Russia, Mongolia, central, eastern and northern Europe, northern part of the China and Japan. According to a survey, about 170,000 cases of humans have been appeared in Europe and Russia since 1990 to 2009 (Suss 2011). This virus has three subtypes that is prevalent across the Eurasian continent i.e. the Western European subtype previously known as central European encephalitis virus, commonly found in the regions of central, eastern and northern Europe (pastoral and woodland), where *I. ricinus* is the main vector; the Siberian subtype earlier called as West

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Siberian encephalitis virus, typically present in Ural region, far-eastern Russia and north-eastern Europe, where *I. persulcatus* is the main vector responsible for disease transmission; and the far-Eastern subtype previously named as Russian spring-summer encephalitis, indigenous in the far-eastern Russia and some woodland of Japan and China. It is also transmitted by *I. persulcatus* (Valarcher et al. 2015).
According to survey of 2000-2019, 51,519 confirmed cases have been reported in Europe, though the number of cases get declined during the years 2014 and 205 but after 2015, instances of cases have climbed again. The main reasons for the prevalence of TBEV are host community, movement of host, environmental conditions and traveling of people around foci area. Overall, mean incidence rate was 3.27 in this entire period (2000-2009) (Wondim et al. 2022).
It is reported in 28 different countries around the globe and recent presence of TBEV virus in north Europe indicates the disclosure of new foci of TBE (Wondim et al. 2022). Its distribution is not constant and the data is still insufficient in some countries i.e., Germany and Austria, where information regarding TBEV virus is inadequate and their reporting habits differ from geographical and historical reasons (Dobler et al. 2012). Therefore, a lot of research needs to be done on this virusotherwise it will get prevalent across the globe and become threat for the human health.

Pathogenesis

Tick bite is considered as a significant source of TBEV infection rather than the consumption of unpasteurized dairy products. After infected tick bite, virus replicate first at the inoculation site, afterwards drain into lymphatic system. Virus has been found in dermal, Langerhans and dendritic cells that is the primary site of infections before enter into regional lymph nodes. Plasma viremia occurs after replication of virus inside the lymph nodes. From this site, virus reach to different tissue i.e., spleen, liver and bone marrow via heamatogenic route that results inflammation, lysis and cellular dysfunctions (Ruzek et al. 2010).
Significant proportion of virus tires are required to cross the blood brain barrier. Patients having small number of TBEV specific antibodies, rarely neutralizes the titres to avoid CNS infection, consequently, virus replicate at neurons target site and cause inflammation, cellular lysis, necrosis, apoptosis and cellular dysfunction. This infection leads microglia and TBEV-specific T lymphocyte migration toward CNS, particularly to the grey matter and prone to immunopathogensis at the infected sites. In lethal state, it also affects spinal cord, brain stem and cerebellum (Mansfield et al. 2009).

Transmission

In every active developmental stage, ticks can be infected with TBE virus. After entering in ticks, this virus localized in tissues, salivary glands and ovaries. It's presence in ovaries indicates that transovarial transmission are common in ticks. Moreover, the virus present in the entire organism, transstadial transmission is also plausible (Ličková et al. 2020).
Larvae are infected with virus via transovarial transmission. Furthermore, larvae and nymph are also get infection while co-feeding the same rodent host and keep their infection after molting in the subsequent stage through transstadial transmission. Once infected, ticks carry that infection throughout their lives. Mammals are infected by tick's bites or contact with the wounds having eggs of infected virus. Virus attached with saliva enter into mammals and reach their organs. Its incubation period is 7-14 days depends on the host species and their immunological conditions. During this entire period, virus multiplies and spread to entire organisms. This make horizontal transmission possible between co-feeding ticks species. Apart from this, transmission is also spread via milk and milk products obtained from the infected host. Additionally, it is also spread through inhalation with dust and blood transfusions (Karbowiak and Biernat 2016). Fig. 1 shows the cycle of TBEV transmission to the host.

Clinical Manifestations

Canine Tick-borne Encephalitis

The common clinical manifestations of tick-borne encephalitis in canines include an elevated body temperature up to 106.5 F and behavioral changes that include denying of food, shyness, apathy and increased aggressiveness. Musculoskeletal disorders are often found in the affected animals, with forelimb and hindlimb motion disabilities being the most common. Severe neurological and brainstem damage is evident from the neurological symptoms such as paresis of the forelimbs or hind limbs, quadriplegia, seizures, convulsions, ataxia, perceptual disorders, hyperalgesia in the neck, hyperesthesia, loss of head sensitivity, facial nerve paralysis, strabismus, anisocoria, nystagmus, miosis, loss of the corneal reflex, and optical neuritis (Valarcher et al. 2015).

Equine Tick-borne Encephalitis

Studies on the prevalence of TBE-specific antibodies in horses have revealed that this species is also susceptible to TBEV infection, though the disease is asymptomatic in the vast majority of cases. The signs of disease reported in individual cases include poor general condition, loss of appetite, anorexia, shyness, nervousness, ataxia, spasms, epileptic seizures, and hyperalgesia in the neck (Klaus 2013).

Ruminants Tick-borne Encephalitis

In Ruminants, Tick-borne encephalitis is usually asymptomatic and do not typically cause problems in the infected host. However, rare descriptions of symptomatic TBE in ruminants also exist (Böhm et al. 2017).

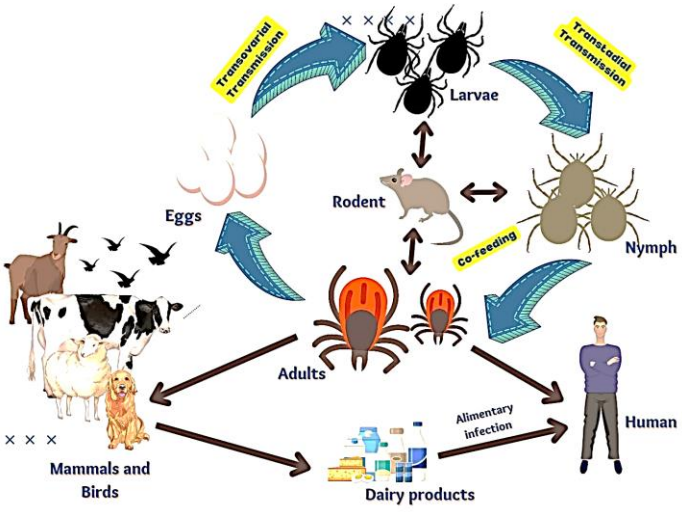

Fig: Tick borne Encephalitis

Fig. 1: Transmission cycle of Tick-Borne Encephalitis Virus

TBE Manifestation in Humans (Tick-borne Encephalitis zoonoses)

TBE virus is one of the principle causes of the central nervous system (CNS) infection in humans. It causes clinical disease in all ages but adults are particularly more vulnerable. TBEV infection is of biphasic nature (Grešíková 1999). The incubation period varies between 2 to 28 days, with an average of 7 days. In the first phase of infection that is the viremic phase which encompasses first two to eight days of infection, flu-like symptoms with an increased temperature, nausea, headache, lethargy and aching back and limbs are most evident. Subsequently, there follows an asymptomatic period and, in 1/3rd of the patients, a second phase of the disease is reported, which is characterized by a sudden onset of fever. This is the phase chiefly affecting CNS and is manifested by clinical symptoms including anorexia, fever, headache, vomiting, photophobia, sensory changes, visual disturbances, paresis, paralysis, or even coma. Other reported symptoms include hyperkinesis of the limbs and face muscles, convulsions, lingual tremor and paresis of the respiratory muscles. This disease might prove fatal a week after the onset of clinical disease (Füzik et al. 2018).

In case of a severe disease observed in 10-20% of the patients, chronic neuropsychiatric or nervous sequelae are observed, such as lack of concentration, depression or paresis of the face or limbs due to chronic myelitis or encephalitis (Chambouris et al. 1989).

Treatment

The TBEV infection has no specific treatment options. When neurological symptoms are present, antiviral therapy is not used as a form of treatment because the virus has already disappeared. The treatment is primarily symptomatic and includes nonsteroidal anti-inflammatory medication. According to the severity of their symptoms, patients typically require hospitalisation and supportive care, which includes giving antipyretics, analgesics, antiemetics, maintaining a healthy balance of water and electrolytes, and giving them anticonvulsive agents if necessary. Intubation and ventilatory support are necessary for patients with neuromuscular paralysis who have respiratory failure. For patients in a coma or experiencing difficulty breathing, reanimation therapies are administered (Böhm et al. 2017). A possible consequence of acute viral encephalitis is cerebral oedema, which worsens the clinical presentation and foretells a poor neurologic outcome. Intravenous mannitol and/or steroids are frequently administered to patients with significantly increased intracranial pressure. Mannitol induces the fluid from an oedematous brain to return to the intravascular space, which strengthens cerebral perfusion pressure, increases circulation volume, and decreases intracranial pressure by cerebral autoregulation. Additionally, it influences the fluidity of the erythrocyte membrane, which enhances blood flow and oxygen delivery by lowering blood viscosity. Five percent of patients with cerebral hypertension experience a rebound phenomenon. When the serum osmolality exceeds 320 mOsm/L, it is often advised to discontinue administering mannitol to avoid complications. No credible (comparative) research supports the use of mannitol in TBE patients, despite the fact that it is a fairly common clinical practise to administer intravenous mannitol to people suffering from extremely increased intracranial pressure (Füzik et al. 2018).

Prevention and Control

The primary methods for controlling TBEV are infection prevention through active immunisation of populations at risk (Christine Klaus et al. 2010) and prevention of transmission from ticks or food products (such as pasteurised milk), wearing light-colored clothing (light colours make ticks easier to spot) having full sleeves and pants tucked into

socks or shoes, using repellents, and carefully checking for ticks over the entire body are the possible options to avoid getting ticks. Avoiding ticks means limiting contact to vegetation, particularly in deciduous and mix forests with a dense understory and a layering of decomposing vegetation on the ground that offers enough humidity for tick formation and survival. However, within a few minutes of attachment, an infected tick's saliva may transfer TBEV since it is present in its salivary glands. The most effective method to prevent the disease in a risk area is active immunization by vaccination. Two TBE vaccines, FSME-IMMUN® and Encepur®, are licensed in Europe. In addition to the European vaccinations, Russia has registered two vaccines (TBE-Moscow and EnceVir) based on the Far-Eastern subtype of Tick born encephalitis virus. The viruses are produced in cells of chick embryo and formalin has been used to inactivate them and aluminum hydroxide is used as adjuvant in both of the vaccines. Another vaccination based on the Far-Eastern subtype of tick born encephalitis virus has been produced and used in China (Riccardi N et al. 2019).

Conclusion

Tick serves as a vector for transmission of tick-borne encephalitis virus and its cycle is affected by certain factors including microclimate, host factor and environmental changes. After infected tick bite to the host, virus replicate first at the inoculation site, and then drain into the lymphatic system. Virus has been found in dermal, Langerhans and dendritic cells that is the primary site of infections before entering into regional lymph nodes. During this entire period, virus multiplies and spread to entire organisms. This makes horizontal transmission possible between co-feeding tick species. The primary methods for controlling TBEV are infection prevention through active immunization of populations at risk leading to prevention of transmission from ticks or food products (such as pasteurised milk), wearing light color clothing (light colours make ticks easier to spot) having full sleeves and pants tucked into socks or shoes, using repellents, and carefully checking for ticks over the entire body.

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3

Babesia microti Studies in México

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INTRODUCTION

In North America, the cases of human babesiosis exceeds 20,000; it is considered as an emerging zoonotic disease mainly caused by *Babesia (B.) microti* (Yang et al. 2021). Wild rodents and hard ticks of the *Ixodes* genus are involved in the life cycle of this parasite. Wild rodents of several genera, including *Peromyscus*, are widely distributed in México (Ceballos 2014) and *Ixodes* ticks are also present in the country (Hoffman and López-Campos 2000). In this respect, *Ixodes (I.) scapularis* not only transmits *B. microti*, it also transmits *Borrelia burgdorferi* the causative agent of Lyme disease (Illoldi-Rangel et al. 2012; Feria et al. 2014). To date, there is no published information on the presence of *B. microti* in wild rodents in Mexico, which are a source of infection in humans. Taking into consideration the One Health focus for controlling parasitic diseases, the objective of this study was to determine the presence of *B. microti* in wild rodents from three Mexican natural parks located in the states of Mexico, Guerrero and Michoacán, through PCR amplification of the 18S rRNA gene.

Etiological Agent

Babesia microti is one of the causative agents of babesiosis in mammals (Kreier and Baker 1987) and a tick vector, generally of the *Ixodes* genus, is involved in transmission of this parasite to mammals. Briefly, when the infected tick bites a mammalian host, generally a wild mouse *Peromyscus* spp. transmits sporozoites to it, which then penetrate a red blood cell; once there, they transform into trophozoites, which generates mature merozoites and these, rupture the red blood cell to penetrate more erythrocytes. When a susceptible tick vector bites the infected mammal, the cycle continues (Westblade et al. 2017). Fig. 1 and 2 show a blood film of a mouse infected with *B. microti* and a simplified life cycle of the parasite, respectively.

Babesia microti Life Cycle

In the life cycle of *B. microti*, the interaction of *I. scapularis* with *Peromyscus* mouse is essential for the maintenance of the parasite in nature. The adult stages of *I. scapularis* feed primarily on deer (*Odocoileus virginianus*), which do not serve as reservoirs for *B. microti*, they feed again in the fall and in the spring, after which the ticks lay eggs. These eggs hatch in the summer, and the larvae feed primarily on wild mice; at this moment, the tick can acquire *B. microti*. The infected larvae overwinter and molt to become nymphs in the spring. Then, the nymphs feed on hosts from May through July. The nymphs that have fed molt into adults in the fall, completing the tick life cycle. In areas where human babesiosis is endemic, the ticks feed primarily on *Peromyscus* wild mice (Kreier and Baker 1987; Telford et al. 1993; Homer et al. 2000).

Material and Methods

Wild mice were captured from Michoacán State, México State, and Guerrero State (Fig. 3) and DNA was extracted from obtained samples and kept in the DNA and Tissue Bank of the Emerging Infectious Diseases Laboratory (IMSS), followed by a descriptive cross-sectional study. For this, DNA was extracted from liver, ear or heart of these rodents, which previously euthanized in accordance with the Norma Oficial Mexicana NOM-062-ZOO (1999). From the samples, the *B. microti* 18S rRNA gene of was amplified, using the Gray type strain of the parasite as a positive control, and a 1.5% agarose gel electrophoresis of the PCR products was carried out to perform purification and product sequencing for comparison with Gen Bank sequences (Persing et al. 1992).

RESULTS

The amplified samples showed 99% similarity to *B. microti*. Regarding the percentages of positivity in 190 DNA's examined by state for *B. microti*, there were 16.9% (14/84) from the State of Mexico; 16.6% (12/71) from Guerrero and 8.6% (3/35) from Michoacán.

The percentages of the 21 positive rodents were as follows: 28.6% for *Peromyscus megalops*, 23.8% for *Peromyscus* sp., 14.3% for *P. maniculatus*, 9.5% for *P. beatae*, 4.8% for *Mus musculus* and 14.3% for *Megadontomys* sp. (Fig. 4).

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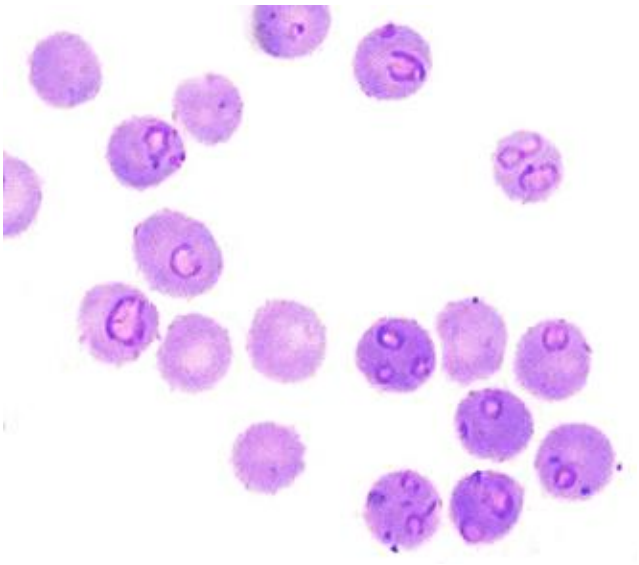

Fig. 1: Blood smear of a mouse infected with the Gray strain of *Babesia microti* stained with Giemsa stain, showing trophozoites (Photograph by Carlos R. Bautista-Garfias).

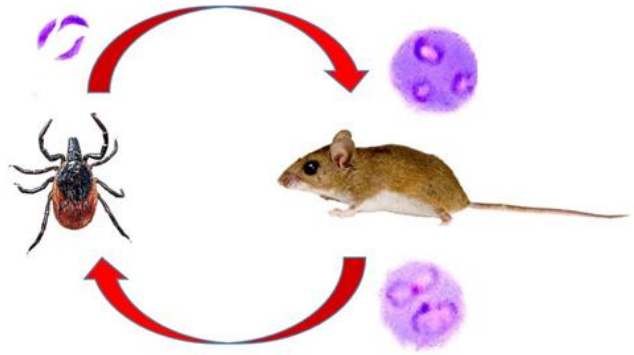

Fig. 2: *Babesia microti* simplified life cycle (Figure designed by Carlos R. Bautista-Garfias). Left: the vector *Ixodes* spp. and a *B. microti* sporozoite; right: wild mouse *Peromyscus* spp. (reservoir of the parasite), and *B. microti* trophozoites inside red blood cells.

Fig. 3: Map of México showing the States where wild rodents were captured for this study.

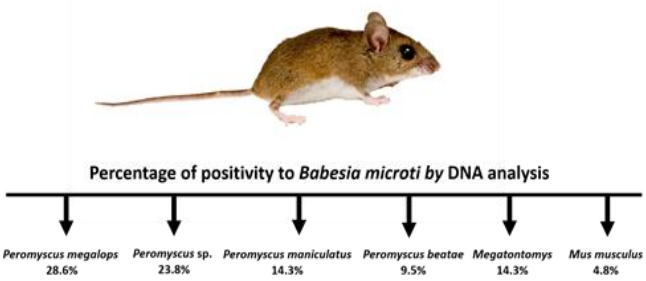

Fig. 4: Percentage of positivity of *B. microti* in wild rodents from three Mexican States.

DISCUSSION

The knowledge on *B. microti* has been increasing in recent years (Gray et al. 2010; Al Zoubi et al. 2016; Arsuaga et al. 2016; Primus et al. 2018; Strizova et al. 2020; Puri et al. 2021; Telford et al. 2021). It also indicates that this parasite may infect small mammals belonging to different families (Gao et al. 2017), which suggests that the problem of babesiosis is complex.

On the other hand, further research on *B. microti* infections needed in Mexico because the only published study on *B. microti* in the country is that carried out in humans in Yucatán (Peniche-Lara et al. 2018). In this context, it must keep in mind that a serious risk for human health is the fact that *B. microti* can be transmitted by blood transfusion (Kumar et al. 2021; O´Brien et al. 2021). Additionally, in a recent study researchers demonstrated that wild rodents from México, such as those of the *Peromyscus* genus, are also infected with *Borrelia burgdorferi*, the causative agent of Lyme disease (Rodríguez-Rojas et al. 2020).

With reference to alternatives for controlling babesiosis, Bautista-Garfias et al. (2005) demonstrated experimentally that using the acid lactic bacteria *Lactobacillus casei* in mice controls infection by *B. microti*, but further research is needed.

Conclusion

The results demonstrated that *B. microti* is present in wild rodents, mainly in animals of the *Peromyscus* genus, which live in natural parks of three states of México. There is a risk that the human population living in these areas, not aware of the problem, and chances are there that they may already exposed to infection by this pathogenic protozoan. At the same time, the population of wild mice infected with *B. microti* and the ticks involved in its transmission is unclear which represent a major threat for human health. It is urgent to carry out epidemiologic studies of *B. microti* and its vectors using One Health approach so that appropriate control measures may be applied (Hopkins et al. 2022).

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4

Trichinellosis: A World Health Problem

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INTRODUCTION

The nematodes of the genus *Trichinella* belongs to the Family Trichinellidae. Likewise, *Trichinella* belongs to the Trichinelloidea superfamily which has particular characteristics different from other nematodes (Wu et al. 1998). Currently in the genus *Trichinella* two main clades are recognized, one that includes the species that are encapsulated in the muscular tissue of the host including *Trichinella (T.) spiralis, T. native*, T6, *T. britovi*, T8, *T. murrelli* and T9 and another in which the species are not encapsulated including *T. pseudospiralis, T. papuae* and *T. zimbabwensis*. It has been pointed out that although there are no clear morphological differences between species and genotypes, yet these can be differentiated (International Commission on Trichinellosis, 2022).

The disease caused by species of the genus *Trichinella* is known as Trichinellosis or trichinosis. It should be noted, according to the International Organization of Epizootics and the International Commission on Trichinellosis, that the worldwide distribution of *T. spiralis* (the best-known species) has been fundamentally influenced by humans, who passively introduced it into the North, Central, and South of the American continent, as well as in New Zealand and Egypt (World Organization for Animal Health, 2022).

Trichinella is a genus of zoonotic nematode that occurs in carnivores and omnivores (mammals, including people, reptiles and birds) and causes the disease known as Trichinellosis, which has been a public health threat for more than 170 years (Murrell and Pozio 2000). In this context, it has been estimated that only in China more than 40 million people are at risk of *Trichinella* infection (Bai et al. 2017).

Etiological Agent

The recent application of molecular techniques has led to the identification of 10 species including *T. spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. pseudospiralis, T. papuae, T. patagoniensis, T. zimbabwensis,* and *T. chanchalensis* and three genotypes including T6, T8, T9 which have not yet been given species status (Zarlenga et al. 2020) (Fig. 1, Table 1).

T. patagoniensis was isolated and identified in muscle tissue from cougar in Argentina (Krivokapich et al. 2012). More recently, a new species, *T. chanchalensis*, was described in wolverine (*Gulo gulo*) from northwestern Canada (Sharma et al. 2020). It is important to note that *T. spiralis*, the most studied species, is an intracellular parasite that does not kill the host cell, but induces transformations in cell structure that benefits the survival of the parasite (Despommier 1990).

Trichinella species infect more than 100 species of vertebrates including mammals, birds, and reptiles. In this respect, it is estimated that 10,000 cases of Trichinellosis have been reported from human worldwide on annual basis, with an average mortality of 0.2% (Pozio 2005; Zarlenga et al. 2006). *Trichinella* larvae are located in muscle tissue and the adults in the small intestine for a long period of time, (International Commission on Trichinellosis, 2022).

From the clinical point of view, the effect of *Trichinella* infection in the pig (the most important host in many countries, including Mexico) Ortega-Pierres et al. 2000) is minimal and practically undetectable; however, trichinellosis is considered an important zoonosis due to outbreaks in humans. It should be noted that most cases of human Trichinellosis in México have been due to the consumption of semi-raw meat from backyard pigs (generally in celebrations and family parties) that do not undergo sanitary inspection (Ortega-Pierres et al. 2000).

Generally, it should be noted that the most important risk factors in the domestic cycle of *Trichinella* include: 1) intentional feeding with food scraps containing pig remains or exposure (intentional or unintentional) to dead pig carcasses or wild animals; 2) allow pigs to feed in garbage dumps; 3) feeding wild animals with carcasses or remains of hunted animals; 4) feeding horses with pig carcasses or animal carcasses; 5) feeding sled dogs carcasses from other animals in the arctic; 6) feeding carcasses as food to fur animals; 7) feeding farmed crocodiles with meat from other farmed crocodiles; 8) feeding young crocodiles with pig carcasses. It is worth to mention that, in the domestic cycle of trichinellosis, there is predominate infection of *T. spiralis* in pigs and synanthropic hosts without affecting the health of these animals significantly, except when the infection by *Trichinella* is severe (International Commission on Trichinellosis, 2022).

Life Cycle of *Trichinella* spp.

The new-born *Trichinella* larvae (NLs) migrate from adult female worms to host lymphatic vessels, then enter in the

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Table 1: *Trichinella* species or genotype, hosts, and world distribution (Table designed by Carlos R. Bautista-Garfias)

Species or genotype	Hosts	Distribution	Reference
T. spiralis	Mammals	Cosmopolitan	Gottstein et al. 2009
T. nativa	Mammals	Arctic and Subarctic regions of America, Europe and Asia	Uspensky el al. 2019
T. britovi	Mammals	Tempered areas of Europe and Asia Northeast and West Africa	Pavic et al. 2019
T. murrelli	Mammals	Tempered areas of North America	Pozio and La Rosa, 2000
T. nelsoni	Mammals	East and South east Africa	Pozio et al. 1997
T. patagoniensis	Mammals	Patagonian region South America	Krivokapich et al. 2012
T. zimbabwensis	Mammals, Reptils	Africa South of Sahara.	Pozio et al. 2007
T. chanchalensis	Mammals	Nothwestern Canada	Sharma et al. 2020
T. papuae	Mammals, Reptils	Papua New Guinea.	Takahashi et al. 2000
T. pseudospiralis	Mammals, Birds	Cosmopolitan	Santrac et al. 2015
T6	Mammals	Arctic and Subarctic regions of America	Reichard et al. 2008
T8	Mammals	Tempered areas of North America	Gottstein et al. 2009
T9	Mammals	Japan	Tada et al. 2018

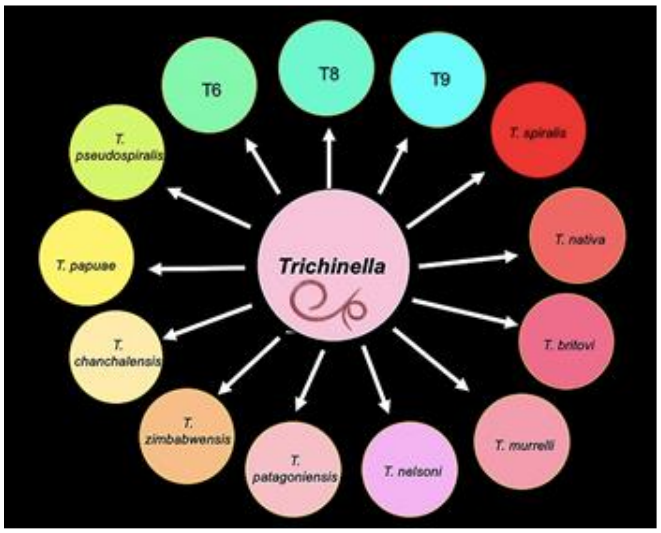

Fig. 1: Known species of genus *Trichinella* (composition by Carlos R. Bautista-Garfias)

blood vessels to be transported to skeletal muscle cells. The NL transform in the muscle cell to stage L_1 larvae. These larvae may survive up to two decades in polar bears and up to four decades in humans. Once the L_1 larvae in muscle tissue are ingested by a new host, they are released from the muscle cells by gastric juices in the stomach; then they reach the duodenum where these penetrate the intestinal villi and transform into adult worms, which mate, and after six to seven days, the females begin to produce NL, whose production continues for at least one to two weeks or longer depending on immune response at intestinal level (International Commission on Trichinellosis, 2022) (Fig. 2). The muscle larvae can be easily recognized in an infected host, while the adult worms are difficult to detect, which can only be obtained from the intestine. It is more difficult to detect NL migrating in the blood of naturally infected host (International Commission on Trichinellosis, 2022).

Transmission

Briefly, *Trichinella* transmission occurs when a susceptible host (carnivorous or omnivorous, including man) eats meat of a *Trichinella* infected host which harbours infective larvae in muscle cells. Then, the life cycle of this parasite begins again as depicted in Fig. 2. (International Commission on Trichinellosis, 2022).

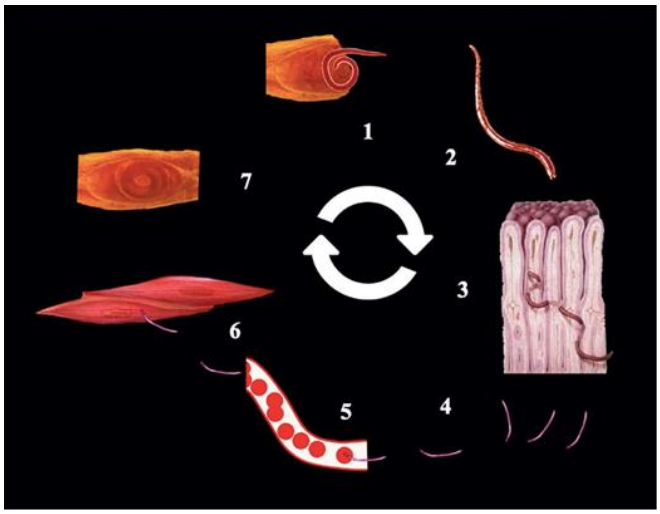

Fig. 2: Biologic cycle of *Trichinella spiralis*: 1) After a muscle cyst is ingested by a new host, the larva is liberated by the gastric fluids of the new host. 2) Infective larvae transform into adults in the intestine. 3) After copulation the female sheds live newborn larvae (NL). 4, 5) NL migrate through lymph and blood. 6,7) NL penetrates a skeletal muscle cell and induces the formation of a nurse cell which will become a muscle cyst. (Figure designed by Carlos R. Bautista-Garfias)

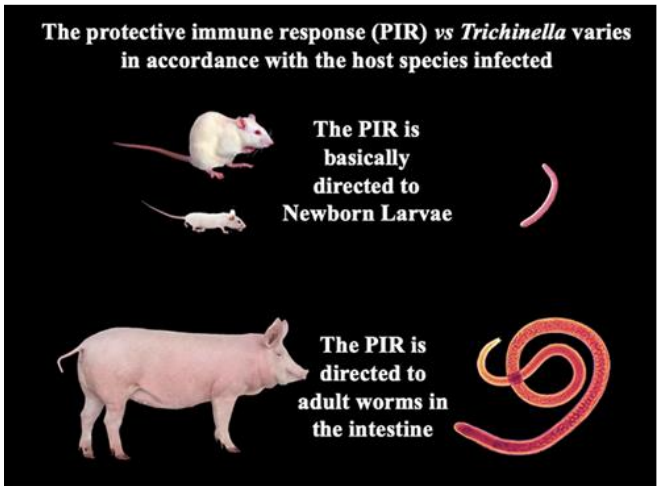

Fig. 3: The protective immune response against *Trichinella spiralis* in rodents and swine (Figure designed by Carlos R. Bautista-Garfias; based on: Murrell 1985; Bell and Wang 1987; Zhang et al. 2018).

Epidemiology of Trichinellosis

T. spiralis parasitize the domestic animals, while the other species in this genus mostly infect wild animals. When there is improper management of domestic and wild animals, other *Trichinella* species are also transmitted from the wild to the domestic environment. Alternatively, *T. spiralis* can also be transmitted from domestic animals to wildlife. In this respect, it should be noted that no systematic epidemiological studies of Trichinellosis have been carried out in some countries such as México. A very limited epidemiological information available regarding the prevalence of *T. spiralis* yet (International Commission on Trichinellosis, 2022).

Pathogenesis/Clinical Signs

Pathogenesis usually refers to humans rather than animals and involves two phases of the *Trichinella* life cycle including an intestinal (or enteric) phase and a muscular (parenteral or systemic) phase. During intestinal phase, symptoms like fever, myalgia, eosinophilia, and diarrhoea occur. In the muscle phase inflammatory and allergic responses due to invasion of skeletal muscle cells by larva migrans may occur. In this phase, either there will be direct damage to muscle cells or indirect stimulation of eosinophils. In this regard, there is a correlation between the levels of eosinophils and muscle serum enzymes such as lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) (Bruschi and Murrell 2002; Dupouy-Camet et al. 2002).

Clinical signs generally are not detectable in animals such as pigs; however, in humans symptoms may appear during the acute phase of Trichinellosis, which include palpebral or facial oedema, and myalgia, which is aggravated by myocarditis, thromboembolic disease and encephalitis (Bruschi and Murrell 2002).

Immune Response of Mammals to *Trichinella* spp. Infection

The immune response of the host against *Trichinella* infection is of both nonspecific and acquired type and depends on the species of infected host (Ottesen et al. 1975; Murrell 1985; Bell and Wang 1987). In mice and rats the protective immune response in a reinfection is directed against new born larvae (Bell and Wang 1987; Zhang et al. 2018), while in swine it is directed against the adults in the intestine (Murrell 1985) as mentioned in Fig. 3. It also depends on the *Trichinella* infective dose (Martínez-Gómez et al. 2011; Wang et al. 2020) and the *Trichinella* species (Wakelin et al. 1994). It must bear in mind that during infection, T. spiralis is also capable of modulate the immune response of the host; for example, depressing the production of effector immune molecules, such as cytokines. (Song et al. 2019; Xu et al. 2021).

On the other hand, based on the acquired immune response against *Trichinella*, several antigens are being evaluated as possible vaccines (Zhang et al. 2018). However, other approaches to induce protection of the host have been developed; for example, the use of *Lactobacillus casei* that generates a non-specific protection against *T. spiralis* infection (Bautista-Garfias et al. 1999; Bautista-Garfias et al. 2001; Martínez-Gómez et al. 2009) and, recently, against *T. britovi* (Boros et al. 2022).

Diagnosis

In order to detect *Trichinella* infection in the hosts, several tests have been implemented, either to observe directly the parasite, or to evaluate indirectly the effector immune molecules (for example, antibodies) elicited by this. In accordance with Ruitenberg et al. (1983), in order to detect *Trichinella* larvae per gram in pigs, the less sensitive test is the Trichinoscopy, while the best techniques *available* are the digestion test (5 x 20g), pooled sample digestion (Van der Giessen et al. 2013; Riehn et al. 2013), and the Enzyme Linked Immunosorbent assay (ELISA) (Venturiello et al. 1998; Gamble et al. 2004). In this context, it has been demonstrated that western blot is a useful diagnostic technique for differentiating *T. spiralis* or *T. britovi* from *T. pseudospiralis* (Gómez-Morales et al. 2018). For diagnosing *Trichinella* infection in human, serological tests, such as ELISA (Bruschi et al. 2001; Gómez-Morales et al. 2008) and Western blot (Yera et al. 2003) have been employed.

Control

The International Commission of Trichinellosis has recommended the following points for the control of Trichinellosis (Gamble et al. 2000; Dupouy-Camet and Murrell 2007):

1- Detection at slaughterhouse level (in order of importance i.e., pigs, horses and game animals.

2- Meat processing by cooking, freezing, or irradiation.

In this respect, China has pointed out the need to carry out effective control measures (for example, educating and informing the public) for controlling Trichinellosis (Liu and Boireau 2002). Contrary to this, when control measures fail due to social, political and economic factors, Trichinellosis re-emerges (Djordjevic et al. 2003). It has been suggested that for controlling Trichinellosis, monitoring *Trichinella* infection in wildlife could help (Van Knapen 2000). The changing global condition such as demographic, climate change, and socioeconomic change affected the parasitic diseases, so there is the need for new transdisciplinary control approaches (Thoisy et al. 2021).

Conclusion and Perspectives

The published information about *Trichinella* and Trichinellosis indicates that this neglected zoonosis is not completely understood. Several advances have been achieved, including, the discovery of new Trichinella species, although their life cycles are partially known only. It is also true that the diagnostic techniques have improved (serological and molecular), and treatment of the disease in humans is effective. However, we do not know how socioeconomic changes, climate change and the continuously growing human population invading wildlife will impact on animal and human trichinellosis, so much research should be carried out under the One Health scheme to implement effective control measures.

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5

Myiasis Infections in Animals and Men

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INTRODUCTION

Myiasis is a condition caused by larval stages of different types of flies belonging to the order Diptera that attack tissues and organs of vertebrate animals, including man. The word myiasis is derived from the Greek word *myia* meaning= fly. On the other hand, this chapter is not an exhaustive review of flies causing myiasis, it refers to some of the most important myiasis, primarily in farm animals of economic interest (Hall and Wall 1995) and secondarily, in man (Francesconi and Lupi 2012; Hosni et al. 2019). In this context, myiasis-producing larvae are important because it produce economic losses in farm animals which are source of infestations in humans. This situation is aggravated by factors such as the growing human population, climatic change, and the lack of proper control measures of myiasis-producing larvae. Under these circumstances, the One Health approach offers a viable control alternative.

Etiological Agents

The major myiasis causing larvae belongs to the *Oestrus ovis*, *Hypoderma* spp., *Gasterophilus* spp., *Dermatobia hominis*, and *Cochliomyia hominivorax*.

Oestrus ovis Linnaeus

O. ovis (Fig. 1) is a species of fly widely distributed in the world. The larvae are obligate parasites of the nasal passages of sheep and goats (Yilma and Dorchies 1991; Hall and Wall 1995; Cepeda-Palacios et al., 1999; Murguia et al., 2000; Yacob et al., 2004) and occasionally affect other species such as man (Hall and Wall, 1995) and dog (Zanzani 2016). The female normally deposits active young (L_1) larvae from early summer or fall in nostrils of host (Fig. 2). Then larvae enter host sinuses, often to the base of the horn and attaching to the

Fig. 1: *Oestrus ovis* adult fly. (Photograph by Carlos R. Bautista-Garfias)

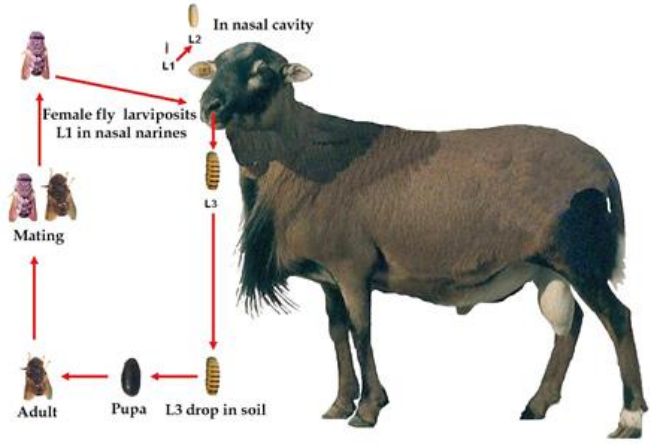

Fig. 2: Biological cycle of *Oestrus ovis*. (Photograph by Carlos R. Bautista -Garfias)

mucous membranes (Fig. 3). Larvae of different stages of development (L_1, L_2, and L_3) can be found here. The larvae reach their maximum development (L_3) in the following spring, with their larval period of 8 to 10 months (Fig. 4) (Hall and Wall 1995).

Generally, the pupal period lasts between three to six weeks, sometimes much longer in areas where low temperatures prevail. Adults can live up to 28 days. The complete development of the parasitic phase, in lambs born in the spring, can be from 25 to 35 days (Hall and Wall 1995).

In the presence of *O. ovis* fly, sheep and goats become very agitated, shaking their heads, thrusting their nostrils into the dust, snorting. In parasitized animals, there is a purulent discharge from the nostrils, vigorous shaking of the head and the animal become emaciated. The infestation by *O. ovis*

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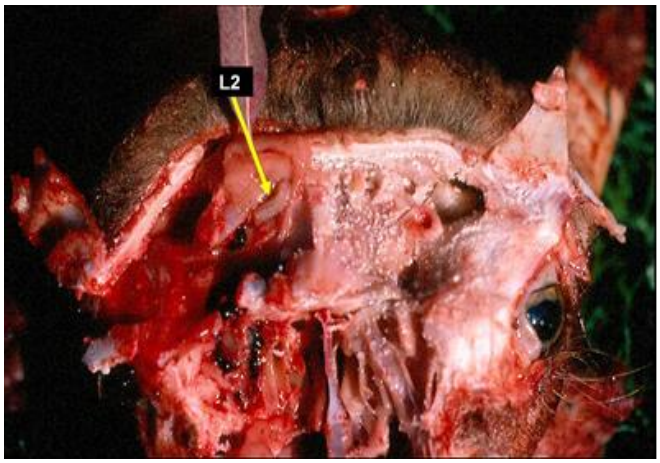

Fig. 3: *Oestrus ovis* L2 in frontal sinus of sheep (arrow). (Photograph by Carlos R. Bautista -Garfias)

Fig. 4: *Oestrus ovis* L_3 goes out sheep nostril. (Photograph by Carlos R. Bautista-Garfias)

larvae generally is not fatal; however, some animals can die within a week or less after the appearance of aggravated signs (secondary infections produced by bacteria) (Horak 1977).
Diagnosis is difficult since it can be confused with the signs caused by other diseases. However, based on the knowledge that sheep and goats mount an immune response against the larvae (Bautista-Garfias 1987; Bautista-Garfias 1996; Tabouret et al. 2003), serological tests can detect IgG circulating antibodies against the larvae (Bautista-Garfias et al. 1982; Bautista-Garfias et al. 1988; Otranto et al. 2004).
On the other hand, there is occasional occurrence of human cases of *O. ovis* infestation infecting the eyes (Beltrán et al. 2006; Singh and Singh 2015; Basmaciyan et al. 2018; Tabuenca-del barrio et al. 2018) and pharynx (Hazratian et al. 2017). With respect to the control, One Health approach has been proposed in order to effectively control sheep myiasis and to increase sheep production (Colwell and Wall 2018).

Hypoderma spp.

Hypodermosis is caused in cattle by the larvae belonging to the genus *Hypoderma*, widely distributed in the northern hemisphere (Hall and Wall 1995; Boulard 2002; Wei et al. 2004). *H. lineatum* (de Villers), the common larva of cattle, is found throughout the U.S., Canada, and northern Mexico. *H. bovis* (Linnaeus) is the larva of north-eastern cattle and is found in Canada and the north-eastern USA. *H. bovis* adults induce a kind of fear or dread in cattle that makes them run uncontrollably, potentially injuring themselves and causing decrease in milk production. Although the adult fly does not bite or sting, it can induce such fear. Adults resemble bees, which are often called "heel flies" (Fig. 5). *Hypoderma* adults are present for four to six weeks from early spring to early summer (Broce 1985). *H. bovis* eggs are attached individually on the flank or lower abdomen; those of *H. lineatum* are glued in rows of 3 to 10 on a single hair on the forelegs, chest, or lower body. They hatch in approximately four days and, after penetration of host´s skin, the larvae causes irritation and exudation. The total production of eggs by a single female fly has been estimated to range between 500 and 800, which dies a short time later, as it has no mouthparts and is unable to feed (Broce 1985).
The larva spends 9 to 10 months migrating as an internal parasite (L_1, L_2) before emerging as L_3 from (Fig. 5) the host to pupate and become a short-lived adult in the following summer (Fig. 6). The active larva L_1 spends much of its parasitic period in migrating through the intermuscular connective tissue to the subcutaneous tissue of the back (loin) (Fig. 7). However, there is an important wintering period in or around the spinal cord in *H. bovis*, or in the mucosa of the oesophagus in *H. lineatum* (Broce 1985). This migration often follows the course of nerves, avoiding blood vessels and muscles. When L_1 reaches the back (loin), it develops into L_2, cutting a one to three mm diameter hole in the skin to breathe through its rear breathing spiracles. At this stage, host reactions give rise to a fibrous cyst that forms around the larva. Shortly thereafter, L_2 transforms into L_3, which is much larger, approximately 25 mm long, brown in colour, and has armour-like features with spines (Fig. 6).
After 6 to 11 weeks, the larva emerges from the breathing hole in the skin of the back, falls to the ground and pupates after burrowing into a dark brown puparium (Fig. 8). The stage, of the development to the adult, lasts approximately 35 days, depending on the climatic conditions but it can be as short as two weeks under optimal weather conditions. The adult then emerges by pushing off the pupal cap, and then comes to the surface to prepare for flight (Broce 1985; Hall and Wall, 1995).
The adult, without effective mouthparts, is a reproductive and dispersal phase that dies approximately six days after emerging. Their success in the distribution of the species depends on prevailing weather conditions, which limit their activity and their ability to find a breeding partner and potential animal host (Hall and Wall 1995).

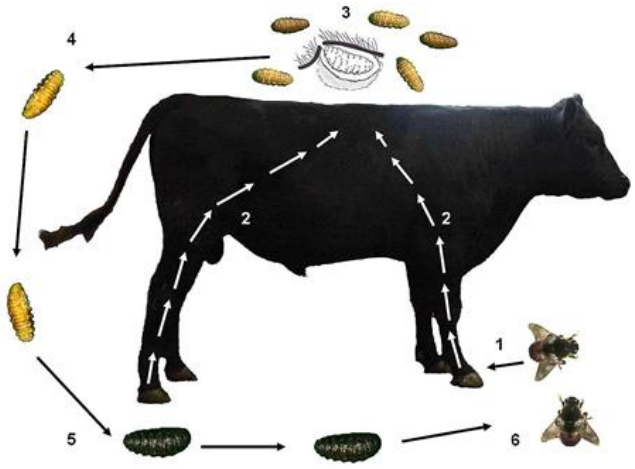

Fig. 5: Life cycle of *Hypoderma* spp. 1, gravid female glues her eggs to host hair. 2, The first instar larvae (L_1) migrate towards the back of the bovine where they pass to the second instar (L_2). 3, A fibrous cyst forms around the larvae; 4, The L2 transform into third instar larvae (L_3) that fall to the ground. 5, L3s transform into pupae. 6, Adults are born from the pupae, which then mate. (Photograph by Carlos R. Bautista-Garfias)

Fig. 6: *Hypoderma* spp. L_3. (Photograph by Carlos R. Bautista-Garfias)

Economic losses for the control and the production costs in USA as estimated by the USDA 1976 were close to 360 million US dollars. Much of this is due to costs of systemic insecticides in beef cattle and non-lactating dairy cattle. The largest losses due to *Hypoderma* larvae are those that are obvious on slaughterhouses such as devalued carcasses, loss of condition, and damage to hides (Broce 1985). As in the case of *O. ovis*, cattle mount immune responses against antigens from *Hypoderma* larvae (Baron and Colwell 1991a), thus serological diagnosis has been shown to be possible (Otranto et al., 2004) and even immunize cattle against *Hypoderma* (Baron and Colwell 1991b).

Hypoderma larvae, occasionally cause myiasis in tissues of human beings i.e. skin (Morgan et al. 1964; Logar et al. 2008), eyes (Lagacé-Wiens et al. 2008), groin and testicular region (Puente et al. 2010), muscles (Starr et al. 2009) and even in the lymph (Scott 1964).

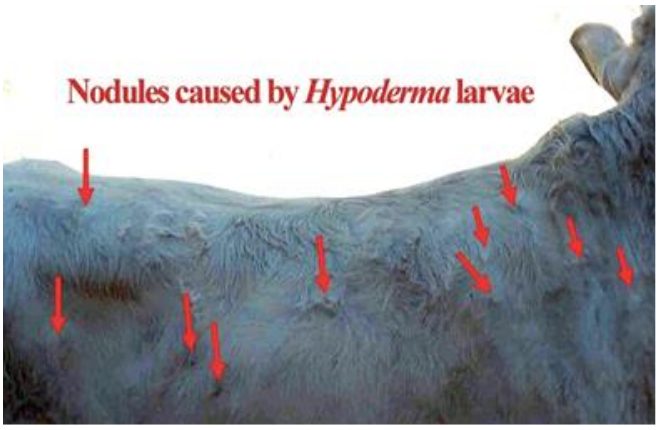

Fig. 7: Nodules produced by *Hypoderma* larvae on back of parasitized cattle (Photograph by Carlos R. Bautista-Garfias)

Fig. 8: *Hypoderma* spp. pupa. (Photograph by Carlos R. Bautista-Garfias)

Gasterophilus spp.

Gastrophilosis in horses, donkeys and mules is caused by the larvae of flies belonging to the genus *Gasterophilus* distributed worldwide depending on its association with the host. The most important species are *G. intestinalis* (De Geer), *G. nasalis* (Linnaeus) and *G. haemorrhoidalis* (Linnaeus) (Broce 1985; Principato 1989; Pandey et al. 1992; Hall and Wall 1995; Otranto et al. 2005).

Adults of all three species have atrophied, non-functional mouthparts and are therefore short-lived. Females begin to oviposit after mating. During this activity, the eggs are attached to the host's body hairs. The site of oviposition varies with the species, and all newly hatched larvae (L_1) penetrate the subcutaneous tissues of the mouth (lips, gums, and tongue) where they spend three weeks. After this time, larvae move to the stomach or small intestine mucosa and transform into second-stage larvae (L_2), which after several months become third-stage larvae (L_3) that detach on their own and go outside with the faeces. Pupation takes place in the upper layer of the soil under the manure. Subsequently, the adults emerge between a few weeks to two months later, depending on the climatic conditions (Fig. 9) (Otranto et al. 2005).

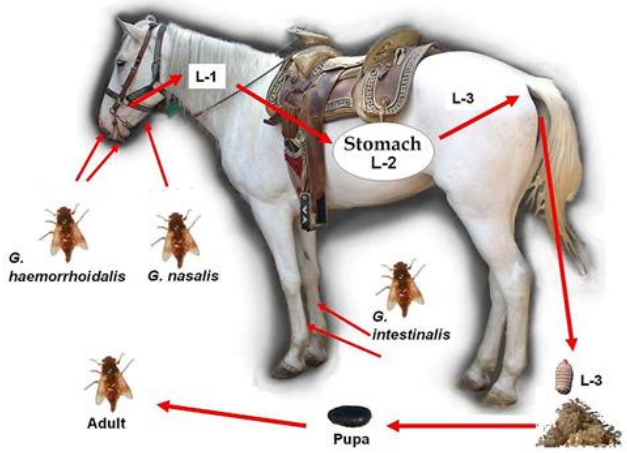

Fig. 9: Life cycle of *Gasterophilus* spp. (Photograph by Carlos R. Bautista Garfias)

Fig. 10: L_3 of *Gasterophilus* spp. (Photograph by Carlos R. Bautista Garfias)

G. intestinalis (the horse fly) females may lay up to 500 to 1,000 eggs. They oviposit as they fly, hovering near the host, occasionally darting toward it to lay an egg. The eggs generally are glued to the internal side of the front legs; however, they can be found in other sites. The general incubation period for horse fly eggs is approximately five days. After a short time in the mouth, they attach to the mucosa of the stomach and remain there approximately for 7 to 10 months, and then L_3 larvae pass out along with the faeces (Fig. 10). Adults are active in early summer (Broce 1985).
G. nasalis (the throat fly) glues its eggs to the hair of the host under the jaw. Each female is capable of producing 450 to 500 eggs and its oviposition activity is extremely troublesome for the affected horses. These eggs can hatch in four to five days. Hatched larvae move along the skin into the horse´s mouth and they penetrate the soft tissue. In approximately 20 days, larvae move toward the stomach to attach to the stomach or duodenum mucosa. Finally, L_3 larvae come out with the faeces. Much of the adult activity takes place in late spring or early summer (Broce 1985).
G. haemorrhoidalis (the nose fly) is a fast flier and females attach their blackish eggs to the hairs on the (upper and lower) lips of horses. Each female usually lays 160 eggs, which hatch in two days stimulated by humidity. The young larvae (L_1), after penetrating the tongue or lips, migrate to the stomach or duodenum. Then, L3 larvae reattach to the wall of the rectum close to the anus for two to three days (Otranto et al. 2005).
Both adults and larvae of *Gasterophilus* species cause damage (Broce 1985). Horse´s reactions to ovopositing females can be violent. L_1 larvae cause irritation when they burrow and move into oral tissue. Larvae adhered to the walls of the stomach and duodenum interfere the process of digestion, and may cause peritonitis (Lapointe et al., 2003). Animals parasitized by *Gasterophilus* larvae gain weight more slowly than non-parasitized horses (Principato 1989).
According to the available literature it is indicated that *Gasterophilus* spp. larvae parasitize almost all horses. *G. intestinalis* is the most prevalent species in USA and the infestation rate is almost 100% (Broce 1985). It is worth to note that studies carried out in central Italy suggest the tendency towards extinction of *G. inermis*, *G. pecorum*, and *G. haemorrhoidalis*, while the most prevalent species are *G. intestinalis* and *G. nasalis* (Otranto et al. 2005). Similarly, a study carried out on donkeys in Morocco showed that *G. intestinalis* and *G. nasalis* are the most prevalent species (Pandey et al. 1992).
The diagnosis of gastrophilosis can be carried out with serological tests in horses and donkeys (Escartín-Peña and Bautista-Garfias 1993). *Gasterophilus* myiasis cases in man are rare such as external ophtalmomyiasis (Medownick et al. 1985), oral myiasis (Townsend et al. 1978) and pulmonary myiasis (Ahmed and Miller 1969).

Dermatobia hominis

The neotropical fly, *Dermatobia (D.) hominis* is a cause of severe losses in the beef, dairy, and bovine leather industries from north-eastern Mexico to north-eastern Argentina (Fig. 11). The life cycle is very complex and requires a flying arthropod to transport its eggs to a mammalian vertebrate, which include cattle, dogs, cats, pigs and man (Sancho 1988; Pereira Da Silva et al. 1998; Brizuela et al., 2003; Maier and Honigsmann 2004; Saraiva et al. 2005). The adult fly is bottle blue in colour. Adults can't feed because of atrophied mouth parts (Sancho 1988).
The life cycle lasts between 100 to 120 days. Larval development is completed in 5 to 10 weeks, after which the mature larvae leaves the host and falls to the ground. After mating, the female lays her eggs on another insect (usually another fly or a mosquito) which transports them to a warm-blooded vertebrate host, after which the larva hatches and penetrates the skin of the new host.

Fig. 11: *Dermatobia hominis* distribution in America, from north-eastern México to north-eastern Argentina. On the left is shown a *D. hominis* L_3 (Figure designed by Carlos R. Bautista-Garfias).

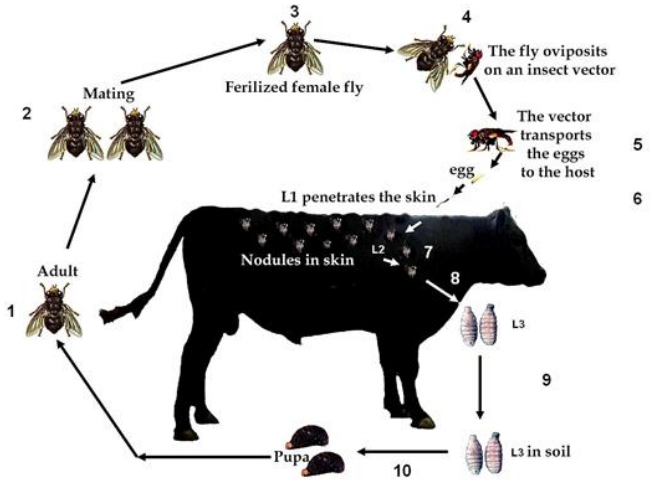

Fig. 12: *Dermatobia hominis* life cycle. 1, The adult fly hatches from the pupa;2, Mating between male and female. 3, The fertilized female captures. 4, An insect vector and oviposit on it. 5, The vector transports the eggs to the host and from each egg. 6, A larva 1(L_1) hatches that penetrates the skin to give rise to nodules where it transforms,7, into larva 2 (L_2) and matures,8, up to larva 3 (L_3) to later fall to the ground, 9, and transform into pupa, 10, from which an imago emerges to continue the cycle. (Photograph by Carlos R. Bautista-Garfias).

The eggs are glued onto other insect so that its flight efficiency is not adversely affected. Almost 50 insect species of carriers have been recorded (half are mosquitoes and one third are other fly species). Egg development requires 4-9 days and hatching is stimulated by increase in temperature, which occurs when the eggs are on a warm-blooded host. At this point, the larvae leave from the egg and enters to the host skin, which occurs between 5-10 minutes (Fig. 12) (Sancho 1988). The third instar larva is elongated and oval in shape, with belts of scattered spines and shows prominent mouth hooks (Fig. 13) (Sancho 1988).

The larvae are located on various parts of the body causing pain to the host. After larva is removed, and in the absence of a secondary infection, the condition resolves approximately in a week. In Brazil, more than 50% of the nodules caused by *Dermatobia* were located on the left side of the body. The preference of the bovine host to rest on its right side could be the reason for this asymmetric distribution (Sancho et al. 1996; Pereira Da Silva et al. 1998; Oliveira-Sequeira et al. 1996).

The mature larva emerges from the mammalian host after three months and pupates on the ground, and after a month, the adult fly emerges (Fig. 12). The L_3 larvae emerge from the host nodules and falls to the soil, then forming a hardened pupa in two to three days. The pupal stage lasts from 4 to 11 (Sancho 1988).

Reports of *D. hominis* myiasis in man are common (Toussaint-Caire et al. 2018; Martínez-Hernández et al. 2019). In America, the countries with the highest infection rates in travellers are Belize, Bolivia, and Brazil (Villalobos et al. 2016).

Cochliomyia hominivorax

Almost all warm-blooded animals, including man and occasionally birds, are hosts for the larvae (screwworms) of *Cochliomyia* (*C.*) *hominivorax*. Cattle, horses, sheep, pigs and dogs are frequently parasitized by this arthropod. If left untreated, screwworm-infested wounds can be fatal (Vargas-Terán et al. 2021).

Before starting the control program of release of sterile males (Davidson 1974) developed by entomologists of the Agricultural Research Service (ARS), Department of US Agriculture (USDA, the screwworm of cattle was widely distributed throughout the tropical and subtropical areas of the American Continent from the Southeast US to northeastern Chile. In 1982, the US was declared free of the screwworm and, then the parasite was controlled towards the south (in October 2000), and Costa Rica was declared free of the screwworm (Kouba 2004). The most successful technique for controlling screwworm was the use of the sterile insect technique (Vargas-Terán et al. 2021).

The adult fly of *C. hominivorax* is approximately two to three time larger than the common house fly and is metallic blue or blue green in colour. Female fly lays eggs on the skin around fresh or necrotic wounds. A wound of skin or mucous membranes is generally required to invade the host tissues. The eggs harch between 12 to 24 hours and the larvae feed

Fig. 13: *Dermatobia hominis* L_3 larvae (Photograph by Carlos R. Bautista Garfias)

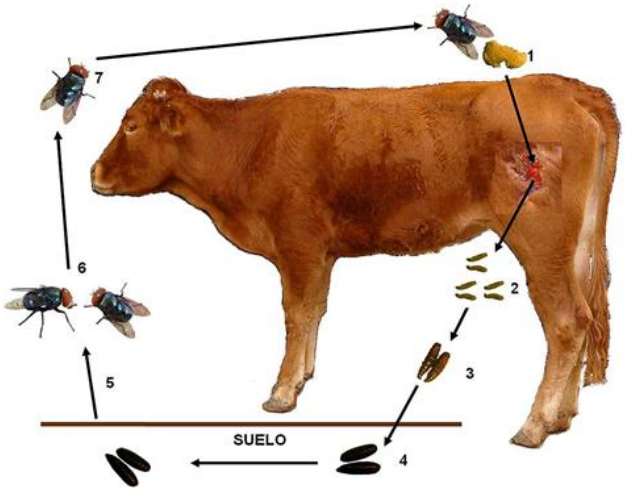

Fig. 14: *Cochliomyia hominivorax life cycle*: 1, The gravid female oviposit in a wound. 2, Larvae (L_1) hatch from the eggs that feed on the wound and then transform into L_2 first and L_3 later. 3, the mature larva (L_3) falls to the ground and buries itself. 4, L_3 pupates. 5, Pupae transform into adults. 6, the male and female mate. 7, The gravid female searches for a wound on a warm-blooded host to oviposit. (Photograph by Carlos R. Bautista-Garfias).

on the wound in a characteristic position (head down and spiracles towards the wound opening). The larvae continue to develop for the next 4 to 10 days, growing to a length of approximately 17 mm. After this time, they fall out of the wound and then transform to pupa in the soil. The pupal stage lasts from a week to three months approximately (Fig. 14) (Vargas-Terán et al. 2021).

Females characteristically mate only once and lay their first set of eggs 5 to 10 days after emergence. They may subsequently lay egg masses every three days during their lifetime. The life cycle during the summer is 24 days on average (Kouba 2004).

C. hominivorax is a true obligate parasite that requires living tissue to feed. It cannot grow on carrion, although an artificial medium for culture has been developed in the laboratory. During feeding, the larva forms characteristic pockets in the affected tissue. Several livestock management procedures such as castration, dehorning, and hot-iron branding, often create oviposition sites (wounds) that attract female fly. The untreated navels of newborn calves in infested areas are frequently attacked. Screwworm-infested wounds are increasingly attractive to gravid flies. Consequently, the syndrome is self-perpetuating in endemic areas and the usual result is death of the host. If *C. hominovorax* populations are not monitored, 20% or more of the animals on a farm may be affected. In the 1980s, ranchers in the USA volunteered to report cases of screwworm myiasis, and in many cases modified their management practices to reduce screwworm problems. In this sense, the breeding programs were altered to produce calves during the winter months (free of flies) and the herds were carefully monitored to facilitate prompt and timely treatment of wounds (Kouba 2004).

In complementary programs, known populations of hematophagous arthropods that attack cattle and similar animals were studied. In this respect, acaricide-impregnated plastic ear tags were widely used to suppress ear tick populations that were later invaded by screwworm (Vargas and Hall 1989; Vargas-Terán 1991; Vargas-Terán et al. 2005, Vargas-Terán 2015; Wyss 2000; Bowman 2006).

C. hominivorax larval infestation in humans generally is a wound myiasis, which can be very severe with penetration and destruction of the underlaying tissue. When the infestation occurs in the nose or ears, the fatality rate is high if untreated (Francesconi and Lupi 2012; Barros and Bricarello 2020; Notejane et al. 2021).

Conclusion

Myiasis in animals and human is caused by the larvae of various species of fly which needs to be controlled as it causes huge economic losses in the animals. The situation may be aggravated by various factors including growing human population, climatic change and lack of proper control measures. One health approach showed its efficacy when a rapid control of the New World screwworm (*C. hominivorax*) outbreak in Florida was achieved in 2016-2017. So, under these circumstances, one health approach offers a viable control alternative.

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6

Impact of Climate Change on Ticks and Ticks-Borne Zoonotic Diseases

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INTRODUCTION

Climate change has emerged as the most serious global threat in the last few decades. It has wide range of impacts limited not only to the environment or the ecosystem but also on the socioeconomics and the politics of the world. It is an inter-governmental issue which needs an organized and cooperative response from all the countries (Dantas-Torres 2015; Abbass et al. 2022). In 2015, United Nations Framework Convention on Climate Change (UNFCCC) in Paris struck an agreement between 195 countries to play their role in fighting the global climatic change by reducing emission of greenhouse gases and limiting the rise in temperature to 1.5°C (Burleson 2016).

The changing earth's climate like global warming, irregular weather patterns, changes in humidity and pressure levels, elevated sea level and melting of glaciers poses sustainable threat to the ecosystem. It causes disappearance of biological communities, changes in biodiversity and alterations in the geographical distributions of species ultimately affecting the human well-being (Dantas-Torres 2015; Pedrono et al. 2016; Khanal et al. 2022). The similar is the case with ticks which spend a major part of their life off from their hosts in the environment (Gray et al. 2009; Nuttall 2021). Their survival in the environment is dependent on the host availability and climatic factors like temperature, humidity, and vegetation coverage (Tomkins et al. 2014; Kaba 2022). Thus, the climate change directly affects the distribution, abundance and the host-seeking behaviour of ticks (Leger et al. 2013).

In the last few decades, the prevalence of ticks has increased showing the positive effect of climate change towards ticks (Cunze et al. 2022). Apart from increased tick prevalence, the impact of climate change on the host's behaviour is also an important factor in the emergence of a disease (Gray and Ogden 2021). Ticks act as vectors for transmission of various diseases including the zoonotic diseases to both the humans and animals. These include bacterial, viral, protozoal and nematode infections collectively referred as tick-borne diseases (Sonenshine and Roe 2014). Both the increased tick prevalence and the rise in magnitude of tick-borne zoonotic diseases are of great concern with life-threatening potential in humans and animals (Cerny et al. 2020; Hromníková et al. 2022; Johnson et al. 2022).

Life Cycle of Ticks

Before we go into the detail of the impact that climate change exerts on ticks and the ticks-borne zoonotic diseases, there is a need for in-depth understanding of tick life cycle. Ticks are the blood sucking ectoparasites of vertebrates which have main four developmental stages, namely eggs, larva, nymph and adult, in their life cycle (Montales et al. 2016). The larvae hatch from eggs, feed on hosts and drop off on the ground where they develop into nymphs. These nymphs again find hosts, feed and again drop off where they undergo final molting into adults. These adults again attach to the hosts where they mate and the female drops off for eggs laying on the ground (Naseer et al. 2021). From the life cycle, it's very clear that most of the ticks' life span is spent in the open environment and are found attached to their hosts only when feeding is required (Dantas-Torres 2010; Estrada-Peña et al. 2012; Cunze et al. 2022). For survival in the open environment, they require certain climatic conditions like high humidity and rainfall to avoid desiccation and a suitable photoperiod and sunshine for proper molting (Belozerov 1982; Estrada-Peña et al. 2013; Gray et al. 2016; Ogden et al. 2021). Thus, any change in climatic conditions directly affects the ticks survival.

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Impact of Climate Change on Ticks

As described earlier, ticks rely on complex set of biotic and abiotic factors for their survival. However, climate is the key factor that determines the prevalence of ticks in a specific area and alters the tick-host-pathogen interactions, thus, opening new areas for ticks invasiveness and pathogenic transmission (Estrada-Peña et al. 2012). Climate change affects the ticks both by direct and indirect means via affecting their survival, reproduction, activity, habitat and their hosts (Ogden et al. 2021). The major impacts of climate change on ticks are discussed below:

Direct Effects

Changes in Geographical Distribution

Climate change has a strong influence over the quality of habitat and hosts abundance for ticks (Simon et al. 2014; Li et al. 2019). It may be either beneficial to the tick growth or may adversely affect the ticks. However, in the last few decades, there has been observed a continuous expansion in ticks geographical distribution even towards higher altitudes (Gray et al. 2009; Jaenson et al. 2012; Leger et al. 2013; Medlock et al. 2013). This is because of increased environmental temperatures along with changes in rainfall patterns which have enabled the ticks to establish new extended areas of their prevalence (Dautel et al. 2008; Keesing et al. 2018). This can be explained with the example of *Ixodes ricinus* tick whose spatial distribution has extended to areas in Europe where it was not recorded previously (Cunze et al. 2022). Furthermore, these climatic changes also favour exotic species in establishing themselves in new areas like an Asian native tick *Haemaphysalis longicornis* is now prevalent in America (Raghavan et al. 2019; Nuttall 2021). Moreover, there are predictions of tremendous increase in global distribution of ticks and inter-continental translocations (López González et al. 2021; Hornok et al. 2022).

Effect on Tick Seasonality, Phenology and Climatic Adaptation

Ticks have a specific pattern of their seasonal activity depending on the weather conditions which favour their host seeking behaviour. These weather conditions include ambient temperature, relative humidity, light intensity and photoperiod (Waladde and Rice 1982; Belozerov et al. 2002; Ostfeld and Brunner 2015; Heath 2021). Warm climate causes an advancement both in the resumption of activity in diapaused ticks as well as the eggs hatching, thus, influencing tick phenology. Over a 19 years period in New York, in the warmer years, *Ixodes scapularis* ticks phenology has been shown to advance by 3 weeks compared to the colder years (Levi et al. 2015). The tick activity of temperate areas is also on rise due to climate warming (Moore et al. 2014; Monaghan et al. 2015). This seasonal effect is more pronounced in the ticks having exophilic behaviour (Estrada-Peña et al. 2012; Ogden et al. 2021). This seasonal effect is evident from the fact that, in Brazil, *Rhipicephalus microplus* tick spends a constant duration of almost 21-23 days on the host irrespective of the season but off the host, this duration is 40-50 days in summer and spring while 70-120 days in winter and autumn (Cruz et al. 2020). Moreover, ticks of the same species have an ability of adaptation to different climatic conditions. This adaptation can be seen in questing behaviour among different populations of same tick species in different areas. Ticks are able to adapt to different climates because of the adaptive evolution and the altered gene expressions in ticks' sensory systems (Simo et al. 2014).

Effect on Tick Reproduction and Development

Climate change is believed to positively affect the ticks' reproduction and development. This positive effect can be seen in terms of increased abundance of ticks in a specific area. This is proved in a study in Russia where an increased abundance of *Ixodes ricinus* ticks was observed over the last 35 years with a 5°C increase in autumn and late summer temperatures (Korotkov et al. 2015). This shows temperature to be the most critical factor for ticks reproduction and development. It affects all the stages of ticks starting from egg laying to questing adults. It has an inverse relation with the duration of ticks development, i.e., the duration is shorter if the temperature is high and vice versa. Thus, the warming earth's climate leads to shortening of ticks life cycle (Ogden et al. 2021). For example, *Ixodes scapularis* tick in Canada takes 3-4 years for completion of its one generation cycle compared to 2 years in USA. Moreover, ticks exhibit behavioural and developmental diapause mechanisms to avoid fatal environmental conditions. Climatic temperature, as the main factor, modulates these mechanisms and as the conditions become favourable, these ticks resume their activity (Ludwig et al. 2016).

Indirect Effects

Effect on Susceptible Hosts

Ticks abundance in a specific area has a strong co-relation with their hosts availability. Any change in the hosts population directly affects the ticks ecology and evolution. These hosts are necessary for the completion of reproduction cycle in ticks (Gilbert 2010; Estrada-Peña et al. 2020). Ticks get their blood meal and, in turn, cause anaemia, weight loss, secondary infections and behavioural modifications in these hosts (Leger et al. 2013). These negative effects of ticks affect the breeding performance and survival of their hosts, thus, leading to alterations in host population dynamics. Moreover, when new tick species invade a new area due to

the climate change, there occur several interactional changes in the community. As a result, some hosts may be favoured while others may be exploited (Tompkins et al. 2011). For example, *Rhipicephalus* (*R.*) *microplus* ticks are specifically the cattle ticks. But New Caledonia invasion by *R. microplus ticks* in 1942 lead to adaptation of rusa deer as their hosts. Initially regarded as poor host, it took almost 250 generations by *R. microplus* ticks to fully adapt to this host and are now existent as separate independent cattle and deer adapted populations (Barré et al. 2001; De Meeûs et al. 2010). This kind of adaptation is the key mechanism which helps ticks in their survival in the changing climate and maintain their biodiversity (Magalhães et al. 2007).

Impact of Climate Change on Ticks-borne Zoonotic Diseases

All the bacterial, viral or parasitic diseases which are transmitted from animals to humans are referred as the zoonotic diseases (Sonenshine 2018). Of all the infectious diseases, 60% are zoonotic in nature (Jones et al. 2008). Transmission of these diseases occurs through different routes like direct contact, inhalation and ingestion or may be vectored by arthropods (Kulkarni et al. 2015). Among the arthropods, ticks transmit the largest number of zoonotic diseases than any other arthropod (Durden 2006). According to CDC in USA, annually 95% of the 50000 notifiable locally acquired vector-borne diseases are tick-borne (Adams et al. 2016; Paddock et al. 2016). These ticks-borne zoonotic diseases are of great public health importance with an increasing worldwide incidence. This increasing diseases' incidence is attributed to the climate change which has direct influence over ticks abundance and survival, host availability and pathogens transmission (Dumic and Severnini 2018). Some of the ticks-borne zoonotic diseases include Lyme disease, tick-borne encephalitis, Crimean-Congo Hemorrhagic Fever, rickettsioses and tularemia (Fritz 2009). These diseases are directly related to ticks for their transmission. Thus, any climate change which affects the ticks either directly or indirectly would certainly have an impact on these ticks borne diseases (Ghafar et al. 2021).

Lyme Disease

Lyme disease or sometimes referred as Borreliosis is a bacterial disease caused mainly by *Borrelia burgdorferi*. It is a zoonotic disease transmitted through bite of infected *Ixodes* spp. ticks (Mills et al. 2010). As described earlier, these ticks pass through three developmental stages and complete their life cycle in 2-3 years depending on the climatic conditions. The climatic conditions resulting from global climate change have resulted in higher ticks prevalence through increased tick survival and host availability (Dumic and Severnini 2018). As a result, Lyme disease cases are increasing across the world. For example, in Canada in 2004, only 40 cases of Lyme disease were reported. During 2009 to 2015, these cases rose from 144 to 917 showing a six-fold increase (Koffi and Gasmi 2019). This increased incidence of the disease in Canada was linked to the northward geographical expansion of *Ixodes scapularis* ticks (Koffi and Gasmi 2019). These ticks rely on white-footed mouse as their primary hosts. Thus, the increased abundance of white-footed mouse favoured by climate change resulted in increased prevalence of *Ixodes* ticks ultimately leading to increased cases of Lyme disease (Mills et al. 2010; Roy-Dufresne et al. 2013). Similarly, the case data over the period of years 2000-2017 in USA indicated an increased incidence of Lyme disease in association with elevated annual climatic temperatures. This climate-disease association was most prominent in the northeast of USA (Couper et al. 2021). In the northeast, there was observed an association between the ticks, rodents and the climate change (Ogden et al. 2018). If this scenario continues in the USA, there is a prediction of 20% increase in Lyme disease incidence in the coming years (Dumic and Severnini 2018).

Tick-borne Encephalitis

It is a viral disease caused by tick-borne encephalitis virus of the *Flavivirus* genus. It is zoonotic in nature with humans acting as accidental hosts while small mammals as the main reservoirs. It affects the central nervous system of the humans and is distributed in Europe, Caucasus, Kazakhstan, Russia and China (Nah et al. 2020; Rubel 2021). In the past few decades, there has been observed a continuous rise in tick-borne encephalitis cases across the globe. It has been recorded even in those areas where it was previously absent (Daniel et al. 2018; Riccardi et al. 2019).

It is typically a seasonal disease linked to *Ixodes ricinus* ticks and particularly their nymphs. The disease transmission between ticks and hosts occurs through different routes like systemic, non-systemic and transovarial methods. In the systemic method, the transmission occurs in a cycle where the infected ticks bite the hosts and transmit pathogens to them. Then, the non-infected ticks bite the infected hosts and take up pathogens with the blood meal and transmit these pathogens to other non-infected hosts while feeding on them, thus, the systemic cycle continues so on. In the non-systemic method, the transmission occurs between infected and non-infected ticks through co-feeding on the same host before the pathogen has established itself in the host for systemic transmission. In the third transovarial method, the pathogens are transmitted from the infected females to the next generation through their eggs (Nah et al. 2019).

Among the various factors that influence the transmission of tick-borne encephalitis, climate change is the most important one. It directly affects the ticks' survival and movement, their reproduction and their ecological interactions (Wondim et al. 2022). The climate change leads to sustained tick-borne encephalitis disease transmission through increased host availability, increased tick abundance and extended periods

of questing which allow co-occurrence of infected and non-infected nymphs and larvae (Nah et al. 2020).

Crimean-Congo Hemorrhagic Fever

It is also a tick-borne zoonotic disease caused by Crimean-Congo hemorrhagic fever virus of the family Nairoviridae. It transmits to humans mainly through the bite of infected *Hyalomma* ticks and is prevalent across Africa, Asia and Europe. Apart from tick biting, this disease can also spread through direct contact with the infected blood and body fluids of patients. Hence, due to its potential threat, it resides in the WHO's list of top eight emerging pathogens and categorized as level 4 biosecurity risk pathogen by CDC (Monsalve-Arteaga et al. 2020; Kuehnert et al. 2021).

As the global prevalence of Crimean-Congo Hemorrhagic Fever is concerned, it is constantly on the rise. There are reports of epidemics in the East Mediterranean countries for the last two decades (Portillo et al. 2021). It has even established itself in the regions where it was previously non-endemic like Turkey, Greece, Iran, India, Georgia and Spain etc. Moreover, apart from geographical expansion, it also possesses a higher incidence rate. For example, since the identification of first human case in 2002 in Turkey, the number grew to over 6300 in 2012. Similarly, huge increase in human cases had also been observed in Iran since the discovery of infection in 1999 (Bente et al. 2013).

The incidence and alterations in geographical ranges of this disease have a triad link with ticks and climatic conditions. Ticks harbour the pathogens and are dependent on climatic conditions for their survival and reproduction. As the conditions become favourable to the tick vectors due to climate change, the tick population grows in number and may establish itself in new geographical areas. As a result, the disease is introduced in new areas and an increase in tick bites occur which ultimately lead to increased pathogenic transmissions (Chinikar et al. 2010; Ahmed et al. 2021).

Conclusion

Climate change is an international issue which is having socioeconomic as well as political impacts. It poses a significant threat to the viability of ecosystem. It is leading towards global warming and irregular weather patterns which affect the biodiversity and cause geographical alterations in the species' habitats. Likewise, ticks are also affected by these changes as they are directly dependent on climatic factors like temperature, humidity, and vegetation coverage for their survival in the environment. Moreover, the host availability to ticks in specific geographical areas is also influenced by the climate change. In the last few decades, the climate change is seen to have favoured the ticks growth. There is seen an increased abundance and prevalence of ticks beyond their normal known geographical boundaries and, hence, an increased magnitude of ticks-borne zoonotic diseases.

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7

Ringworm Among Cattle

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INTRODUCTION

Dermatophytosis was first discovered by Gurby during the first half of 19th century. He found *Microsporum audouinii* in human who suffered from tinea capiti (Gräser et al. 2000). Ringworm, dermatophytosis, dermatomycoses or tinea, all refer to the same disease, which is caused by keratinophilic fungi called dermatophytes. A total of six genera may cause ringworm infection, including *Trichophyton*, *Microsporum*, *Epidermophyton*, *Arthroderma*, *Nannizzia* and *Lophophyton*. However, according to formal classification, a total of three genera which is involved in causing the infection. They also attack the superficial keratinized tissues of the nail, claws, skin, and hair of animals and human (Gudding and Lund 1995; Al-Ani et al. 2002; Pal 2007; Dalis et al. 2019; Begum et al. 2020). In addition, *Trichophyton (T.) verrucosum* is an infectious agent of cattle dermatophytosis (Gudding and Lund 1995; Shokri and Khosravi 2016). Besides, *T. mentagrophytes* were also reported among the animals (Shams et al. 2009). This disease is responsible for causing public health problem and large economic losses across the world which include, reduction of milk and meat and production losses besides damage or low grade type of skin structure (Eman-abdeen 2018; Dalis et al. 2018). It is zoonotic pathogen (ElAshmawy and Ali 2016) that is transmitted from animals to humans either via the direct contact with a diseased animal, or indirectly via contact with a contaminated environment. However, contact with arthrospores or conidia are the main rout of transmission of the disease. The high occurrence of ringworm was recorded in winter season. Because, fungal spores grow best in high humidity leading to increase susceptibility of the hosts to ringworm infection (Nooruddin and Singh 1987). However, chances of infection are more in housed animal (Al-Ani et al. 2002; Radostits et al. 2007; Dalis et al. 2014). Infection with dermatophytes is characterized by the development of ring-shaped lesions which becomes alopecic. Direct microscopic examination, culture, Wood's lamp examination, histopathology, PCR assay are mostly used for diagnosis of the infection (OIE 2013). However, molecular test along with culture results showed as gold standard approaches for detection of the infection (Abd-Elmegeed et al. 2020). In this chapter, we highlighted the etiology, epidemiology, pathogenesis, clinical signs, diagnosis, treatment and control of the infection among cattle.

Etiology and Epidemiology

Conventionally "dermatophytes" are identified in the imperfect fungi or Deuteromycota in three anamorphic genera including: Epidermophyton, Trichophyton, and Microsporum. These are recognized as asexual or imperfect stat. But the teleomorphic state which is "perfect or sexual state " has been described for some species. Dermatophytes are classified in the genus Arthroderma, and phylum Ascomycota (Markey et al. 2013). However, they are regarded as fungi that use keratin for growth. According to many researches about 40 dermatophyte species were recognized so far and only, three genera i.e., *Trichophyton*, *Microsporum* and *Epidermophyton* are identified to be pathogenic for animals and human (Weitzman and Summerbell 1995; Smith 2011; Eman-abdeen 2018). The species of dermatophytes that affected animals are called ectothrix such as the septate hyphae attacking the hair fragment and skin structure into arthrospores and these from a sheath around the infected structures. Besides, these microconidia and macroconidia are created in the laboratory cultures. Macrocoindia of *Trichophyton* spp. is characterized by enlongated, cigar-shape with approximately parallel sides. The *Microsporum* spp. tends to yield boat or spindle shaped. Whereas, macrocoindia of *M.nanum* characterized by having pear-shaped and usually two-celled (Markey et al. 2013). According to habitat there are three main types of dermatophytes, called zoophilic (animal), geophilic (soil), and anthrophilic (man). Meanwhile, most bovine dermatophytosis caused by *T. verrucosum* belong to zoophilic (animal), while *T. mentagrophytes* may also causing cattle dermatophytosis along with *Microsporum (M.) canis*. There is difference between dermatophytes species from diagnostic examination and culturing. Furthermore, *T. verrucosum* can remain infective in environment for long periods of almost (5-7) years (Eman-abdeen 2018).
T. verrucosum can grow at 37 $\dot{C}^0$, while both M. canis and T. mentagrophytes cannot grow at this temperature. *T. verrucosum* needs vitamins requirement such as Thiamine and inositol (Eman-abdeen 2018). Socioeconomic status,

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lifestyle, migration, and drug therapy are the main causes of change in the epidemiology of ringworm (Ameen 2010). Dermatophyte-infection have a several range of host species, but it is most frequently reported in those areas where animals are housed in dense groups, particularly indoors (Radostits et al. 2007). The route of transmission of the infection is through contact with infected inanimate objects or direct contact. Furthermore, carrier animals are the source of infection (Radostits et al. 2007). Fungal diseases will emerge if the immune system of the host is weak (Shokri and Khosravi 2016). In addition, the occurrence and distribution of ringworm is also influenced by host factors (stress, age, management and transportation), climate condition and geographic area (Al-Rubiay and Al-Rubiay 2006). However, the factors such as species, numbers and age of animal besides environmental aspects will serve a significant role in the rate of infection (Eman-abdeen 2018). Furthermore, a study conducted by Marai et al. (1999) showed that the rate of ringworm infection among cattle was higher in foreign breed than in native breed. According to studies conducted by Pascoe (1979) and Shams et al. (2009) the prevalence rate was higher in the young animals. Another study by Abd-Elmegeed et al. (2020) showed higher infection rates in male animals as compared to female animals (Abd-Elmegeed et al. 2020). Many studies reported cattle infection with *T. verrucosum* in the Asian countries, including Iraq (Hussein et al. 1989; AL-Samarrae 2009), Iran (Shams et al. 2009; Shokri and Khosravi 2016), Turkey (Ozkanlar and Kirecci 2009), Saudi Arabia (Khaled et al. 2015) and Egypt (Abou-Gabal et al. 1976; Bagy et al. 1986; Abd-Elmegeed et al. 2020). The prevalence of fungal infection were also found significant in European countries, including United Kingdom (Oldenkamp 1979), Norway (Stenwig 1985), Germany (Berlin et al. 2020), and Italy (Atzori et al. 2012). Season plays a role in the intensity of the disease transmission, for example (Al-Ani et al. 2002; Radostits et al. 2007, Dalis et al. 2014; Abd-Elmegeed et al. 2020) showed that the incidence rate of the disease was peaked in winter. Table 1 shows the prevalence rate of bovine ringworm infection in various countries.

Pathogenesis

Dermatophytes invade in the keratinized tissues, chiefly the hair fibers and stratum corneum, and causing the hydrolysis of the fiber structure, and breaking off of the hair, which ultimately leads to alopecia (Radostits et al. 2007). The body of animal host shows hypersensitivity reaction against the metabolic products of the pathogen leading to development of lesion. However, the host mounts an inflammatory response that is harmful to the fungus, so the dermatophyte moves away peripherally towards normal skin. It ultimately leads to the development of circular lesions with alopecia having healing at the center and inflammation at the edge. (Markey et al. 2013). The importance of epidermis pH in the growth of dermatophytosis is usually known (Radostits et al. 2007).

Clinical Signs

Among cattle, ringworm infection ranges from small focal lesions to extensive pathogenesis involving the entire body (OIE 2013). Characteristically, the lesion is a heavy, grey-white crust that is elevated perceptibly above the skin. The lesions are circular, almost 3 cm in diameter and are commonly found on the neck and head, particularly around the eyes and face. However in severe diseased animals, it may be observed over the whole body (Apaydin and Atalay 2007). In addition, the clinical signs usually resolve spontaneously during 2 to 4 months (OIE 2013). However, according to Guo et al. (2020) the skin lesion was reported in different body sites. The highest rate was 38.71% in head, and lowest rate was 9.68% in whole body (Fig. 1).

Diagnosis

The diagnosis of bovine dermatophytosis is generally based on history, close physical examination, clinical signs, direct microscopic examination, Wood's lamp examination and histology of the tissues (Apaydin and Atalay 2007; Swa and Sanka 2012) However, molecular diagnostic test along with culture results showed as gold standard approaches for detection of the infection (Abd-Elmegeed et al. 2020). Traditional method for detection of the infection in dermatophytes suspected lesions by using 20% KOH (Ellis et al. 2007). Dermatophyte organisms can be cultured on several fungal media, including dermatophyte test medium (DTM) and Sabouraud agar (SDA) (with cycloheximide and antibiotics). These are usually incubated at room temperature (20–-28ºC). While, *T. verrucosum* needs higher temperatures. However, colonies often become visible within 7-14 days (OIE 2013). Fungal cultures, is important to recognize the source of dermatophytosis and targeting preventive measures appropriately. Culture may also be needed in either the diagnosis is uncertain, or the infection is resistant to standard therapy (OIE 2013). *T. verrucosum* is usually characterized by very slow growing white, cottony, non-pigmented reversed side colonies having heaped up, and button like appearance with folded areas (Dalis et al. 2014; Eman-abdeen 2018). In contrast to microscopical picture, *T. verrucosum*-agent appear as septated hyphae and microconidia with existence of *chlamydospores* which arranged in chain (Eman-abdeen 2018). However, molecular diagnostic test along with culture results showed as gold standard approaches for detection of the infection (Abd-Elmegeed et al. 2020). Molecular tests such as PCR have been efficiently used for investigation of the organisms which proved to be more specific, accurate and stable than phenotypic characterization(Graser et al. 2000).

Treatment and Control

Ringworm causes a self-limiting infection showing natural recovery in mild cases. While, different antifungal such as topical iodine and Sulphur preparation are applied for

Table 1: shows the main differentiation between the two genera including Trichophyton spp and Microsporum spp. by microscopic examination.

	. *Trichophyton* spp.		*Microsporum* spp	
	Macroconidium	Microconidia	Macroconidium	Microconidia
Macroconidia	Relatively insufficient or lacking among various species. If existing they are elongated and pencil or cigar-shaped. Their walls are smooth and thin; where distributed by septa into 3-8 cells		Large thick-walled and separated into numerous cells by transverse septa. They are boat or spindle-shaped.	
Microconidia	Generally, these are several in number and borne singly along the hyphae or in grape-like clusters.		Moderately insufficient or lacking. If existing these are tear-shaped and borne singly on the hyphae.	

Table 2: Bovine ringworm's prevalence rate in different countries

Locations	Prevalence rate	References
Central region of Iraq	21.2 %	(Hussein et al. 1989)
Ninevah, Mosul, Iraq	26.5%	(Arslan et al. 1998)
Baghdad, Iraq	68 %	(AL-Samarrae 2009)
Diyala, Iraq	90 %	(Jameel 2015)
Ningxia, China	15.35 %	(Guo et al. 2020)
Different parts of Jordan	30.6 %	(Al-Ani et al. 2002)
West Bank of Jordan	59.3 %	(Ali-Shtayeh et al. 1988)
Ankara, Turkey	33.33 %	(Sever et al. 2017)
Barcelona, Spain	25 %	(Cabanes et al. 1997)
Nweze, Nigeria	12.6 %	(Nweze 2011)
Thamar, Yemen	11 %	(Golah et al. 2012)
Brazil	58.3 %	(Duarte et al. 2013)

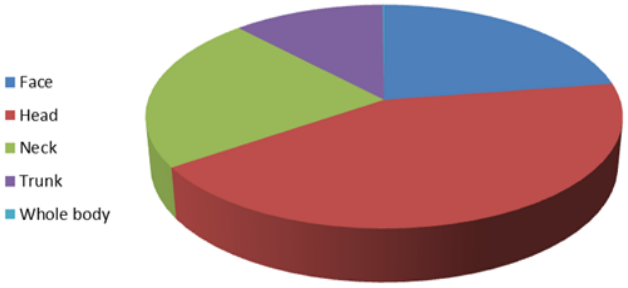

Fig. 1: Distribution of Ringworm in different body regions

treatment of severely affected lesions. Some researchers also recommended the removal of scales and crust before applying the ointment preparation. In addition, there are systemic antifungal treatments but may left some residues which has harmful or toxic effect on animals or human body (Araújo et al. 2009). Furthermore, plant fungicides like chlorhexidine and captan, iodide shampoos and tinctures, 5 per cent lime sulphur, enilconazole, thiabendazole, sodium tolnaftate, and fluorides (toothpaste) are also used for topical treatment. Sodium iodide and *T. verrucosum* vaccine may also be used to treat the infection by intravenous and intramuscular injection, respectively. In addition, griseofulvin used orally to treat the infection (Pandey 1979; Apaydin and Atalay 2007). On the other hand, ivermectin significantly can be used to treat the disease (Jameel 2015). In recent studies, natural antifungal plants have been developed, because these are effective, have low cost, easily applied under field conditions and less toxic. Lemon grass, garlic, ginger, acacia, datura, a triplex, neem, black seed, eucalyptus, basil and alfalfa are some types of natural plant. Recent study by Eman-Abdeen and El-Diasty (2015) showed that Clove oil proved highly effective antifungal activity against the infection invitro and can be used as a topical spray and ointment for treatment of ringworm. Failure to control an outbreak of dermatophytosis is frequently due to the widespread contamination of the environment before treatment is attempted. In addition isolation, treatment of infected animals, cleaning and disinfection of stables are need (Radostits et al. 2007). Vaccination has an important role to prevent the infection among cattle and horses (Radostits et al. 2007). Both innate and adaptive immune mechanisms are involved in the response to the infection. Moreover, it has been found that antigens of *M. canis* and numerous species in the genus of *Trichophyton* stimulate both humoral and cell-mediated immune responses (Pier et al. 1992; DeBoer and Moriello 1993). Among cattle, *T. verrucosum*-agent is the main cause of the infection; rarely *T. equinum*, *T. mentagrophytes* and *M. canis* are isolated from lesions of the infected animals (Stenwig 1985; Radostits et al. 2007). The goal for the

prevention of cattle dermatophytosis is to obtain an effective vaccine against *T. verrucosum* infection. Both live attenuated and inactivated vaccine" for the agent have been developed. In most of Europe, there are currently four available dermatophyte vaccines (Lund and DeBoer 2008). However, the main common method for assessment of vaccine safety and efficacy and characterization of the immune response involves the target animal species. A few studies have used heterologous challenge strains indicating some degree of cross reactions(Lund and DeBoer 2008). In Norway, there is a program to eradicate bovine dermatophytosis in herds by vaccination, isolation of infected animals, good hygiene and disinfection of contaminated stables. In one region of Norway, over a period of 8 years, where 95% of flocks participated, the infection rate of the disease reduced from 70% to 0% (OIE 2013).

Conclusions

The disease is commonly known by several names including ringworm, dermatophytosis, dermatomycoses or tinea. *T. verrucosum* is the main cause of bovine dermatophytosis. The main route for spread of infection from animals to humans is through direct contact. Molecular assay along with culturing serve as a gold standard approaches for diagnosis. The high incidence of the infection is usually recorded in winter season. The occurrence and distribution of ringworm is influenced by host factors (stress, age, management and transportation), climate condition and geographic area. Vaccination has an important role to prevent the infection among cattle and horses. Natural antifungal plants i.e., clove oil proved highly effective against the infection and can be used as a topical spray and ointment for treatment of ringworm.

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8

Tick Bites and Red Meat Allergy

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INTRODUCTION

Red meat allergy, also known as an alpha-gal syndrome (AGS), is symptomatically associated with the consumption of glycan galactose-alpha-1,3-galactose (alpha-gal) (Chung et al. 2008).

Alpha-gal is a carbohydrate present in mammals except for humans and Old-World monkeys. The gene (GGTA1) responsible for the synthesis of the enzyme (alpha-1,3-galactosyltransferase) that is involved in the glycosylation of alpha-gal is absent in humans and Old-World monkeys. Therefore, immunocompetent persons can show anti-alpha-gal antibodies in a natural way Galili et al. 1987; Singh et al. 2021). Symptoms of red meat or mammalian meat allergy include angioedema, anaphylaxis, and gastrointestinal (GI) symptoms such as abdominal pain, nausea, diarrhea, heartburn, joint pain and pruritus (Iweala et al. 2018; Mabelane et al. 2018; Wilson et al. 2019). These symptoms occur 3-8 hours after the consumption of mammalian meat (beef, pork, or lamb) or other mammalian-derived products (gelatin, dairy products and pharmaceutical products containing alpha-gal). The delayed onset of the symptoms is due to the time taken for the digestion of lipids and protein containing alpha-gal and entry of alpha-gal into the blood circulation. Due to the delay in symptoms, it is difficult for doctors and clinicians to diagnose it as a food allergy (Flaherty et al. 2017).

Ticks are responsible for different allergic reactions in different countries across the world. The tick *Ambloymma (A.) americanum* is the vector for Rocky Mountain spotted fever and is also responsible for red meat allergy in the United States (Van Nunen et al. 2019). Similarly, red meat allergy is a tick-induced hypersensitivity reaction and is associated with anaphylaxis, angioedema, and urticaria. In this disease, IgE antibodies are produced against alpha-gal and cause hypersensitivity reactions in humans. Red meat allergy is different from other food allergies as IgE-mediated responses are produced against a carbohydrate (alpha-gal). While in other food allergies IgE mediated reactions are produced against proteins or other ingested allergens. Antibodies production against alpha-gal in red meat allergy is associated with tick bites rather than the ingestion of some allergen (Commins et al. 2011).

Association Between Tick Bites and Red Meat Allergy

The increased levels of specific IgE and IgG antibodies against alpha-gal epitope are characteristics of AGS or red meat allergy patients, and most of the individuals with red meat allergy who may have withstood the mammalian meat for several years can develop alpha-gal sensitization after tick bites (Platts-Mills et al. 2015; Kollmann et al. 2017). It is discovered that the different tick species, especially the most abundant *Ixodes (I.) ricinus* species in Europe, contain alpha-gal in their cement and salivary glands (Hamsten et al. 2013). The process of inducing sensitization to this epitope by tick bites and, ultimately, mammalian meat allergy is not fully understood yet. It is evident that only the alpha-gal exposure is not responsible for the IgE response; it may be due to the ticks' salivary proteins containing alpha-gal antigens or may be due to the prostaglandin E2 (PGE2) in the saliva (Carvalho-Costa et al. 2015).

There is an association between tick bites and red meat allergy, and is reported worldwide. Concentrations of alpha-gal IgE in the blood of patients decrease as they avoid the recurrent tick bites, and the level of decrease varies from person to person (Commins et al. 2011).

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In the U.S., it was observed that there were similarities in the geographical distribution of the reported patients of alpha gal syndrome and Rocky Mountain spotted fever (Commins and Platts-Mills 2013; Crispell et al. 2019). The tick *A. americanum* is responsible for the transmission of the causative agents of these diseases *(Rickettsia* and *Ehrlichia).* In this preview, it was hypothesized that the lone star tick (*A. americanum)* is the cause of sensitization to alpha-gal (Commins et al. 2009; Commins et al. 2011). Other reports also give evidence that the high titer alpha-gal IgE is associated with more than two tick bites, and the titers are low in the individuals avoiding tick bites, suggesting the relation of ticks as sensitizing agents (Hashizume et al. 2018). Initially, it was stated that alpha-gal transmitted to human hosts by mature ticks is derived from mammals during blood meal, but latter evidence showed that larval ticks transmitted alpha-gal that was never fed mammalian blood (Stoltz et al. 2019).

Worldwide Distribution of Ticks-induced Mammalian Meat Allergy

Alpha-gal in the meat is responsible for the production of IgE in the human host. Data on the red meat allergy after tick bites have been reported (Van Nunen et al. 2007).

In Europe, the prevalence of IgE production to alpha-gal has been found to be 5.5% in Denmark, 15.7% in Spain, and 24.7% in a rural region of northeast Italy (Joral et al. 2022). More than 5000 cases have been reported in the U.S. The work about this disease started when a cancer patient in the U.S. developed a hypersensitivity reaction to cetuximab (a medicine used in the treatment of cancer). During the clinical processes, there was a low risk of allergy against the drug but in the cases from the specific region of the U.S. developed, severe drug hypersensitivity reactions. Later, researchers found that the patients, who showed allergic reactions, already had IgE antibodies that bound with the alpha-gal present in the murine portion of cetuximab (Chung et al. 2008).

The number of cases increased with hypersensitivity reactions after eating red meat in the U.S. In these cases, many individuals who have been consuming red meat for years never developed symptoms before (Commins et al. 2016). The IgE response developed against the alpha-gal present in red meat. It was noted that both drug-induced and meat-induced allergy individuals belonged to the same area abundant with lone star tick (Steinke et al. 2015).

Fig. 1 shows the occurrence of red meat allergy reported for the first time in different areas of the world.

Many cases were also reported in Australia having a history of tick bites. The first research on tick bites causing red meat allergy in Australia was published in 2007. Starting from those days, this disease is turning into a global issue and is influencing almost all continents. In Australia, two species of ticks *I. holocyclus* and *I. australiensis* responsible for causing red meat allergies (Binder et al. 2021).

Clinical Features of Red Meat Allergy

The disease shows similar kind of symptoms in children and adults. Angioedema, GI symptoms and most severe anaphylaxis causing adverse meat allergies contribute almost 65.6% (Fischer et al. 2016). Nearly 10 % of the cases that are sensitive to red meat also react to the gelatin obtained from mammals. Intravenous or intramuscular administration of gelatin may increase the chances of anaphylaxis and may be the initiation of red meat allergy. Clinical reactions were reported when gelatin was administered orally and through the intravenous route and few cases were reported with positive gelatin tests and negative red meat tests (Mullins et al. 2012).

The role of co-factors in red meat allergy is very important. Knowledge about the factors that increase the impact of mammalian meat allergy is important to know for the safety purposes. These factors, individually or with the synergism, increase the severity of alpha-gal sensitivity reactions to red meat (Wölbing et al. 2013). The major contributing factors of the disease include consumption of a high amount of allergen, alcohol intake with food, use of spices (chili & capsicum), physical activity, use of anti-inflammatory non-steroidal agents, to be in the premenstrual period, and cooking impacts (Versluis et al. 2016). Moreover, the level of alpha-gal is different in many products, such as egg and pork kidneys have high levels of alpha-gal and increase the chances of sensitivity. The milk obtained from cows also has alpha-gal, and the sensitivity of alpha-gal has vanished on heating this milk. Hence, pasteurization of this milk makes it tolerable (Commins et al. 2014).

Process of Development of Red Meat Allergy

The development of red meat allergy via tick bites is an example of the initiation of an allergy. It is a phenomenon in which climatic change (High tick population, increased tick bites), inheritance, host immune shifts due to parasites and the presence of a pathogen in ticks (rickettsiosis) are involved (van Nunen and Sheryl A 2018).

As it is evident from the fossils that the process of development of red meat allergy due to tick bites started 28 million years ago. The enzyme responsible for the production of alpha-gal was inactivated in our ancestors at that time, this is why the human body gets alpha-gal as a pathogenic particle, and alpha-gal IgE antibodies are produced, hence giving defense to the pathogenic bacteria, coated viruses and protozoa that contain alpha-gal (Galili 2013).

As per available literature, alpha-gal is an external particle for humans that prepares them after bites from ticks and initiates the pro-allergy Th2 cells cytokines in the humans that starts the preparation of anti-alpha-gal antibodies (Abs) by the IgG and ultimately the IgE Abs from B cells (Ferreira and Silva 1999). Proteins from the ticks are glycosylated, which promotes this process leading to an increase in immunity. So, when IgE class Abs to ticks proteins are

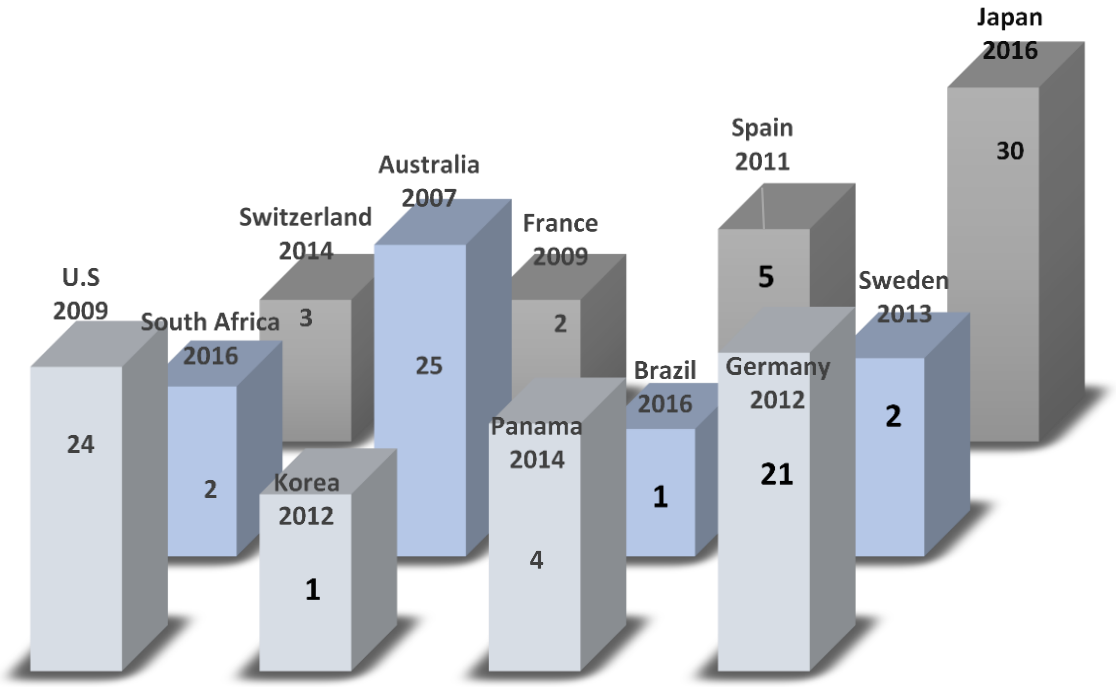

Fig. 1: Graphical representation of number of cases reported first time in different countries (Van Nunen and Sheryl A 2018).

generated at the same time, alpha-gal IgE Abs are also produced. The IgE production mechanism is activated by the tick bites against the alpha-gal, and when this person consumes mammalian meat, the IgE production starts against the alpha-gal present in meat, and hypersensitivity reactions occur, causing red meat allergy (Dorey 1998).

The last important thing in the red meat allergy reactions is the delay in the occurrence of these reactions. This procrastination in the appearance of the symptoms is because of the time required for the transport of alpha-gal from the gastrointestinal tract to the blood circulations. Glycoproteins, as well as glycolipids, also contain alpha-gal. The complete breakdown of lipids takes many hours, and after that, the absorption of chylomicron having alpha-gal starts in the small intestine into the lymphatics and then into the bloodstream stimulating basophil mediators' production in the blood (Commins et al. 2014).

Management of Red Meat Allergy

To date, there is no cure for this disease, but to get rid of this disease, prevention strategies are adopted. Avoidance of mammalian meat, mammal-derived things, and sometimes dairy is advised for the patients (Patel and Iweala 2020). Evidence showed that more tick bites increase the level of IgE in the blood, and the prevention of tick bites reduces the amount of IgE in the patients and also the sensitivity to red meat (Kim et al. 2020). In a study, 12% of patients who avoided tick bites for nearly five years reduced their IgE level to less than 0.1 IU/mL and included red meat in their meals successfully (Commins et al. 2016).

No study is conducted yet showing the relationship between the use of red meat and dairy products influencing the levels of IgE in red meat allergy patients. Another observation also supports this concept when some patients developed mild or no symptoms and tolerated red meat on an event, severe sensitivity reactions to red meat appeared in the same patients in another event. This difference is not due to the quantity of meat used but due to the level and quality of alpha-gal present in meat or may be due to the inclusion of co-factors and current bites from the ticks (Iweala et al. 2018). Following preventive measure should be taken to reduce the chances of red meat allergy.

Avoiding Meat from Mammals

Firstly, the new cases reported of red meat allergies are strictly instructed to skip mammalian meat such as Lamb, pork, beef, and venison. Organ meat, specifically pork kidney, also causes sensitivity reactions, so it should be excluded from the diet (Fischer et al. 2014). Meat rich in fats is also associated with the severity of reactions and symptoms. Alpha-gal is not decomposed by heating meat, but the fat content is decreased, which minimizes the severity of the reaction (Apostolovic et al. 2014). Other mammalian meats and products should not be consumed. Some cases also develop signs of red meat allergy when air droplets arising from the heating of meat are inhaled, but no document has been published yet.

Avoiding Dairy Products

Products from dairy, such as cheese and milk, are not recommended in red meat allergy patients on a daily basis because nearly 81-90% of cases do not show reactions to these products (Levin et al. 2019). Some experts' opinions and research work suggest the complete avoidance of these products in the cases who are not consuming meat and still, there is no significant decrease in symptoms (Commins 2016).

Non-dairy and Mammalian Derived Products

Non-dairy and mammalian-derived products may also pose a risk of allergy when mammal-derived ingredients are mixed in these foods. A major risk factor is the availability of non-labeled products. In the market, some of these items mentioned that alpha-gal content (cetuximab) is included, while in some, it is missing (glycerine) because of the reason obtained from the mammals. Mammalian-derived bovine serum albumin does not consist of alpha-gal, so being obtained from mammals does not mean that it consists of alpha-gal (Thall and Galili 1990).

The occurrence of hypersensitivity reactions in individuals who have removed all known forms of alpha-gal from their diets is due to the presence of a hidden form of alpha-gal in those foods. Special attention is given to foods that contain high levels of mammalian-derived lipids, particularly when they are associated with exercise, alcohol, sickness, and menses etc (Scott 2020).

Foods high in fat and added fats are also linked with the severity of reactions. Lard is used in food preparations, gravy, and sauce. It is also used as a flavor enhancer. Mammal-derived fat such as suet and tallow are also used in food preparations. Different types of sausages contain casings (a chemical that contains alpha-gal) obtained from the pig gut. Turkey and chicken sausages also resulted in sensitivity reactions in some cases. Carrageenan, as well as gelatin, are commonly used foods additive obtained from mammals and contain alpha-gal (Scott 2020). Gelatin is an important content of gelatin desserts and its sensitivity is common in patients, but in many cases, it is tolerated if present in low quantity in daily uses (Caponelto et al. 2013). Carrageenan is obtained from reddish esculent seaweed and is commercially used in food preparation as a thickening and stabilizing agent. The chances of developing symptoms after eating these products are very low (Chauhan and Saxena 2016). The problem is that it is a plant-origin food that is alpha-gal-free foods. So, the cases who are avoiding the diets but still have sensitivity should be analyzed for carrageenan use.

Medical Therapies of Red Meat Allergy

In the drug therapy, long active oral antihistamine (fexofenadine) is preferably used two times a day. Another feasible method that can be used is the application of short-active oral antihistamines, as many cases have endured the Unisom and SleepMelt tablets (Scott 2020). Those cases who are avoiding specific foods but still showing gastrointestinal tract signs and symptoms are advised to use oral solutions of cromolyn. It is recommended four times a day with a dose range of 100-200 mg (Scott 2020).

Red meat allergy individuals having severe and recurrent sensitivity with asthma can be treated with oral corticosteroids. Omalizumab has been used successfully for the control of continued reactivity in some patients, and those individuals added small amounts of red meat in their meals showing no harm (Scott 2020). In a study, six cases were using Metformin during the preparations of gastric bypass surgery, started consuming dairy products, and then included mammalian meat in their meal (Samavedam et al. 2016). In another research, it is evident that Metformin's impact on the unfolded protein response can change the cytokine environment and potentially reprogram the immune system (Samavedam et al. 2016).

Therapeutic Prevention

Alpha-gal is a component of many drugs and medicines and can be dangerous in some new therapies for the persons who are allergic to alpha-gal (Galili 2013).

- Because of the alpha-gal present in cetuximab, dangerous reactions appeared by its intake.
- Vaccines such as measles and mumps as well as zoster contain alpha-gal can cause allergic reaction in the person sensitive to alpha-gal (Stone et al. 2017).
- Gelatin is also mammalian derived and is component of vaccine, tablet, capsule and implants (Mullins et al. 2012).
- Antivenom against snakes, scorpions, spiders, jellyfishes etc. also contain alpha-gal and cause sensitivity reactions in the red meat allergy patients when used (Fischer et al. 2017).

Expert's Opinion

Knowledge about red meat allergy to professionals in healthcare is important to diagnose and manage this disease. In the regions abundant in the population of ticks and where bites from the ticks are usual, mammalian meat allergy is in the process of recognition and diagnosis. Alpha-gal IgE tests are suggested in these areas. A magazine having mammalian meat allergy-related information for the patient's families and healthcare providers should be developed. Similar to other food allergies, avoid exposure to allergens and tick bites. Proper labeling of ingredients in the food obtained from mammals, medicines, drugs, and vaccines is recommended for mammalian meat allergy cases. Manufacturing of porcine products with no alpha-gal will give a source of 'sensitivity-free' food and medicines. A detailed understanding needs to be developed of the chances of reactivity for the different products that contain small concentrations of mammal-derived ingredients (Scott 2020).

Conclusion

Red meat allergy is different from other conventional food allergies. Tick bites play a role in triggering this disease, but this association is not fully proven yet. It has been diagnosed across the world but is more prevalent in areas abundant with ticks' population. The role of alpha-gal in the development of mammalian meat allergy after tick bites has strong scientific evidence. The reactions might appear immediately when the medicines (containing alpha-gal) are given via the parenteral route, and there is a delay in the appearance of the symptoms from 3-6 hours if meat from mammals, dairy, and other mammal-derived products are consumed via the oral route. The best management of this syndrome is to avoid further tick bites, mammalian meat, and other mammal-derived products.

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9

An Overview of Psittacosis

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INTRODUCTION

Psittacosis is a zoonotic infection caused by Chlamydia (C.) psittaci (Fig. 1), which is an obligate intracellular bacterium (Hans and Olivia 2016). The term 'psittacosis' originated from the Greek word psittakos, which is used for parrots and was first used by Morange in 1895 (Morange 1895). Direct contact with diseased birds primarily transmits the infection and induces a broad-spectrum of symptoms with varying severity. Psittacosis is also regarded as 'parrot fever' and 'ornithosis' and the birds are considered as a prime epidemiological reservoir for this disease (Fig. 1) Formerly, only the word 'psittacosis' was used but then, another term 'ornithosis' was proposed in order to distinguish the infection in fowls from the infection in psittacine birds.Both of these conditions are now considered similar (Andersen and Vanrompay 2008). Although infection in the birds from the order Psittaciformes (parakeets, parrots, lories, cockatoos, and budgerigars) and Galliformes (chickens, turkeys, pheasants) are more often observed, but the disease can infect every bird species. This has been reported in 467 species from 30 different orders of birds (Stewardson and Grayson 2010). Hence, bird exposure is considered as the major risk factor for its transmission to humans. The bird exposure may occur through direct contact with the diseased birds, or inhalation of aerosolized organisms in faeces, urine, eye, and respiratory secretions. The bird-human contact may happen in veterinary hospitals, pet shops, and bird shows (Halsby et al. 2014), while the person-to-person transmission of psittacosismay also happen but is occasional (Stewardson and Grayson 2010).

Etiology

C. psittaci belongs to family Chlamydiaceae, and order Chlamydiales (Kaleta and Taday 2003). The Chlamydiaceae family comprises of two genera i.e. *Chlamydophila* and *Chlamydia*. Formerly, genus *Chlamydia* was known to have nine species (Laroucau et al. 2009). But according to the revised taxonomy of Chlamydiaceae family, the genus *Chlamydia* now consists of 11 species i.e. *C. psittaci*, *C. pecorum*, *C. felis*, *C. caviae*, *C. abortus*, *C. pneumonia*, *C. suis*, *C. trachomatis* and *C. muridarum* and newly discovered species, *C. avium* and *C. gallinacean* (Sachse et al. 2014). *C. psittaci*, having multiple genotypes, is gram-negative, obligate intracellular bacteria that resides in both, birds and mammals. Successful sequencing of these genotypes by using genotype-specific real-time PCR can help in detection, as well as epidemiological research. Being animal host specific, every genotype can be transmitted to humans and can induce infection (Stewardson and Grayson 2010).

Epidemiology

Generally, psittacosis is considered sporadic (Grayston et al. 1986; Marrie et al. 1987). But, outbreaks of disease may occur as Ritter reported the first outbreak of psittacosis (Jordan and Prouty 1956). He observed seven cases of atypical pneumonia which occurred after contact with parrots and finches at his brother's house. Other early outbreaks that happened in Europe and Faroe Islands were found to have a connection with sick parrots and fulmar petrels (Grayston et al. 1986; Saikku et al. 1985; Palmer 1982). Despite the fact that all groups and genders can be affected by psittacosis, the incidence of this infection is seen to attain a peak in middle-aged people having an age of 35 to 55 years (Yung and Grayson 1988). Still, psittacosis is considered a rare zoonotic infection. Due to this reason, there is no ample awareness regarding this disease among the people and health care providers (de Gier et al. 2018). According to CDC (Centers for Disease Control and Prevention), psittacosis is a notifiable disease in the United States. The estimated reported cases are less than 10 per annum and underdiagnosis and underreporting are thought to be the reasons behind the reporting of such a small number of cases. The individuals who are more likely to have exposure to the birds are generally considered more susceptible of acquiring infection. Bird exposure may occur at veterinary hospitals, pet shops and bird exhibitions and occupational exposure can also occur in the people working in the poultry industry (de Gier et al. 2018).

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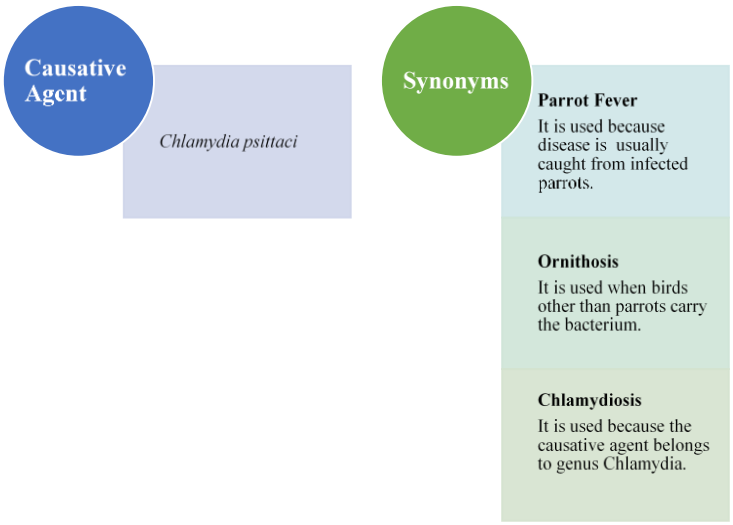

Fig. 1: Facts regarding Psittacosis.

Modes of Transmission

Bird to Bird

Psittacosis is regardedas "Avian Chlamydiosis" (AC) in birds. *C. psittaci* is found in nasal discharges and faeces of birds which harbors the infection. Sickbirds, as well as, asymptomatic birds may give out the bacteria alternatively for many months. Birds don't develop immunity against it and so, there is a chance to acquire the infection again (Balsamo et al. 2017).

Bird to Humans

C. psittaci is transmitted through the air passageway. Apart from the direct transmission through droplets, the indirect transmission of bacteria may occur by inhaling the aerosol of faeces of infected birds (Saito et al. 2005). It is reported that some patients experienced the symptoms without having ahistory of bird exposure (Ito et al. 2002) and even momentary exposures can cause symptomatic infection (Rehn et al. 2013).

Person to Person

It is believed that psittacosis is hardly transferred via direct human-to-human contact because none of the studies show evidence regarding its transmission among individuals (Hughes et al. 1997; Ito et al. 2002; McGuigan et al. 2012; Wallensten et al. 2014; Ojeda Rodriguez et al. 2022).

Other Animals to Human

Parrots and ornamental birds are usually considered as the source of psittacosis. However, some other birds and animals, like pigeons, poultry species and even mammals, have also been observed as the source of infection in humans (Haag-Wackernagel and Moch 2004; Fenga et al. 2007; Verminnen and Vanrompay 2009; Deschuyffeleer et al. 2012). *C. psittaci* transmission to humans from non-avian sources is probably not known, however, it has been reported in the case studies of some pregnant women who had a history of exposure to abortion products from sheep, abattoir workers, shepherds, and laboratory staff members (Barnes and Brainerd 1964; Anderson et al. 1978; Hyde and Benirschke 1997; Meijer et al. 2004). There are also some case reports in which humans who hadcontact with ill foals were infected with psittacosis (Chan et al. 2017). Fig. 3 shows various routes from where human may get the infection.

Pathogenesis

According to recent research employing a bovine model, C. *psittaci* initiates infection of the alveolar epithelial cells upon inoculation to the host (Knittler et al. 2014). The infection spreads due to the multiplication of bacteria within the host's epithelial cells. This elicits a host immune response resulting in a large inflow of neutrophils along with the release of chemokine and interleukin-8 (Knittler et al. 2014).

The acute-phase reaction brought about by chemokines causes the activation of an inflammatory cascade and reactive oxygen species. This further results in the recruitment and aggregation of immune cells and phagocytes from the bloodstream to the site of infection. This is considered to cause the hematogenous spread of *C.* Psittacithrough the disintegration of the alveolar-capillary membrane and tissue damage (Knittler et al. 2014). This inflammatory cascade and infection hinder the transfer of oxygen within the alveoli resulting in hypoxemia and

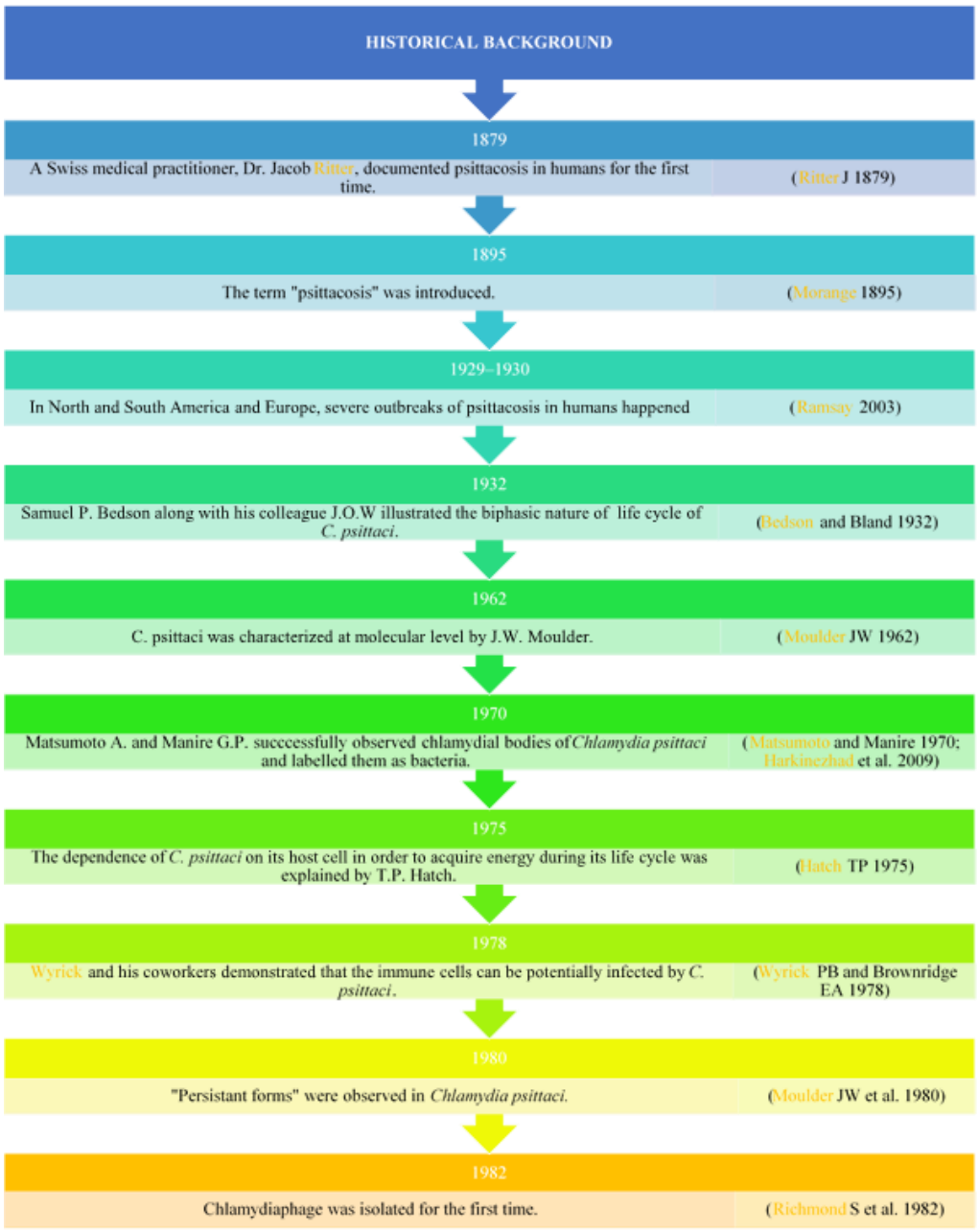

Fig. 2: A bird eye view of historical background is described.

alveolar hypoventilation (Knittler et al. 2014). The hematogenous spread of *C. psittaci* which resulted in various pathological changes in the body have been shown in Fig. 4.

Histopathology

The developmental cycle of *C. psittaci* involves two forms. The organism comprises of a larger metabolically active intracellular reticulate body and an extracellular infectious elementary body (Chu et al. 2022).

The extracellular infectious elementary body is endocytosed into the cell when it comes in contact with the cell membrane receptor of the host cell, dodging the host immune response. As a result, a metabolically active reticulate body is formed when the endocytosed elementary body increases in size (Grimes 1987; Peeling and Brunham 1996).

The reticulate bodies use host cells' ATP and form further new reticulate bodies upon binary fission. These inclusion reticulate bodies reorganize to form an intermediate state. Ultimately, elementary bodies are formed and released by

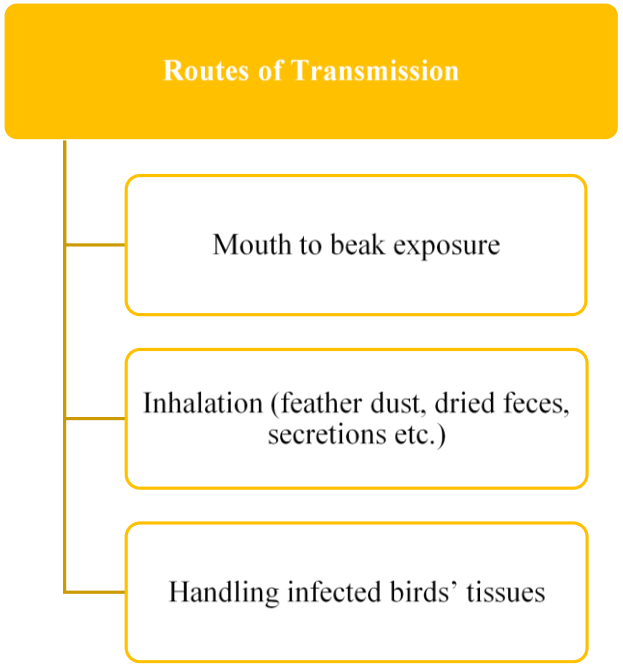

Fig. 3: Possible routes of infection transmission to humans

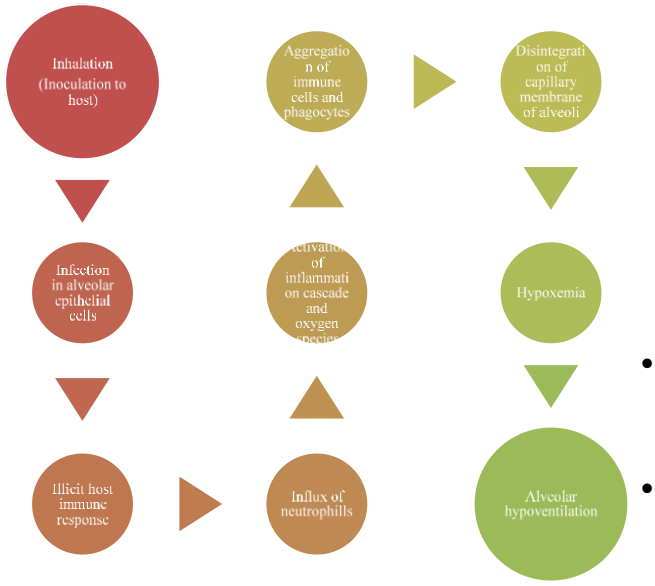

Fig. 4: Hematogenous spread of *C. psittaci* resulting in various pathological changes in the host body

cell lysis and reverse endocytosis and this release of elementary bodies are considered as a cause of silent and chronic infection (Peeling and Brunham 1996).

New host cells are infected with these released elementary bodies. In this way, the disease cycle propagates and spreads to other organ systems of the body via a hematogenous route (Vanrompay et al. 1995; Knittler and Sachse 2015). The infectious cycle of *C. psittaci* involving the formation of reticulate and elementary bodies have been shown in Fig. 5.

History of Patient

Although there is a strong connection between bird exposure and psittacosis, yet it is not compulsory for diagnosis. This is considered accuratefor areas where there is an abundance of undomesticated birds. In Australia, two outbreakshappened in the areas that were located amidst large avian flora (Williams et al. 1998; Telfer et al. 2005). Diagnosis mostly dependson taking adetailed history involving the medical history, travel history, occupation andhobbies of the patient, along withstrong suspicion of infection (Chu et al. 2022).

Clinical Manifestations

Despite the respiratory symptoms of *C.psittaci* infection in humans, there can be other clinical manifestations that can extremely differ. Infection can influence multiple organ systems as it spreads after replicating in the respiratory system. The average incubation period of infection is about 5-14 days (Beeckman and Vanrompay 2009).

The onset of symptoms is usually sudden. Headache is usually mentioned along with fever, nausea, diarrhea, cough and myalgias (Yung and Grayson 1988). Other signs of psittacosis include disoriented mental condition, photophobia, mild stiffness in the neck, hepatomegaly, splenomegaly and pharyngitis (Stewardson and Grayson 2010). Fig. 6 shows the clinical manifestation of psittacosis infection in the host.

Diagnosis

Lab Investigations

- *White Blood Cell Differential count*: Slight decrease inleukocyte count manifest initial phase of infection. Leukopenia can be noticedin the acute phase of infection (Longbottom and Coulter 2003).
- *Red Blood Cell Count*: During the course of infection, hemolysismay lead to anemia (Longbottom and Coulter 2003).
- *Liver Function Tests*: Sometimes, there can be high levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), besides gamma-glutamyltranspeptidase (GGT) (Longbottom and Coulter 2003). Elevated levels of CRP (C- reactive protein) can also be observed (Longbottom and Coulter 2003).
- *Culture*: *C. psittaci* is isolated from respiratory tract secretions (sputum, throat swab etc.) and can be cultured on Minimum Essential Medium (MEM) (Favaroni et al. 2021).
- *Serology*: This method is usually applied to confirm psittacosis. Following serological tests are available for diagnosis of psittacosis:

Microimmunofluorescence Test: IgG-specific and IgM-specific antibodies are detected by MIF test.

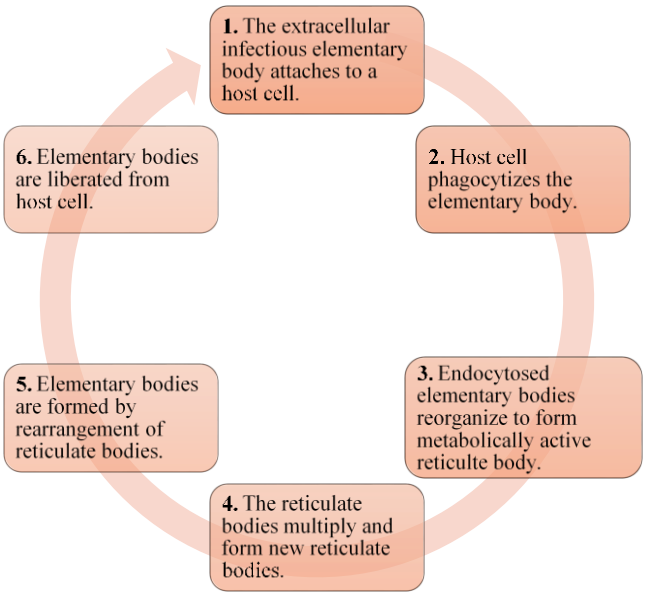

Fig. 5: Infectious cycle of *C. psittaci*

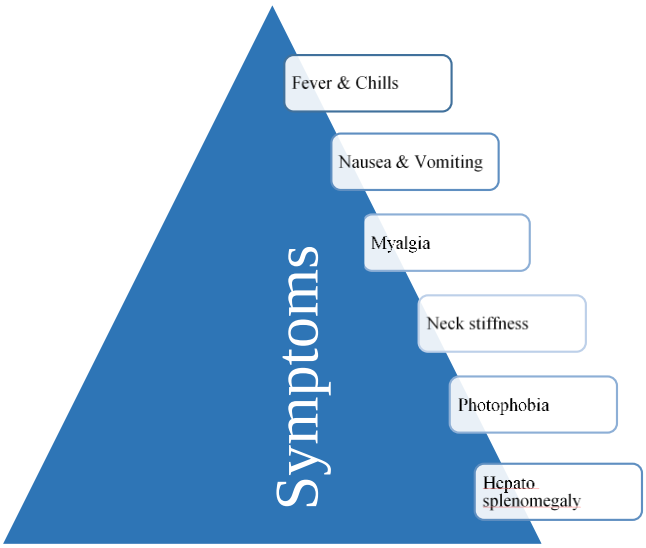

Fig. 6: Clinical manifestation of psittacosis infection

In the initial stage of diagnosis, there is a positive result of IgM. The positive rate may reach up to 80–95 % (Mi et al. 2015).
Complement-fixation test (CFT): If paired serum titers elevate at a four-time speed while detecting specific antibodies then a diagnosis is required (Mi et al. 2015).
Microimmunofluorescence (MIF) is considered more sensitive than the complement-fixation test (CFT) (Mi et al. 2015).

Imaging:

- *Chest X-Ray*: Around 80% to 90% of patients exhibit abnormal chest x-rays. These involve migratory infiltrates and pleural effusions (Yamato et al. 1992).

Magnetic Resonance Imaging (MRI): MRI is usually advisedfor diagnosing neurological issues associated with psittacosis (Mi et al. 2015).

Nucleic Acid Amplification

PCR helps in the rapid detection of psittacosis patients as it allows us to find out the source of infectionby genotyping. It is highly sensitive only in the acute phase and is mild in chronic cases (Nieuwenhuizen et al. 2018).

Prognosis

The prognosis of psittacosis may be influenced by the severity of clinical disease and the comorbidities of the patient. In addition to this, prognosis also relies on the duration of treatment and management. (Hogerwerf et al. 2017). The mortality rate is approximately 1%, despite of antibiotics treatment (Chin 2000).

Treatment

Psittacosis is primarily treated by antibiotics. Tetracycline and doxycycline are two antibiotics that are usually recommended and considered effective against this disease without contraindications. Most patients show improvement within48 hours (Yung and Grayson 1988). Intravenous doxycycline can be used in cases where antibiotics cannot be administered orally. The recommended dosage of doxycycline is 100 mg PO or IV for 10 to 14 days. Azithromycin can also be used ininfants. Erythromycin and azithromycin are recommended for pregnant patients and can also be used in cases where doxycycline is contraindicated (Chu et al. 2022).
Fluoroquinolonescan also be prescribed at times but these are less effective than tetracyclines and azithromycin (Chu et al. 2022).

Differential Diagnosis

There aremany disorders which may have similar symptoms as psittacosis or parrot fever. A comparison can be beneficial for differential diagnosis. The differential features of psittacosis infection have been mentioned in Table 1.

Complications

The psittacosis-infected patients may present several manifestationsas a consequence of its hematogenous spread after the first inoculation. *C. psittaci* infection may lead to respiratory failure, hepatitis, pneumonia, pancreatitis, endocarditis, DIC (Disseminated Intravascular Coagulation) and encephalitis. The fulminant course of psittacosis may lead to multiple organ failures (Chu et al. 2022).

Table 1: Differential Features of Psittacosis Infection with Various Other Disorders

References		(Moghadami 2017)	(HamidrezaHonarmand 2012; BiyankaJaltotage et al. 2021)	(Ticona et al. 2021; Jain et al. 2022)	(Penn 1994; Yeni et al. 2021)	(Yagupsky and Baron 2005)
Signs	Pericarditis	✓	✓	✓	✓	✓
	Hepatomegaly	✗	✓	✗	✓	✓
	Leukopenia	✗	✗	✗	✗	✗
Symptoms	Myalgia (muscle pain)	✓	✓	✓	✓	✓
	Malaise	✓	✓	✓	✓	✓
	Fever and chills	✓	✓	✓	✓	✓
	Abdominal pain	✓	✓	✓	✓	✓
	Nausea and vomiting	✓	✓	✓	✓	✓
Differential diagnosis of Psittacosis		Influenza	Q fever	Pneumonia	Tularemia	Brucellosis

Prevention and Control

There are no vaccines available so far against this infection (Stidham et al. 2019). So, for now, strategies for minimizing the spread of these bacteria are the only way to control the disease (Smith et al. 2005). Therefore, people should be guided in dealing with birds and birdhouses in order to restrictthe spread of disease (Schlossberg et al. 1993). The use of personal protective equipment (PPE) such as gloves, masks, etc. must be assured while dealing with diseased birds and their cages. The veterinarians and healthcare providers must be consulted if the birds are doubted for carrying the infection (Chu et al. 2022).

Public Health Significance

Psittacosis, being zoonotic in nature (Gaede et al. 2008; Andersen and Vanrompay 2000; Seth-Smith et al. 2011), has distinct importance in public health, as parrots are kept in our houses, in schools and nursing homes on regular basis (OIE Terrestrial Manual, 2008). Proper knowledge and guidance about the clinical signs and course of the diseaseshould be provided to people who are susceptible of acquiring disease, along with the healthcare professionals (Balsamo et al. 2017). This must cover the public awareness aspect regarding the proper handling of birds, the use of personal protective equipment, and disposable particulate respirator usage. In order to figure out the sources of disease, there should be coordination between the healthcare personnel and the public health department for the guidance of industry and the public in tracking down all the dealings involving birds. The sick birds should be tagged, quarantined and isolated along with the implementation of appropriate cleaning and infection preventive guidelines (Balsamo et al. 2017). All these suggestions highlight the importance of general public awareness and the role of health care providers in the control of this zoonotic disease. So, an initiative involving general public awareness and cooperation between veterinarians and public health authorities is highly required for the prevention of this disease (Chu et al. 2022).

Future Perspectives

On-time reporting of disease and development of commercial vaccines are the biggest challenges related to psittacosis in future, as no human or avian vaccines are developed and commercialized yet. However, immunization with genetically modified DNA plasmid consisting of *C. psittaci* ompA gene induced partial immunity in SPF (specified pathogen free) budgerigars and turkeys. DNA immunization can be done even if maternal antibodies are present which triggershumoral and cell-mediated immune responses similar to those in usual body infections. So, it is high time that safe and effective vaccine against psittacosis must be developed. Studies have also shown the effectiveness of ovotransferrin against *C. psittaci*, when administered in turkeys. It potentially decreased the concentration of bacteria in the air and significantly lowered the mortality rate. So, the administration of ovotransferrin (OvoTF) in poultry is suggested as it can be a groundbreaking antimicrobial approachin near future (Van Droogenbroeck et al. 2011).

Conclusion

Increasing incidence of various zoonotic infections is one of the burning issues around the globe. However, psittacosis as a zoonotic disease is still overlooked. It is regarded as a reportable disease in many countries but still, it is an underreported condition. Even the usual laboratory investigationsdo not involve the diagnostic tests required for psittacosis. Moreover,the serological tests cannot give confirmatory diagnosis if a single serum sample is provided. The proportion of reported cases as compared to the actual ones is very low. So, we can say that the estimated impact of psittacosis on public health is still not clear.The bird-human contact is undeniable as man has been domesticating birds for ages. Moreover, the expansion of poultry industry over the past few years has made this contact more often but bird owners, public, poultry farmers and even medical practitioners have insufficient understanding of this infection. Therefore, raising general awareness for psittacosis is required which will promote the timely

reporting of this disease. Devising effective vaccines and specific diagnostic strategies are the needs of time and required to control this zoonosis.

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10

Rocky Mountain Spotted Fever

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INTRODUCTION

Rickettsia (R), is a small obligatory, intracellular gram-negative bacterium that infect both humans and animals (Dunning Hotopp et al. 2006). In 1909, Howard Ricketts was the first person to discover the genus Rickettsia (Ricketts 1909). On the basis of serological features, it has been classically classified into three distinct groups including the typhus group (TG), spotted fever group (SFG), and the scrub typhus group (STG). Both TG and SFG are under the genus Rickettsia and the STG is under the genus Orienia (Tamura et al. 1995; Dumler et al. 2001; Bermúdez 2018). There are only two species of TG rickettsiae: *R. prowazekii*, which is transmitted by louse; and causes a disease named epidemic typhus, and *R. typhi*, which is transmitted by flea and causes a disease named murine typhus. While there are more than twenty species of SFG and all species are transmitted by hard ticks except two species including *R. akari*, being transmitted by mites, and *R. felis* being transmitted by flea (Greene and Breitschwerdt 1998; Foil and Gorham 2000; Centers for Disease Control and Prevention, National Center for Infectious Diseases 2002). *R. rickettsii*, is the causative agent for Rocky Mountain spotted fever (RMSF) and comes in the group Rickettsia (Williams et al. 2007).

The differential features of TG and SFG group involve the polymerization of actin, type of outer membrane proteins and difference in the optimal growth temperature. The TG group cannot polymerize the actin and enter the host cell cytoplasm, have type B outer proteins and show optimal growth at 35°C while the SFG group can polymerize the actine and enter the host cell nucleus, have type A and B outer proteins and show optimal growth at 32°C (Fournier and Raoult 2007). The last difference is the difference in the ratio of genomic G-C, which is 29% in case of TG, while it is 32% -33% in case of SFG (Gillespie et al. 2007).

Like other bacteria, Rickettsiae have both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and they secrete substances, generate energy and perform all other living activities. Rickettsiae are transmitted to the host during biting and the blood meal by the infected ticks. It is transmitted from the site of bite by the bloodstream to infect the endothelium and sometimes to the vascular smooth muscle cells. Rickettsia species in their target cells can multiply by binary fission and cause direct damage to heavily parasitized cells (Walker and Ismeil 2008).

Rocky Mountain Spotted Fever (RMSF)

Many studies approved that the RMSF was found in America before humans arrived there and this is due to the hard tick that transmits the *R. rickettsia* through trans-ovarian way. The hard ticks have acquired the infection from feeding and biting of infected animals and they lay infective eggs and the pathogen was transmitted to the whole generation of tick (Burgdorfer 1963). In North America, human infections with *R. rickettsii* have been recorded, and was named Rocky Mountain spotted fever (RMSF). It is named as fiebre maculosa Brasileira' in Brazil and fiebre de Tobia in Columbia (Oteo et al. 2014). RMSF is an acute fatal bacterial disease that infects humans of different ages and dogs. It is transmitted by the bite of an infected hard tick in two ways: by trans-ovarian and trans-stadial transmission (Walker and Raoult 2000; Savic 2019). The disease is characterized by fever, chills, rash, and muscle aches (Warner and Marsh 2002). RMSF is still considered as the most virulent disease among all human infectious diseases, mainly in young people in North and South America (Warner and Marsh 2002; Bermúdez 2018).

History of RMSF

Firstly, the RMSF was identified as black measles and was reported for the first time in the late 1890s, in Idaho and Rocky Mountain, so it was named Rocky Mountain spotted fever (Ricketts 1909; Azad and Beard 1998; Centers for Disease Control and Prevention website 2017). In 1906 Howard Ricketts discovered that RMSF was a bacterial infection that was transmitted to humans by hard ticks (Thorner et al. 1998). Initially, the disease was localized at Rocky Mountain, and then the disease has been observed throughout different regions of America (Centers for Disease Control and Prevention 2022). The disease spread to various countries such as Colombia, Brazil, Mexico, Costa Rica, Argentina, and Panama (Razzaq and Schutze 2005; Dantas-Torres 2007). Over the past 20 years, the

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incidence of RMSF has been continuously rising in the United States, reaching a peak in 2012. The mortality rate has been shown elevated in older patients more than sixty years of age, in individuals who have been lately diagnosed, and in those who do not receive doxycycline drug as a treatment (Holman et al. 2001; Biggs et al. 2016).

Synonyms for RMSF

RMSF disease is also known with various names such as tick-borne typhus fever, tick fever, black measles, black fever, Mexican spotted fever, and New World spotted fever (Harwood and James 1979).

Vector for RMSF

The common vectors for the transmission of RMSF are hard ticks, mainly *Dermacentor* (*D.*) *andersoni* (Rocky Mountain wood) and *D. variabilis* (American dog tick) (Levin et al. 2017; Ismael and Omer 2021). These two species of ticks are considered as the common species in the northwestern states and the eastern United States. Ticks need several factors to complete their life cycle for the hatching of eggs and molting which include a suitable host, suitable humidity, oxygen, appropriate temperature, and a proper place (Estrada-Peña et al. 2012). Various species of hard tick act as the vector for RMSF and this depends on the geographical area for example; there are three common species of hard ticks in North America including *Rhipicephalus* (*R.*) *sanguineus* (brown dog tick), *D. variabilis* (American dog tick) (Fig. 1) and *D. andersoni* (Rocky Mountain wood tick) (Fig. 2). Both *Amblyomma* and *Rhipicephalus* act as the main vectors for RMSF in Central and South America, mainly in Costa Rica (Oteo et al. 2014; Levin et al. 2017; Ismael and Omer 2021). Additionally, several species of hard ticks have been reported in America such as *Amblyomma imitator, Amblyomma parvum, Amblyomma americanum, Haemaphysalis leporispalutris,* and *Dermacentor nitens* (Labruna and Mattar 2011).

A hard tick has a complex and long-life cycle; involving four morphological stages during their life cycle including the egg, larva, nymph, and adult (Fig. 4). The adult female lays eggs, which is then converted in to larvae. The process of converting each stage is called molting. Hard ticks remain on the host for a short period or during their whole life cycle. During this time, they consume various numbers of blood meals during biting (Walker and Raoult 2000; Golezardy 2006; Williams et al. 2007). The hard tick's life cycle begins once an engorged adult female tick found a proper area to lay her eggs. Normally, the hatching of eggs occurs within one to four weeks. The released larvae are very small in size, light in color and have six legs. Larvae are responsible to find a new animal host for feeding on blood and complete its life cycle (Brumin et al. 2012; Tian et al. 2020).

The host is infected with disease, when the larvae attach to the suitable host and feed on its blood by using its mouthparts (including chelicerae and hypostome), leading to the initiation of infective stage into the host blood (Varela-Stokes et al. 2009). After that, the larvae drop off on the ground and molt to the second stage of the tick called a nymph, which may form one or more nymphal stages and the number of molting differs according to the species of hard ticks and environmental conditions such as temperature and optimal humidity (Walker and Raoult 2000; Tian et al. 2020). The nymph stage then feeds on the host and as usual, they drop off again on the ground and molt to adult males and females. Both nymph and adult stages are brown and have eight legs, and an adult female feed on the host till become engorged. Engorged females lay thousands of eggs on the ground and these depend on the species of hard ticks and environmental conditions (Sen et al. 2012).

The Role of Dogs in RMSF

R. sanguineus (brown dog ticks) infect both humans and animals (Demma et al. 2005; Yaglom et al. 2018). It was identified for the first time during the RMSF outbreak as a potential vector of *R. rickettsii* in North America, and the role of stray dogs has been suggested as reservoirs and primary hosts for the Rhipicephalus at the same time (Demma et al. 2005; Nicholson et al. 2006). In North America, brown dog ticks that transmit the *R. rickettsii* can transmit many other pathogens such as *Anaplasma* spp., *Babesia* spp, *Bartonella* spp., and *Ehrlichia* spp. (Higuchi et al. 1995; Mathew et al. 1996; Wikswo et al. 2007; Diniz et al. 2010). Due to these characteristics, there are increasing outbreaks of RMSF in countries that have a large number of stray dogs (Yaglom et al. 2018).

Pathogenesis of RMSF

The causative agent of RMSF, *R. rickettsii* infects and replicated within the endothelial cells that line the small blood vessels, causes systemic vasculitis and is the main cause of skin rash and petechial lesions on the skin (CDC 2019). The bacteria cause direct injury to microvascular lining and damage to vascular endothelial cells. The endothelial cells release more prostaglandins which may increase the vascular permeability and escape of high amount of fluid into the neighbor tissues resulting in edema and loss of blood volume (Rydkina et al. 2006; Zhou et al. 2022). Injury and damage of blood vessels lead to the inflammation known as vasculitis and this cause bleeding and clotting in vital organs, mainly the brain. Many other pathological changes may occur due to host response to RMSF such as encephalitis, myocarditis, and interstitial pneumonitis (Sahni et al. 2021; Zhou et al. 2022). The severity of the infection and clinical signs depend on several factors including age, sex, body color and history of chronic disease i.e., diabetes mellitus. (Pearce and Grove 1987; Parola et al. 2003).

Fig. 1: Adult tick of *Dermacentor variabilis* (female) (Biggs et al. 2016)

Fig. 2: Adult tick of *Dermacentor andersoni* (Female)(Biggs et al. 2016)

Fig. 3: Adult of *Rhipicephalus sanguineus* (Female) (Biggs et al. 2016)

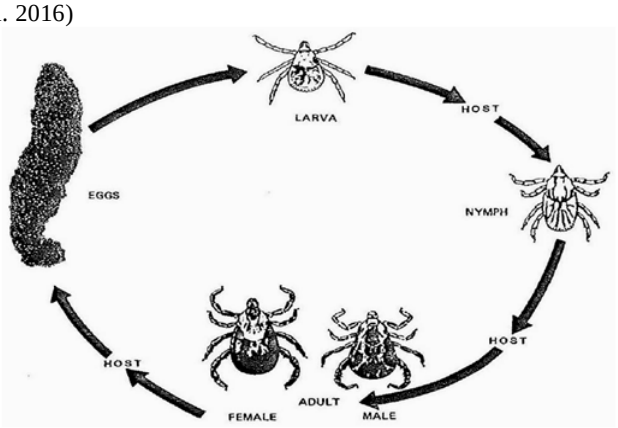

Fig. 4: Life cycle of Hard Tick (Varela-Stokes et al. 2009)

Clinical Symptoms of RMSF

Usually, the incubation period of RMSF ranges from 2 to 14 days following a tick bite. Most tick bites are painless, and some people may not even remember getting bitten, while in Brazil one case was reported with an incubation period ranging between 1-21 days. RMSF is characterized by nonspecific clinical signs such as fever (37 ºC -39ºC), headache, muscle pain, vomiting, and nausea. It may lead to rash, breathing difficulty, abdominal pain, seizure, and shock if not treated correctly (Paddock and Childs 2003; Gottlieb et al. 2018). The typical rash usually appear following 2-4 days of fever and in some cases may appear between 1-6 days. The rash initially appeared as small flat, pink papules on the ankles and wrist and then distributed to the legs, arms, and body trunk (Fig. 5). By the end of the first week, the rash develop into a maculopapular rash with central petechiae (CDC 2000; Regan et al. 2015; Lindblom 2016; Elzein et al. 2020). In RMSF, a skin rash may be not obvious in patients with dark skin (Kirkland et al. 1995; Rathi and Rathi 2010). Children also showed similar signs of RMSF as in adults. A study found a serious case of RMSF in the child sixteen months old, presented with persistent high-grade fever lasting longer than a week and a skin rash that dramatically involves the palms and soles of the feet (Fig. 6 and Fig. 7) (Inamadar and Aparna 2019). The skin rash appears early in children as compared to adults (Purvis and Edwards 2000; Murali et al. 2001). The common symptoms in children include facial swelling, swelling of legs and generalized body edema, enlargement of the liver and spleen, pneumonia, hyperemia, and vasculitis of the eyes (Fig. 8) (Azad and Beard 1998; Chapman et al. 2006; Agahan et al. 2011).

Diagnosis of RMSF

1. Clinical Diagnosis

At the early phase of disease, it is very difficult to differentiate between RMSF and other diseases that have the same clinical signs such as high fever, chills, fatigue, and myalgia. Therefore, its unable to suspect the RMSF at beginning of the disease, because of no specific signs, while in the advanced stage of the disease is easy to differentiate between RMSF and other diseases, because of special petechial skin rash and eschar formation (Paddock and Childs 2003; Gottlieb et al. 2018).

Fig. 5 (A & B): Rash on the upper and lower limbs C. Eschar in the arm (Elzein et al. 2020)

Fig. 6: Sixteen months old child with a clear rash on their palms (Inamadar and Aparna 2019)

Fig. 7: Sixteen months old child with a clear rash on their foot soles (Inamadar and Aparna 2019)

Fig. 8: Hyperemia found in the child's eye (Inamadar and Aparna 2019)

The early diagnosis depends on the history of the disease such as patients having tick bites (specific skin lesion) and previous exposure to the endemic region where for RMFS (Chen and Sexton 2008)

2. Laboratory Diagnosis

Serological test such as indirect immunofluorescence antibody assay (IFA) is considered the main standard test for the diagnosis of rickettsial specie. Antibodies are commonly detected after the onset of infection between 7-10 days. The sensitivity and specificity of IFA are about 94-100% and 80 % respectively and it depends on the time of blood collection (before or after 14 days of infection). The second is enzyme-linked immunosorbent assay (ELISA), which is also used for the detection of antibodies (Ehrlichiosis, 2004; Biggs et al. 2006).

Immunofluorescence staining test, is used to detect both fatal and non-fatal types of RMSF, by taking a biopsy from the skin rash for detection of *R. rickettsii*, and it has been proved by many studies to be sensitive and specific (70%-100%) respectively (Walker 1995; Demma et al. 2005).

Histopathological method, is used for the detection of skin rickettsial antigen. It is done by taking a biopsy from the skin rash, followed by the preparation of a smear and staining with eosin and hematoxylin stain. The infiltration of mononuclear cells which surrounds the vascular system of skin are shown under the microscope (Sexton 2011).

Immunohistochemical staining test, is another test used for the detection of RMSF. The sensitivity and specificity of this test ranges from 70-100 % respectively. It is also used for the detection of skin rickettsial antigen as in histopathological method while under the microscope it appears as focal lesion (Kao et al. 1997; Stewart and Stewart 2021).

The Polymerase Chain Reaction assay (PCR) is highly effective for detection *R. rickettsii* DNA in skin rash biopsy than in blood samples and this is due to *R. rickettsii* being concentrated more in skin rash in advanced stages of disease than in the blood sample (Demma et al. 2005; Institute of Medicine US 2011; McQuiston et al. 2014).

Treatment of RMSF

The recommended drug for the treatment of all types of rickettsiae infection is doxycycline which should be prescribed immediately after RMSF is diagnosed. Doxycycline is highly effective on intracellular bacteria and

its use is safe in children, therefore, doxycycline is recommended as a specific treatment for RMSF by the American academy of pediatrics community. Rickettsiae has resistance to many antibiotics that have lower activity on intracellular bacteria such as cephalosporins, aminoglycosides and trimethoprim-sulfametoxa- zoleand penicillins (Todd et al. 2015; Biggs et al. 2016). Doxycycline is recommended for the effective treatment of RMSF for adults and children (Minniear and Buckingham 2009; Todd et al. 2015; Biggs et al. 2016).

The recommended dosage of doxycycline, for adults, is about 100mg every 12 hours which may be given orally or IV. For children, it is 4mg and should be divided into two dosages and given every 12 hours (orally or IV). Doxycycline should be given for three days as a minimum, while in the severe cases, it should be given at least 5-10 days. In the case, of patients allergic to doxycycline, chloramphenicol is the second drug of choice for RMSF (Thorner et al. 1998; Thomas et al. 2009; Todd et al. 2015).

Prevention and Control

Till now there is no available vaccine for RMSF. Therefore, to decrease the morbidity and mortality of RMSF in endemic regions, it should be diagnosed properly and suspected patients should avoid to visit endemic areas in spring and summer seasons (Helmick et al. 1984; Drexler et al. 2014). Early steps of prevention include the protection from the bite of ticks, reducing contact with tick population, mainly from forested, and grassy regions and finally ticks that are adhered to the body should be removed carefully (Centers for Disease Control and Prevention Tick Removal 2016).

Conclusion

RMSF is a zoonotic tick-borne disease found worldside that infects humans (including adults and children) and dogs and is transmitted by hard ticks. It is considered as one of the main public health issues because of its high prevalence and effects. It is not promptly recognized and diagnosed, and may leads to death. The two factors that leads to death include the delayed or incorrect diagnosis of the case because of no early specific sign and the delayed treatment of cases with doxycycline because if a patient does not receive doxycycline during the first five days may lead to many systemic complications. Finally, the suspected patients who have a history of tick bites, or have fever and skin rash in an endemic region should be treated carefully. Save people's life from RMSF in endemic regions, is depending on the early accurate diagnosis and correct treatment to prevent the occurrence of fatal complications. It is the responsibility of the public health sector to prevent and control the disease in the endemic regions by reducing the tick population and reducing stray dogs because dogs play an important role in the RMSF.

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Eimeriosis in Small Ruminants in Basrah Province/Southern Iraq

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INTRODUCTION

Livestock is one of the most important sources of the economy for any country. So, it is necessary to ensure the good health of animals, their development and prosperity and to preserve it from wasting and death. This is only possible by the periodic examination to ensure that it is free from bacterial, viral and parasitic diseases and focus on giving vaccines on time. Parasitic infections among small ruminants play a significant role in animal death and productivity, and *Eimeria* is one of the parasitic protozoa with a wide spread epidemiology among all animals, including small ruminants. The rates of its spread among animals have increased recently, and the reason for this is the spread of random grazing and the dependence of shepherds on feed from contaminated sources. Another reason for the increased infection is the mixing of animals in the same barns and lack of ventilation leading to the massive spread of sporozoites and emergence of new species that did not exist previously. Therefore, it is necessary to give the the utmost importance to this subject, and to follow up on the frequency of *Eimeria* between this region, and to find solutions to eliminate the parasitic infection. The emergence of new types of *Eimeria* was noted when it was detected at the molecular level. Formerly, eimeriosis was thought to be caused by the obligatory intestine intracellular apicomplexan protozoan parasite *Eimeria spp.* (Yakhchali and Rezaei 2010). The disease rapidly spread throughout the world and afflicted many animals, costing both individual farmers and the ovine business very badly (Reeg et al. 2005). *Eimeria spp.* is a parasite that infect several domestic animals, with the site of infection being the gut and occasionally other organs, including the liver and kidney (Levine 1973). Taxonomically, *Eimeriaspp.* has been placed in the *Eimeriidae* family including more than 1,000 species and the genus *Eimeria* comprising the majority of species affecting domestic animals as well as birds. There is total 15 species known to infect the sheep, however, *Eimeria* (*E.*) *ovinoidalis* and *E. crandallis* are the two most dangerous species (Catchpole et al.2000). There are 17 species known to have been found in goats, although the pathogenic species *E. arloingi* and *E. ninakohlyakimovae* are particularly common (Cavalcante et al. 2012). In life cycle, Oocysts are excreted in the faeces of infected animals and require favourable environmental conditions, such as temperature > 15°C and relative humidity > 80%, to mature into Sporulated oocysts that are capable of infecting other animals in the same field (Daugschies and Najdrowski 2005). Additionally, the principal route of transmission of disease between animals is through the ingestion of contaminated food and water containing oocysts (Fitzgerald 1980).

Historical Preview

The first discovery of *Eimeria spp.* was documented in 1674 by Antonie Van LeevnHook, who examined parasitic cysts in gall bladder ofrabbits. Then, schizogonous stages was descript by Schneider in 1875.Later, avian *Eimeria* oocysts was described by Leuckartin 1879.Schaudinn documented the whole life cycle of the parasite in 1900; thereafter, *Eimeria* was regarded as a distinct species from Eimerian, and the term *Eimeria* was first recorded in 1902 by Stiles and Liihe. The first discovery of *Eimeria spp.* in goats was documented by Marotelin 1905, who give it the name *Coccidiaumarloingi* having the Micropyle. The pathogenic aspects were clearly described by Johnson in 1930 and Tyzzeret al. 1932 (Soulsby 1974).

*Eimeria spp.*in Sheep and Goats

Different species of *Eimeria* found and describe in sheep and goats around the world (Sweeny et al. 2011). In sheep, Fifteen species of *Eimeria* was described by Soulsby (1982), like: *E. ahsata*was described by Honess(1942), *E. ovina* by Levine and Ivens (1970), *E. ovinoidalis*by by Yakimoff (1933), *E. crandallis* by Honess(1942), *E. faurei* by Moussuand Marotel(1902),*E. gilruthi*by Martin (1909) and Chatton (1910), *E. gonzaelzi*by Reichenowand Carini (1937), *E. granulosa* by Christensen (1938), *E. hawkinsi* by Ray (1952), *E. intricata* by Spiegl (1925), *E. pallida* by Christensen (1938), *E. parva* by Kotlân et al. (1951), *E. punctate* by Landers (1955) and *E. weybridgensis* by Norton and catchpole (1976). There are several species of goats have also been reported including *E. ninakohlyakimovae*, *E. hirci*, *E. caprina*, *E. caprovina*, *E. alijevi*, *E. africiensis*, *E.*

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christenseni, E. punctatae, E. kocharli E. jolchijevi, E. apshronica, E. capralis, E. masseynsis, E. charlstoni, E. minasnsis and E. arloingi. *E. arloingi*and *E. ninakohlyakimovae*are considered as the highly prevalent pathogenic species (Silva and Lima 1998; Chartier and Paraud 2012). In Iraq Leiper (1957) first documented the *Eimeria spp*. in sheep, then Mirza (1970) recorded *E. ahsata, E. ninakohlyakimovan, E. intricate, E. faurei, E. carandailis, E. parva* and *E. granuolosa. E. ovinoidalis and E. Pallida* was first mentioned by Yakob et al. (1989).

Geographical Distribution and Prevalence

Eimeria has a worldwide distribution in sheep and goats, and it is difficult to define a specific geographical split between a single or numerous genus and species. As a result, sporadic occurrences of a single species with severe pathogenic consequences have been seen. Otherwise, some species have no pathogenic effect under normal conditions, and several publications have documented the occurrence of *Eimeria spp*. in sheep and goats around the world. Factors such as management, sanitary conditions, temperature, agroecology, climatic and environmental conditions, and the immunological response of the host, dosage of infection, and sampling duration can all affect the occurrence and distribution of Eimeriosis in different places (Khodakaram-Taftiand Hashemnia2017).

In Poland, 4.6-60% prevalence of *Eimeriaspp.* was recorded in sheep (Gorski et al. 2004), whereas in Austria the prevalence was 97-100% (Platzer et al. 2005), 43.1% (Reeg et al. 2005) and 37.61% (Hashemnia et al. 2014) and 74.8% prevalence was reported in Brazil (Berto et al. 2013). China, Zimbabwe, and Egypt recorded 91.5% in adult sheep and lambs, respectively (Kaya 2004; Yakhchali and Golami 2008; Mohamaden et al. 2018). In USA the prevalence of *Eimeriaspp.* in goat was 97% (Kahan and Greiner 2013), while in India it was 96.66% (Kaur et al. 2017), 65.07% in Egypt (Mohamaden et al. 2018), 55.99% in Pakistan (Rehman et al. 2011) and 73.91% in Brazil, respectively (Macedo et al. 2019).

In Iraq, distribution of *Eimeria spp.* varies according to the periods, regions and breed of sheep and goats. In Baghdad province the prevalence in sheep with Eimeriosis was 79.09% (Abd Al-Wahab,2003), while, in Diwaniya province it was reached to 1% in lambs as recorded by Dawood et al. (2008). On the other hand, Kalef and Fadl (2011) reported a prevalence rate of 49% in Baghdad province and Mohammed (2013) reported a prevalence rate of 67.5% in sheep in AlMuthana province. In Diyala province, the infection rate of 86.09% was recorded in sheep and 87.30% in goat (Mineet 2014), while Al-Sadoon(2018) recorded a prevalence rate of 84.16% in sheep in Wasit province. The rate of infection with *Eimeriaspp.* was affected by the way the farm was run and the number of cases of was found lower in large and closed farms. This did not necessarily mean that these farms had intensive systems, but it's likely because these farms used stricter hygiene measures and de-parasitization methods. Other factors, like differences in immunological competence due to differences in nutritional status, could have also played a role (Knox and Steel 1996). Furthermore, inadequate hygienic sanitation may be regarded as a risk factor for Eimeriosis, as it can increase the duration and amount of infection/exposure and the incidence of infection owing to contaminated food and water. Furthermore, stress may also promote immunosuppressant conditions. The presence of non-cemented floors, a closed housing system, and a large herd size, resulted in the greater contamination of overcrowded animals and feeding and watering troughs(Altaf and Hidayatua 2014). Furthermore, there may be statistically significant differences between a body condition score and *Eimeriaspp*. infection; for example, Khan et al. (2011) found a greater infection rate in sheep with low body ratings compared to those with superior body ratings. On the other hand, there are positive connections between conditions such as temperature and the severity of infection in semiarid and subhumid regions (Balicka-Ramisz 1999). This correlation might be related to the effect of temperature on *Eimeriaspp*. sporulation rates (Graat et al. 1994). This correlation explained that temperature effect on sporulation rates of the *Eimeria spp.* (Graat et al. 1994). The breed susceptibility differences also affect the *Eimeria spp.* infection. Indigenous goats in Zimbabwe were found to be resistant to Eimeriosis, while Angora and wild goats were found to be more likely to get clinical Eimeriosis than dairy breeds goats (Chhabra and Pandey 1991).

Pathogenicity

Many factors affecting on the Pathogenicity of *Eimeria* such as thedose of oocysts ingestion, host cells destruction, location of parasite in hosttissues, stage of infection, general condition and age of host, and degree of immunity which may be acquired or natural (Kaneko et al.2008; Moreet al. 2011). Gregory et al. (1983) looked at sheep that had been infected with *E. crandallis* and *E. bakuensis.* They found that these parasites can cause the host cell to go through mitosis and can sometimes divide at the same time as the host cell. During an *E. crandallis* infection, parasites can also divide continuously at the same time along with the epithelial cells of the host. Cox (2009) discovered that heavy *Eimeria spp.* infections result in schizonts found in mucosa and submucosa cells with high destruction and haemorrhage when compared to light infections that affect intestinal mucosa with local absorption. On the other hand, some *Eimeriaspp.* infections resulted in superficial development with villi atrophy, that might be due to a decrease in epithelial cell lifetime and the surface area accessible for absorption, resulting in a lower feed efficiency. Typically, infection with different species of *Eimeria* at same time was common in the field and cause a sever pathological effects (Blood and Radostitis 1989).

Catchpole et al. (1975) detected that mixed *Eimeriaspp.* infection in sheep resulted in prolonged patency and increased oocyst production with or without clinical signs. In general, *E. ovinoidalis* is regarded as one of the most virulent species in sheep (Gregory et al. 1989; Abakar 1996). In goats, *E. arloingi*and *E. ninakohlyakimovae* are the most common pathogenic species (Cavalcante et al. 2012). Stress and environmental variables are key predisposing factors in *Eimeria* pathogenesis, and a research has shown that these factors are linked to recurrent outbreaks of Eimeriosis (Gul 2007). Sometimes lambs and kids that treated with corticosteroids can convert subclinical infections to acute clinical infection (Gasmir 2005). On the other hand, schizonts growth cause damage in the caecum, which cause most numerous and mucosal polyps in sheep (Taylor and Catchpole 1994).

Clinical Signs

Different experimental studies showed different clinical signs in lambs and kids infected with Eimeriosis without prominentdifferences when used inoculated doses (Dai et al. 2006).The initial clinical symptom of Eimeriosis infection include the abrupt acute diarrhoea with bad odours and stools including mucus and blood, as along with an increasing loss of body weight (Blood and Radostitis 1989). According to a study,palemucous membranes, weakness, staggering, dyspnea, dehydration, andrecumbency were also reported in diseases animals(Mohamed et al.1990). While Abakar (1996) noted an appetite, dullness, pale mucous membranes, and minor pyrexia as clinical indications of acute Eimeriosis, leading to a disruption of the digestive system resulted in the release of water, electrolytes, and protein (Reid et al. 2012). Several lambs may eventually die on dehydration because of diarrhea and lose of appetite while, some lambs die with profuse watery diarrhea (Taylor et al.2007).

Diagnosis

Eimeriosis may be diagnosed in sheep and goats based on a case history, clinical indicators, gross lesions, necropsy results, and microscopic analysis of faeces by flotation method using various floatation liquids. So, a necropsyand recognized schizonts in lesions make a positive diagnosis (Levine1973). In the acute phase of Eimeriosis, the presence of a large number of sporozoites may lead to the tissue loss, resulting in the formation ofmerozoites that are failed to locate and invade new cells in order to grow before any oocysts form (Gregory et al.1983). Typically, *Eimeria* can easily be diagnosed through faecal examination using floatation technique (Levine 1961; Menezes and Lopes 1995).

Molecular Characterization of Eimeria spp.

The use of available tools in molecular biology is important to detect any parasitic infection that may infect human and animals and is important in modern Veterinary Diagnostic Parasitology comparing with the techniques used in past (Zarlenga and Higgins 2001). So, in the past, studies that looked for *Eimeriaspp.* used either traditional characteristics or a combination of traditional characteristics and other methods, such as the electrophoretic variation of enzymes in avian *Eimeriaspp.*, which uses variation in DNA sequences. The PCR-based assay has also been described, which could be used to identify *Eimeria spp.* (Viljoen and Nel 2002). The development of novel DNA-based diagnostic tests might expedite and simplify the identification of *Eimeriaspp.*, while the application of the PCR technique is changing the detection of pathogens (Erlich et al. 1991). According to Al-Sadoon (2018), the molecular study revealed the highest infection rate of *Eimeria spp.* of sheep at Wasit-province, Iraq via PCR on sheep faecal samples (84.16%), and phylogenetic tree analysis of the common four Eimeria species (*E. ovinoidalis, E. crandalis, E. ahsata, and E. weybridgensis*) has been disclosed employing multiplex PCR. The total infection rate of *Eimeria spp.* through PCR analysis showed a significant increase between species and included 57.42% positive samples, with *E. ahsata* having a higher infection rate (53.44%) followed by *E. ovinoidalis* (29.31%), *E. weybridgensis* (12.93%) and *E. crandallis* (4.31%), respectively.

Molecular characterization of *Eimeria spp.* by Shaheed (2021) in Basrah Province, Iraq

This study foundeleven *Eimeria spp.* in sheep and six *Eimeria* species in goats, respectively. This recognition depends on the shape and structure of isolated oocysts under microscope as: *E. ovinoidalis, E. crandallis, E. ahsata, E. weybridgensis, E. bakuensis (ovine), E. intricata, E. faurei, E. pallida, E. granulosa, E. parva* and *E. marsica*in sheep, while *E. arloingi, E. ninakohlakimovae, E. hirci, E. christenseni, E. aspheronica*and *E. capralis*in goats. Sporulation time of isolated oocysts was recorded by using Sugar solution in flotation, maturation, growth and diagnosis of *Eimeria* as a substitute method to potassium dichromate and formalin, that usually use in sporulation of *Eimeria spp.* The sugar is known as a nutritional substance with no caution or side effects compared to the potassium dichromate which is a carcinogenic substance while the formalin is also reported to be a harmful chemical to the human respiratory system. The results were astonished by using the sugar solution, as the rate of sporulation was estimated of 100% compared to the potassium dichromate which was observed giving a lower rate of only 30% of sporulation. In addition, the characteristic of *Eimeria* were very clear as a cyst that sporulated in the sugar solution compared to the cysts where sporulated in the potassium dichromate which was unclear under light microscope.The time of sporulation was continued from 1 day to 5 weeks with sugar solution, in comparison to 7 to 12 days with potassium dichromate. The result showed *E. bakuensis* and

E. parva of sheep and *E. arloingi, E. ninakohlakimovae, E. hirci, E. christenseni, E. capralis*of goats need three days or more to begin sporulation, while the other *Eimeria* species need less than three days to begin sporulation. According to the result of phylogenetic analysis there were nine *Eimeria spp.* recognized from twenty-five PCR positive fecal sample of sheep. *E. ovinoidalis, E. ahsata, E. crandallis, Eimeria spp. voucher* and *E. bovis* infected the cattle, *E.hirci* and *E. christenseni* infected the goats and *Eimeria labbeana*-like infected the birds and were recorded as a new species, and sheep infected with nonspecific species which was first record as a new species of *Eimeria* at Basrah province. It can be noticed that all isolates of *Eimeriaspp.* showed 92.54-99.51% similar identity with *Eimeriaspp.* isolated from different countries and recorded in GenBank, and it showed close association with the isolates detected from Iran and Jordon.

A- Evolutionary Relationships of *Eimeria spp.* Isolated in BasrahProvince, Iraq

The Neighbor-Joining method was applied to generate an estimate of the evolutionary history of the taxa that were investigated, and the bootstrap consensus tree that was derived from 500 different iterations of the analysis was selected in order to symbolize the evolutionary history of the species. When a bootstrap replicate is done, branches that belong to partitions that haven't been replicated in more than 50% of them are collapsed. Next to the branches are the percentages of duplicate trees in which related taxa were grouped together in the bootstrap test (500 times). The evolutionary distances were calculated using the Jukes-Cantor method. The research used 24 nucleotide sequences with codon locations 1^{st}+2^{nd}+3^{rd}+Noncoding in units of the number of base substitutions per site. All spots with blanks or missing information were taken out. In the end, there were a total of 268 locations in the dataset. MEGA7 was used to do an analysis of evolution. Fig. 1 shows phylogenetic analysis of *Eimeria spp.* isolated from small ruminants by using bootstrap consensus tree and Fig. 2 shows phylogenetic tree by using Neighbor-Joining method. Molecularly, all species found and recorded for the first time inBasrah province by using novel primers. Likewise, the normal host of *E. labbeana*are birds but it was isolated from sheep showing greater similarity with other strain submitted at GenBank from Iran, Jordon and Turkey. The results showed that these were neighboring countries and movement of animal in these countries by following import and export laws allowed the transmission of *Eimeria* and other parasitic infections.The evolutionary history of the studied taxa was figured out by using Neighbor-Joining method (Saitou and Nei 1987). The history of detected isolates was shown by the bootstrap consensus tree figured out from 500 replicates (Felsenstein 1985) and evolutionary distances were found using the Jukes-Cantor method (Jukes and Cantor 1969). Table 1 shows the percent identity of detected isolates with sequences available in GenBank.

B- *Eimeria* species detected in sheep

Eimeria ovinoidalis: Oocysts with an ellipsoidal form, smooth wall, colourless to pale-yellow, no polar cap, present inconspicuous micropyle, mean size 26.5±0.8× 20.3±0.8 having range 27.5 – 20 × 21.5 – 15 µm with sporulation period 1-3 days (Fig. 3).

***Eimeria crandallis*:** Oocysts are subspherical to broadly ellipsoidalshape and has smooth wall, with a micropyle, which may be distinct or indistinct and a micropylar cap, pale yellowish in color. Mean size 25.0±1.1×19.1±0.8 having range 27.5 – 18.5) × (20 – 12.5 µm and 1-3 days assporulation time (Fig. 3).

Eimeria weybridgensis: Oocysts are ellipsoidal to subspherical shape,a smooth wall, colorless or pale yellow. micropyle and polar cap present,mean size 31.0±1.5×20±0.7, with range 34.5 –24.5 × 24 – 20 µm, and 1-3 days as sporulation time (Fig. 4).

***Eimeria parva*:** Oocyst's shape is spherical to subspherical, smoothwall colorless to pale yellow, Polar cap absent, Micropyle absent, mean size 18.9±1.0× 15.6±1.0, with range 22-10 × 18-7.5 µm and 3-5 days as sporulation time (Fig. 4).

Eimeria ahsata: Oocysts are ellipsoidal shape, a smooth wallyellowish brown color, with distinct polar cap, and micropyle. mean size36.4±1.8× 24.1±1.3, with range 42.5-27.5×25–22.5 µm and 2-3 days as sporulation time (Fig. 5).

***Eimeria faurei*:** Oocyst is oval, pale-yellowish-brown in colour, coated with a smooth layer, no polar cap and prominent micropyle, mean size 32.1±0.6× 23.2±0.7, with range 37–22.5×27-20 µm and sporulation period 1-3 days (Fig. 5).

Eimeria bakuensis: Oocysts are ellipsoidal shape, pale yellowishbrown, micropyle and micropylar cap present, sporozoites lying head to tail in sporocyst, mean size 31.4±0.9× 18.9±0.6, with range 36 –20 × 24 – 15 µm, and 2-4 days as sporulation time (Fig. 6).

***Eimeria marsica*:** Oocysts are ellipsoidal shape, colorless slightlygreyish or pale yellow with smooth wall, with micropyle (indistinct) which may have an inconspicuous micropylar cap, mean size 22.7±0.4 × 15.7±0.7, with range 22.5-18.5×15-8 µm and 3 days as sporulation time (Fig. 6).

***Eimeria intricata*:** Oocyst are ellipsoidal shape or slightly ovoid, brownish yellow to dark brown in color, with thick wall that is granular and transversely striated, micropyle in the outer layer, a micropylar cap, mean size 48.0±2.3× 37.7±1.8, with range 56 – 40 ×41 – 30 µm, and 1-3 days as sporulation time. (Fig. 7).

Eimeria granulosa: Oocysts are urn-shaped, with a large micropleand micropylar cap at the broad end, yellowish-brown in color with twosmooth layers, mean size 33.6±1.4×22.1±1.4, with range 35 - 22 × 25 - 17.5 µm, and 1-2 days as sporulation time. (Fig. 7).

Table 1: Sequence identity with Accession number of strain in GenBank

	Student code	Sequence code	Identity	*Eimeria spp.*	Accession number At GenBank
1	G2	C2	92.45%	*Eimeria bovisHS02; HS18*	MZ562402.1
					MZ562419.1
2	G18	M2	93.30%	*Eimeria ahsata HS06; HS01*	MZ562403.1
					MZ562406.1
3	G6	M3	99.28%	*Eimeria crandallis HS03; HS10; HS16*	MZ562407.1
					MZ562412.1
					MZ562417.1
4	G16	M4	99.28%	*Eimeria crandallis HS07; HS08; HS09*	MZ562409.1
					MZ562410.1
					MZ562411.1
5	G5	M5	99.05%	*Eimeria crandallis HS21*	MZ562421.1
6	G19	M11	98.02%	*Eimeria christenseni HS17*	MZ562418.1
7	G20	M12	99.46%	*Eimeria hirci HS11*	MZ562413.1
8	G12	M13	99.01%	*Eimeria christenseni HS23*	MZ562405.1
9	C3	M14	97.98%	*Eimeria faure HS05*	MZ562408.1
10	C4	M15	98.47%	*Eimeria ovinoidalis HS12*	MZ562414.1
11	O6	M17	96.92%	*Eimeria sp.* RY-2016a HS04; HS13; HS14; HS20; HS22	MZ562400.1
					MZ562401.1
					MZ562415.1
					MZ562420.1
					MZ562422.1
12	S1	M19	93.80%	*Eimeria christenseni HS15*	MZ562416.1
13	S4	M20	99.28%	*Eimeria ovinoidalis HS19; HS24*	MZ562404.1
					MZ562423.1

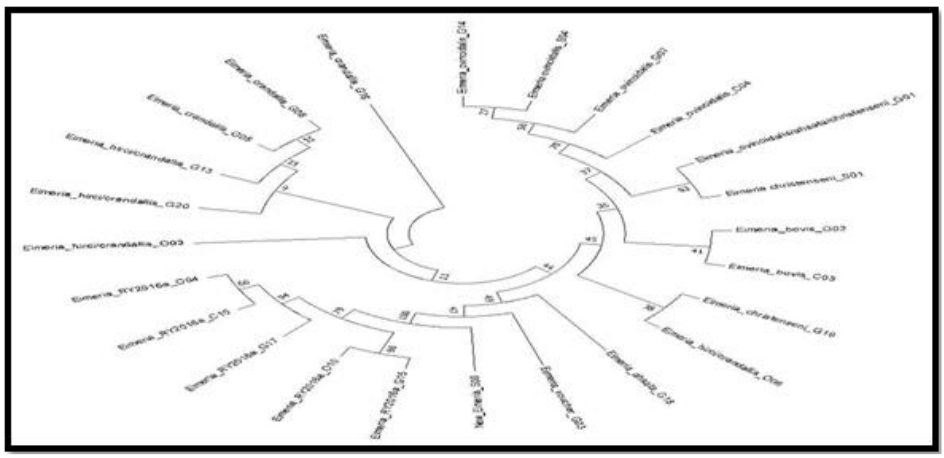

Fig. 1: Phylogenetic tree analysis (bootstrap consensus tree)

***Eimeria pallida*:** Oocysts are ellipsoidal, smooth wall colorless topale yellow or yellowish green, Polar cap absent, Micropyle absent, mean size 19.8±0.6×16.8±1.2, with range 20–12 × 15 - 8 µm, and 1-3 days as sporulation time (Fig. 8).

Eimeria Species Detected inGoats

***Eimeria ninakohlyakimovae*:** Oocysts are ellipsoidal or slightly subspherical, thin-walled, colorless, without micropyle or micropyle cap mean size 23.5±1.0×16.0±1.2, with range 24.3–20×19.5–14 µm and sporulation time is 1–4 days (Fig. 9).

Eimeria christenseni: The oocysts are ovoid or ellipsoidal, colorless topale yellow, with a micropyle and micropyle cap. mean size30.1±1.6×17.1±0.3, with range 44-27×31-17 µm and sporulation time is 3-6 days (Fig. 9).

Eimeria aspheronica: Oocysts are ovoid, greenish to yellow brown,with a micropyle but without a micropyle cap, mean size 24.6±0.3×17.5±1.2, with range 37-24 ×26-18 µm, and sporulation time is 1–2 days (Fig. 10).

Eimeria hirci: Oocysts are ellipsoidal to subspherical, light brown tobrownish yellow, with a micropyle and micropyle cap, mean size22.8±0.3×14.2±1.1, with range 23-18×19-14 µm, and sporulation time is 1–3 days (Fig. 10).

***Eimeria arloingi*:** Oocysts are ellipsoidal or slightly ovoid, with athick wall. a micropyle and micropyle cap present, mean size 29.2±1.6×17.1±1.1, with range 42-17×19-14 µm and sporulation time is 1–4 days (Fig. 11).

Eimeria capralis: Oocysts are ellipsoidal with a distinct micropylecap, but without micropyle having mean size 29.5±1.5×19.6±0.3**,** with range 34-25× 24.5-19.5 µmand 5 days as sporulation time (Fig. 11).

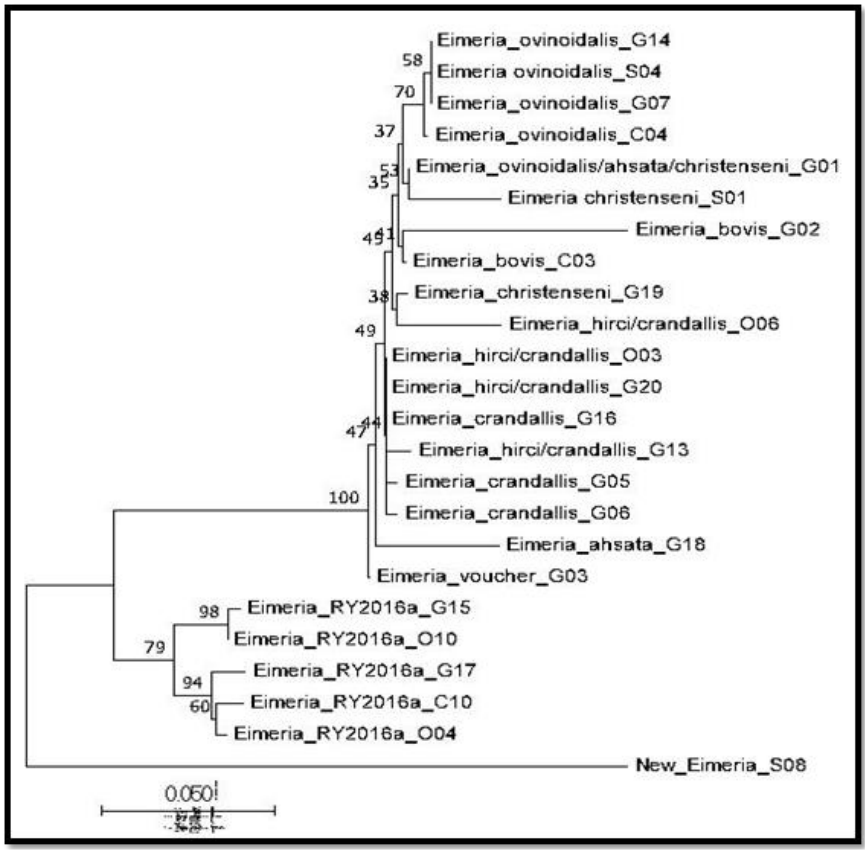

Fig. 2: Phylogenetic tree analysis (Neighbor-Joining method)

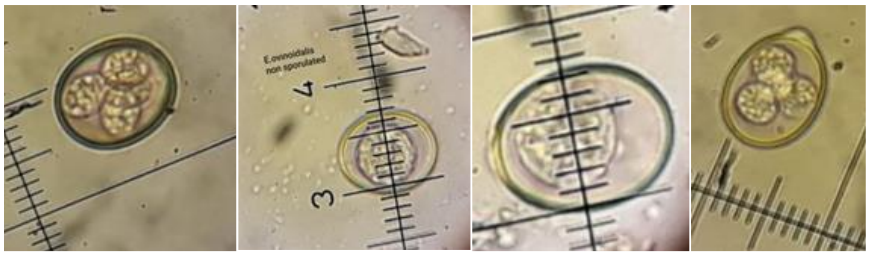

Fig. 3: Sporulated andnon sporulated Oocyst of *E. ovinoidalis* and*E. cran*

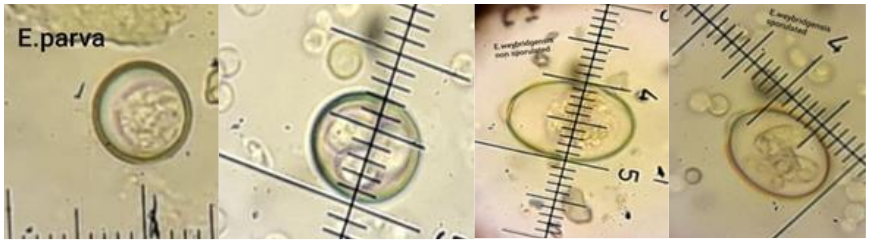

Fig. 4: Sporulated andnon sporulated Oocyst of *E. parva* and *E.weybridgensis* (40X)

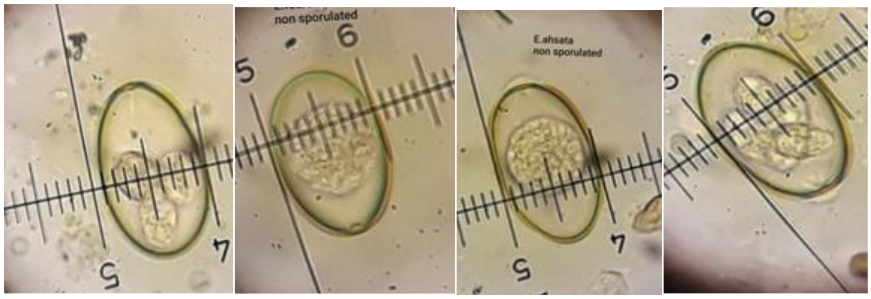

Fig. 5: Sporulated andnon sporulated Oocyst of *E. faurei* and *E. ahsata* (40X)

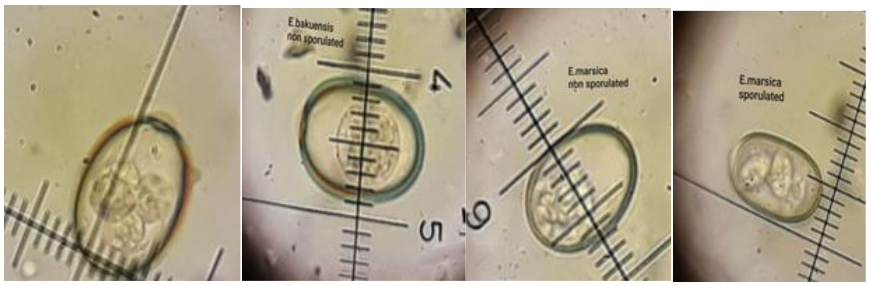

Fig. 6: Sporulated and non sporulated Oocyst of *E. bakuensis* and*E.marsica* (40X)

Fig. 7: Sporulated and non sporulated Oocyst of *E. granulosa and E. intricata* (40X).

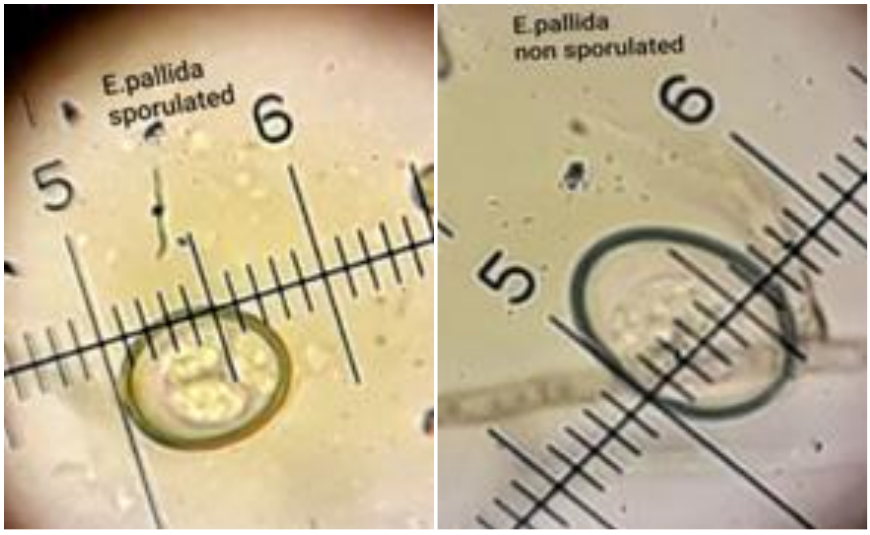

Fig. 8: Sporulated and non sporulated Oocyst of*E.pallida*(40X)

Fig. 9: Sporulated and non sporulated Oocyst of *E. christenseni and E. ninakohlyakimovae* (40X)

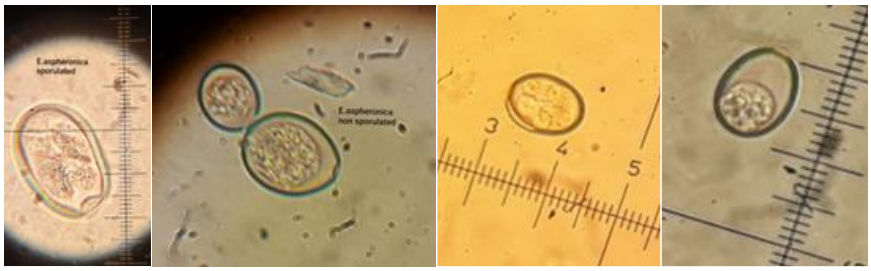

Fig. 10: Sporulated (100X) and non sporulated (40X) Oocyst of *E. aspheronica and E. hirci*(40X)

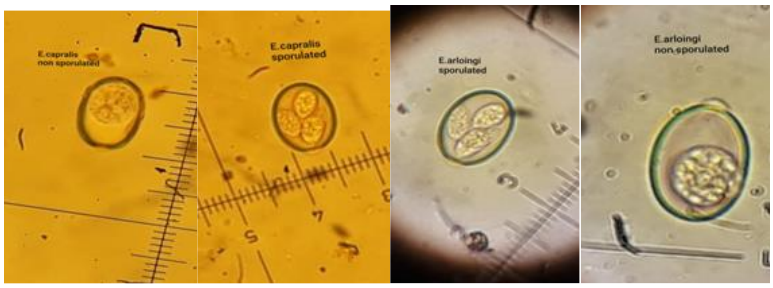

Fig. 11: Sporulated and non sporulated Oocyst of *E. capralis and E. arloingi*(40X)

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12

Ehrlichiosis: Tick-borne Malady

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INTRODUCTION

Ehrlichiosis or especially canine ehrlichiosis is an important tick-borne disease with worldwide distribution ranging from Brazil to the United States of America (Dumler et al. 2001; Dumler et al. 2007; Heitman et al. 2016). *Ehrlichia canis* was first identified in Algeria in 1935 by Donatien and Lestoquard (Harrus et al. 1998). It was discovered upon examination of dogs showing signs of anemia and fever. Formerly, it was called Tropical Canine Pancytopenia, but later more appropriately renamed to Canine Monocytic Ehrlichiosis (Huxsoll et al. 1970). Commonly ehrlichiosis is associated with signs including fever, fatigue and myalgia (Buller et al. 1999; Dumler and Walker 2014). In this chapter, various aspects of Ehrlichia like its history, life cycle, transmission, pathogenesis, clinical signs, symptoms, treatment, control, and prevention of ehrlichiosis are discussed in the following sections.

An Ehrlichia infection affecting platelets was first identified in the US in 1978. Its causative agent was identified to be *Ehrlichia platys* which was later renamed *Anaplasma platys.* It caused a clinical syndrome of cyclic infectious thrombocytopenia in canines (Harvey et al. 1978).

Several species of *E. canis* were discovered in dogs over the span of the 1980s to 1990s. However, improvement in molecular genetics later proved that these were species of *Anaplasma* or *Neorickettsia* (Dumler et al. 2001). That is why to date only *E. canis* is the single species that has been isolated from dogs in Europe (Keysary et al. 1996; Aguirre et al. 2004). Many other species of Ehrlichia including *E. chaffeensis, E. ewingii, E. muris,* and *E. ruminatum* out of which only *E. muris* was found in *Ixodes* ticks in Russia and Slovakia (Shpynov et al. 2006; Spitalska et al. 2008).

E. chaffeensis is a major etiologic agent that causes ehrlichiosis in humans (CDC, 2010). It has been identified as the most-wide spread tick-borne disease of humans in the Southern United States (Beall et al. 2012). A few cases of *E. ewingii* infection are also reported infrequently and most of them were discovered in patients already having a history of immune incompetence (Buller et al. 1999; Chapman et al. 2006; Thomas et al. 2007; Allen et al. 2014; Dumler and Walker 2014).

The life cycle of Ehrlichia has been outlined in Fig. 1 & 2. Briefly, Ehrlichia is not transmitted from adult female ticks to the eggs. The newly laid eggs of the ticks are uninfected. These eggs grow into uninfected larvae. When these larvae grow on infected reservoir hosts they become infected after a blood meal. Infected nymphs are formed from these uninfected larvae. These nymphs have the ability to infect a new host and they can accidentally affect human hosts too. After infecting new hosts, the infected nymphs grow into infected adults. These adults can transmit infections to new hosts. When residing on a host the adult female ticks lay uninfected eggs on the hosts nullifying the expression of vertical transmission of Ehrlichia in ticks.

The Ehrlichia are taken up by ticks through blood meal as elementary bodies (Fig. 2). These elementary bodies reside in the gut and then migrate to the salivary glands of the tick. When this tick bites a healthy person the Ehrlichia are transmitted to the host as elementary bodies. These elementary bodies are phagocytosed by neutrophils. Inside neutrophils, these elementary bodies are developed in morulae with reticulate bodies. The reticulate bodies keep developing until the cell bursts releasing elementary bodies into the blood of the host from where it is once again taken by the ticks (Ganguly and Mukhopadhayay 2009).

Disease in Animals

Ehrlichia canis can cause disease in dogs of all breeds irrespective of their sex, age or breed. However, German Shepherds and Siberian Huskies are found to be more prone to ehrlichiosis. These breeds also show poor prognoses for recovery (Nyindo et al. 1980; Harrus et al. 1997). Different strains of ehrlichia cause disease in both animals and humans as shown in Table 1.

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Table 1: Etiological agents of different types of Ehrlichiosis in different hosts.

No.	Diseases	Host	Pathogen	References
1.	Human Granulocytic Ehrlichiosis	Human	*Ehrlichia chiffeensis, Ehrlichia ewingii*	(Ganguly and Mukhopadhayay 2009)
2.	Human Monocytic Ehrlichiosis	Human	*Ehrlichia chiffeensis, Ehrlichia ewingii*	(Ganguly and Mukhopadhayay 2009)
3.	Canine Monocytic Ehrlichiosis	Dogs	*Ehrlichia canis*	(Harrus et al. 1997)
4.	Heartwater	Ruminants	*Ehrlichia ruminantium*	(Allsopp 2010)

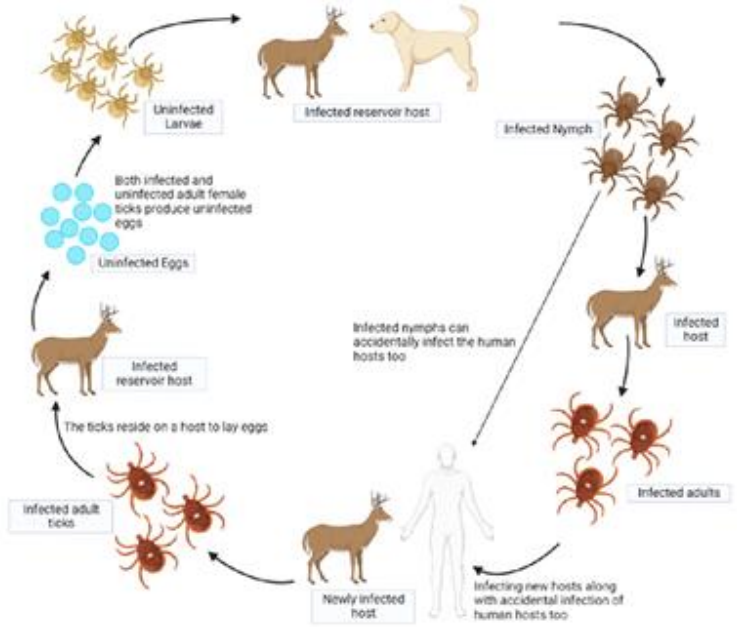

Fig. 1: The infection cycle of Ehrlichia

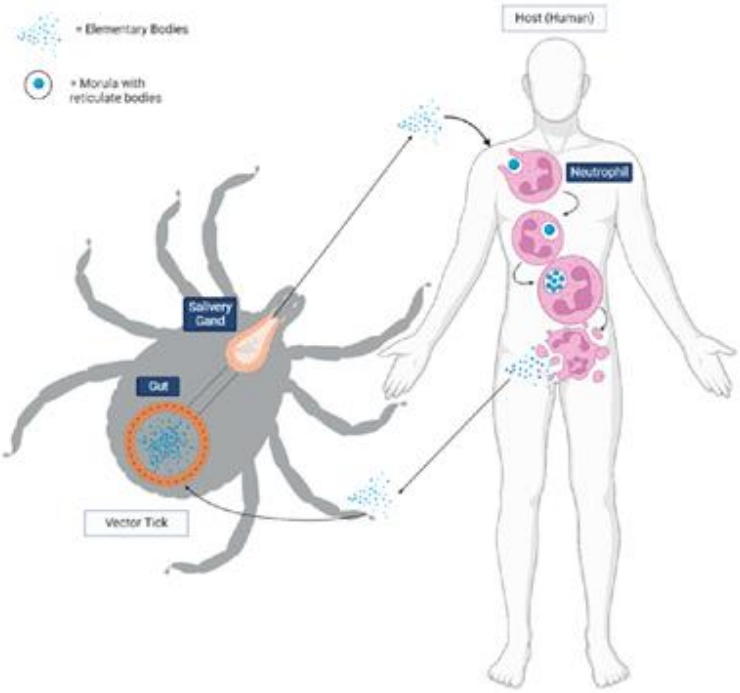

Fig. 2: The life cycle of Ehrlichia

Pathogenesis

The *E. chaffeensis* pathogen mainly affects vertebrates. This pathogen targets the mononuclear phagocytic cells. Mostly, monocytic cells are found to be affected due to an infection but several other cells have also been described by many researchers that are influenced during an Ehrlichia infection. The other cells infected by *E. chaffeensis* include

metamyelocytes, lymphocytes, promyelocytes, atypical lymphocytes, and band and segmented neutrophils (Maeda et al. 1987; Abbott et al. 1991; Dumler et al. 1993; Paddock et al. 1997). The morulae of Ehrlichia are found in the cells of an infected person. There may be 1 or 2 morulae in a cell. This number can go up to 15 morulae in the leucocytes of a person with below-average immune competence (Paddock et al. 1993; Barenfanger et al. 1996; Martin et al. 1999).

Histopathologically, bone marrow is the most researched tissue for checking the pathogenic effects of Ehrlichia but no consistent pathogenic patterns have been seen in this disease until now. However, researchers have found the bone marrow in a normocellular or hypercellular state along with myeloid hyperplasia or megakaryocytes, both may also occur together sometimes (Standaert et al. 1998; Dumler et al. 1993; Grant et al. 1997).

In human monocytic ehrlichiosis (HME), the cytopenia associated with diseases is not a direct result of infection. The disturbed cell count is rather attributed to peripheral events like sequestration, cellular destruction mostly by phagocytosis of infected and some non-infected cells too and consumption of the cells (Harkess et al. 1989; Dumler et al. 1993).

Pathological signs seen in Ehrlichia are often found in patients suffering from some immune-compromising disorders along with Ehrlichia. These signs include edema of the lungs, diffuse alveolar damage, interstitial alveolar hemorrhage, and intra-alveolar hemorrhage (Dumler et al. 1991; Paddock et al. 1993; Marty et al. 1995; Paddock et al. 1997; Fordham et al. 1998). Perivascular infiltrates may also be found in several organs including meninges without any evidence of endothelial damage or thrombosis. Lymphohistiocytic infiltrates are the dominant type of infiltrates often found in organs (Marty et al. 1995; Paddock et al. 1997; Walker and Dumler 1997).

Focal necrosis may also be seen in the liver, spleen, and lymph nodes in the bodies of patients suffering from *Ehrlichia chaffeensis* (Dumler et al. 1991; Paddock et al. 1993). Diffuse hemorrhages may also be discovered in visceral organs including the urinary bladder, kidneys, meninges, and diaphragm (Marty et al. 1995; Paddock et al. 1997).

Signs

E. canis infection can produce a variety of clinical signs in dogs after infection depending upon the strain that has infected the animal. The signs may also vary according to the immune response produced by the host's body. Infestation with ticks and other flea-borne pathogens can also cause the signs to deviate from the typical ones or may affect the severity of the infection. Even sometimes it happens that sometimes dogs do not show any clinical signs or laboratory findings related to the diagnosis of Ehrlichia infection despite being carriers of *E. canis* (Harvey et al. 1978; Greig et al. 1996; Egenvall et al. 1997; Harrus et al. 1997; Varela et al. 1997 Breitschwerdt et al. 1998; Egenvall et al. 1998; Goldman et al. 1998; Lilliehöök et al. 1998; Neer 1998; Breitschwerdt 2005; Leiva et al. 2005; Komnenou et al. 2007; Diniz et al. 2008; Tabar et al. 2009; Little 2010).

Diagnosis

Ehrlichia canis aggregates or morulae are rarely detected through blood smear microscopy. Only 4- 6% of clinical cases of ehrlichiosis were found to have blood smear morulae discovered by microscopy. However, the likelihood of detecting morulae increases if microscopy of the buffy coat is performed instead of whole blood (Mylonakis et al. 2003). Further confirmation techniques like PCR must be used for surefire identification of Ehrlichia. An expert cytologist may also be able to identify Ehrlichia morulae by microscopy of lymph node aspirates under oil immersion field views. This technique is also not very effective for diagnosis and has only a 50% chance of success (Mylonakis et al. 2003; Mylonakis et al. 2011). Different diagnostic tools for Ehrlichia are whole blood smear microscopy, buffy coat smear microscopy, cytology of lymph nodes, and PCR technique (Fig. 3).

Transmission

Many of the ticks found in homes, gardens, and pastures are also responsible for Ehrlichia transmission. Out of many examples of such agents involved, *Rhipicephalus sanguine* complex of ticks is the top suspect. It is one of the ticks mostly found indoors. It may also be common in many other places. Other ticks may also be involved in Ehrlichia transmission in other places like gardens and grassy areas. This suggests that the dogs can become infected with Ehrlichia being transmitted through these ticks at any time and in any place. Even in the backyard, gardens, and grassy places found near houses, there is a risk that there might be a population of ticks there that can transmit Ehrlichia. So, whenever a dog is involved in any activity that demands going to or being near a grassy area, it is at risk of getting in contact with ticks and hence becomes a target of Ehrlichia infection (Bremer et al. 2005).

E. canis can be also transmitted among dogs through the bite of brown dog ticks *R. sanguineus* (Bremer et al. 2005).

Treatment

The tetracycline group of antibiotics shows promising results when used for treating ehrlichiosis in dogs or canine monocytic ehrlichiosis. The drug of choice from this to be used against Ehrlichia is doxycycline. Two dosing methods can be used to administer doxycycline to the dogs. The first method is a once-a-day dosing system. A single dose of 10 mg/kg should be given to the dog orally once a day. The second method is a twice-a-day dosing system. In this system, the per day 10 mg/kg dose of doxycycline for the dog is divided into two parts of 5 mg/kg. This dose is then two times a day with an interval of 12 hours between two doses instead

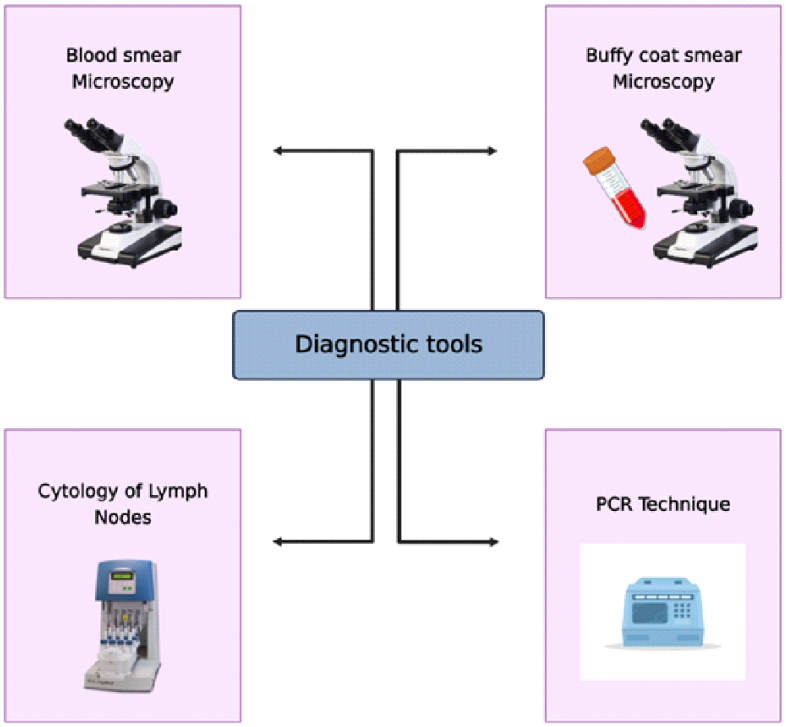

Fig. 3: Different diagnostic tools for Ehrlichia

of giving one single dose for 24 hours. These doses either as a single per day dose or twice per day doses should be continued for at least 4 weeks for complete recovery of dogs from Ehrlichia. This treatment regime for Ehrlichia promises a good prognosis. Hence, effective response can be seen in Ehrlichia sick dogs once this treatment protocol is implemented (Harrus et al. 1998; Harrus et al. 2004; McClure et al. 2010).

The prolonged treatment time of 4 weeks is strictly recommended to completely cure the dog from ehrlichiosis. During experimental investigations, it was proved that shortening this treatment period causes unforeseen circumstances in terms of prognosis and recovery. Several dogs that were experimentally infected with Ehrlichia and given doxycycline for a shorter period even at recommended doses, did not recover completely. Instead of recovering completely, these dogs became subclinical carriers of Ehrlichia (Wen et al. 1997; Breitschwerdt et al. 1998; McClure et al. 2010).

Prevention

Until the present time, researchers have been unable to create a definitive vaccine against Ehrlichia in dogs for its sure-fire prevention in felines. Especially the topic of prevention of *E. canis* infection has seen a lot of debate but chances of a vaccine coming up against infection of this pathogen are still slim. However, recent studies have shown some hope. A certain strain of *E. canis* has shown promising results as a vaccine when in attenuated form. It is expected that in near future a vaccine will be made from this strain for commercial use (Rudoler et al. 2012).

Quick action upon discovering that a dog has become infested with ticks can also help in saving the dog from getting infected with Ehrlichia. Under normal conditions, it is a general consideration that the infectious agents take 24-48 hours to travel from the salivary gland of ticks to the host's bloodstream. Hence an injection of a preventive drug at that time will save the dog from getting infected with Ehrlichia (Nicholson et al. 2010). However, recent studies have brought us the bad news that some Ehrlichia agents like *E. canis* are transmitted from the tick's salivary glands to the host's bloodstream more rapidly than the other pathogens that follow the general 4-48 hours transmission time rule (Gray et al. 2013). *E. canis* is also a problematic pathogen as it can re-infect dogs even after they have recovered from the infection once. This happens because no persistent immunity is developed against this pathogen in the host's body (Harrus et al. 1997).

Control

To prevent dogs from getting ehrlichiosis the focus must be shifted to control of its transmission agents. So, the control of tick populations that are transmitting Ehrlichia will ultimately control the prevalence of these diseases in dogs.

To save a dog from getting Ehrlichia infection, it is necessary to save them from getting in contact with these Ehrlichia-transmitting ticks. Some measures to be taken for saving dogs from the attacks of these ticks are:

- Keep the dogs away from large fields. Large fields usually have a high chance of having ticks. Once a dog gets infested with even a single tick then this tick will be transmitted to indoor housing areas and its population will grow into large numbers rapidly. This will not only make the infested dog sick, but it will also pose a threat to other dogs living in nearby areas (Sainz et al. 2015).

Table 2: Vectors involved in Ehrlichia transmission.

No.	Common name	Scientific name	Reference(s)
1.	Dog tick	*Dermacentor variabilis*	Anderson et al. 1993; Roland et al. 1998; Kramer et al. 1999
2.	Western Black-legged tick	*Ixodes pacificus*	Kramer et al. 1999
3.	Castor bean tick	*Ixodes ricinus*	Alekseev et al. 2001

- Dogs should be prevented from getting infested by ticks even if they live in an area where the tick population is abundant. This objective is harder to achieve but it can be achieved by regularly treating dogs with acaricidal drugs (Torres 2008; Pereira et al. 2009).
- Registered tick repellents like pyrethroids and several preparations of diazinon can be used to keep the ticks away from dogs (Sainz et al. 2015).

Control of these ticks is also possible by keeping in mind the temperatures at which the ticks are active and keeping the dogs indoors at that time to prevent them from getting infested with the ticks. In the case of *R. sanguineus* ticks, they are active only when the temperature is above 10-12 ℃ but below this temperature, these ticks are mostly inactive. Hence, at lower temperatures dogs are somewhat safe from the infestation of these ticks and in turn from Ehrlichia infection (Sainz et al. 2015).

Similarly, *I. Ricinus* ticks become active when the temperature rises above 6℃. So, these ticks are more active as compared to the *R. sanguines* ticks and hence require more intense measures for saving dogs from being infested with them (Gray et al. 2013).

Disease in Humans

Zoonotic ehrlichiosis in humans is a potentially fatal tick-borne disease. In humans, ehrlichiosis can be caused by infectious agents like *Ehrlichia chaffeensis* or *Ehrlichia ewingii*. The first case of human monocytic ehrlichiosis was diagnosed in 1991 and its etiological agent was discovered to be *E. chaffeensis* (Dawson et al. 1991). Later in the year 1992 cases of granulocytic ehrlichiosis were also diagnosed and reported. These cases of ehrlichiosis were different from the ones reported in the past because the infectious agent involved in causing diseases this time was found to be *E. ewingii* (Dawson et al. 1991; Fishbein et al. 1994; Paddock and Childs 2003; Chapman et al. 2006).

Transmission

Centers for Disease Control and Prevention in 2010 and 2014 reported that in humans, ehrlichiosis is majorly transmitted only through tick bites. The main culprit involved in the transmission of ehrlichiosis in humans is a tick named the lone star tick along with several other species of ticks as shown in (Table 2). The scientific name of this tick is *Amblyomma americanum*. Transmission of Ehrlichia solely happens through bites of this tick and hence Ehrlichia is most prevalent in the regions where the lone star tick population is the highest. This tick is most commonly found in southeastern, south-central, and northeastern parts of the United States (Paddock and Childs 2003; Beall et al. 2012).

These lone star ticks are particularly very effective agents of Ehrlichia transmission. Their effectiveness increases because of characteristics like being aggressive non-selective feeders and having the ability to bite and transmit infections throughout all stages of life (Childs and Paddock 2003). Centers for Disease Control and Prevention in 2010 stated that the adult and nymph stages are however the major culprits of Ehrlichia transmission. The feeding seasons of these stages coincide with the peak infection seasons of Ehrlichia. This peak is achieved during hot weather ranging from the month of May to July (Paddock and Childs 2003; Dumler and Walker 2014).

American Academy of Pediatrics in 2015 released a statement claiming that transfusion and transplantation of organs like liver and kidney have also been reported as a medium for transmitting *E. chaffeensis* (Antony et al. 1995; Paddock and Childs 2003; Dumler and Walker 2014; Sachdev et al. 2014). Only one such case of *Ehrlichia ewingii* transmission has been reported to occur when a young boy went through the transfusion of platelets (Regan et al. 2013).

Zoonosis

Many wild and domestic animals serve as reservoirs for Ehrlichia pathogens. These animals then serve as the basis for the zoonotic transmission of Ehrlichia to humans through ticks. An example of such a wild reservoir animal is the white-tailed deer scientifically named *Odocoileus virginianus*. This deer has been found to be naturally infected with *E. chaffeensis* and is thus involved in maintaining its enzootic cycle (Yabsley et al. 2002; Childs and Paddock 2003; Paddock and Yabsley 2007). Similarly, just like the white-tailed deer, domestic dogs are also involved in the zoonotic transmission of Ehrlichia by serving as reservoirs maintaining the enzootic life cycle of the pathogen. Domestic dogs are majorly found to be the reservoirs of *E. ewingii* (Yabsley et al. 2002; Beall et al. 2012). The dogs can also serve as transport carriers. The pet or stray dogs once infected can carry the pathogen closer to human populations making them more prone to being infected with Ehrlichia (Childs and Paddock 2003; Paddock and Childs 2003).

Along with Ehrlichia, some animals can also serve as potential hosts for the lone star ticks making the transmission of Ehrlichia from animals to humans possible. This category includes a large number of animals. Some examples of such animals are domestic dogs, birds, rabbits, goats, wild turkeys, red foxes, opossums, canids, and raccoons (Childs and

Paddock 2003; Paddock and Childs 2003; Paddock and Yabsley 2007).

Signs and Symptoms

In humans, ehrlichiosis has non-specific symptoms that begin to appear after 7 to 14 days of incubation period post-exposure to the infectious agent (Dumler and Walker 2014). In humans, the commonly observed signs of Ehrlichia include fever, headache, chills, nausea, myalgia, and malaise (Buller et al. 1999; Dumler and Walker 2014).

Severe illness in the case of ehrlichiosis is indicated by some characteristic signs. In adults, the signs seen with increasing severity of ehrlichiosis usually included confusion, lymphadenopathy, diarrhea, and cough. However, the signs of severe illness were seen to differ in children as compared to adult patients. In children, the severity of ehrlichiosis was marked by the appearance of edema on the hands and feet. When laboratory diagnostic tests for further studying the pathological effects of Ehrlichia infection were conducted, some new facts were unveiled for the researchers. Ehrlichia also affected the blood profile of its hosts. This disturbance was seen as leukopenia and thrombocytopenia during the blood analysis of the patients. Along with these blood tests, the conduction of serum analysis also revealed increased serum levels of hepatic aminotransferase (Dumler and Walker 2014).

According to reports from the Centers for Disease Control and Prevention 2010, a large number of cases of *E. chaffeensis* infection in children were marked with the appearance of a rash. The rash was seen in less than 1/3rd of the ehrlichiosis cases of adults. The rash seen in ehrlichiosis started as a maculopapular rash in the early stages of infection. However, as the infection progressed the rash also changed its state from maculopapular to petechial (Harkess et al. 1991; Paddock and Childs 2003; Chapman et al. 2006; Dumler and Walker 2014). American Academy of Pediatrics 2015 confirmed in its reports that the rash had some characteristic appearance areas on the human body. The rash was typically seen on the trunk. The rash started to appear 7 days after symptoms developed in the patient. The rash often kept itself limited to the trunk and did not spread to the hands or feet of the patient (Chapman et al. 2006). It was also observed during diagnostic studies that the rash is commonly seen during *E. chaffeensis* infections. Rashes were rather rare to be seen when a person was diagnosed to be infected with *E. ewingii* (Chapman et al. 2006).

E. chaffeensis infections lead to the appearance of severe signs. *E. chaffeensis* infections have been reported to lead to death in 1 to 3% of cases out of all *E. chaffeensis* infections. The death of a patient can occur as early as during the second week of infection by *E. chaffeensis* (Paddock and Childs 2003; Chapman et al. 2006). However, *E. ewingii* infections are much less severe than *E. chaffeensis* infections. *E. ewingii* causes milder signs during infection. There are no deaths reported due to *E. ewingii* infection (Paddock and Childs 2003; Dumler and Walker 2014).

Treatment

Ehrlichiosis usually appears like any other infection and the signs can vary from mild to moderate and severe. Generally, people are hospitalized for treatment of Ehrlichia depending upon the severity of signs. Around 50 to 70 % of the people infected with Ehrlichia are hospitalized for treatment (Fishbein et al. 1994; Paddock and Childs 2003; Chapman et al. 2006).

According to the American Academy of Pediatrics 2015, it is necessary to begin the treatment of Ehrlichia as soon as the signs and symptoms appear. Laboratory confirmation should not be regarded as a reason to delay the treatment (Chapman et al. 2006; Todd et al. 2015). American Academy of Pediatrics 2015 has recommended beginning treatment within 5 days after post appearance of signs of Ehrlichia infection to expect a better prognosis for the recovery of the patient as compared to the situation where treatment is withheld or delayed beyond this time frame (Fishbein et al. 1994).

Unjustified delay in treatment or giving no treatment to an Ehrlichia patient at all can lead to severe consequences as the disease progresses. It can lead to the failure of important organs like kidneys. The nervous may also be affected due to Ehrlichia infection. It can also lead to issues like Adult Respiratory Distress Syndrome (ARDS) and Disseminated Intravascular coagulation-like syndrome (Dahlgren 2011; Dumler and Walker 2014).

Prevention

Since the main agents for Ehrlichia transmission in humans are the ticks, the main efforts of reducing ehrlichiosis depend on the effective control of tick populations and the elimination of its reservoirs (Childs and Paddock 2003). Dogs can also serve as reservoirs for both ticks and *E. ewingii* so it is recommended for pet dog owners to be careful that their dogs should not come in contact with infection or become a reservoir of ticks. This objective can be achieved by using acaricide-containing collars, veterinary ectoparasite control drugs, or by using topical applicants against tick attachment and infestation (Pereira et al. 2009).

The American Academy of Pediatrics has recommended in 2015 that starting treatment of Ehrlichia-infected patients at the earliest opportunity after appearance signs is a good measure to save human lives but prevention is still better than cure. The best option to prevent ehrlichiosis infections in humans is to avoid tick bites. The people and pets visiting Ehrlichia endemic and tick-infested areas should be checked for ticks to prevent the transfer of ticks. Regular checkups of people and pets should be made customary as a preventive measure for reducing Ehrlichia transmission. As there is no

vaccine or prophylactic drug against Ehrlichia, it is necessary for humans to reduce their exposure to ticks to prevent infection. An important measure that people can adopt to prevent tick bites is to wear full-covering clothing impregnated with permethrin. Furthermore, using repellents *n,n-diethyl-m-toluamide* (DEET) is also effective to avoid tick bites (Chapman et al. 2006; Brett et al. 2014).

Conclusion

Ehrlichia might seem a moderate disease but can result in fatality if left untreated. Ehrlichia affects a wide variety of animals. The major impact of Ehrlichia is seen in our beloved pet dogs. This disease can not only kill a dog, but it can also lead to disease in humans too if the dog is affected by an Ehrlichia strain with zoonotic potential.

Such a situation makes it necessary for humans to take special care of preventing Ehrlichia transmission from infected dogs. In absence of a vaccine, the best method for preventing Ehrlichia infection is by preventing tick infestation in dogs. If there is no agent to transmit Ehrlichia then there will be no spread of infection. This prevention is far better than a medicinal cure because even after a dog has fully recovered from Ehrlichia, it still remains a carrier of Ehrlichia. This puts the other dogs, animals, and even humans around it at risk of an Ehrlichia infection.

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13

Fascioliasis

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INTRODUCTION

Fascioliasis is a zoonotic disease caused by a trematode parasite belongs to the genus *Fasciola* (Bargues et al. 2016). It has a wide range of geographical distribution and found across the world (Charlier et al. 2020). *Fasciola* species are commonly known as liver flukes, as they are leaf shaped (David 1990). The flukes are hermaphrodite and are mainly confined to the bile ducts of the liver or gall bladder of infected hosts. They cause liver-rot in ruminant hosts, that may lead to the death (Khoramian et al. 2014).

Fascioliasis has been listed as a neglected zoonotic disease by the World Health Organization (Mas-Coma et al. 2018). It has a predictable impact on livestock production (Kalu 2015). According to an estimate, over 600 million animals were infected with it worldwide, having an annual estimated economic loss to nearly $3 billion (Toet et al. 2014). The public health risk with Fascioliasis among people was estimated to be about 17 million cases worldwide (Mas-Coma et al. 2009a), and up to 180 million at risk of infection (Mas-Coma et al., 2018).

The global increase in human fascioliasis prevalence rates are greatly correlated with a high proportion of infected ruminant hosts (Ashrafi et al. 2014; Diyana et al. 2019).

Flukes of the genus *Fasciola* has complex life cycle. Their larval stages depend on Lymnaeids snails as an intermediate host for their growth and development (Munita et al. 2019). A wide range of mammals including cattle, sheep, goat, horse (Taylor et al. 2013), buffalo, camel, deer and human serve as the definitive host (John et al. 2019). Donkey and mules can also harbor the flukes and become a reservoirs host (Meray Sierra 2020). In addition, the fluke was reported from pigs, alpacas, kangaroos, wallabies, and rabbits (Alemneh 2019). The occurrence of Fasciolaiasis depends on several factors related to the biology of vectors and parasites, and the management of animal herds (Khoramian et al. 2014). The distribution of each *Fasciola* species depends on the availability of intermediate hosts of Lymnaeid snails (Prasad et al. 2008).

Fascioliasis appears in two forms, acute or chronic, depending on the extent of the disease and the required time for its occurrence (Radiostis et al. 2007). The main economic impact of fascioliasis is the condemnation of the infected liver, along with a decrease in productivity and a reduction in the growth rate of infected animals (Usip et al. 2014).

Fascioliasis is an emerging disease in many countries, especially when there is a tradition of eating uncooked vegetables harboring the infective metacercarial stage (Ashrafi et al. 2006a). So, it is regarded as one of the foodborne diseases with greater pathogenic effects mostly in the acute phase of infection during 3-4 months (Chen and Mott 1990). Global changes appear to have a correlation with the emergence of fascioliasis such as importation/exportation and livestock management (Mas-Coma et al. 2009b), environmental anthropogenic modifications, travel (Ashrafi et al. 2014) and alteration in human diet traditions (Ashrafi et al. 2006a).

Etiology

Fascioliasis is considered as food and water borne zoonotic infection caused by digenean trematodes of the genus *Fasciola* (Alemneh 2019). *Fasciola (F.) hepatica* (Linnaeus 1758) and *Fasciola (F.) gigantica* (Cobbold 1856) are the common and more prevalent flukes causing infection in human and animals (Admassu et al. 2015; Amer et al. 2016). *F. hepatica* is nearly distributed throughout all continents, while *F. gigantica* is mostly restricted to the parts of Asia and Africa (Meray Sierra 2020).

Taxonomy

The parasitic tapeworms belong to the Phylum platyhelminths involve two classes: Class Cestoda (the tapeworms) and Class Trematoda (the flukes). The class Trematoda is further divided into two main subclasses, Monogenea (direct life cycle) and Digenia (involving intermediate host). The trematodes belong to the family Fasciolidae comprising of the parasites of major veterinary importance. According to Urquhart et al. (1996) the taxonomic classification of *Fasciola* is as follows;

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Trematoda
Subclass: Digenia
Order: Echinostomida
Family: Fasciolidae
Genus: *Fasciola*
Species: *F. hepatica*, *F. gigantica*

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Morphology

F. hepatica and *F. gigantica* can be distinguished morphologically based on characteristics of their body length and width (Ashrafi et al. 2006b; Itagaki et al. 2009). The adult fluke of *F. hepatica* is large flattened and leaf-like, anteriorly provided with cone shaped projection followed by a pair of prominent shoulder, with wider and rounded posterior end (Hendrix and Robinson 2006). Flukes are grayish brown in color changing to gray when preserved (Wagari 2021). The adults possess two suckers for attachment. The oral sucker at the anterior end surrounds the mouth and the ventral one, situated on fluke´s ventral surface (Urquhart et al. 1996). The flucks´ tegument is absorptive and armed with backward directed spines, together with the suckers, assist to preserve the parasitic position in the bile ducts by an effective mechanism (Smyth 1994). The muscles lie directly under the tegument, and the organs are packed in a parenchyma since they lack the body cavity. The digestive system starts with oral opening leading into a pharynx, esophagus and a pair of blindly branched intestinal ceca. A large number of ciliated flame cells together forms the excretory system, and the waste metabolic products pass through a connected tubular system and exposed externally. The simple nervous system consists of two anterior ganglia and a pair of longitudinal trunks arising from them (Urquhart et al. 1996; Rickard 2001).
Based on geomorphology, *F. hepatica* is short and possess broad shoulders, whilst *F. gigantica* is elongated and with narrower body (Mas-Coma and Burger 1997; Lotfy and Hiller 2003). *F. hepatica* measures "30- 20 mm × 10 mm" and *F. gigantica* measure "27 to 75mm" × 12mm" (Brown 1980). When hybridization of both species occurs within the host's body, subsequent offspring have intermediate phenotypes (Vara-Del Rio et al. 2007; Beesley et al. 2018). Due to the presence of the intermediate form combining of morphological and molecular techniques for distinguishing of *Fasciola* species is critical especially in regions where fluke species overlap (Haridwala et al. 2021). Both species have the ability to reproduce sexually or through self-fertilization (Shoriki et al. 2014).

Egg

The eggs of *Fasciola* spp. are large in size, oval, yellow brown in color, with a thin shell and possess a distinct operculum. The eggs of *F. hepatica* measure up to "130 to 150 µm" by "60 to 90 µm" (Hendrix and Robinson 2006), and in *F. gigantica* measures up to "120 -180 µm" by "80 - 110 µm" (Phalee et al. 2015). Eggs consist of a fertilized ovum with vitelline cells surrounded with proteinous shell (Andrews 1999). The ova contain one cell stage embryo surrounded by a group of oval body yolk cells. Development of eggs to reach maturation in both *Fasciola* species required 12-16 days, and the miracidia hatch within 4 days after maturation (Hussein et al. 2020).

Miracidium

It has an elongated conical body with a broad anterior end and tapering posterior end, and swims at great speed (Malek 1980). The outer surface cover with numerous cilia, except in lateral connection regions of epidermal plats. These cilia appear longer on the apical parts of both anterior and posterior end than the rest parts of the body, and remain viable for about 9-12 hours (Hussein et al. 2010).

Cercaria

It has a large heart shaped body and simple long tail. The body covered with thick wall and is surrounded by tiny spines all over its surface (Hussein et al. 2010).

Metacercaria

It is spherical white color cyst directly infective to the definitive host. With time it becomes yellow and darker in color after 1 or 2 days, the cyst measures up to "0.26 to 0.30 mm" in diameters, and protected by thick wall capsules of double outer and inner layer for protection against environmental impacts (Phalee et al. 2015).

Transmission

The transmitted vectors for *Fasciola* spp. are amphibious freshwater lymnaeid snails (Mas-Coma et al. 2009a). It was estimated that nearly 30 species of lymnaeid snail are recognized as intermediate hosts for *Fasciola* spp. globally (Vázquez et al.2018). *Galba truncatula* is the common lymnaeid act as a transmitter for *F. hepatica* in endemic temperate and subtropical areas (Artigas et al 2011; Bargues et al 2020).
Different *Lymnaea* species including: *L. cousin, L. columella, L. ollula, L. natalensis,* and *L. viridis* act as an intermediate host for *Fasciola* spp. (Hussein and Khalifa 2008). Both *Radix (R.) auricularia* and *R. natalensis* lymnaeid snails that live in the subtropical and tropics area, can transmit *F. gigantica* (Mas-Coma et al. 2009b). *Biomphalaria alexandrina* has also been reported as a transmitter for *F. gaigantica* (Farag and El Sayad 1995).
Other cosmopolitan freshwater lymnaeid snails which are responsible for transmission of *Fasciola* spp. in different areas include: *Radix rubiginosa, Austropeplea tomentosa, Pseudosuccinea columella, Stagnicola corvus,* and *Hinkleyia caperata* (Vázquez et al. 2018). The high transmission capacity of vectors is connected to the duration and persistence of the life span of the infected snails after infection (Mas-Coma et al. 2001).
Humans act as the incidental hosts for liver flukes (Alemneh 2019). Ingestion of freshwater wild plants including watercress is the main source of infection to humans (Mas-

Coma et al. 2018). In spite of watercress, various freshwater plant species might be involved in *Fasciola* transmission and human infection, which depend mainly on geographical distribution of those plants and the dietary traditions of peoples in that region (Mas-Coma et al. 1999). Water had been mentioned as another source for infection in human, either directly by drinking or indirectly by contaminating vegetables, fruits, and kitchen utensils (Chen and Mott 1990). Humans also become infected with fascioliasis after eating raw dishes prepared freshly from an infected liver with immature flukes (Taira et al. 1997).

Epidemiology

Previous studies revealed that fascioliasis has a higher spreading capacity, which is greatly related to the biological ability of intermediate lymnaeid hosts and the fluke adaptation capacity (Mas-Coma et al. 1999). Due to the ability of parasites and snails to develop in diverse adaptation strategies, the transmission rates become higher (Mas-Coma 1996).

F. hepatica is distributed commonly in Europe (Robinson and Dalton 1999), temperate regions of Asia, Africa, Oceania and America, while *F. gigantica* is mainly restricted to Africa and Asia (Lotfy and Hillyer 2003; Mas-Coma et al. 2009a). Both fluke types appear to be present in the same geographical areas especially in some subtropical and warm temperate regions in Africa and Asia (Mas-Coma et al. 2009b; Kalu 2015).

The larval stages of fasciolids species as well as their intermediate host snails, are highly dependent on climate features, so changes in environmental conditions have an impact on liver fluke infection (Fuentes et al. 2001). The dissemination of fascioliasis to a new geographical area is essentially related to the distribution of intermediate lymnaeid hosts, the presence of an infected definitive host, and the presence of appropriate environments for the snail vector. High lands areas with acid soils, poorly drained marshy grazing field and waterlogged are frequently estimated to be appropriate for their propagation and providing high endemic areas for the development of fascioliases (Ayele and Hiko 2016).

Up to 50% of infective overwinter metacercariae might remain viable on pasture and infect grazing livestock and capable to infect livestock hosts following grazing in next spring (John et al. 2019). Their survival is mainly dependent on dampness and diffident temperature, as they can tolerate repeated freeze-thawing action (Boray and Enigk 1964).

Metacercariae of *Fasciola* species might remain viable for more than one year, occasionally for up to two years with infectivity to induce infection in definitive hosts. Additionally, metacercariae from different livestock species origins do not show significant differences in definitive host infectivity (Valero and Mas- Coma 2000; Valero et al. 2002).

The occurrence of fascioliasis in humans has increased in the past 20 years, due to the global increase in the number of infected humans and animals (Alemneh 2019). Previous studies have demonstrated the significant role of human in the spreading of fascioliasis, especially in hyperendemic zones (Esteban et al. 1997), particularly where outdoor defecation is practiced (Mas-Coma et al., 1999), or where the correct services for waste and sewage disposal are absent (Hillyer and Apt 1997).

Life cycle

Fasciola spp. have a complex life cycle requiring the mammalian definitive hosts and a freshwater snail as an intermediate host (Vázquez et al. 2018). In subtropical areas, infection persist during the whole year but significantly slow down during winter (López Lemes et al. 1996). The essential point in trematode life cycle is that, one egg of trematode ultimately develops into hundreds of adults, when it passes through paedogenesis phenomenon in the body of snail intermediate hosts (Alemneh 2019).

The flukes are oviparous: the mature adult in bile ducts of definitive host lay eggs with an operculum. Eggs are transported from the bile medium to the small intestine where they mix up with feces (Nyindo and Lukambagire 2015).

In ruminant, eggs are dropped with feces on to the pasture, and undertake embryonation to the pyriform ciliated larva called a miracidium. Hatching of embryonated eggs can happen in response to the outside stimuli such as light, humidity and temperature (Vázquez et al. 2018). The developing free-swimming ciliated miracidia must find a suitable lymnaeid snail intermediate host for its further development (Urquhart et al. 1996; Graber et al. 2005). It was believed to use chemotactic and phototactic movements for vector finding in less than 24 hours (Vázquez et al. 2018). Upon contact, the miracidia mechanically attack the soft tissues of snail hosts by the effects of proteolytic enzymes and their penetrating styles (Zhang et al. 2019).

The entire penetration process occurs within thirty minutes and later on the miracidium loss its tail and cilia and changes to an elongated saclike structure named sporocyst, that contain a number of germinal cells. These cells undergo a development to the next stage, the redia which migrate to the hepato-pancreatic region of the snail and ultimately leads to the formation of cercaria. The second generation of redia may form during unfavorable environmental conditions. The cercaria is the young flucks with long tail arises actively from the snail in considerable numbers. The majority of infected snails die prematurely due to the disruption in their hepato- pancreas (Urquhart et al. 1996; Rickard 2001; Graber et al. 2005).

The development of flukes inside the snail required about 6 weeks depending on the environmental temperature (Beesley et al. 2018). The asexual development of parasite inside the snail refers as “clonal expansion”; a single miracidium can produce nearly ten to seventy hundred cercaria (Graczyk and Fried 1999).

Finally, the cercaria locates the wet leave of vegetations by negative geotactic movements and attach themselves, shed their tail and metamorphose into metacercariae.

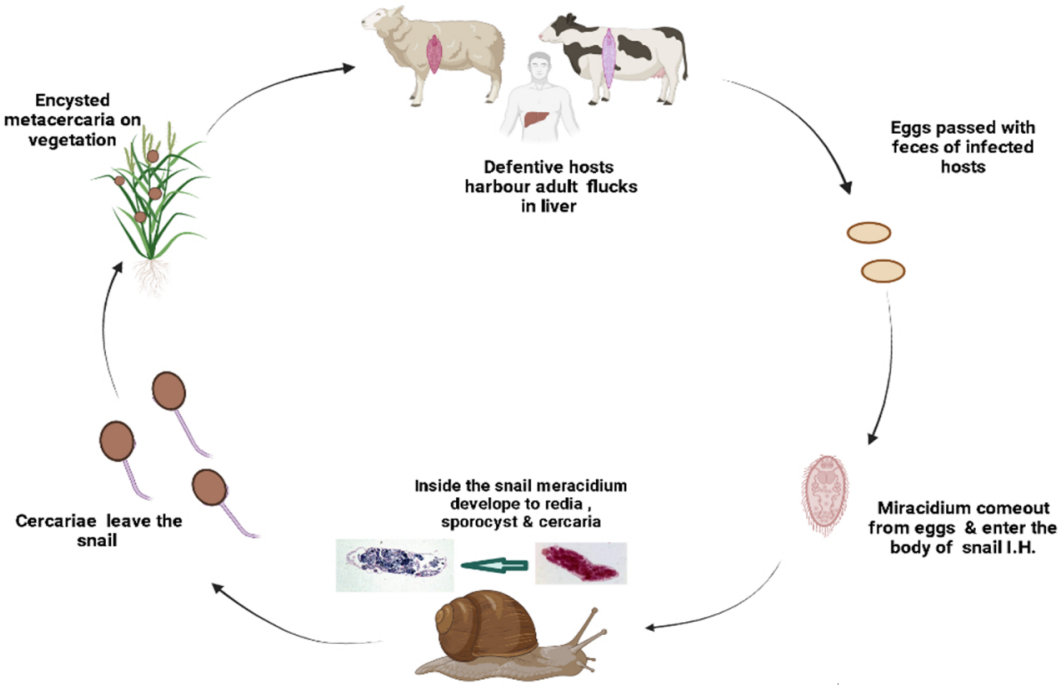

Fig. 1: Life cycle of *Fasciola* spp.

Encysted metacercariae have a great possibility of survival (Nyindo and Lukambagire 2015).

Metacercariae are the infective form of flukes and upon ingestion by suitable definitive hosts, an immature fluke might liberate. During mastication the outer cyst layer is removed, and inner cyst ruptured in the intestine depending on the enzymatic hatching mechanism, which is activated by suitable oxidation reduction and CO_2 system provided by intestinal environment (Urquhart et al. 1996; Rickard 2001; Graber et al. 2005).

The juvenile flukes burrow through the wall of the small intestine and temporarily settle in the peritoneal cavity for several hours (Atalabi and Lawal 2019). Afterward it migrates and penetrate the liver during four to six days, wounder there for another four to seven weeks leading to the entrance in the bile ducts, settle down and lay eggs after sexual reproduction. The life cycle reinitiates, when it lives for several years (Urquhart et al. 1996; Rickard 2001; Graber et al. 2005). The adult worm produces various number of eggs per day in different definitive hosts; reports have shown that in cow, it extruded 25,000 eggs and in sheep 12,000 eggs (Valero et al. 2002). Fig. 1 demonstrate different life cycle stages of *Fasciola* spp.

Pathogenesis

Pathogenesis occurs in two phases: the first phase is acute fascioliasis that occurs after liver penetration by enormous parasitic stages within a short period of time, and migration through the liver parenchyma. Its outcome is the severe liver damage and hemorrhage with subsequent sudden death mainly in sheep. The second phase is chronic fascioliasis, happen when fewer numbers of fluke result in infections over the long period of times even in weeks or months. The adult flukes reach the bile ducts, and result in the damage of the biliary mucosa by their cuticular spines. Sometimes, acute and chronic infections can occur simultaneously. The subclinical form is a common type of fasciolosis, occurring as a result of infection with low numbers of fluke, which accompanying reduction in weight gain and wool quality (Hayward et al. 2021). In both acute and chronic phases, the disease demonstrates high pathogenicity and immunosuppressive capacity (Valero et al. 2003; Girones et al. 2007).

Other pathogenic effects concurrent with fascioliasis include traumatic hepatitis and hemorrhage caused by juvenile flukes and fibrosis of the migratory tracts that eventually calcifies, caused by adult flukes. Moreover, anemia and hypoalbuminemia might occur (Roberts and Suhardono 1996; Javid et al. 2011).

In humans, the complexity of fascioliasis is sometimes related to the capability of the flucks to invade vital organs, leading to the significant outcome and even death of the patient (Mas-Coma et al. 2014).

Furthermore, the metabolites release from the liver flukes into the host circulatory system associated with anemia, increases the concentration of serum enzymes and dysfunction of the adrenal and thyroid glands (Sharma et al. 2011).

The pathogenicity of liver fluke infection can be affected by numerous factors including the breed of host, body

condition, dietary status and the burden of infection (Chauvin et al. 2001).

Clinical Signs

Fascioliasis is associated with significant morbidity and mortality in livestock (Hosseini-Safa et al. 2019). Acute phase often distinguished by sudden death of up to 10% of the flock, due to high levels of blood loss from physical damage to the liver. Typical clinical signs primarily in sheep and goats include reduced appetite, abdominal pain, depression, anemia, weight loss, and sudden death in a few days. Secondary bacterial infection of liver by *Clostridium noyvi*, during the acute phase resulting in clostridial necrotic hepatitis (Lalor et al. 2021).

During the chronic phase, additional clinical signs appear, such as inappetence and lower weight gain, anemia, and ascites (Urquhart et al. 1996; Rickard 2001), decrease in milk yield, diarrhea, and submandibular edema (Fufa 2009). Emaciation during chronic fascioliasis is prominent, especially in more susceptible animals and ewes during the advanced gestation period. The Inflammatory mediators arising from liver damage could have an effect on early pregnancy (Sargison and Scott 2011). Liver fluke infection is also considered as a predisposing risk factor for mastitis (Mavrogianni et al. 2014). The flukes incidentally infect the peritoneal cavity, lungs, subcutaneous tissue, lymph nodes, eye and other locations (Hosseini-Safa et al. 2019). In humans, various complex clinical disorders appear including severe neurological, psychiatric and ophthalmological conditions (Mas-Coma et al. 2014), during the acute phase of infection caused by migration of numerous juvenile parasitic stages (Gonzalez-Miguel et al. 2019).

Diagnosis

In endemic areas, rapid and accurate diagnosis for animal fascioliasis is considered as a successful prevention and treatment measure. Although there is significant progress in the application of new therapeutic agents, little attention has been paid to confirm the diagnosis of fascioliasis in animals (Amiri et al. 2021). Fascioliasis has been diagnosed by parasitological, immunological and molecular methods (Atalabi and Lawal 2020).

Generally, fascioliasis is diagnosed by fecal testing and finding eggs of parasitic flukes in stool, bile or duodenal fluid through wet mount and/or concentration techniques such as formalin- ether.

The expertise of the examiner and the number of parasite eggs in the stool sample are the main disadvantages associated with previous diagnostic techniques. In addition, a number of serologic procedures such as IFA, IHA and ELISA are relevant for diagnosis of fascioliasis during different stages of the disease (Hamoo et al. 2019).

Serological methods give the advantages for early diagnosis of fascioliasis, however circulating antibodies could persist in the blood for several months after effective treatment (Salimi-Bejestani et al. 2005; Arifin et al. 2016).

Moreover, the nucleic acid-based techniques appear to be expectant for diagnosis of recent fascioliasis (Rojas 2014; Davies Calvani et al. 2018). Various molecular procedures are applicable for diagnosis of fascioliasis, i.e., nested PCR provide higher sensitivity than existing diagnostic methods, when fascioliasis could be detected in the feces of infected sheep two weeks post infection (Martınez-Perez 2012; Beesley et al. 2018). Furthermore, sequencing the whole genome, and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay can also be used for diagnosis (Hamoo et al. 2019). Loop mediated isothermal amplification (LAMP) is an alternative technique, because molecular diagnostic techniques using PCR are not available everywhere (Martınez-Valladares and Rojo-Vazquez 2016). It is with low-cost and simple performing test, which permits quick amplification of small amount of DNA with high sensitivity (Amiri et al. 2021) and can be applied for diagnosis of a variety of zoonotic helminths including *Fasciola* species (Ai 2010). It has been recognized to be more sensitive and specific by detecting fascioliasis one-week post infection in experimentally infected sheep (Martınez-Valladares et al. 2016).

Treatment

The recommended treatment depends on the nature of the disease. Some of the existing anti helminthic drugs are not effective against immature flukes, so these are not recommended during acute flukes outbreak. The commonly used flukicides is Triclabendazole, which is effective against both immature and adult flukes (Ahmed et al. 2005). Triclabendazole is also an efficient drug available for human treatment (Gandhi et al. 2019).

Control

The control strategy should be directed at the application of preventive measures rather than a curative basis. The effective control measures include the treatment with appropriate anthelmintics drugs to decrease the number of parasitic flukes in the host body and the number of fluke eggs in the pasture, reduction in the number of snail intermediate host by using mollucicided and improvement of drainage (Ahmed et al. 2005; Fufa 2009). Other control measures include the development of management system (housing, grazing practice and animal watering), reduce snail population by drying the marshy or wet areas or using biological control methods like, introducing the frogs and birds (Alemneh 2019).

Conclusion

Fasciolosis is a common parasitic infection which affects the ruminant productivity by its direct or indirect losses.

Different factors including change in climatic condition and human activities play a role in further spread and distribution of liver flukes. Great concerns should be directed against resistance to flukicides to reduce the number of parasites that led to restrictions in their use. The drug residues in animal products i.e., meat and milk are another issue that restricts anthelmintic usage at any time, due to the long withdrawal period of some products. Moreover, increase in the frequency of liver fluke infection among animals adversely leads to rise the infection rates in human at different regions of the world.

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14

Global Review of Human Taeniasis

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INTRODUCTION

Human taeniasis is zoonotic cestodal infection caused by worms from the Taeniidae family. Although the disease has widespread distribution, communities in the developing nations bear the most of its burden. Taeniidae family possesses three species that may infect people; *Taenia (T.) asiatica* (also known as the "Asian tapeworm"), *Taenia (T.) solium* ("pork tapeworm") and *Taenia (T.) saginata* (sometimes called "beef tapeworm") (Ito et al. 2004).

The adult tapeworm of these three species is exclusively found in the small intestine of humans. For *T. saginata*, cattle serve as the intermediate host, whereas pigs are the larval hosts for Asian tapeworm and pork tapeworm. Humans develop disease by eating *T. solium* eggs from their environment and act as aberrant intermediate host. Although pain in abdomen and loss of weight have been observed, human taeniasis is mostly asymptomatic (Garcia et al. 2003; Flisser et al. 2011; Tembo and Craig 2015), though carriers may experience some discomfort when they see segments in their feces, particularly of motile *T. saginata* (Garcia et al. 2003). Perforation in gall bladder, swelling of appendix, and bowel blockage are infrequent complications of intestinal taeniasis (Hakeem et al. 2012; Kulkarni et al. 2014; Atef and Emna 2015; Li et al. 2015).

Human health burden is caused by larval infection of swine cestode (*T. solium*). Ingestion of fertile eggs of *T. solium* causes an abnormal cyst formation in numerous regions of the body of human. Cysts most commonly appear in the subcutaneous tissue, muscles, ocular system, and brain. The formation of a single or multiple cysts within the central nervous system - often the brain – are responsible for inducing nervous signs (Garcia et al. 2014).

According to a study conducted in various regions of world in 2010, disease in humans produced by swine tapeworm was culpable for 503,000 disability-adjusted life years (DALYs) lost per year (Murray et al. 2012). This is certainly an understatement of the total burden, considering that NCC may be responsible for thirty percent epilepsy occurrences in the prevalent regions (Rajshekhar et al. 2006; Ndimubanzi et al. 2010; Bruno et al. 2013). Swine tapeworm is also predicted to be the cause of 28,000 (95% CI 21,000-37,000) fatalities worldwide each year (Torgerson et al. 2015). Human taeniasis prevention and care are essential to control human cysticercosis, which will lead to decrease in epilepsy cases (Garcia et al. 2014).

Global Distribution

In the majority of North America, Australia, Europe, and New Zealand, *T. solium* has been successfully retained; although, disease transmission has been documented from some regions of Europe and North America (Sorvillo et al. 2011; Zammarchi et al. 2013; Devleesschauwer et al. 2017). Swine tapeworm is most prevalent in the developing nations, with the parasite endemic throughout African, and Asian countries, as well as in Latin America (Braae et al. 2015; Coral-Almeida et al. 2015). *T. saginata* is more widely distributed, including findings from Europe (Dorny and Praet 2007), New Zealand, Australia (Howell and Brown 2007), and other parts of the developing countries (Flisser et al. 2011).

Human taeniasis prevalence varies greatly across endemic countries, with a current meta-analysis indicating prevalence of 13.9% in Africa, 17.25% in Latin America, and 3% in Asia (Coral-Almeida et al., 2015). Prevalence of human taeniasis is low in USA, Canada and Australia, but the disease is re-emerging (Fig.1).

Diagnosis of Cases of Human Taeniasis

These estimates were made on the basis of a number of different diagnostic techniques. These have varying degrees of specificity (Sp) and sensitivity (Se) in the detection of taeniasis (Allan et al. 2003).

Adult *Taenia* carriers are traditionally diagnosed with the aid of microscope by observing ejected eggs in the feces. Despite the ease of this diagnostic procedure in resource-poor situations, a key disadvantage is the microscopy sensitivity, which is limited due to the irregular nature of released eggs. The reported sensitivity estimates vary from 3% (Allan et al. 1996) to 52% (Praet et al. 2013). Moreover, while microscopy has a high species specificity, speciation needs the examination of ejected proglottids, as *Taenia* eggs seem similar underneath the light microscope (Wilkins et al. 1999; Allan and Craig 2006).

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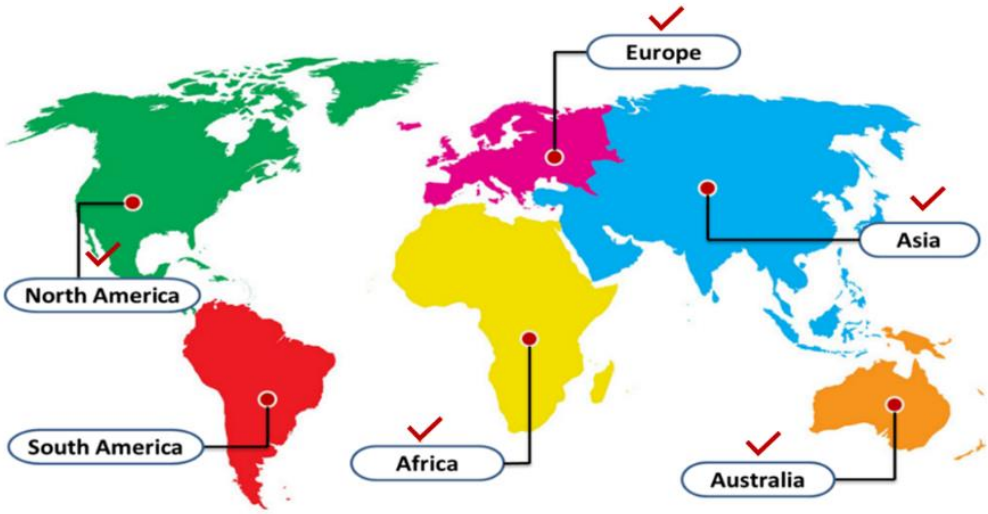

Fig. 1: Cosmopolitan distribution of human taeniasis

Fecal antigen (copro-Ag) detection is based on the identification of unique antigens in feces and, does not depend on active release of eggs or proglottids like microscopy to identify infection. It has now been effectively proved to diagnose *Taenia* spp. carriers in a range of settings. In a field experiment in Mexico, copro-Ag ELISA had a specificity/sensitivity (Sp/Se) of 99.0%/98.0%, whereas microscopy had a sensitivity of 38.0%, respectively (Allan et al. 1996).

One disadvantage of the presently offered fecal ELISAs is that they cannot distinguish between pork and beef tapeworms (Allan et al. 1996). Furthermore, cross-reactions with other gastrointestinal parasites such as, *Trichuris trichiura*, *Ascaris lumbricoides*, and some protozoa have been documented (Praet et al. 2013). DNA-based diagnostics have now been developed to provide species-specific diagnosis. A quick nested PCR test that used markers based on the reported *T. solium* oncospheral protein (Tso31) gene sequences exhibited 97%-100% sensitivity and 100% specificity, even under field settings (Mayta et al. 2008).

Given the fundamental challenges involved with diagnostic tests using faecal material, particularly in terms of health hazards and public acceptance, serological identification of mature *Taenia* carriers has a clear position. This was accomplished using an immunoblot technique for detecting antibodies against excretory and secretory antigens of swine tapeworm. When employed to test sera with confirmed infection status, including sera from beef tapeworm carriers and *Echinococcus* infected persons, the assay obtained a Se/Sp of 95%/100%, respectively (Wilkins et al. 1999).

However, use of local antigens limited the test's applicability outside the laboratory, and antigens have recently been generated in a baculo-virus system for application in different tests (Levine et al. 2004). rES33 and rES38 proteins are now being employed in an enzyme-linked immunoelectrotransfer blot (EITB) format in a current eradication programme against cysticercosis in Peru, with both demonstrating great sensitivity of 97.0% and 98.0% (rES33) and specificity of 100% and 91.0% (rES38), in field testing (Levine et al. 2007).

Treatment of Human Taeniasis

Adult *Taenia* spp. infections respond to the common anthelmintic medicines including tribendimidine (200 mg one per-oral dosage) (Steinmann et al. 2008), niclosamide (2 g/person), praziquantel (5-10 mg/kg, single per-oral dose) (Pearson and Guerrant 1983; Pearson and Hewlett 1985) and albendazole (3400 mg/person for three successive days) (Steinmann et al. 2011). Three times dose of albendazole can completely cure *Taenia* spp. cases, while praziquantel and niclosamide had effectivity rates of 95% and 85%, respectively (Pawlowski et al. 2005).

Praziquantel and niclosamide are the most effective antiparasitic medications against *Taenia* infection, and praziquantel seems to be an economical option @ $0.05-0.1 for a man/woman as a single dose (Engels et al. 2003). A few adverse outcomes of praziquantel have been reported, including stomach discomfort, laziness, and diarrhea (Raso et al. 2004); nevertheless, it is revealed that it may be due to potential of praziquantel to penetrate within brain, there may be nervous implication due to stimulation of undetected latent NCC (Flisser et al. 2003). In spite of the findings, no adverse outcomes were recorded in a research conducted in Tanzania in which school students were given the drug (praziquantel) in the region where schistosomiasis and cysticercosis were co-endemic (Braae et al. 2017). Albendazole therapy, which also crosses the blood brain barrier, may result in neurological adverse effects (Sotelo and Jung 1998); while niclosamide has low systemic penetration and hence has no impact on NCC (Pawlowski 2006).

Control Strategies of Human Taeniasis

Preventive chemotherapy refers to the taeniasis treatment to reduce the parasite load in a specified population and could be executed in three different ways. 1) Mass drug administration (MDA) is the treatment of entire population of a designated region at specified periods, regardless of physical state. 2) Targeted chemotherapy treats the specified risk group areas at specific intervals, whereas 3) selective chemotherapy examines persons and cures them based on their clinical state (Gabrielli et al. 2011). Many studies that have been conducted to examine the application of MDA for pork tapeworm (Keilbach et al. 1989; Diaz et al. 1991; Del Brutto et al. 1996; Allan et al. 1997; Sarti et al. 2000; Garcia et al. 2006; Wu et al. 2012; Ash et al. 2015). Most studies were found a decrease in taeniasis occurrence, while the impact on cysticercosis (human as well as porcine) were more diverse (Thomas 2015).

Data from modelling indicate that one-time MDA programmes rarely results in long-term suppression of *T. solium*, with fast reductions in frequency accompanied by a rapid recovery to earlier levels (Kyvsgaard et al. 2007). However, when MDA was used in conjunction with other techniques such as pig immunization and/or oxfendazole therapy, a persistent decline in porcine cysticercosis and human taeniasis was documented (Kyvsgaard et al. 2007; Assana et al. 2010; Okello et al. 2016).

Selective chemotherapy is considered as an important part of pork tapeworm control (Montresor and Palmer 2006; Pawlowski 2008; Penrith 2009), particularly with more health coverage (Sarti and Rajshekhar 2003) and with modeling data indicating that this treatment results in significant decrease in disease frequency (Kyvsgaard et al. 2007). Two trials in the field have been conducted till now that involve selective chemotherapy. Both of these trials were undertaken in combination with targeted MDA in school. A significant reduction in neurocysticercosis was observed in research conducted during eight-year interval (Medina et al. 2011). Another survey in Tanzania revealed more than 77% decrease in occurrence of *Taenia* infection within 22 months (Braae et al. 2017).

Vaccination against *T. solium* larval invasion in the swine host have been developed now, and two of them including SP3VAC and TSOL18, displaying great effectiveness in swines from both natural and experimental threats (Lightowlers 1999; Plancarte et al. 1999; Huerta et al. 2001; Gonzalez et al. 2005; Sciutto et al. 2007a; Sciutto et al. 2007b; Morales et al. 2008; Silva and Costa-Cruz 2010; Lightowlers 2010; Morales et al. 2011; Jayashi et al. 2012). One disadvantage of available vaccine choices is that none kills preexisting cysts; consequently, it is advised that swine vaccination must be administered in combination with oxfendazole at a dosage of 30.0 mg/kg to influence porcine cysticercosis illnesses established pre-immunization.

When employed in a field study in Cameroon, this combo of TSOL18 immunization and high therapeutic dose of oxfendazole treatment provided full protection from infection (Assana et al. 2010). TSOL18 vaccine (Cysvax) has been marketed with cooperation from the University of Melbourne, GALVmed, Indian Immunologicals Limited, and commercial manufacturing has begun. Permission for its usage in India is now in process, with certification across Africa likely by 2020 (Thomas 2015). Cattle vaccination against *T. saginata* has some efficacy with the TSA9/TSA18 vaccine displaying excellent effectiveness in preventing cattle from infection (Rickard et al. 1981; Lightowlers et al. 1996; Lightowlers et al. 2000; Harrison et al. 2005). However, this vaccine is not presently explored on commercial scale since the existing clues do not reveal that it is financially feasible (Lightowlers 2006).

Anthelmintic therapy can be used to treat the larval form of *T. solium* and using oxfendazole (30 mg/kg) exhibits the highest effectiveness (Gonzales et al. 1996; Gonzalez et al. 1997; Gonzalez et al. 1998; Gonzalez et al. 2001; Sikasunge et al. 2008). Oxfendazole have no recorded negative effects (Gonzalez et al. 1998), and is now approved in several countries, and presently being manufactured particularly for pigs as Panthic 10% (Thomas 2015). Bovine cysticercosis responds to praziquantel (Thomas and Gönnert 1978; Pawlowski et al. 1978; Harrison et al. 1984), and protection over re-infection seems to extend at least 3 months. Despite its effectiveness in bovines, praziquantel has still not been prepared for large ruminants.

Multi Host Intervention as One Health Approach

There are several ways to combat both beef and pork tapeworm using approaches that address human as well as animal hosts (WHO 2015). Pig vaccination along with MDA result in rapid and consistent reduction in prevalence of *Taenia* infection in humans as well as in pigs (Kyvsgaard et al. 2007).

Pigs were followed employing EITB strip diagnostic tests for 18 months (US Centers for Disease Control, Atlanta, GA, USA). The findings showed that living in a treated area after the interventions was an important measure against porcine cysticercosis (Garcia et al. 2006). Recently, The Bill & Melinda Gates Foundation funded a wide-scale experiment to eradicate pork tapeworm from a vast region of remote Peru. Human MDA (2 g nicolsamide, three rounds per year) is provided in conjunction with pig vaccination (TSOL18) and antiparasitic therapy effectively removed swine tapeworm from the pig host in (105/107) experimental rural areas and parasitic elimination persisted for one year post-treatment (Garcia et al. 2016).

Porcine vaccine (TSOL18) and antiparasitic therapy were recently paired with MDA programme of humans (triple albendazole dose 400 mg in two rounds) in Lao PDR, where an earlier quick decrease in human *Taenia* infection was persisted during the two years of research (Ash et al. 2015; Okello et al. 2016).

Conclusion

There are numerous critical elements of human taeniasis treatment and control, exploring significant potential and problems of existing therapeutic and diagnostic techniques. There is a need for further scaling-out of successful pilot control programs in order to assess their long-term impact and cost-effectiveness in good way, primarily in Asian and African countries. There is a dire need of integrating research findings into government policy and community-level action, allowing vulnerable communities throughout the globe to address the effects of taeniasis in a better way.

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15

Giardiasis: Aqua-borne Ailment

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INTRODUCTION

Giardia is a genus of flagellate protozoan parasites. It is one of the most common parasitic agents affecting the GIT tract in both animals and humans. It is a cause of waterborne diarrhea worldwide. The disease caused by Giardia is known as Giardiasis or lambliasis. Giardiasis may manifest as asymptomatic colonial growth of protozoa and acute or chronic diarrhea. The common model organism of Giardia observed for studies is *Giardia lamblia* (Leung 2011). It is also the protozoal pathogen most commonly isolated from intestines, worldwide (Eisenstein et al. 2006; Daly et al. 2010). Giardia species like *Giardia (G.) duodenalis* inhabit portions of several mammals' small intestines like the duodenum and jejunum. This species has 8 genetic groups ranging from A to H. These groups are separated by host distribution and specificity (Cacciò and Lalle 2015; Kirk et al. 2015). *G. duodenalis* is another name used for the same organism called *G. lamblia* and *G. intestinalis* (Boutrid et al. 2018; Vivancos et al. 2018; Horton et al. 2019). A characteristic lesion manifested by the *Giardia* infection is atrophy of intestinal villi (Dawson 2005; Huang and White 2006; Halliez and Buret 2013; Robaei et al. 2014; Liu et al. 2018; Bartelt and Kaplan 2018). This leads to the characteristic sign of giardiasis i.e., diarrhea (Naz et al. 2018).

Etiology

The causative agent of Giardiasis in humans is *Giardia (G.) lamblia*. It has two forms in terms of morphology. These forms include trophozoite and cyst. The trophozoite has a median body with two symmetric nuclei placed at the anterior end of the body. It has four pairs of flagella. The surfaces of the median body of trophozoite are dorsally convex and ventrally flat. The ventral surface of trophozoite also contains an adhesive disc also known as a spiral organelle (Einarsson et al. 2016). The trophozoite has a pear-like shape. It is 5 to 10 µm wide and 12 to 20 µm long. The *Giardia* cyst is a smooth-walled structure with an ovoid shape. The width of the cysts ranges from 7 to 10 µm while its length is about 8 to 12 µm (Leung 2011).
Out of eight genotypes of *G. lamblia* ranging from A to H (Fink and Singer 2017; Burnett 2018; Leder and Weller 2019) the first two (A and B) parasitize both animals and humans, While the last six genotypes (from C to H) are only found in animals. Animals affected by A and B genotypes include pets like cats and dogs, livestock animals, and wild animals too. Similarly, the genotypes from C to H are a cause of Giardiasis in livestock cattle, beavers, and pet animals like cats and dogs (Cama and Mathison 2015; Minetti et al. 2016; Fink and Singer 2017; Burnett 2018; Leder and Weller 2019).

Life Cycle

Depending upon its morphological forms (Fig. 2) the life cycle of giardia is also divided into two distinct phases (Fig. 1). These two phases include a proliferating stage of the trophozoite phase and an infectious stage of the cyst (Fink and Singer 2017).

1. The hosts ingest the cysts of giardia either through contaminated faeces, food, water, or any other edible.
2. These cysts then hatch into trophozoites in the small intestine followed by its replication
3. The life cycle of giardia completes when these trophozoites mature into cysts and are shed through feces to be taken up by another animal (Adam 2001).

Pathogenesis

The pathogenic potential of *Giardia* cysts is too high that even with ingestion of a small number of cysts, the clinical disease may occur (Kucik et al. 2004; Burnett 2018). Once the cyst is ingested its excystation happens in the duodenum section of the small intestine (Lebwohl et al. 2003; Kucik et al. 2004; Kalyoussef and Goldman 2010) possibly due to its

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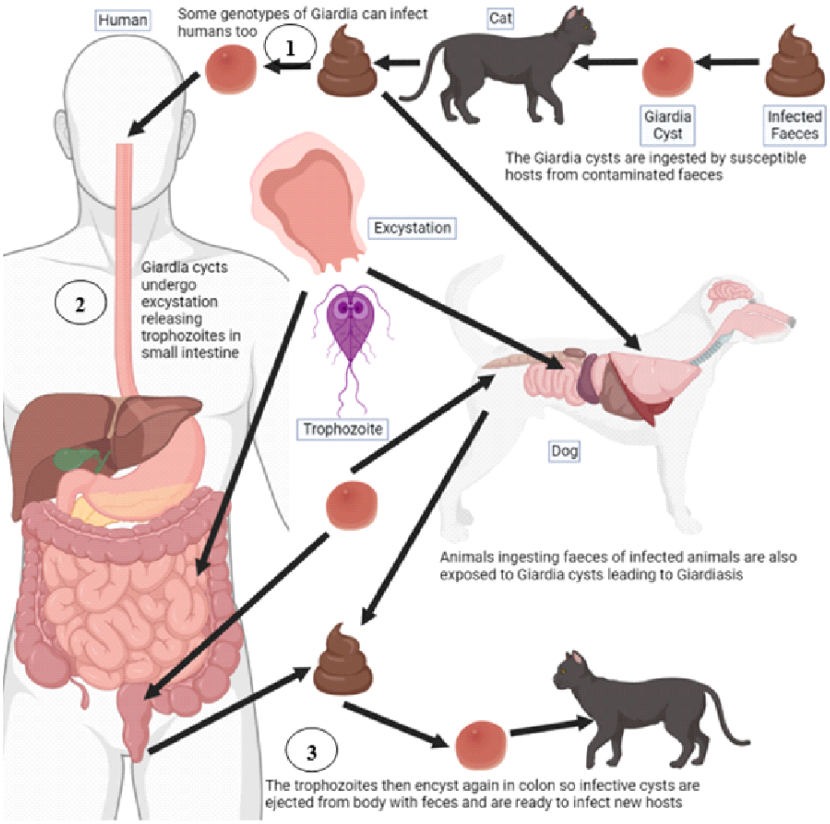

Fig. 1: Life cycle of *Giardia* and its transmission among hosts of different species.

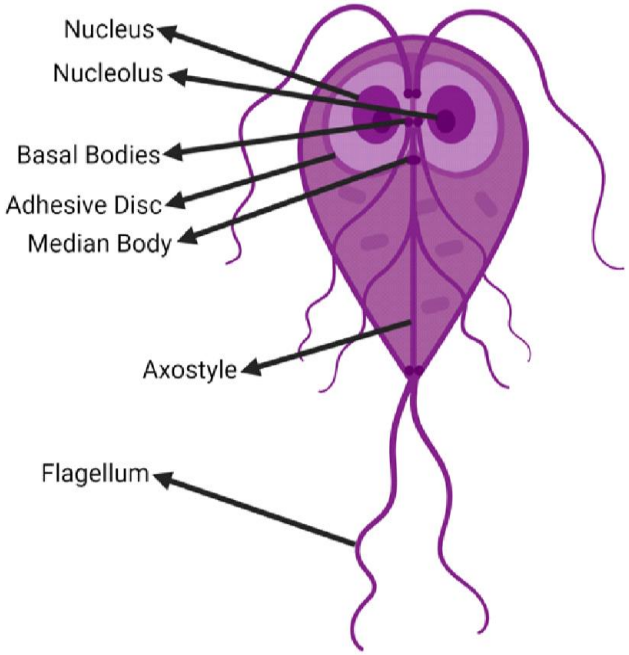

Fig. 2: Morphology of *Giardia* Trophozoite.

exposure to the strong gastric acid from the stomach, bile, and proteases from the pancreas (Lebwohl et al. 2003; Robaei et al. 2014). A nuclear division already happened during the maturation of the cyst before excystation, so excystation results in the production of two motile trophozoites (Halliez and Buret 2013; Bartelt and Kaplan 2018).

The main predilection site for these trophozoites is the proximal part of the intestine so they are found in the duodenum and jejunum (Fig. 3). Usually, these trophozoites attach themselves to the enterocytes with the help adhesive discs found on the ventral surface of their bodies (Romero et al. 2015). Although uncommon but presence of trophozoites in the terminal portion of the intestine, the ileum, has also been reported (Heagley and Jakate 2012).

The pathogenic action of *Giardia* begins in its trophozoite stage. This happens because the trophozoite begins damaging the intestinal lumen wall. *Giardia* destroys the intestinal mucosa leading to the shortening of the brush border of microvilli. Microvilli brush border shortening may or may not be accompanied by villous atrophy during giardiasis. A deficiency of disaccharides began to appear and the host immune response is also activated. Activation of immune response results in increased permeability of intestines. An increased intestinal permeability leads to an increase in anion and fluid secretion into the intestines which in turn affects and changes the microflora of the intestine. Modified microflora serve as a stimulatory factor for enhancing the pathogenicity of *Giardia*. This results in the apoptosis of enterocytes leading to the loss of function of the intestinal barriers (Dawson 2005; Huang and White 2006; Halliez and Buret 2013; Robaei et al. 2014; Liu et al. 2018; Bartelt and

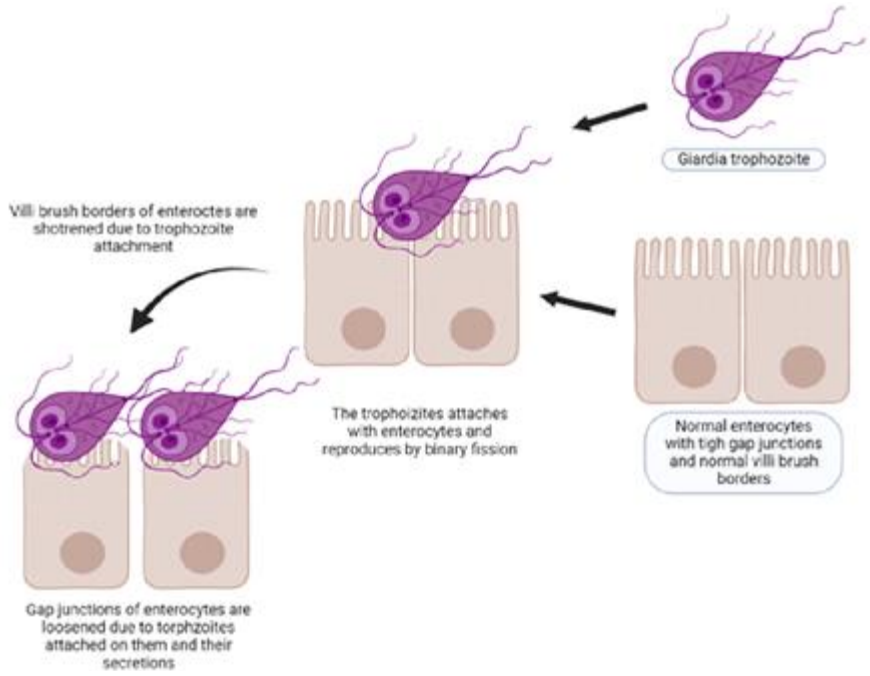

Fig. 3: Pathogenic action of Giardia in the host's body.

Kaplan 2018). The main agent suspected to be the cause of all this destruction is an enzyme secreted by trophozoite, the cysteine protease (Liu et al. 2018). Mainly *Giardia* trophozoites are extracellular parasites. This means they do not damage the cells lining the small intestine (Adam 2001; Halliez and Buret 2013; Einarsson et al. 2016) instead they tend to proliferate while being attached to the microvilli (Adam 2001). The trophozoites disrupt the epithelial cell junctions of the intestine altering the gastro-intestinal motility. They also release lectins and thiol proteinase enzymes that have a cytopathic effect on intestinal cells (Leung et al. 2019) In the small intestine, the trophozoites double their numbers within 9 to 12 hours by reproducing through binary fission (Lebwohl et al. 2003; Leung 2011).

After maturation, these trophozoites are passed from the small intestine to the colon along with the ingesta. In the colon, these trophozoites then encyst (Fink and Singer 2017). These cysts are then readily ejected from the body along with faeces. These are actively infective right after their ejection from the host's body. Hence, they are responsible for the further transmission of *Giardia* (Adam 2001; Naz et al. 2018). The cyst wall is a very useful structure for surviving in harsh environmental conditions outside the host's body. The cyst can survive for weeks to about a month while facing harsh conditions such as moist weather and water as cold as 4℃ (Adam 2001; Naz et al. 2018).

Clinical Signs

After a *Giardia* cyst enters the body of the host, it takes about 3 weeks for the signs to appear (Kucik et al. 2004; Dawson 2005; Biggs et al. 2016). Usually, the *Giardia* infection progresses asymptomatically. Clinical signs may appear in 25% to 50% of the infected hosts (Lebwohl et al. 2003; Biggs et al. 2016; Leder and Weller 2019). Clinical signs are usually seen in young ones infected with *Giardia*. Infection in adult hosts progresses without any clinical signs in most of the cases (Biggs et al. 2016). The asymptomatic carriers keep shedding its cyst for 6 months post-infection (Pickering et al. 1984; Romero et al. 2015). Clinically affected individuals present a typical sign of acute or chronic diarrhea. At the beginning of the infection, the stools are just loose and watery but as the disease progresses the odor of stool becomes foul and its consistency turns to greasy (Naz et al. 2018).

Some general signs of disease include;

- Fatigue (shown by lethargy)
- Anorexia
- Abdominal pain
- Flatulence
- Asthenia
- Bloating
- Weight loss (Adam 2001; Pietrzak et al. 2005; Naz et al. 2018).

The signs like abdominal aches and asthenia are more commonly observed in younger patients as compared to adult ones (Almirall et al. 2013). Symptoms like headache, chills and fever may also appear during Giardiasis although these are rarely seen (Leung 2011). The appearance of blood, mucus or leucocytes in faeces has never been observed (Leung 2011; Minetti et al. 2016). These symptoms usually subside in 2 to 4 weeks after the appearance of the first clinical signs (Lebwohl et al. 2003; Leder and Weller 2019).

Diagnosis

Giardiasis can be confirmed by a faecal examination of the suspected individual. If *Giardia* trophozoites or cysts are seen during the microscopic examination of the stool sample, infection is confirmed (Leung et al. 2019). Usually, stool examination gives 50% to 75% sensitivity because the sample is taken once while cysts are excreted at irregular intervals. This sensitivity can be increased by over 90% by taking multiple samples for 2 to 3 days (Kucik et al. 2004; Leung 2011; Minetti et al. 2016). Real-time PCR can be also used for diagnosing Giardiasis as it gives 100% specificity and 98% sensitivity (Soares and Tasca 2016; Mero et al. 2017; Parčina et al. 2018).

Treatment

For treating giardiasis, the primary effort should be to correct dehydration and imbalance of electrolytes. Actively providing symptomatic treatment against giardiasis helps in the alleviation of clinical signs and reducing their duration, which in turn prevents complications from occurring while reducing disease transmission at the same time (Leung et al. 2019). European Scientific Counsel Companion Animal Parasites reported in 2018 that a 25 mg/kg oral dose of Metronidazole twice a day for 5 days has been proven sufficient to treat giardiasis in cats and dogs (ESCCAP 2018).

Disease in Humans

Introduction

Giardiasis is one of the most common protozoal infections in humans. Its causative agent is *Giardia (G.) lamblia*. Some common conditions caused by Giardiasis include water-borne diarrhoea, food-borne diarrhea, traveler's diarrhea, and day care center outbreaks. According to the World Health Organization giardiasis is one of the most neglected diseases that are associated with unhygienic conditions and poverty (Savioli et al. 2006).

Etiology

Only two genotypes or assemblages of *G. lamblia* namely A and B are generally presumed to be culprits of giardiasis in humans (Halliez and Buret 2013). This general assumption was proved to be untrue when some recent reports proved the role of the E genotype in human giardiasis. These reports came from Australia, Brazil, and Egypt (Moein and Saeed 2016; Fantinatti et al. 2016; Zahedi et al. 2017). The assemblage C was also found in giardiasis patients in Slovakia and China (Liu et al. 2014; Štrkolcová et al. 2015). The assemblage F was reported in human infection in Slovakia (Pipiková et al. 2020). The assemblage D was also reported in some travellers from Germany after they visited the South-eastern parts of Asia (Broglia et al. 2013).

Transmission/ Zoonosis

Giardia is usually transmitted to human via faeco-oral route and direct contact. Zoonotic transmission of disease can also happen but rare cases have been reported so far (Hlavsa et al. 2005). Giardiasis infection begins in humans when cysts are ingested from contaminated water bodies or through direct contact with an infected person. Lack of proper hygiene management and application of sufficient sanitation measures also plays a vital role in transmission. Recently it has been observed that the day cares for children are serving as shelters for *Giardia* populations to flourish and transmit into new hosts. This transmission happens when the day care nurses tend to handle babies and change their diapers without properly maintaining hygiene and handwashing protocols (Reses et al. 2018).

Prevalence

In developing countries, the prevalence of giardiasis is too high that about 33% population of these countries is affected by it. The prevalence of Giardiasis for different age groups is given in Table 1.

From the aspect of development status of a country, the prevalence of giardiasis is given in Table 2.

Even in well-developed countries, some specific groups of people have been identified as at-risk individuals for getting infected with *Giardia* as given in Table 3.

Clinical Signs / Symptoms

In humans, the incubation period of *Giardia* is about 2 weeks after that the clinical signs begin to appear. The severity of giardiasis is highly variable in humans and sub-clinical infection is also common. The appearance of signs in different states of infections is given in Table 4.

Treatment

Firstly, restoring the optimal hydration and electrolyte balance of the patient is important. This minimizes the severity and duration of infection. Patients of very young or very old age are less tolerant to fluid loss and electrolyte imbalance so they require extra care. One way of achieving this rehydration besides IV infusions is with oral rehydration solutions (Leung et al. 1987; Leung and Robson 1989; Issenman and Leung 1993; Chow et al. 2010).

Along with managemental protocols, a regime of drug-based treatment should also be followed to treat giardiasis. This regime includes the drugs of choice against *Giardia* as given in Table 5.

Table 1: Giardiasis prevalence according to age groups (Zajaczkowski et al. 2018)

No.	Age Group	Percentage of Giardia infected
1	Children	8%
2	Adults	2%

Table 2: Giardiasis prevalence in different economic conditionsn (Dixon et al. 2011)

No.	Type of Country	Prevalence Rate
1	Developed	2% - 7%
2	Developing	20% - 30%

Table 3: Risk of contracting giardiasis among different groups of people (Coffey et al. 2021).

No.	Group of people	Risk of getting in contact with faeces
1	People with gay sexuality	During sexual activity
2	Day care workers	While changing diapers and handling children
3	Professionals dealing with human faecal material like lab workers, prostate examiners	While performing their duties
4	Wilderness travellers	May come in contact with faeces of animals
5	International travellers	May come in contact due to unhygienic conditions during traveling

Table 4: Signs and symptoms in different states of giardiasis

No.	State of Disease	Signs and Symptoms	Reference
1	Acute	Diarrhoea, Nausea, Cramps, Vomiting, Fatigue and Weight loss	(Cacciò and Lalle 2015)
2	Chronic	With acute clinical signs Or without any clinical signs and symptoms	(Muhsen and Levine 2012; Escobedo et al. 2014)

Table 5: Dose regimen of different drugs for the treatment of giardiasis (Petri 2005; Robertson et al. 2010; Bartelt and Kaplan 2018)

No.	Drugs Generic name (Brands)	Dose	Dose Frequency	Route
1	Metronidazole (Flagyl)	15 mg/kg/day (Max 750 mg/day)	Twice a day for 5 to 10 days	Oral
2	Tinidazole (Tindamax, Fasigyn)	50 mg/kg (Max 2 g)	Single dose a day	Oral
3	Nitazoxanide (Alina, Allpar)	7.5 mg/kg	Twice a day for 3 days	Oral

Tinidazole has less side effects than other drugs on this list, so it is considered safe for use in children of age 3 years and above (Leung 2011; Biggs et al. 2016).

Control Methods

Controlling *Giardia* is not very easy because its cysts are well-built to last in harsh environmental conditions. The cysts also remain unaffected by disinfecting agents like chlorine used for cleaning water. However, Iodine can be used against cysts but it needs 8 hours to make the water safely consumable. Boiling water for 10 minutes is an easy method to eliminate the cysts. Travelers that do not have the facilities to boil water may use National Safety Foundation standard rated 53 or NSF standard-rated 58 filters to make water safe for drinking by reducing cysts in the water (Adeyemo et al. 2019).

Conclusion

Giardiasis is an important disease of both animals and humans marked by diarrhea and weight loss. Usually, it is asymptomatic in adult patients but despite showing no clinical signs the infected person can shed cysts in their faeces for several months. Such characteristics make it difficult to control the spread of Giardiasis. It is more prevalent in developing countries where they have fewer resources to maintain proper sanitation and hygienic protocols. The control of giardiasis is very difficult because it is transmitted through edibles and develops strong cysts to survive in harsh conditions. Still, the use of simple hygienic measures like boiling water for 10 minutes before consumption can eliminate the protozoal cysts.

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16

Dermatophytosis

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INTRODUCTION

Dermatophytosis is a chronic contagious disease caused by a class of pathogenic fungus called dermatophytes (Bitew 2018). It is also known as ringworm when the lesion takes the shape of a circle in which the center of the lesion is clear and surrounded by the inflammatory reaction. Tinea is an alternative name of dermatophytosis based on the affected body site, such as Tinea unguium where dermatophytes infected nail (Chang et al. 2022). Dermatophytosis is commonly cutaneous in nature and limited to the superficial layer of skin, nails and hair of human (Vishnu et al. 2015) due to the inability of the fungi to tolerate human body temperature (37ºC), acidic properties of skin (pH 4.7) and the antifungal activity of blood proteins in immunocompetent individual (Martinez-Rossi et al. 2012; Al-Janabi 2014).

Currently, dermatophytosis is a significant disease across the world with a public health issue in numerous countries mainly in third world countries (Nweze and Eke 2016). Several factors considered as risk factors for the occurrence of the dermatophytosis in developing countries, including crowding, low socio-economic position, insufficient health services, poor hygiene, and the exchange of footwear, clothing and barbershop supplies among people (Moto et al. 2015). Dermatophytosis can be caused by almost 40 species of fungus typically in the genera *Microsporum*, *Trichophyton* and *Epidermophyton*. It is transmitted directly through contact with infected humans or/and animals or indirectly via contact with fomites (Degreef 2008; McBain et al. 2016). The lesion of the dermatophytosis typically, is an itchy, erythematous, scaly, circular plaque on the skin (Mora-Montes and Lopes-Bezerra 2017). Clinical symptoms of dermatophyte infections may be mild to severe based on the virulence factors of the species, the immunological status of the host, the affected region, and the external environmental factors. These fungal infections are associated with high morbidity however, they are rarely related to a fatal consequence (White et al. 2008; Bitew 2018). Eventually, most cases of dermatophytosis require about 2-4 weeks to be treated and may take many months in cases of onychomycosis (nail infection) and tinea capitis (Hay 2018).

Etiology

Dermatophytes are filamentous, keratinophilic fungi naturally found in soil (Zhan and Liu 2017). Dermatophytes species have the ability to produce different enzymes such as keratinases, adhesins, lipases, phosphatases, DNases, and non-specific proteases playing an essential role in attachment and invasion to the stratum corneum of skin (Martinez-Rossi et al. 2012).

In the past, dermatophytes were divided into three genuses, namely *Trichophyton* (*T*), *Epidermophyton* (*E*), and *Microsporum* (*M*), however, with the new diagnostic tools, three new genera of the dermatophytes were discovered namely *Nannizzia*, *Lophophyton*, and *Arthroderma* (Begum et al. 2020). The *Trichophyton* and *Microsporum* species can cause infections in human and animals. Although, the only pathogenic species of the *Epidermophyton* genus recognized to cause dermatophytosis is a *E. floccosum*, which only infects human. The term "dermatophytoids" refers to species of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* that live in soil and are rarely or never known to cause infection, for example *T. terrestre* (Distribution 2005).

On the other hand, the dermatophytes can divide into three groups based on their usual niche (Fig. 1). The first group is anthropophilic which is transmitted from one person to another by direct contact, i.e., *Microsporum langeronii* and *Trichophyton interdigitale*. Occasionally, some anthropophilic species cause ringworm infection in animals such as *Trichophyton rubrum* has been reported to cause an

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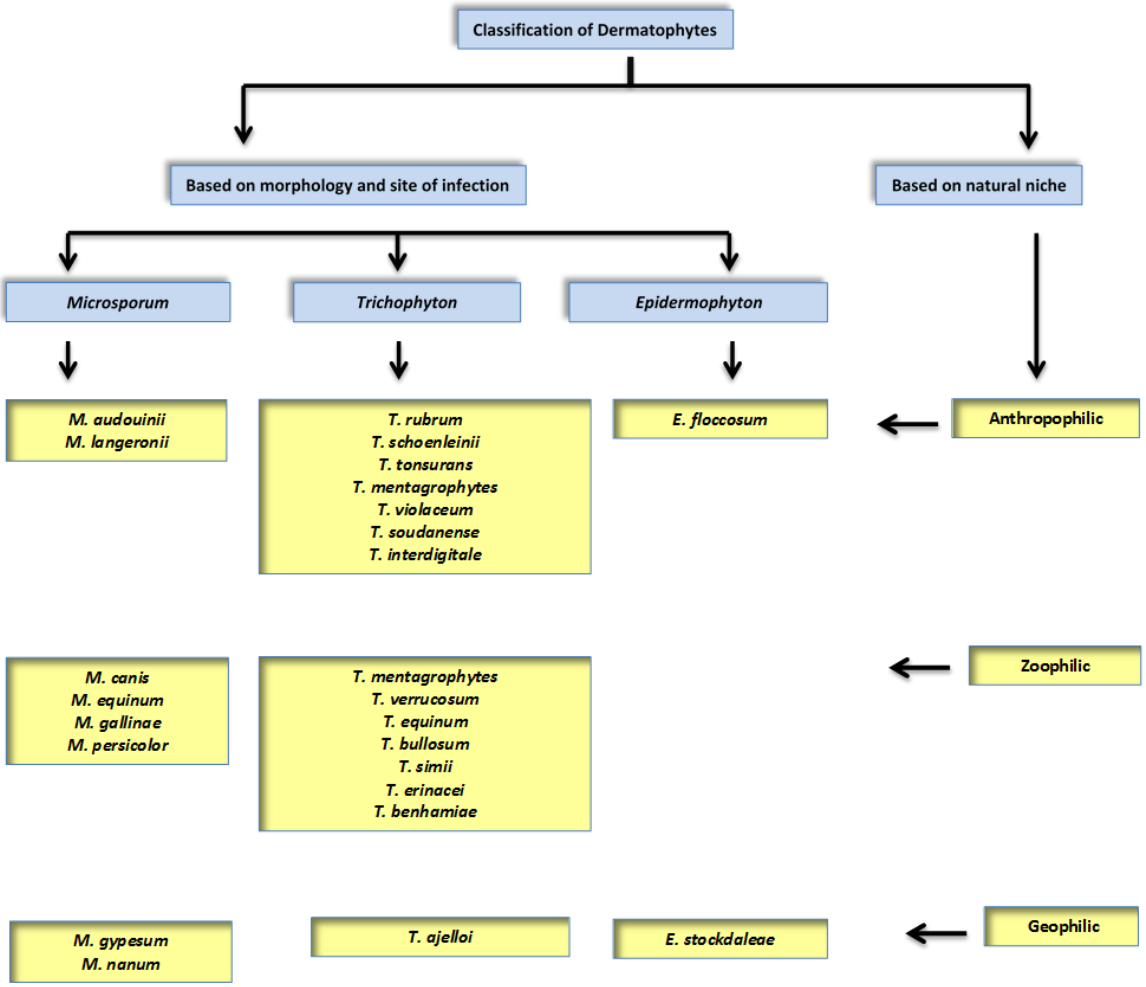

Fig. 1: Classification of dermatophytes according to the morphological characteristics and usual habitat

infection in dog (Georg 1960; Simpanya 2000). The second group is zoophilic which is transmitted from animals to human or other animals such as *Microsporum canis* and *Trichophyton mentagrophytes* which generally affect dogs and cats. The last group is geophilic which as saprophytes living on the keratinous resources in soil, and transmitted to person through contaminated soil i.e., *Microsporum gypseum* (Mancianti et al. 2003).

Epidemiology

Dermatophytosis, as a common superficial skin infection, is distributed around the world, with a higher prevalence in tropical and subtropical regions because of high temperature and humidity (Jartarkar et al. 2022). Nevertheless, it is commonly approved that between 20-25% of people worldwide are affected by dermatophytosis (Ameen 2010). The ascending of recalcitrant dermatophytosis might be associated with epidemiological change in pattern of growth of the pathogens resulting in enhancing persistence and the evolution in the dermatophytes genotypes which is increasing their virulence as well as pathogenicity, and drug-resistant species dramatically have appeared due to the widespread use of inadequate dosages of potent antimycotic drugs (Agarwal et al. 2014; Jartarkar et al. 2022).

Over the past few year's studies concluded that the prevalence and spectrum of infection have increased simultaneously with changing of migration, tourism patterns, socioeconomic conditions, and interaction with animals. In addition, rare species have been isolated in different countries (Lakshmanan et al. 2015). For instance, endemic dermatophytes to Asia and Africa (*T. soudanense*, *T. violaceum*, *M. audouinii*) increased in occurrence in North America and Europe because of the migration. Furthermore, tinea pedis is most common in Northern Europe and Central America, and in contrast, *M. canis* or *T. verrucosum* (zoophilic dermatophytes) are more frequent in Europe and Arab countries. Moreover, the frequency of *M. canis* infection in Mediterranean countries have increased which causes tinea capitis in infants (Mora-Montes and Lopes-Bezerra 2017). In the developing countries few

studies focused on the etiology of the dermatophytes infection as less data about epidemiological changes is available. Subsequently, findings from a specific location of a country cannot regard as a precise reflection of the total dermatophytosis of that country. It is challenging to make an accurate assessment of the dermatophytes prevalence in overall countries of the world (Ameen 2010).

Predisposing Factors

The ability of dermatophyte species to produce different proteolytic enzymes (i.e., keratinases and mycelium) and the contagiousness of dermatophytosis which can spread through direct contact with animals and fomites are the major predisposing factors to cause infection (Stollery 2007). Some extrinsic factors can also relate to the high incidence of dermatophytosis, such as low socioeconomic status increases the risk of infection by these fungi compared to high socioeconomic status which is likely associated with poor hygiene and poor medical care. Superficial infection of human skin is stimulated by humid and high temperature in tropical and subtropical regions and are exaggerated by the sweating, wearing of occlusive clothing and footwear. The occurrence of infection is related to the type of geographical location i.e., infection is mostly developed in rural areas than in urban areas (Coulibaly et al. 2018). The prevalence of onychomycosis due to *T. rubrum* increased by chronic diseases or disorders as reported in chronic venous insufficiency and diabetic patients (Da Silva et al. 2014; Eba et al. 2016). The use of antibiotics, steroid drugs and advanced age, are also enhancing the skin infection. Moreover, there are evidences of a genetic or family susceptibility to dermatophytosis, as some of these peoples have autosomal recessive (caspase recruitment domain containing protein 9) CARD9 deficiency (Lanternier et al. 2013). According to a study, dermatophytes have the capacity to infect deep layers of skin and other adjacent organs, such as lymph node. The majority of these deeply infection cases has been reported in patients with human immunodeficiency virus syndrome (HIV) and patients who are taking immunosuppressive therapy. Eventually, with the same factors all individuals are not equally predisposed to infection (Da Silva et al. 2014).

Dermatophytosis

In humans, dermatophytosis is also referred as tinea or ringworm, and is named according to the sites of the body affected as shown in Table.1. For example, tinea manus and tinea pedis referred to the hands and feet infections, respectively (Warnock 2012). Additionally, infection can transmit from one site of the body to another, i.e., tinea capitis (scalp dermatophytosis) can transmit to facial region and causes tinea faciei (facial dermatophytosis) (Zhan and Liu 2017).

Transmission

Dermatophytes are transmitted to the hosts through penetration in the injured skin, burns, and scars. Dermatophytes are abundant in different ecological niches and all three groups of dermatophytes can infect humans and produce dermatophytosis (Segal and Frenkel 2015). Zoophilic and anthropophilic groups are generally transmitted among hosts by conidia or arthrospores. It has been reported that some spores can survive in salt water for at least one year and in suitable environments for up to 1-2 years (Distribution 2005).

The zoophilic group are transferred from animal to people by direct contact with subclinically infected or sick animal, mostly pet animals (dog, cat). In sick animal, the shaft of the affected hair is fragile and hair fragments comprising arthrospores are powerful in increasing dermatophytes infection. Furthermore, non-infected pet animals can passively transmit arthrospores on their hair. Indirect transmission may arise by contaminated toys, brushes, and collars. Arthrospores are widely spread by dust particles, even in room without entering pet animal (Frymus et al. 2013). This type of skin disease is an occupational infection of Veterinarians, abattoir and tannery workers, farmers, and pet owners particularly the teenagers who care the infected cat and dog (Samanta 2015). Animal is commonly an asymptomatic carrier of dermatophytes because of the pathogen adaptation to the immune system of the host subsequently; zoophilic species cause severe inflammatory reactions. Most species are specific to only one host, like *T. verrucosum* to cattle, *M. canis* to cat, or *T. erinacei* to hedgehog (Gräser et al. 2018). As a result of improvement of hygiene, new lifestyle, and generalization of animals domestication, it is possible that, these pathogens will shift from zoophilic (*T. mentagrophytes*, *M. canis*) to anthropophilic species (*T. rubrum*, *T. tonsurans*, and *T. violaceum*), which are transmitted by unknown methods and cause mild infection in human (Zhan et al. 2015).

From human to human, the indirect transmission of dermatophytes such as *T. schoenleinii* via lost hair strands and desquamated skin cells is most common than the direct transmission. The transmission may happen through contaminated hats, combs, and hairbrushes. The transmission among family members may occurred horizontally between household members or vertically between the generations (from mother to grandchild). The vertical transmission of infection is much more common than the horizontal spread. *T. schoenleinii* can survive in homes for numerous generations without appropriate cleaning (Samanta 2015). It has been shown that shared wet surfaces (patios, balconies, showers, bathtubs) and shared tools may contribute to the transmission of dermatophytes among family member, as dermatophytes groups can persist on a variety of surfaces for up to 18 months (Jazdarehee et al. 2022). Other sources of infection are fitness studios, mats in sports facilities, public pools, hotels, and mosques (Tlougan et al. 2011; Yenişehirli et al. 2012; Watanabe et al. 2017).

Table 1: Clinical manifestations of dermatophytosis

Type of tinea	Sites of infection	Clinical features	Causative agents	References
Tinea capitis (scalp ringworm)	Scalp and hair shaft	Well demarcated or irregular alopecia and scaling. When affected hairs break a few millimeters from the scalp black dot alopecia is made. Follicular pustules with extensive purulent discharge, mainly when zoophilic species invade hair follicles deeply	*T. tonsurans* *M. ferrugineum* *T. violaceum* *T. soudanense* *M. canis* *M. audouinii*	(Havlickova et al. 2009; Fuller et al. 2014; Lova-Navarro et al. 2016)
Tinea faciei (Facial ringworm)	Glabrous (hair less) skin of the face	Erythematous, itchy, disc-shape, peripheral scaling lesions with healing of the foci in the center	*T. rubrum,* *T. mentagrophytes*	(Stollery 2007)
Tinea corporis (Body ringworm)	Glabrous skin of the arms, legs, and trunk	Redness, scaly, erythematous papulosquamous lesions with central sparing and accentuated margins	*M. canis* *T. rubrum* *T. verrucosum* *T. tonsurans*	(Havlickova et al. 2009; Segal et al. 2013)
Tinea pedis (Foot ringworm, Athlete's Foot)	Foot	Interdigital form (most popular): peeling, maceration, erosion, fissures chiefly in the space between third and fourth digits. Squamous hyperkeratotic form: dry, diffuse scaling, and non-inflammatory keratosis of the entire foot sole	*T. interdigitale* *T. rubrum* *E. floccosum*	(Degreef 2008)
Tinea manus (Hand ringworm)	Dorsum, or palm, interdigital folds of one or both hands	On the palm, there is a fine, partially collarette-like scaling, which highlights lines of the palm. On the dorsum and fingers the lesion similar to tinea corporis with erythemato-squamous lesions	*T. rubrum*	(Stollery 2007)
Tinea unguium (Onychomycosis, nail infection)	Toe and finger nails	Small yellowish discoloration of the nail plate to complete crumbly decay of it	*T. tonsurans* *T. rubrum* *T. violaceum* *M. gypseum* *T. soudanense* *E. floccosum* *T. interdigitale*	(Degreef 2008; Havlickova et al. 2009)
Tinea barbae	Beard, mustache area and eyebrows of adult man	Erythema with superficial inflammation, scaling, and pustules quickly penetrates into the hair follicles deeply, creating soft, infiltrated, furunculoid nodules. The lesion is covered with follicular pustules	*T. verrucosum* *T. mentagrophytes*	(Tosti et al. 2015; Vazheva and Zisova 2021)
Tinea cruris (Groin ringworm, "jock itch")	Inguinal region, sub-mammary folds in fatty women	Itchy and enflamed rash in the inguinal area. It is frequently found in young men of tropical area. Axillary infection can be seen as an analogous tinea form in woman	*T. rubrum* *T. mentagrophytes* *E. floccosum*	(Stollery 2007; Degreef 2008; Havlickova et al. 2009)
Tinea Incognito	Face and intertriginous areas	Erythematous, well demarcated lesions with pustules and a squamous margin. It is modified case of dermatophytosis following the use of systemic or topical steroids	*M. gypseum* *T. rubrum*	(Jacobs et al. 2001; Yu et al. 2010; Dutta et al. 2017)
Tinea nigra	Palms, soles and elsewhere	A single brown to black non-scaling macule.	*T. rubrum*	(Degreef 2008)

Incubation Period

Incubation period of disease ranges from one to two weeks in human (Distribution 2005).

Diagnosis

The rapid and proper diagnosis of etiological agents and mode of infection is crucial for accurate treatment and inhibition of further spread (Rezaei-Matehkolaei et al. 2013). Diagnosis is made using the patient history, physical inspection, microscopic investigation of skin scrapings and hairs from the lesions, fungal culture, Wood's lamp examination, and histopathological inspection of the tissues (Distribution 2005; Tosti et al. 2015).

Potassium Hydroxide (KOH) microscopy (Wet mount preparation)

The direct visualization of hyaline, septate, and branching hyphae under the light microscope is an essential method for the diagnosis of dermatophytes. Scrapings of kin should be obtained from the active border of the lesion, nail scrapings are usually taken from the subungual debris, and hairs sample should be pulled from the affected area without breakage. The hairs that are scaly, broken, and glow under a Wood's lamp are the ideal ones for collection (Distribution 2005). The small fragments of the specimen are placed on a clean microscope slide, a coverslip is placed, and heated to remove non-fungal materials as heating accelerates the maceration of the skin scale and makes it easier to see the

hyphae among the keratinocytes. A few drops of 10- 20% KOH put to the edge of the coverslip (Ponka and Baddar 2014). The wet mount preparation is then inspected under a microscope. Hyphae rounding up into arthroconidia are diagnostic, but hyphae alone could be caused by other fungi, including contaminants. On the surface of the affected hairs shaft, arthroconidia can be visualized externally (ectothrix) or internally (endothrix) (Mohamed Shalaby et al., 2016).

Fungal Culture

If Potassium Hydroxide microscopy does not provide adequate information, culture is the most reliable test for accurate diagnosis of dermatophyte species. Specimens for culture involve skin, hair, and nails. During identification of asymptomatic carriers, other methods such as, hair brushing, using adhesive tape for sample collection, or rubbing the lesion with a sterile toothbrush or moistened cotton swab may also be effective. Colonies develop in five days to four weeks, based on the pathogens (Distribution 2005).
Morphology of colony can differ with the medium. Sabouraud peptone-glucose agar (Emmons' modification) amended with cycloheximide and chloramphenicol is commonly used (Weitzman and Summerbell 1995). Species of dermatophyte can be distinguished by their colonial characteristics (the appearance of microconidia and macroconidia) on Sabouraud glucose agar, range of growth temperature, limited nutritional tests, cycloheximide resistance, and biochemical test such as urease production. Differential media as bromocresol purple-milk solids glucose and phytone yeast extract agar can be helpful during differentiation from negative result (Distribution 2005; Dowd, 2007: Vermout et al. 2008). Dermatophyte test medium (DTM) is another isolation medium containing a pH indicator-phenol red. After incubation at room temperature for 5-14 days, the color of the media turns from yellow to bright red when the dermatophytes utilize proteins resulting in ammonium ion release and an alkaline environment (Jartarkar et al. 2022).

Wood's Lamp Examination (Ultraviolet light, Black light)

Wood's lamp examination may be useful in making the diagnosis of some dermatological disorders. In addition, it has lately been used as a diagnostic tool for certain skin cancers. Robert Willams Wood made Wood's lamp in 1903 and for the first time, it was used in dermatological practice for the finding of hair fungal infection (Gupta and Singhi 2004). Wood's lamp produces an invisible long-wave ultraviolet radiation which is named black light at the wavelength of 340-450 nm (Suraprasit et al. 2016). Dermatophytes that cause fluorescence mostly belongs to the *Microsporum* genus. For example, *M. audouinii*, *M. canis*, *M. ferrugineum*, and *M. distortum* shows blue-green light, while, *M. gypseum* shows dull-yellow light (Gupta and Singhi 2004). A value of Wood's lamp is limited in detecting some dermatophytes like *T. rubrum*, *T. metagrophytes*, and *T. violaceum* in tinea capitis as they are non-fluorescent under wood's lamp. For that reason, the lack of fluorescence does not certainly eliminate tinea capitis as most *Trichophyton* members, are non-fluorescent with the exception of *T. schoenleinii*, which shows dull-blue light (Suraprasit et al. 2016). Some practical caution should be kept in mind to avoid misdiagnosis in use of a Wood's lamp. The lamp must perfectly be allowed to warm up for about one minute. The examination lab should be totally dark and the inspector should get dark adapted in order to see the contrast obviously. The light source should be 10 cm away from the lesion. Avoid washing the affected area or applying topical medicaments before exposing it for Wood's lamp examination as it may produce false negative results (Gupta and Singhi 2004).

Histopathological Examination (Skin and nails biopsy)

Histopathological examination of the affected area is occasionally helpful, especially in onychomycosis. Microscopically, the species of dermatophyte cannot be detected. There is no distinctive histopathological lesion related to dermatophytes. The microscopical section reveals the degenerating and dead mycelium, cellular debris at the centre, and hyphae at the peripheral of the lesion. In *T. schoenleinii* infection, the concave, cup-shaped yellow crust (scutulum) is observed on the atrophic epidermis. The epidermis may appear unaffected to mildly hyperkeratotic with patchy parakeratosis. Spongiosis and microabscesses in the stratum corneum may be seen. A perivascular infiltration of inflammatory cells can be present in the upper dermis, depending on the infecting species. Branching, septate hyphae can be visualized best in the stratum corneum with a special stain such as periodic acid–Schiff (PAS) with diastase predigestion, Grocott methenamine silver and calcofluor white (CFW) stains (Jartarkar et al. 2022), Although, they may also be seen in Hematoxylin and Eosin stained preparations. The diagnostic sensitivity can be increased with biopsy which is not always possible to conduct especially in human patients suffering with diabetes (Samanta 2015).

Molecular Biology

Molecular methods have been established to provide more fast and precise alternatives to pre-existing diagnostic methods due to overlapping phenotypic characteristics, variability, and pleomorphism (Li et al. 2008). According to a number of studies, the rate of dermatophytosis detection is increased by 10-19.5% when Polymers Chain Reaction (PCR) techniques were used instead of the fungal culture approach. However, the result of the PCR assays may differ

Table 2: Summary of systemic antifungals in dermatophytosis

Class	Active agents	Mechanism of action	Dose (adult)	Duration of use	Contraindications
Imidazole (Azoles)	Ketoconazole	Block lanosterol 14-α demethylase resulting in the inhibition of synthesis of ergosterol, and impairment of fungal cell membrane permeability	200-400 mg/day	3-6 weeks (Tinea capitis) 4 weeks (Tinea cruris) 4 weeks (Tinea pedis) 6 months (onychomycosis)	- Acute or chronic hepatic disorders - Adrenal insufficiency -Hypersensitivity reaction to ketoconazole
Triazoles (Azoles)	Fluconazole	Block lanosterol 14-α demethylase	150-450 mg/week	3-6 weeks (Tinea capitis) 2-4 weeks (Tinea cruris) 4-6 weeks (Tinea pedis) 3 months (fingernails) and 6 months (toenails) onychomycosis	- Severe liver disease - Use with caution in patients sensitive to other azoles
Triazoles (Azoles)	Itraconazole	Block lanosterol 14-α demethylase	200 mg/day	4-8 weeks (Tinea capitis) 1 weeks (Tinea cruris) 1 week (Tinea pedis) 1 week/months (onychomycosis)	Patient with congestive heart failure (CHF)
Allylamine	Terbinafine	Inhibiting the enzyme squalene monooxygenase which is involved in the synthesis of sterol in fungi. This inhibits fungal sterol biosynthesis by decreasing ergosterol levels	250 mg/day	3-4 weeks (Tinea capitis) 1 weeks (Tinea cruris) 2 weeks (Tinea pedis) 6-12 weeks (onychomycosis)	None
Benzofurane	Griseofulvin	Disruption of mitotic spindle and inhibition of fungal mitosis	500 mg/day	6-8 weeks (Tinea capitis) 2-4 weeks (Tinea cruris) 4 weeks (Tinea pedis) 6-9 months (fingernail) and 12-18 months (toenail) onychomycosis	Patients with porphyria or hepatocellular failure

The data from (Finkelstein et al. 1996; De Beule and Van Gestel 2001; Johnson and Kauffman 2003; Stollery 2007; Newland and Abdel-Rahman 2009; Pires et al. 2014; Fuller et al. 2014; Kaul et al. 2017; Hay 2018; Sonthalia et al. 2019; Jartarkar et al. 2022).

based on the origin of the clinical sample, sample preparation, selection of the target sequence, and laboratory conditions (Gordon et al. 2016). The rapid detection of etiological agents accurately in clinical cases relating to dermatophytosis occurred by employing specific primers, followed by interpretation of the results based on the amplicon size in agarose gel (Verrier and Monod 2016). Conventional PCR technique is a simple and low cost molecular technique for application. Real-time PCR-based methods expand the possibilities of multiple simultaneous species recognitions and limit the risk of contamination, whereas methods employing post-PCR techniques prolong the turnaround time and may increase the contamination risk (Jensen and Arendrup 2012).

Treatment

Dermatophytosis is treated with different topical and systemic antifungal drugs (Gupta and Cooper 2008). Topical treatments are indicated for localized and mild dermatophytes infections while systemic drugs (Table 2) are recommended for more extensive (chronic) infections or where application of a topical drug is not possible. Combination of local and systemic treatments is preferred to obtain a better clinical and mycological therapy. In addition, for preventing the appearance of drug resistance different group of antifungals can be used (Jartarkar et al. 2022). For the accurate treatment, asymptomatic dermatophytosis such as onychomycosis or tinea pedis should be considered specifically, individual with tinea capitis and tinea corporis should be closely inspected for possible infections or as carriers of an animal source such as those found on pets, in order to ensure that the optimal therapeutic measures are taken (Zhan et al. 2015). A wide variety of topical medications are available, in shampoo, lotion, gel, and cream formulations. A majority of the agents are of the ‘azole’ and ‘allylamine’ family. Families of these agents are known for their high efficacy against the dermatophyte infection. Topical drugs applied once or twice daily (Gupta and Cooper 2008). An ideal treatment should have a low cost, rapid onset of effect, low relapse rate, high cure rate, high anti-inflammatory action, minimal systemic absorption, minimal side effects, and safe to be used in lactation, pregnancy, renal and hepatic failure (Jartarkar et al. 2022).

Conclusion

Dermatophytosis is a frequent skin disease causes by keratinolytic fungi called dermatophytes. Causative agents responsible for dermatophytosis are generally classified into anthropophilic, zoophilic, and geophilic groups from the *Trichophyton*, *Epidermophyton*, and *Microsporum* genera. Recently, due to immigration from tropical areas, increased international tourism, and interaction with animals (particularly dog and cat) the frequency of dermatophytosis in humans has dramatically increased during the past 20 years. Additionally, taking immunosuppressive drugs is a predisposing factor that makes people more susceptible to developing dermatophytosis. The frequency and severity of each dermatophyte infections are variable in a particular region based on the host, pathogens, and environmental conditions. It is essential to note that due to the contagiousness of the dermatophyte infection, spreading can occur from person to person, from animal to human, even from one area to another within the same body of an infected person. The flaky, annular with central clearing appearance is a typical lesion in an immunocompetent individual; however, the lesions can be deep and extensive in immunocompromised person. In general, treatment of dermatophytosis requires long duration to acquire effective result. Various antifungal drugs are used in the treatment of dermatophytosis. However, the most vital factor for control of the infections is maintenance of appropriate hygienic conditions. Almost all varieties of dermatophytosis require at least 2-4 weeks to be treated, whereas onychomycosis and tinea capitis could take up to 6 months.

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17

Bovine Trichomoniasis

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INTRODUCTION

Reproductive diseases are a significant cause of reduced productivity in cattle breeding systems. Infectious diseases are usually endemic and result in less efficient reproduction, infertility, miscarriage, and reduced productivity. These diseases are typically asymptomatic or subclinical, complicating their identification (Campero et al. 2003). Trichomoniasis is one of the livestock's most common protozoal diseases, and the most widely known trichomonad in veterinary medicine is *Tritrichomonas (T.) foetus*, the etiologic agent of bovine trichomoniasis.

Bovine trichomoniasis is a venereal protozoan disease that occurs in many geographic areas worldwide, with most cases occurring in intensively managed cattle farms (Florin-Christensen and Schnittger 2018). This causative agent is *T. foetus*, a flagellated protozoan that occurs solely in cattle genitalia (Yao 2013). In infected cattle, there is vaginitis, endometritis, infertility, miscarriage, and early embryonic death (Martin et al. 2021). Mazzanti first discovered it in 1900, and since then, much work has been done on its incidence, especially in the United States and Britain. Emmerson (1932) reported the first case of bovine trichomoniasis in Pa McNutt in the U.S.A., and Walsh and Murray reported the disease in Iowa in 1930 (Danan and Teschke 2015). Several protozoan species occur in the bovine reproductive system, like the preputial cavity in bulls. These protozoa include *T. foetus*, which may be zoonotic, and cause opportunistic infections in humans (Yao 2012).

The trophozoites of *T. foetus* are transmitted among bulls and cows during coitus, causing metritis and early embryonic death in cows, but infected bulls typically are without clinical signs (Parthiban et al. 2015). Infected cattle with trichomoniasis might experience mild "vaginitis" or "endometritis," or the infection can be as serious as causing severe inflammation throughout the whole reproductive tract. Other complications may include pyometra in pregnant cattle, inability to be pregnant, and decreased calving ratio (Alobaidii et al. 2021). Sexual intercourse is the primary transmission mode of *T. foetus* from infected to healthy animals, most commonly via natural mating (BonDurant 2005). The bulls get infected while breeding infected cows and stay symptomless carriers of the infection (Fig. 1). However, the protozoan can subsist in the raw and processed semen of breeder bulls and be transmitted via artificial insemination (AI) (Eaglesome et al. 1995). Also, the protozoan endures freezing in liquid nitrogen, where the protozoa-contaminated semen is preserved (Yao et al. 2011). Hence, artificial insemination cannot eliminate the disease but can reduce the prevalence rate, as reports indicate that AI substantially reduced the incidence of trichomonosis and other venereal infections (Van Bergen et al. 2006). Other means of transmission are also possible. For example, Goodger and Skirrow (1986) reported that unsanitary estrus detection through vaginal examinations led to the transfer of *T. foetus*, carried via contaminated gloves, from infected to non-infected cows.

The transmission of *T. foetus* by insects, such as flies, was reported by Clark et al. (1977), as insects can transmit infection among cows. Also, infection is possible through direct contact between a healthy cow's vulva and that of an infected cow and passive transmission through a healthy bull's penis. Some females maintained infection up to 9 weeks postpartum through a normal pregnancy (Skirrow et al. 1985). *T. foetus* decreases cattle productivity by increasing reproductive losses and reducing conception rates. Bovine trichomoniasis causes a sustained breeding season (Adeyeye et al. 2012). The protozoa were also documented to cause human infections in immunocompromised and immunosuppressed individuals, including meningoencephalitis and peritonitis (Yao 2012), as mentioned in Fig. 1.

Differential diagnoses of bovine trichomoniasis include anaplasmosis, bovine viral diarrhea, brucellosis, campylobacteriosis, chlamydiosis, infectious bovine rhinotracheitis, leptospirosis, and neosporosis.

These diseases may cause clinical signs, including infertility, vaginitis, pyometra, abortions, and vaginal discharge, which should be excluded (Florin-Christensen and Schnittger 2018).

Morphology of the Agent

T. foetus has a pyriform or ovoid trophozoite stage about 8–18 µm long and 4–9 µm wide (Issa 2014). The locomotive activity of the trophozoite occurs via several structures,

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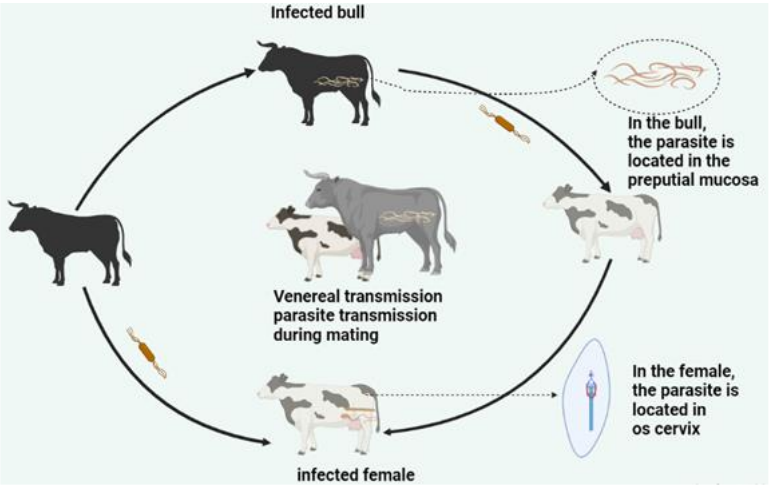

Fig. 1: The life cycle of bovine *T. foetus*.

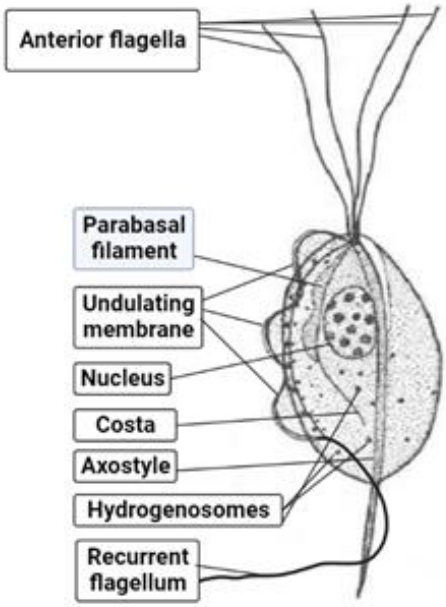

Fig 2: A *Trichomonas foetus* trophozoite.

like the undulating membrane and four flagella. The flagella are located in the cell's apical pole and originate from the basal bodies or kinetosomes. Three similar-length flagella are directed forward, while the fourth flagellum (the recurrent flagellum) is directed toward the cell's posterior part, is associated with the undulating membrane, and stretches beyond the undulating membrane's posterior end (Benchimol 2004). Cattle (*Bos indicus* and *B. taurus*) are the usual hosts of *T. foetus*. The number of flagella after examination under a phase contrast microscope or after staining is an essential morphological feature that can assist in differentiating *T. foetus* from other flagellated bovine parasites.

Nevertheless, non-*T. foetus* trichomonads are invariably challenging to distinguish from *T. foetus*, depending on morphology (Pereira-Neves et al. 2003). Trichomonads are highly motile and are about the size of leukocytes. *Trichomonas vaginalis* has four flagella on the anterior side, while *T. foetus* has three anterior flagella and one recurrent flagellum (Benchimol et al. 2006) (Fig. 2).

Prevalence

Bovine trichomoniasis is a significant problem worldwide. In Iraq, *T. foetus* infection was first reported in cows in Nineveh province, with a higher infection rate in >2–4-year-old cows and early embryonic death (Alobaidii et al. 2021). The protozoan disease is widespread, affecting many cattle herds in North and South America, parts of Europe, Africa, Asia, and Australia (Guven et al. 2013; Yao 2013; de Oliveira et al. 2015). Trichomoniasis is prevalent in Argentina, reducing pregnancy rates by 15%–25% (Campero et al. 2003). The within-herd prevalence rates of trichomoniasis in bulls are 26.4% in South Africa (Pefanis et al. 1988), 30.6–50.0% in Australia, and 5.8–38.5% in California (Skirrow et al. 1985). Many studies have reported infected bulls with *T. foetus* in the United States of America (Szonyi et al. 2012), Argentina (Mardones et al. 2008), Spain (Mendoza-Ibarra et al. 2012), Austria (McCool et al. 1988), the Republic of Transkei (Pefanis et al. 1988), Colombia (Griffiths et al. 1984), Tanzania (Swai et al. 2005), Nigeria (Bawa et al. 1991), Canada (Waldner et al. 2013), and Argentina (Molina et al. 2013). Australian surveys have shown infection rates of about 8.4%. About 10.7% of cows were infected with *T. foetus* in a sizeable Californian dairy farm (Goodger and Skirrow 1986). Northern Spain was considered a hotspot of infection since natural breeding is still implemented (Mendoza-Ibarra et al. 2012). Compared to other livestock diseases, The rate of *T. foetus* infection is expected to be low in the United States. Hence, control of the disease is not unified at the federal level, leading to the enactment of different regulations among states (Martin et al. 2021). Twenty-six states had trichomoniasis control/management program regulations in place to curtail the spread of this disease as of 1 April 2014 (Yao 2015). The herd size and bull:cow ratio are vital for infection prevalence (Mardones et al. 2008). Factors

associated with a high bovine trichomoniasis rate in a herd include the herd size. So, the infection hazard is higher in large herds that share grazing, have a significant number of bulls with a high ratio of 4 years or older bulls, and a high ratio of bulls to cows (Szonyi et al. 2012).

Pathogenesis and Pathology

The underlying factors affecting the loss of the embryo or fetus are not accurately identified. However, some of these mechanisms include the adverse effects of enzymes released by the protozoan, the effect of antiparasitic inflammatory reactions in the uterus, and the parasite's direct mechanical activity (Campero and Cobo 2006). Cyto-adherence and cytotoxicity are thought to be the principal mechanisms (Petropolis et al. 2008).

The concentration of *T. foetus* in the cervicovaginal mucus changes during the estrus cycle, and the highest concentration is observed a few days prior to the estrus phase (Schuster and Schaub 2001). The uterus was believed to be the primary infection site, but several studies of naturally infected cows indicate that the os cervix is the preferred site. Placentitis and a uniform pattern of placental and fetal lesions are also seen. The fusional stage of abortion is associated with variation in the pathogenicity of *T. foetus* strains. The infective threshold number of organisms or the host's immune condition is unknown and should be further studied. Bovine trichomoniasis causes abortion, usually during early gestation (BonDurant 2005). A scant purulent preputial discharge may be observed within the first two weeks of infection. Older bulls seem to become permanent *T. foetus* carriers, possibly due to the growth of epithelial crypts in the preputial cavity (Walker et al. 2003). It is rare for abortions due to *T. foetus* to occur after six months of gestation. The cow or heifer usually recovers spontaneously when the placenta and fetal and placental membranes are eliminated following abortion. However, chronic catarrhal or purulent endometritis, which may cause permanent sterility, may occur if a part of the placenta or membrane remains. Sometimes, the abortion fails to occur following fetal death, and maceration results in the uterus (Schlafer and Foster 2016). There is a lack of research on how *T. foetus* affects the conceptus and causes abortion. However, there is a possible role of tumor necrosis factor (TNF) in malaria-induced abortion, and lymphokine-mediated cytotoxicity is perhaps essential in bovine trichomoniasis (Yule et al. 1989).

Microscopic lesions in aborted fetuses consist of pyogranulomatous bronchopneumonia and necrotizing enteritis with trichomonads invading the tissues. Specifically, pulmonary air passages contain many neutrophils, macrophages, multinucleated giant cells, meconium, and trichomonads located extracellularly and phagocytized. Small focal collections of lymphocytes and plasma cells are observed in the interstitium. Multiple trichomonads are dispersed in the aborted fetuses' interlobular septal connective tissue and aggregated in the fetuses' interlobular septal and subpleural vessels. Additionally, fetuses may have pronounced focal hemorrhage in interlobular septa and airways of some pulmonary lobules. Mild focal epithelial degeneration to diffuse necrosis and loss of epithelium might occur in the gastroenteric tract. Fetuses may have marked mucosal, submucosal, and subserosal hemorrhage. Also, the forestomach, abomasum, and small and large intestines may contain thrombotic lesions. Multiple large intraepithelial vesicles comprising fibrin strands and erythrocytes occur in the mucosa of the rumen and omasum overlying hemorrhagic foci (Schlafer and Foster 2016).

Tritrichomonas foetus in Bulls

Infection with *T. foetus* is limited to the reproductive system and, in bulls, the preputial cavity and urethral orifice (Michi et al. 2016). Bulls are the natural carriers of the parasite (Higgins 2006). Young bulls are either more tolerant to *T. foetus* or can eliminate the infection more efficiently. Bulls 1–2 years old are refractory to infection (Michi et al. 2016). The parasite survives in fresh, pure, or diluted semen that has been refrigerated and can resist cryopreservation, and transmission through AI with contaminated semen is probable (BonDurant 2005). Feces are commonly found in the preputial cavity of bulls since they tend to mount each other. The feces may comprise trichomonad species other than *T. foetus*, such as *Pentatrichomonas hominins* and nonpathogenic species of *Tetratrichomonas* (Campero et al. 2003). The possibility of *T. foetus* contagion between males is considered very low.

Chronically infected bulls are considered asymptomatic carriers for years since the clinical signs of the disease are not apparent, but bulls infected with the acute form have lesions and discharge in the genital organs for a short time (González-Carmona et al. 2012). Unlike female cattle, histopathological changes in bulls are absent, and unlike female cattle, bulls do not self-cure without prior vaccination (Higgins 2006). Previous studies have been unable to detect lesions associated with *T. foetus* infection. Tests such as the mucus agglutination test and the ELISA test have limited use in diagnosing the parasite since they are not adequately sensitive and specific, and infected bulls do not develop enough immune responses for serological diagnoses (Voyich et al. 2001).

Rhyan et al. (1999) detected *T. foetus* in the superficial layers of the penile and preputial epithelium in histological sections of the reproductive tracts of bulls infected with *T. foetus*. However, they failed to detect the parasite's invasion of these structures' basement membrane or dermis. The absence of the parasite's invasion of these tissues may explain the limited immunologic reaction in *T. foetus*-infected bulls. Significantly higher amounts of specific antibodies in the preputial secretions of infected bulls than non-infected bulls resulted from local antigen uptake, processing, and antibody deposition. The absence of pathologic changes and the immune response's inability to eliminate the parasite from

the preputial cavity led to chronic infection, particularly in older bulls.

Several studies have tried to determine the correlation between the age of bulls and infection risk and concluded that as the bull ages, the chance of *T. foetus* infection increases (Rae et al. 2004). Investigators of *T. foetus* have likewise argued that the growth of crypts in old bulls is a cause of age-related vulnerability to *T. foetus* (BonDurant and Honigberg 1994). Several studies have proposed different susceptibility levels of cattle breeds to *T. foetus* infection (Rae et al. 2004).

Tritrichomonas foetus in Cows

Cows are more susceptible to *T. foetus* infection as only 103 trichomonads are required to establish infection in female bulls (Higgins 2006). It was shown that an infected bull could infect previously uninfected susceptible nulliparous cows by a single service with a 95% infection rate. Transmission from infected cows/heifers to bulls appears less efficient (Yao 2015). The late-gestation abortion by trichomonads supports the observed occurrence of "carrier cows." The cows can deliver normal calves and maintain infection throughout pregnancy and six to nine weeks postnatal, becoming an infection source for bulls (Yule et al. 1989).

Infection can be self-limiting in cows, and the parasites can be cleared from the reproductive tract after about three months (Yule et al. 1989). Most gestations are lost approximately 2.5 weeks postconception when maternal recognition has taken place, but embryonic death might happen at any time until five months of gestation (BonDurant 1985). However, later in gestation, embryonic or fetal loss results in abnormally long interservice intervals (2–5 months). Fetal deaths at approximately 50 to 70 days post-coitus have been reported, and deaths as late as eight months' gestation may occur (BonDurant 1985). After a variable period of infertility after the initial exposure, cows regain their fertility, even though infected bulls breed them. This suggests that infected cows develop an immune response to the parasite that reduces their susceptibility to subsequent infection for some time, possibly as long as six months, the fetal membranes are retained, and a chronic catarrhal or purulent endometritis usually results (Anderson et al. 1994).

After the parasite has initially multiplied in the vagina, it remains in the uterus, and the cells' number in the vagina may change during the estrous cycle. This fluctuation may be influenced by the cycle type, regular or prolonged (Mancebo et al. 1995). Chronically infected cows with *Tritrichomonas foetus* were carriers of the infection for as long as ten months (Mancebo *et al.* 1995). Also, chronic infections were observed throughout normal pregnancies, with the ability to isolate *T. foetus* for as long as nine weeks.

Diagnosis

Due to the insidious nature of *T. foetus* infection, the parasite occurrence on cattle farms often goes undetected until a substantial loss has already occurred. Infection in females often goes undetected due to early abortion resulting in re-exposure of females to males, increased calving to conception intervals (BonDurant 2005), and smaller, less developed calves due to the shortened weaning season. The most common practice for detecting infection within a herd is the demonstration of a live *T. foetus* by culture scrapings from the preputial smegma in sexually rested bulls (Higgins 2006).

1. Causative agent identification

The tentative diagnosis of trichomoniasis as a reason for reproductive failure on a farm depends on the clinical history, signs of early miscarriage, and recurrent or irregular estrous cycles. However, the infection is confirmed by the manifestation of *T. foetus* in placental fluid, an aborted fetus's stomach contents, vaginal mucus, endometrial washings, inflammatory discharge due to pyometra, or preputial smegma. The most dependable sample to diagnose infected herds is the washings or scrapings of the prepuce or vagina (Corney 2013). The most common diagnostic method is the visualization of motile trichomonads in a saline preparation of the vaginal fluid, which must be done after 10 to 20 minutes of sample collection. Otherwise, the trichomonads will die. The parasites are 10–20 μm long and 5–15 μm wide, near the size of a leukocyte, and may move actively or be observed beating their flagella without the organism's movement (Schwebke and Burgess 2004).

2. T. foetus identification by direct examination or in culture

Many techniques are used to diagnose *T. foetus* with different levels of specificity and sensitivity. An example is the detection of *T. foetus* in Giemsa-stained vaginal smears under the microscope. However, this method cannot detect infections with low parasite numbers. Another way is to grow the parasite in different culture media (Parker et al. 2001), such as Diamond's or Claussen's media, allowing the protozoa to grow *in vitro* until a sufficient number of parasites facilitates detection by light microscopy (Anderson et al. 1994). One drawback of this method is that it takes two to seven days and does not differentiate different *Tritrichomonas* species (Ginter Summarell et al. 2018). Smegma samples taken either by preputial lavage or scraping seem to be most satisfactory for diagnosing infected bulls and yielding comparable numbers of organisms (Michi et al. 2016). It is preferable to rest bulls sexually for at least seven days before collecting samples to increase the concentration of organisms in the preputial cavity. *T. foetus* trophozoites are microscopically distinguished by their jerky, rolling movement, three anterior flagella, and an undulating membrane (Anderson et al. 1994). Proper diagnosis of *T. foetus* relies on correct collection and handling of samples, suitable growth media and conditions, and proper organism identification by microscopic examination.

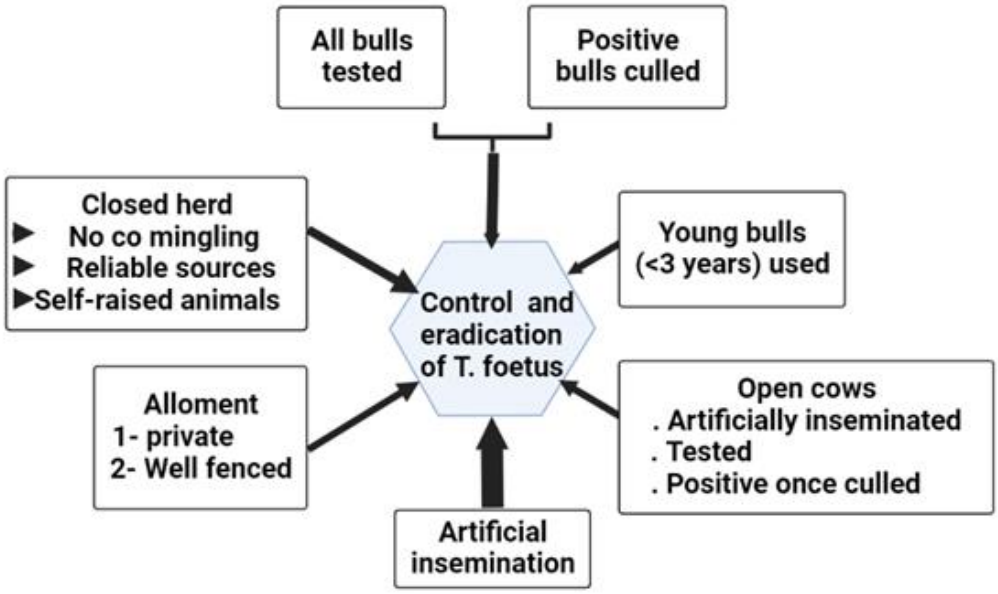

Fig. 3: An integrated approach for controlling and eradicating *T. foetus* infections. The increasing thickness of the arrows indicates the increasing importance of each approach.

In samples where the concentration of organisms is sufficiently high, it is possible to further characterize the organisms by phase contrast microscopy (Skirrow and BonDurant 1990) or staining methods (Lun and Gajadhar 1999) to help visualize vital diagnostic features of *T. foetus.*

3. Polymerase Chain Reaction (PCR)

An alternative test that can detect *T. foetus* infection is the polymerase chain reaction (PCR) diagnostic assay, which is of particular value if the number of organisms in the culture remains low (Ginter Summarell *et al.* 2018). The PCR widely detects *T. foetus* DNA using primers such as TF1, TF2, TF3, and TF4. This technique was about 90% sensitive, using TFR3 and TFR4 primers for *T. foetus* detection (Mukhufhi et al. 2003; Alobaidii et al. 2021). PCR has provided vital improvements over the culture techniques, such as enabling the detection of pseudocysts (non-motile forms) (Pereira-Neves et al. 2011), short duration, and high specificity. However, PCR techniques still encounter many challenges (Ginter Summarell et al. 2018). To minimize false positive results, the authors utilized a complementary DNA enzyme immunoassay to efficiently discriminate between false-negative amplification products and *T. foetus* DNA (Higgins 2006).

4. Serological Tests

Serological tests like mucus agglutination and ELISA can be applied to diagnose *T. foetus.* However, these methods have limited use since they are not highly sensitive or specific, and bulls do not develop adequate immune reactions for serological diagnoses (Voyich et al. 2001).

Control and Prevention

Strategies for preventing and controlling bovine trichomoniasis depend upon the distinctive epidemiologic characteristics of bovine trichomonosis. In this sexually transmitted infection, bulls are asymptomatic carriers and are a permanent source of infection, while infections are usually temporary in cows and heifers (Florin-Christensen and Schnittger 2018).

Bovine trichomoniasis is best controlled by proper management (Fig. 3). All bulls in the herd and subsequent replacements should be tested for trichomonads at least three weekly intervals before being used for breeding. Infected bulls should be removed from the herd and replaced with young (≤ 2 years) virgin bulls (Fort et al. 2016). Alternatively, AI can control the transmission of *T. foetus* effectively, but a complete change from natural services to AI may not be practical. If the cow herd was exposed to *T. foetus*, cows should be examined, and all those with recent pregnancy loss or pyometra should be culled. A cow herd exposed to trichomoniasis can be divided into two groups; pregnant cows should be observed for abortion, and nonpregnant cows should be rested sexually for at least four months to eliminate the T. foetus organisms immunologically from their urogenital tracts (BonDurant and Honigberg 1994). After successful calving, cows in the infected group also should be given sexual rest for a 90-day postpartum interval, or no less than two normal estrous periods after the breeding season begins, before being moved into a herd with uninfected cattle (Mancebo et al. 1995). Trichomoniasis can be prevented by testing all additions to an established herd. Because testing procedures for individual cows are not well established, additions to established herds should be limited

to animals from familiar herds or virgins. If that is not possible, all other female additions should be tested by culture on multiple samples before entering the herd. One commercial "bacterin-type" vaccine and several experimental antigen vaccines (Skirrow and BonDurant 1990) have been shown to induce an immunity *T. foetus* in female cattle vaccinated before breeding.

Conclusion

Bovine trichomoniasis is a sexually transmitted host-specific disease of cattle that continues to pose a severe economic loss on cattle production due to infertility and abortion. The disease's asymptomatic nature, particularly in the bull, makes diagnosis complex and challenging. The infection can be diagnosed by direct smear examination, culturing, and molecular or serological techniques. The control and eradication of *T. foetus* can only be done by culling positive bulls upon testing.

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18

Babesiosis in Cattle

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INTRODUCTION

Cattle is an important dairy and meat producing animal playing an important role in the economy (Saunsoucy 1995; Suarez and Noh 2011; Suarez et al. 2018). *Babesia* is a protozoan parasite belonging to the genus piroplasmida, causes a deadly disease in livestock and farm animals and is transmitted by the ticks. Because the illness has direct economic effects like decreased milk output, loss of body weight, and animal death, it poses major issues for both animal life and farm economies (Menshawy 2020). It also exerts secondary costs associated with treatment and prevention (Guswanto et al. 2017). The several regions of Africa, Australia, America, and Asia, particularly India, have a great impact on the cattle industry (Bock et al. 2004; Bal et al. 2016; Hashem et al. 2018). It affects and spreads in tropical as well as subtropical countries (Beugnet and Moreau 2015; Rozej-Bielicka et al. 2015). It causes lack of appetite, fever, anemia, ceasing rumination, and increases in heart and respiratory rates. In later stages, it may lead to hemoglobinuria, a yellowish mucous membrane, and the death of animal (Wagner et al. 2002; Zintl et al. 2003; Demeke et al. 2018; Mezouaghi et al. 2019). According to (Silva et al. 2010), the Ixodidae tick can transmit the babesiosis infection to several animal species. *Babesia* (*B.*) *bovis* and *B. bigemina* are the two most important babesia species in cattle (Zintl et al. 2013). *B. divergens*, is one of the main babesia species that causes bovine babesiosis, and raised concerns among international health authorities (OIE). *Rhipicephalus* and *Ixodes* tick species can transmit babesiosis to cattle depending on the disease's type (Jabbar et al. 2015). *B. bovis and B. bigemina* can be transmitted by number of vectors including *Rhipicephalus* (*R.*) *microplus, R. annulatus,* and *R. geigyi*, whereas *R. decoloratus* and *R. evertsi* can only be transmited by *B. bigemina*. *Ixodes* (*I.*) *ricinus* typically transmits *B. divergens* (Bock et al. 2004; Gohil et al. 2013).

Etiology and Morphology

Babesiosis is also known by the various other names i.e., Piroplasmosis, Texas fever, and Red water fever (Sahinduran, 2012). The genus Babesia includes the two main species which are *B. bovis* and *B. bigemina* Belonging to the phylum Apicomplexa and class Sporozoasida (Allsopp et al. 1994; Radostits et al. 2006). Furthermore, the taxonomical classification of Babesia species was based on the phylogenetic analysis of 18s rRNA (Criado-Fornelio et al. 2003). Babesiosis in bovine is caused by several species of babesia i.e., *B. bovis*, *B. bigemina*, and *B. divergens* are the three most prevalent pathogenic species (Kaandorp 2004; Radostits et al. 2007; Fakhar et al. 2012). *B. bovis* infection can result in more serious illness than *B. bigemina* (Gubbels et al. 1999). The parasite *B. bovis* is located in the core of the RBCs. Its dimensions are 1.1-1.5 x 0.5-1.0 m. While *B. bigemina* is longer than other species and can be seen in pairs. It has a pear-like form. It is 1-1.5 m wide and 3-3.5 m long (Soulsby 1986; El Sawalhy 1999). According to (Jerram and Willshire 2019) and (Alvarez et al. 2019), *B. divergenis* has a small, thin, and obtuse angle (Fig. 1). Moreover, *B. major*, *B. ovata*, *B. occultaus*, and *B. jakimovi* can also infect the cattle (Menshawy 2020).

Life Cycle of Bovine Babesiosis

All species belonging to the genus *Babesia* have shown same life cycle stages with minor differences. Some species showed transovarial transmission (Babesia spp. sensu stricto) while other may be transmitted through transstadial route (*B. microti*) (Saad et al. 2015). Their life cycle can be completed in three main stages:

- Gametogony: fusion and formation of gametes occur in the gut of the ticks.
- Sporogony: It is asexual reproduction taking place in the salivary gland of tick
- Merogony: It take place in the vertebrates (Fig. 2) (Otify 2011; Abdela and Jilo 2016). Binary fission is the way of multiplication inside the red blood cells, and causing considerable pleomorphism followed by the gametocyte formation. The conjugation of gametocyte take place in the tick gut followed by the multiplication and migration to the different tissues such as salivary glands. Furthermore, the continuous development occurs in the salivary glands. The transovarial transmission may happen at this stage (Gray et al. 2010). The host will be infected when the larvae sucks the blood. The larvae transform in to the nymph after molting which is then converted in to adult. Host may get the

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Fig. 1: Babesia parasites inside red blood cells

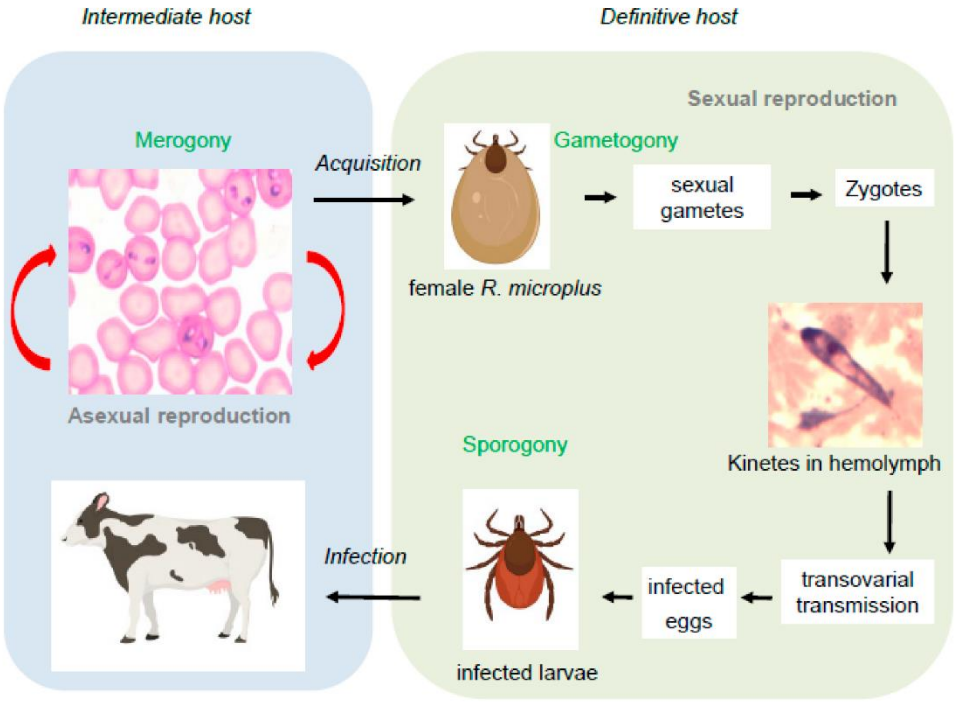

Fig. 2: Babesia Species life cycle (Gallego-Lopez et al. 2019)

infection, when vector takes a blood meal (Uilenberg 2006; Simuunza 2009; Lefevre et al. 2010; Mandal 2012; Schnittger et al. 2012; Ozubek et al. 2020).

Host Range

Out of hundred types of *Babesia* spp., only eighteen species can cause infection in domestic animals (Suarez and Noh 2011). Babesiosis mainly affects cattle, goats, sheep, horses, dogs, cats and human (Hamsho et al. 2015; Gray et al. 2019). *B. bovis* and *B. bigemina* have recently been detected in deer. The primary host for *Babesia* spp. is cattle while all other animals are considered of little epidemiological distribution (CFSPH, 2008).

Geographic Distribution

Babesiosis in cattle is present across the world due to presence of vector. However, tropical and subtropical locations frequently experience it (CFSPH, 2008). The

highest prevalence of babesiosis is found in areas where ticks vector is present excessively. They are especially important in Australia, Africa, Asia, and the United States. Even while *B. bovis* typically inhabits the same habitats as *B. bigemina*, only a small number of other tick species have the ability to transmit both species. Additionally, the regional distribution of these ticks varies with the area. For instance, the two tick species can serve as a biological vector, *B. bigemina* is widely distributed in Africa (Spickler et al. 2010; Pohl 2013).

Risk Factors

Host Factors

Host factors which mainly affect the presence of disease include breed, age and immune status of the animals (Jabbar et al. 2015).

- Regarding the age of the host, the infection rate among young animals is low due to innate resistance, which is boosted by maternal antibodies passed on to calves via colostrum. This resistance gradually deteriorates, leaving the animal vulnerable to disease (Fadly 2012).
- Regarding breed, *Bos taurus* is more susceptible to babesia infection than *Bos indicus* (Radostits et al., 2007). Besides that, native breeds have higher resistance to babesiosis than foreign breeds. Because tick populations have been exposed to nature for a long time, they have developed either an innate ability or an innate resistance to progress a good immune system to the tick (Wodaje et al. 2019).
- In endemic areas, young animals can acquire passive immunity from dams via colostrum and often suffer the transient infections with mild symptoms. This infection is enough to activate active immunity and make the host a carrier for a long time. Active immunity is in charge of the carrier's persistence and premunity. These animals can be infected naturally or through chemotherapy and still have a strong immune system (Taylor et al. 2007). According to susceptibility to *B. bovis* infection, *Bos taurus* were classified into three phenotypes: 1- susceptible animals which may experience clinical signs leading to death, 2- animals having mild clinical signs, and 3- animals that are resistant and having few clinical signs (Benavides and Sacco 2007).

Pathogen Factor

Pathogenicity varies greatly depending on the strain. Because of the wide variety of strains, *B. bovis* is typically more virulent than *B. bigemina* and *B. divergens* (CFSPH 2008). Through rapid antigenic variation, various blood parasites can keep the host immune system alive (Bock et al. 2004).

Environmental Factors

The prevalence of clinical babesiosis can be varied according to seasonal variation, which also influenced by the peak of tick population. The largest prevalence occurring directly after the summit of the population of the tick. Regarding weather conditions, temperature is the most crucial factor affecting on the activity of the tick. Increase in temperature can cause the increase of the disease happenings (Menshawy et al. 2018). Cattle infection reaches the top in the summer season (El Moghazy et al. 2014; El-Bahy et al. 2018). Main economic losses happen in those places where marginal occurrence of disease is present because the population of the tick is mostly variable according to the conditions of environment (Radostits et al. 2007; Demessie and Derso 2015).

Transmission

Babesia species are biologically transmitted by vectors via transovarian transmission (first generation) and transsadial transmission (transmission of infection from egg until the adult) (Demessie and Derso 2015; Enbiyale et al. 2018). Babesiosis can be transmitted to cattle by a biological tick vector (Boophilus spp.). *Boophilus* ticks can transmit both *B. bigemina* and *B. bovis*, with nymphs and adults transmitting *B. bigemina* but only tick larvae transmitting *B. bovis* (Esmaeil et al. 2015). It is also mechanically transmitted by infected needles and syringes, blood transfusion, and surgical instruments (Menshawy 2020). *R. micropuls* (formerly *Boophilus micropuls*) and *R. annulatus* are tick vectors of *B. bigemina* (formerly *Boophilus annulatus*). Competent vectors include *R. decoloratus*, *R. geigyi*, and *R. evertsi*. *R. microplus* and *R. annulatus* are tick vectors of *B. bovis*, and *R. geigyi* can also act as its competent vector (Bock et al. 2004; De Vos and Potgieter 2004; Yadhav et al. 2015). Transplacental transmission of babesia species in cattle has also been demonstrated (De Vos and Potgieter 2004; Spickler and Anna Rovid 2016).

The Babesia species can develops and distribute throughout the organs of the ticks, infecting the salivary glands or eggs. When infected tick bites a cattle, it transferred the infection to the final host (Government and State agencies bord 2013).

Pathogenesis

There are two principal mechanisms of producing acute disease by babesia which are hemolysis and circulatory disturbance (Carlton and McGavin 1995). Sporozoites enter the host directly after tick bite and infect the erythrocytes. Within the body of the host, sporozoites will then progress into piroplasm inside the infected RBCs. This will produce 2 or 4 daughter cells and they will then leave the host cell to infect other RBCs (Hunfeld et al. 2008). They will invade other erythrocytes and can cause intravascular and extravascular hemolysis (Carlton and McGavin 1995). The rapid division of the parasite in the cells can cause rapid destruction and then haemoglobinaemia, hemoglobinuria, and fever. This can be very acute and cause death in a few days. During this process, the PCV falls to less than 20% and this will cause anemia. Clinical signs can be detected during

the stage of parasitemia. At this stage, up to 45% of the red cells are infected according to Babesia species (Urquhart et al. 1996). Hemolysis also invades the release of many pharmacologically active agents (ex: proteolytic enzyme), which affect microcirculation (vasodilation, increased permeability) leading to hypotension and edema, and affect blood (viscosity, coagulation and adherence) leading to ischemia (congestion and degeneration change in tissue/organ) (Ahmed 2002). The main consequence of the disease is anemia due to hemolysis. The secondary mechanism is electrolyte imbalance. Liver and kidney degeneration are caused by lack of oxygen and perhaps by immune pathologic reaction. The kidney tubule epithelium damage will lead to impair ion exchange, which will result in hydrogen ion retention and cause acidosis (Enbiyale et al. 2018).

Clinical Signs

Incubation period ranges between eight and fifteen days in natural infection. Before the onset of other clinical signs fever (>40°C) usually appears (OIE 2010). The clinical signs are different according to the age and species of the animals, parasite strain, immunological status, concurrent infection with other pathogens, and genetic factors in the dose of the inoculated parasites. Most cases have been detected in animals less than 9 months of age usually staying asymptomatic (Anon 2008).

Babesiosis clinical signs include emaciation, ataxia, loss of appetite, stop rumination, loss of body weight, progressive hemolytic anemia, jaundice (Icterus), yellowish color of conjunctival as well as vaginal mucous membranes in more advanced cases; hemoglobinuria, problems in the heart and respiratory rates, and a decrease in milk yield. In some cases, fever during an infection causes abortion in cattle. Patients experience general circulatory shock and, in some cases, nervous symptoms due to the sequestration of the infected RBCs in cerebral capillaries (Zintl et al. 2003; Khan et al. 2004; Akande et al. 2010; Chaudhry et al. 2010; Rashid et al. 2010; Terkawi et al. 2011; Onoja et al. 2013; El Moghazy et al. 2014; Bhat et al. 2015; Masih et al. 2021).

Dark red urine is one of the clinical signs of babesia (Yadav et al. 2004). The main clinical signs of *B. bigemina* are fever, hemoglobinuria, and anemia (Zintl et al. 2013).

Diagnosis

Detection of active cases of babesiosis is based mainly on several diagnostic techniques as follow:

Microscopic Examination

The conventional model of babesiosis examination is a direct examination under a microscope. It is used to identify the agent in the infected host. This is accomplished by examining thick and thin films and then staining them with Giemsa or Romanowsky stain. Thick films can detect parasites as few as one parasite out of 106 RBCs (Kahn 2005). Microscopic examination is still the most cost-effective and time-efficient technique for identifying Babesia parasites (Hamoda et al. 2014). Giemsa-stained thin blood smears are the traditional and gold standard for identification (Nayel et al. 2012) and serve as an ideal method for species differentiation. It is adequate for detecting acute infections but has lower effects in cases of low parasitemia in carriers (Criado-Fornelio et al. 2009; Bal et al. 2016; Shang et al. 2016; Masih et al. 2021).

Serological Examinations

To detect antibodies in subclinical cases and avoid the drawbacks of microscopic examination, the Indirect Fluorescent Antibody Test (IFAT) and Enzyme-Linked Immunosorbent Assay (ELISA) are used (El-Fayomy et al. 2013). These tests have low sensitivity and frequently fail to distinguish between chronic and acute infections (Mahmoud et al. 2016). These tests produce false-positive and false-negative results due to cross-reactive antibodies (Esmaeil et al. 2015). Another point to consider is that antibodies persist even months after infection, implying that no active infection exists. As a result, these will be unable to reveal the precise prevalence at a given time (Abdel Aziz et al. 2014). The most common test for detecting antibodies in babesia species is IFAT (Chaudhry et al. 2010). Anonymous (2008) described a complement fixation (CF) test for detecting antibodies to *B. bovis* and *B.bigemina*.

Molecular Diagnosis

Molecular diagnosis is used to identify nucleic acids which is considered as an indirect identification. However, both sensitivity and specificity are very high (Mosqueda et al. 2012). The most sensitive and specific technique for the detection of babesiosis is (PCR) Polymerase chain reaction (Vannier and Krause 2009; AbouLaila et al. 2010) and useful for the detection of infection in the early stage. It has been reported that the PCR technique is much more sensitive than microscopy for the identification of babesiosis. It is an important test for confirmation in some cases for regulatory testing (Shams et al. 2013; Sharma et al. 2016; Bal et al. 2016).

Differential Diagnosis

Like many other infectious diseases, babesiosis also causes fever and anemia. Anaplasmosis, theileriosis, trypanosomiasis, leptospirosis, rapeseed poisoning, and chronic copper poisoning can be counted as a differential diagnosis of babesiosis. Rabies and other encephalitis's can also be considered in cattle with CNS signs (Spickler and Anna Rovid 2016).

Treatment

The successful treatment of babesiosis is dependent on the use of effective drugs and early detection (Vial and Gorenflot 2006). Trypan blue, which was first used against *B. bigemina* but has no effect on *B. bovis*, was one of the most effective drugs used to treat bovine babesiosis. It is rarely used because it discolors the flesh of animals. In the tropics, diminazene aceturate is currently used as a babesiacide. It has been withdrawn from the market in Europe for marketing reasons (Sayin et al. 1997). Imidocarb, which is primarily used in animals, is another effective drug for treating babesiosis. This drug can also be used to prevent babesiosis and anaplasmosis. Imidocarb can linger in tissues for a long time (Hashem et al. 2018) However, acridine and quinuronium derivatives can be used as effective drugs as well. Many European countries used the babesiacides quinuronium sulfate, amicarbalide, diminazene aceturate, and imidocarb diproprionate against bovine babesiosis for several years, but quinuronium sulfate and amicarbilide were withdrawn due to manufacturing safety issues (Vial and Gorenflot 2006). The combination of imidocarb dipropionate and oxytetracycline is the most effective treatment for Babesiosis in small ruminants (Ijaz et al. 2013). Beside this, in severe cases, supportive therapy is also required (Zintl et al. 2013). Vitamin E can also be used as a supportive therapy because it reduces the oxidative effect of babesia by increasing antioxidant activity (Abdel Hamid et al. 2014).

Prevention and Control

In the world, several countries have not completely controlled bovine babesiosis, despite the availability of live attenuated vaccine (De Vos and Bock 2000; Florin-Christensen et al. 2014). This can confirm the quick action for crucial vaccines to prevent the development of acute disease as well as parasite distribution into non-endemic areas. Bovine babesiosis control is currently under threat because of climate changes that act on vector development and expansion (Dantas-Torres 2015; Sonenshine 2018).

Control of this disease is created by accurate diagnosis, perfect treatment, and prevention of babesiosis (Mylonakis 2001). Animals after recovering from infection remain immunized. The parasite can persist in the peripheral blood for several years in *B. bovis* cases and for many months in *B. bigemina*, and no signs are apparent during this carrier state, so the animal should be monitored and treated after infection to prevent the distribution of disease to other animals (El Sawalhy 1999). Prevention and control of babesiosis can actively be maintained by the following methods: immunization, chemoprophylaxis, and vector control (Suarez and Noh 2011; ILRAD 1991). The combination of these three methods is also a choice. Tick control by vaccination has been stated as a useful way in Australia (Lightowlers 2013). A research has reported that using combined chemotherapeutics is more effective for parasite elimination and results in decreasing the risk of drug resistance (Pritchard et al. 2013). The advantages of mixing of the chemotherapeutics include highly effectiveness, reduction in the dose (which may lead to reduced side effects) and lowering of drug resistance. According to the US reports, Babesiosis can be controlled and eradicated by eliminating the host tick(s). This will be done by using acaricides every two to three weeks. In those countries where eradication is not applicable, tick control can reduce the incidence of disease (APHIS 2010). Chemotherapy is another important method for controlling bovine babesiosis, either in the field or to control artificially induced infections. Chemotherapy is critical in some parts of the world to eradicate and prevent babesiosis. Infected animals should be treated with antiparasitic drugs as soon as possible in countries where the disease is endemic. The success of disease treatment is dependent on early diagnosis and proper administration of the drug of choice (Fernandez and White 2010; Georgiou et al. 2015). Use of living attenuated vaccine is the cornerstone to control and prevent babesiosis in many countries like Argentina, Israel, and Australia. However, this live vaccine is not cheap to produce and has many limitations (Brown et al. 2006; Florin-Christensen et al. 2014; Costamagna et al. 2016; Aranda et al. 2017; Suarez et al. 2018). Vaccines are provided in frozen form. Live babesia vaccines are not completely safe. A single dose can immunize animals against babesiosis over life (Saad et al. 2015).

Immunization of the animals in a prophylactic way has been stated as the most efficient way to decrease losses happened by bovine babesiosis. Live attenuated vaccine from the *B. bovis* or *B. bigemina* strain is used to immunize cattle in many countries. These vaccines are important due to having safety issues such as the potential effect for virulence in adult animals, contamination possibly occurring with other etiological agents, and blood protein hypersensitivity reactions (OIE 2015).

Conclusion

Babesiosis is a severe disease not only in cattle and other domestic and wild animals but also in human beings. It has significant impacts on both the economic and medical processes. It can cause impairment in the trade of animal products such as milk, meat, and hide by decreasing their quality. It has been reported that imidocarb and diminazene aceturate used as a treatment of babesiosis for many years, but nowadays, several compounds are progressed and assessed as a treatment. This can offer a good point for disease control. Controlling tick-borne diseases is important in developing livestock health services products. Control strategies can be different from country to country and place to place and the most important ones are vaccines and drugs.

Recommendations

- Knowledge, as well as awareness, should be given to the owners about the transmission way, prevention, and control of babesia.
- Governments and organizations should give attention to control and eradicate babesiosis in order to improve the economy.
- The surveillance system is important in Kurdistan Region to prevent bovine babesiosis.

New drugs and vaccines should be developed to eradicate the carrier states.

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19

Hymenolepiasis

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INTRODUCTION

Two cestodes species are known for producing hymenolepiasis in human beings, namely, *Hymenolepis diminuta* and *H. nana*. Out of these, *H. nana* is the main culprit that affects humans worldwide in most cases. It especially affects the children living in areas with lower hygiene standards. *H. nana* infections are frequent in countries with mild and tropical weather. They are usually asymptomatic, whereas heavy infections can present many gastrointestinal symptoms and allergic responses. *H. nana* carries out a monoxenic life cycle with a single final host, which can be a man, mice, or rats. Also, this cestode can be carried out in a heteroxenic cycle in which an arthropod is involved (Kim et al. 2014; Cabeza et al. 2015; Panty et al. 2017).

H. diminuta, on the other hand, mainly affects rodents mostly, though it may also infest humans by chance. It is one of the non-invasive parasites as it lacks the tapeworm scolex hooks that injuriously invade the host body. Despite this non-invasive behavior, it is still a threat to the host as its metabolic secretions hinder the normal functioning of the host's alimentary tract. *H. diminuta* carries out only an heteroxenic cycle. This is a zoonotic cestode parasitizing the small intestine of rodents (definitive hosts). Humans can become unintentionally intermingled in cestodes life cycle upon ingestion of insects infested with infective parasites (Kapczuk et al. 2018; Panty et al. 2020).

Etiological Agents

Almost all cestodes, or tapeworms (class Cestoda in the phylum Platyhelminthes), are parasitic as adults in the intestinal tract of vertebrates. They are bilaterally symmetric, usually flattened dorsoventrally, and lack a body cavity (Smyth 1994). The cestodes are broadly classified as pseudophyllidean and cyclophyllidean cestodes. *Hymenolepis* species (spp.) fall into the cyclophyllidean group, which is characterized by the presence of four cup-like structures in the scolex/head called suckers. The suckers are either armed (presence of hook-like structures) or unarmed (no hooks). *Hymenolepis* spp. is armed with the presence of a single round of hooks around the suckers (Kandi et al. 2019).

The disease known as Hymenolepiasis in humans is produced by the infection with either of two parasitic cestode species: *H. nana* or *H. diminuta*. *H. nana* adult size 15 to 40 mm in length. The second one is also known as the rat tapeworm and the adults measure 20 to 60 cm in length (Fig. 1,2) (Al-Olayan et al. 2020).

The scolex of *H. nana* bears a retractable rostellum, armed with a single circle of 20 to 30 hooks (Fig. 2). The neck is long and slender, and the proglottids are wider than they are long. Genital pores are unilateral; each mature segment contains three testes. Gravid segments break off from the strobila and disintegrate, releasing eggs 30 to 47 µm in diameter. The oncosphere is covered with a thin hyaline outer membrane and an inner thick membrane, with polar thickenings that bear several hair-like filaments embedded in the inner membrane (Schantz, 1996).

The body of *H. diminuta* has three sections of it is body: a scolex also called the head, neck, and a strobilus. It has four suckers and at scolex, it has an apical organ, but it does not have rostellar hooks. In both male and female sexual organs, the strobilus is detached into a proglottid (Arai 1980; Deines et al. 1999; Pappas, 2000).

Life Cycle *of Hymenolepis* spp.

The *Hymenolepis* spp. has two types of life cycles, direct and indirect life cycle (Fig. 3). In the case of humans, the source of infection is the ingestion of food contaminated with embryonated eggs of parasites and water which is contaminated with feces. Inside the human, which is a definitive host, the parasite followed the direct life cycle for its propagation (Ito and Budke 2021). Upon arrival in the stomach, the eggs which are in the infective phase hatch due to the action of gastric and biliary juices which soften the walls of the egg and result in the release of oncospheres.

Citation: Aguilar-Marcelino L, Aguilar-Figueroa BR, Oropeza-Guzmán G, Mendoza-Galvez B, Bautista-Garfias CR and Viladomat GRC, 2023. Hymenolepiasis. In: Aguilar-Marcelino L, Younus M, Khan A, Saeed NM and Abbas RZ (eds), One Health Triad, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. 3, pp: 122-127. https://doi.org/10.47278/book.oht/2023.86

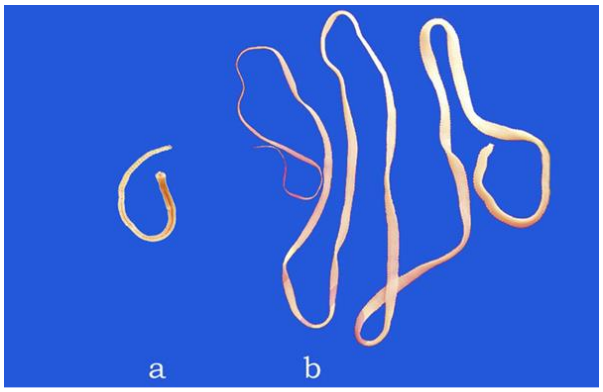

Fig. 1: Comparative size between *Hymenolepis nana* (a) and *H. diminuta* (b) (Composition by Carlos R. Bautista-Garfias).

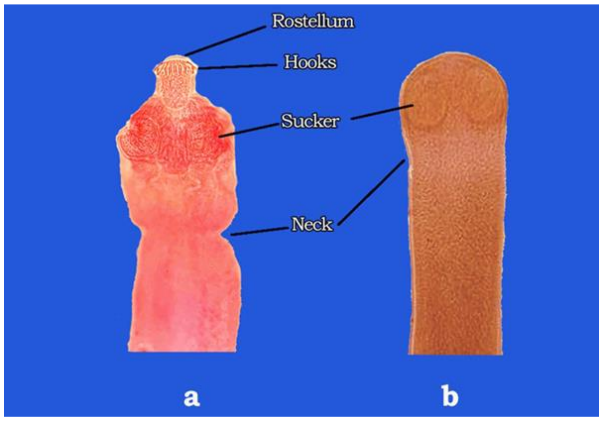

Fig. 2: Adults: (a) *Hymenolepis nana*, and (b) *H. diminuta* (Composition by Carlos R. Bautista-Garfias).

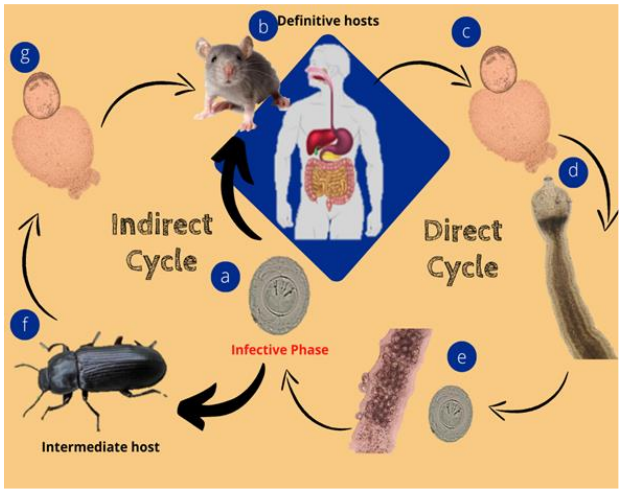

Fig. 3: *Hymenolepis* spp. life cycle. (a) Embryonated egg in the external environment (b) Definitive hosts: human and rodents. (c) Cysticercoid larvae develop in small intestine microvilli. (d) The adult phase develops in the ileum. (e)Eggs released from gravid proglottids (f) Arthropod intermediate host: *Tenebrio*. (g) Cysticercoid larvae develop in insects (Composition by Belén Mendoza-Galves).

The oncospheres once released start penetrating microvilli of the small intestine (doubtful). On the fifth day of the life cycle, the oncosphere is now a cysticercoid larva that is able to move through the jejunum and ileum and transformed into the adult phase. The gravid proglottids are now detached and release eggs that infect other or the same host through feces (Gutierrez and Ruiz 2014).

The indirect cycle requires two hosts to complete the cycle (the definitive host and the intermediate host). This occurs mainly in rodents and occasionally in humans by accidental ingestion of coprophagous arthropods (Galán-Puchades 2015), more commonly flour beetles, belonging to the genera *Tenebrio* and *Tribolium*, as well as flea larvae such as *Xenopsylla cheopis*, *Ctenocephallides canis*, and *Pulex irritans* which are intermediate hosts, these, in turn, have been infected by feeding on fecal matter containing the eggs of *Hymenolepis* spp., harboring the cysticercoid larvae stage, which settles in the hemocoel of the insect until it is ingested by its host definitive, where the cysticercoid larvae are released, migrates to the ileum and settles to complete its adult stage (Al-Mekhlafi 2020).

The host can get the infection through autoinfection, in which the eggs are not passed through the feces and grow into the adult phase inside the same host intestine. Only those people who get infected through this mechanism have slow intestinal movements which give parasites a long period to stay in the body (Galan-Puchades 2015).

Diagnosis

The diagnosis of hymenolepiasis may be: clinical, parasitological, or molecular, although the first after it has been carried out by an experienced medical practitioner, requires a confirmative laboratory test. It has to bear in mind that the majority of infections are asymptomatic.

Clinical

It is based on clinical signs such as crampy abdominal pain, diarrhea, anorexia, and anal pruritus. The affected person may also exhibit dizziness, irritability, sleep disturbance, and seizures (Kandi et al. 2019).

Parasitological

Eggs in fecal samples can be identified by performing a microscopic examination of the sample (Galos et al. 2022). A simple test tube floatation technique (FLOTAC) is a reliable qualitative test reliable method for copro-diagnostic purposes and can be effectively performed to detect the presence of nematode and cestode eggs of *H. diminuta* and *H. nana*. *H. nana* infection can be differentially diagnosed by measuring 30 × 47 µm in diameter parasite eggs. These eggs when observed in stool slide appear to have double membranes. On the other hand, *H. diminuta* eggs are measured to be 70 × 80 µm in diameter (Fig. 4) (Steinmann et al. 2012).

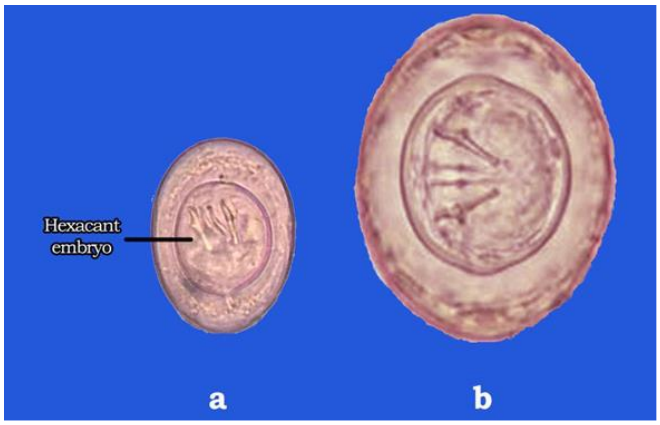

Fig. 4: Eggs: (a) *Hymenolepis nana* and (b) *H. diminuta*. (Composition by Carlos R. Bautista-Garfias).

Molecular

Sharma et al. (2016) carried out Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) studies of the nuclear ribosomal internal transcribed spacer 2 (rDNA-ITS2) gene markers. The researchers found that both *H. nana* and *H. diminuta* displayed distinct restriction patterns when digested with one of the enzymes namely RsaI, HaeIII, or HhaI. The annotated rDNA-ITS2 sequences from the two species turned out to be different in the length; a clear demarcation was also seen between the secondary folded structures of the two species along with length difference in helices. The pyrimidine-pyrimidine mismatches and sites of motifs occurrence were also found to be varying. Yang et al. (2017) got the molecular diagnosis of *H. nana* and *H. diminuta*, evaluated in rats by amplification of the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal RNA gene and the mitochondrial cytochrome C oxidase subunit 1 (COX1) gene, through PCR.

Epidemiology

With an estimated 50-75 million human carriers worldwide *H. nana* and *H. diminuta* probably are the most common cestode parasites of humans. Afghanistan, Argentine, Africa, Asia, Australia, Central and South America, India, Italy, Spain, Mexico, North America, and southern and eastern Europe are the endemic areas for these cestodes with prevalence rates going from as low as 1% to as high as 30%. The institutionalized populations are most prone to infections with prevalence rates reaching up to 8% in patients suffering from immunity or nutritional issues. In children, *H. nana* can have a prevalence of 5–25%. In contrast to this *H. diminuta* is distributed multi-ethnically and its prevalence in some parts of India is found to be up to 1%. Children that are exposed to rodents and stored cereals or grains have the highest chances of getting infected (Besedina 1970; Buscher and Haley 1972; Ghadirian, 1972; Cabeza et al. 2005; Guerrant et al. 2011; Burton et al. 2013; Mega et al. 2013; Abrar et al. 2015; Cabada et al. 2016; Cabada et al. 2017; Bennet et al. 2020). Throughout the Northern Territory of Australia, *H. nana* remains endemic, predominantly infecting Indigenous children less than 5 years of age (Hamid et al. 2015).

Panti-May et al. (2020) to get a more accurate estimate of human cases that got infected with *H. diminuta*, a literature review of published records was conducted. This review was from the literature about human infection with *H. diminuta*. An overview explaining human infections with this parasite. From an exhaustive list of 80 countries, one thousand five hundred and sixty-one published records of infection with *H. diminuta* were identified. The review displays an estimated number of *H. diminuta* infection cases in humans with an overview of the current prevalence rate, symptoms, geographic distribution, diagnosis of the disease, mechanism of exposure to infective stages, and approaches for the treatment of this underestimated tapeworm with zoonotic potential (Nasir et al. 2004).

Panti-May et al. (2020) with the aim to describe the role of rodents as a potential zoonotic source of infection, conducted a morphological and molecular survey on cestodes in rural "Paraiso" and "Xkalakdzonot" villages from Yucatan, Mexico. *H. nana* infected to 7.8% of children from Paraíso, *H. microstoma* was isolated in 4.4% of *Mus musculus* from Paraíso, and *H. diminuta* in 15.3% of *Rattus rattus* from Xkalakdzonot villages (Goudarzi et al. 2021).

Parasitic infection is a major health issue that affects humans in developing countries. (Kheirandish et al. 2014) in a study people working as staff in fast food shops, roast meat outlets, and restaurants of Khorramabad and southeast of Kerman provinces (Western Iran), people were selected and then checked for the infestation of parasites. The percentage of intestinal parasites found in this study is as follows: *Giardia lamblia* 2.9%, *Entamoeba coli* 4.3%, *Blastocystis* sp. 1.4%, and *H. nana* 0.5%-2.5% (Willcocks et al. 2002; Kheirandish et al. 2014; Panti-May et al. 2020a; Panti-May et al. 2020b). *H. nana* (2.4%) a helminthic parasite is found as the most common parasite of the intestine in a study of the southeast of Kerman province southeastern Iran. Many authors logistic regression proved that *Hymenolepis* is associated with parasitic intestinal infections which spread through drinking water and residential status (rural/urban) (Daryani et al. 2015; Sadeghi et al. 2019; Khojasteh et al. 2021). Near the southeastern coast of continental North America, wild animals have been found for specimens of *Peromyscus polionotus* and species *hymenolepidid* have been found in the old field mouse and these cestodes are attributed to *Hymenolepis*. *H. folkertsi* n. sp. It belongs to a diverse genera *Peromyscus* which has 56 unique species in the Nearctic. Recent research and evidence show that the diversity of tapeworms is due to the huge variety of hosts including small rodents of the family Cricetidae, murid, and geomyid in sympatry (Abbaszadeh et al. 2020). The distribution of Hymenolepiasis around the world is shown in Fig. 5.

Fig. 5: The worldwide *Hymenolepiasis* distribution. (Composition by Germán R. Colmenares Viladomat).
Regions with Hymenolepiasis Regions free

Factors Involved in the Transmission of *Hymenolepis* spp.

With no requirement for an intermediate host in its life cycle makes *H. nana* one of its kind and unique cestode. Both man and rodents can act as final and intermediate hosts simultaneously for this parasite. Some arthropods can also serve intermediate hosts including fleas and grain beetles. The eggs present in the contaminated hands, fomites, soil, water, and food can serve as a source of infection in humans if ingested. This is the reason for the high prevalence of these parasites in populations with low hygiene standards and a high number of rodents. Sometimes the accidental ingestion of insects containing this parasite can also lead to the transmission of infection to humans. The factors like the seasons of the year or the socio-economic conditions may favor the transmission of *H. nana*. Lack of hygiene plays a vital role in spreading infection. The precarious housing conditions and the presence of animal feces in public parks. Most children are affected due to the lack of good hygiene habits. Consuming unwashed and dirty vegetables or fruits is also a factor that supports the spread of infection. It is a well-known fact that vegetables are irrigated with sewage making them a suspect of harboring parasitic agents (Loján-Neira et al. 2017; Chitsaz et al. 2018; Murillo-Zavala et al. 2018).
H. diminuta causes disease in humans less frequently than it does in animals. Various larvae and adult insects are susceptible to infection with this parasite. The ingestion of this parasite's eggs by insects (e.g. flour beetles or larvae of fleas) leads to the formation of the cysticercoid larva inside their body cavity. Humans may have a chance of infection if they orally consume these larvae along with raw or undercooked insects that were already infested. Oral ingestion is the only route of transmission. The prevalence of this parasite mainly occurs in individuals living in areas with lower hygiene standards, the presence of rodents in living premises, and a history of careless behavior with animals. It is especially important in areas where insects are commonly consumed as food (Martínez-Barbabosa et al. 2012; Melhorn 2016).

Control

The use of oral praziquantel (single dose of 20-25 mg/kg for children as well as adults) is the most common way of treatment. After using the drug, a copro-parasitoscopic follow-up is done after 3 weeks. Besides this nitazoxanide is used as an alternative treatment if the parasitosis is 82% (Apt 2013).
The drug praziquantel works by increasing the permeability of the tegument of the helminth resulting in the rupture of the tegument and death of the helminth occurred (Cruz and Camargo 2001). While the mechanism of action of nitazoxanide is the inhibition of tubulin, which causes destabilization in the tubulin-microtubule balance, thus causing the parasite to lose cell homeostasis and thus detachment and death (Scarcella et al. 2007).
The side effects that praziquantel generates in greater proportion are headache, abdominal pain, nausea, dizziness, drowsiness, and rarely fever, hives, and seizures, for which research has been carried out in the search for an alternative treatment for the elimination of the parasite reducing the adverse effects or that they do not alter the daily life of the patient (Pabón 2014).

Alternatives for Controlling Hymenolepiasis

The use of medicinal plants like spice and culinary herb "cinnamon" is an alternative method to control hymenolepiasis. Different studies reveal that the bark of cinnamon has organic extracts, proanthocyanidin tannins, and trans-cinnamaldehyde. Since this knowledge is not enough and further studies are required on the antiparasitic properties of *Cinnamonum* spp. and some action of this plant is shown in some infections of cestodes (Castañeda-Ramírez et al. 2020).
On the other hand, the use of extracts from edible mushrooms for medicinal purposes has become more evident today and they have been shown to help reduce or eliminate the number of parasites in certain infections. A study by Velazco-Cruz (2017), evaluated the hydroalcoholic extract of the edible mushroom *Pleurotus ostreatus* in rodents infected with *H. diminuta*. The (ECS-1123) strain of the edible mushroom *P. ostreatus* was obtained from the mycological strain collection of the Tropical Fungi Laboratory (Colegio de la Frontera Sur located in Tapachula, Chiapas, Mexico) under the prior authorization of Dr José E. Sánchez. The hydroalcoholic extract was obtained by the maceration method, it was administered orally to a batch of rats and its activity was evaluated at the egg and adult levels. Obtaining a reduction at the egg level of 29.8% and 67.56% at the adult level at a concentration of 8 mg/mL.

Conclusion

H. nana is the main culprit that causes hymenolepiasis in humans across the world. It especially affects the children living in areas with lower hygiene standards. It is important

to emphasize having a three-party vision of a global one-health triad that lets humans, animals, and the environment join forces to understand their interrelationship in maintaining the ecosystem. The concept of "One Health" presented an idea that was already known to man for more than a century, human and animal well-being are interdependent and connected to the health of the ecosystems in which they co-exist. We accepted and applied this approach as a collaborative goal of the global effort to understand the risks faced by human and animal health as well as the well-being of the ecosystem as a whole unit.

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20

Lyme Disease and Relapsing Fever

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INTRODUCTION

Lyme disease is a prevalent tick-borne infection in the United States (Roberts et al. 1998). Lyme disease and relapsing fever are caused by various species of genus *Borrelia* causing different pathological problems. The causative agent for Lyme disease is a spirochete bacterium called *Borrelia* (*B.*) *Burgdorferi* (*sensu lato*) strain, while relapsing fever is caused by Relapsing Fever *Borrelia* (RFB), which is a spiral-shaped bacterium (IGeneX Inc 2015). Lyme disease infection is transmitted by tick *Ixodes* (*I.*) *Ricinus.* The most common tick born disease in Europe is Lyme Borreliosis. Spirochetes do not have any effect in the transmission of the disease to humans even though they have been isolated from mosquitoes, flies and fleas. In Europe, deer and rodents serve as the key reservoir for *B. Burgdorferi* on which *I. Ricinus* ticks usually prey (Stańczak et al. 1999). Almost 11 known genostrains of genus *Borrelia* are considered to be pathogenic. The clinical signs of relapsing fever are similar as that of Lyme disease and caused by a species of *Borrelia* called Relapsing Fever *Borrelia* (RFB). Three Lyme disease stages are known; early localized, early dissemination, and late. Erythema migrans which is a red ring-shaped rash at the site of tick bite is the sign for early localized disease (Cervantes 2018). Early localized symptoms might include flu, headache, fever, malaise, myalgia, and arthralgia (Bransfield 2018). Disseminated stage has symptoms similar to early stage, with the most common symptom of several lesions of erythema migrans, flu, lymphadenopathy, arthralgia, myalgia, ophthalmic conditions, lymphocytic meningitis, and palsies of the cranial nerves (Bransfield 2018). Arthritis is the most common pathological condition caused by these pathogens that affects large knees and joints (Arvikar and Steere 2015). The diagnosis of the disease with clinical signs and symptoms are difficult because the signs are not specific (Shapiro 1995). Lyme- disease can be diagnosed by exposure to the bites of ticks, typical signs, serological tests for anti-Bb antibodies and physical findings (Murray and Shapiro 2010). The treatment of the disease includes the use of antimicrobial drugs depending on the age of the patient and the stage of the disease (Antony 2018).

Etiology

Lyme disease or Lyme borreliosis is a vector-borne disease caused by different species of spirochete bacteria known as *B. Burgdorferi sensu lato,* which is transferred by the infected tick bite (Stanek et al. 2012). Different species of ticks transfer the disease, with the *I. ricinus* being the most common vector of the disease (Stańczak et al. 1999). It is a gram-negative, spiral-shaped, slowly growing, micro aerobic, spiral-shaped bacterium. The cells of the bacteria divide about every 12-24 hours (Żarnowska and Prymek 1995; Zajkowska 2005; Oliveira et al. 2010). Among 11 genospecies that are transferred by ticks and affect wild animals, 3 species can infect humans, including *B. burgdorferi sensu stricto, B. garinii, and B. afzelii*, mostly prevalent in European countries. *B. burgdorferi sensu stricto* also exist in North America, while, *B. garinii, B. afzelii, B. bissettii, B. valaisiana* and *B. lusitaniae* appear in the Asian countries which are pathogenic to humans (Aguero-Rosenfeld et al. 2005). The main reason for different clinical manifestations of Lyme disease in Europe and United States is the presence of different spirochete genospecies in these two continents (Wang et al. 1999). Different genospecies of Borrelia attacks different organs and body parts. *B. burgdorferi sensu stricto* affects the joints and causes Lyme arthritis. *B. garinii* is responsible for neuroborreliosis, and *B. afzelli* causes limb dermatitis (Zajkowska 2008).

Epidemiology

Lyme borreliosis is a tick borne and endemic disease in North Asia, Europe and North America (Owecki and Kozubski 2007). The disease is prevalent in areas with high forested geographies including Scandinavia, Germany, Slovenia and Austria (Rydz-Stryczewska 2007). Australia, Africa, South America and southern states of the United States are considered as free from lyme disease (Owecki and Kozubski 2007). Northeastern and upper Midwestern region of the United States is the most common places in the North America for the occurrence of Lyme disease (Berry et al. 2017). Fig. 1 shows the distribution of Lyme disease due to distribution of the *Ixodes* ticks, primarily *I. scapularis* that transmit the causative agent of Lyme disease in the United States (Murray and Shapiro 2010).

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Fig. 1: Reported Cases of Lyme disease -- United States, 2019 (Centers for Disease Control andPrevention 2019).

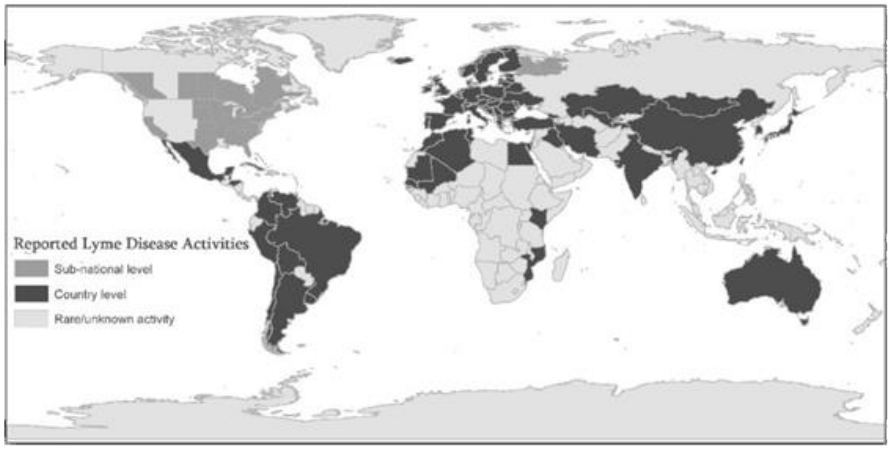

Fig. 2: Geographic Extension of Lyme Disease activities (Ozdenerol 2015).

Lyme disease has also extended into many countries worldwide beyond the endemic foci. Fig. 2 shows Lyme disease activities around the world, which include diagnosed cases of the disease, presence of infected ticks, infected animals, and positive human blood samples for *Borrelia* (Ozdenerol 2015).

Molecular Biology

Immunological study of *B. burgdorferi* (North American strains) shows two surface proteins including outer surface protein A (OspA, 30 to 32 kD) and outer surface protein B (OspB, 34 to 36 kD) (Karami 2012). Like flagellar antigens, the 41-kD antigen is also found in the flagellum. Nowadays, all the isolates have 4 to 9 pieces of extrachromosomal plasmid DNA. Protein may code by plasmid which are crucial for the pathogenicity since the loss of infection of the isolates are abundantly distributed in the laboratory. Thus, they have a relation with the loss of specific plasmid in culture (Barthold et al. 2010). In recent studies, it has been found that similar to relapsing fever, *B. burgdorferi* can differ its antigenicity using different methods and genome modifications (Barbour 1991). Borrelia cells have an average size of 0.2 to 0.5 µm by 4 to 18 µm. The flagella which are periplasmic in nature and have an origination form either end of the spirochete and wind around the protoplasmic cylinder, giving the shape and motility to the organism, in contrast to the peptidoglycan layer that shaped the other bacterial organism (Fig. 3) (Karami 2012). The flagella role is established by inactivation of a gene known as flab that encodes the flagellar protein, filament protein (FlaB) (Karami 2012). The bacterium produced do not have periplasmic flagella and are rod-shaped and non-motile. Alternatively, the motility of bacteria which have external flagella is hindered in viscous substances (Groshong and Blevins 2014).

Pathogenesis of *B. Burgdorferi*

The pathogenicity of *B. burgdorferi* depends on several factors, including the spirochete's motility, cytotoxicity, lymphocyte stimulation and spirochetes resistance to activate completely in the specific antibodies (Sobieszczańska 1994). *B. burgdorferi* can be transferred from the infection site to various parts of the body through blood, lymph and by peripheral nerves. As the tick-bites are the main sources of infection, the inflammatory symptoms are getting visible

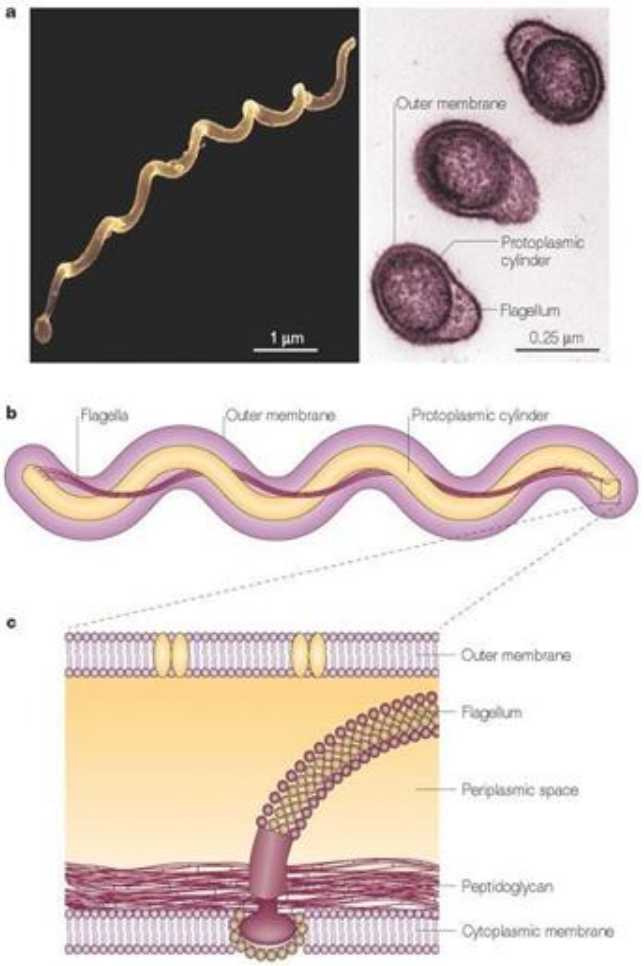

Fig. 3: Structure and morphology of *B. burgdorferi* (Rosa et al. 2005).

more quickly at the site of bite which is an indication that dissemination is more effective in tissues than blood (Fig. 4) (Zajkowska et al. 2000; Zajkowska and Hermanowska–Szpakowicz 2002). *B. burgdorferi* spirochetes can connect to endothelial cells and cross the endothelial layer into the extracellular matrix. The bacteria hide from the defense mechanism of the host as well as antibiotics by localization in the extracellular matrix, utilization of fibrocytes and B-lymphocytes (Zajkowska et al. 2000). The bacteria show tropism to the connective tissue of the heart, synovial membrane, vascular endothelium and to tendon and ligament attachments (Grzesik et al. 2004). Superficial outer surface proteins play an important role in the survival of the bacteria which protect membranous proteins against the action of antibodies (Zajkowska and Hermanowska–Szpakowicz 2002). Bb spirochetes are capable of modifying both cellular and humoral immunological response, and are able to decrease the phagocytotic action of the host. The bacteria disturb cytokines and antibody secretions by aggregation with tissue proteins and fibroblasts. *B. burgdorferi* might attack and destroy T and B lymphocytes (Zajkowska et al. 2000). Complement system can be activated by classical or alternative pathway after the attack of bacteria on the host, while the action of antibacterial is only activated in the existence of specific anti-B antibodies. Microbial adherence might happen independently in the presence of antibodies (Tuchocka 2002). In the Lyme disease pathogenesis, spirochetes fusions with glycosaminoglycans, heparin and heparan sulfate will be able to fuse spirochetes with endothelium. Moreover, decorin which is skin proteoglycan can be figure out by bacterial lipoproteins (Grzesik et al. 2004).

Clinical Manifestation and Infection Course

Chronic Lyme disease has a diverse clinical picture (Rolla–Szczepańska 2007). This disease can be divided into three main stages; early localized, early disseminated, and late stage (Fig. 5) (Tylewska–Wierzbanowska et al. 2008). Early localized Lyme disease is characterized by an expanding, circular red rash known as erythema migrans (EM) which appears around 1 to 28 days after tick exposure in endemic areas (Flisiak and Pancewicz 2008). The second stage which usually develops around 3 to 12 weeks after infection. General malaise, fever, neurological feature such as head ache and cardiac symptoms like chest pain, palpitations and dyspnea are the general features of early disseminated stage (Muhammad and Simonelli 2018). Late Lyme disease appears months or years after infection. The typical characteristics of late stage of the disease include neurological and rheumatological involvements (Yeung and Baranchuk 2018).

Erythema Migrans (EM)

Erythema migrans occurs in nearly 60% of the infected individuals regardless of the age and sex. EM is an oval, red or blue rash that appears at the site of tick bite (Fig. 6). A few weeks after the tick bite, EM starts to increase in diameter. The maximum diameter that EM might reach is as large as 70 centimeters (Nau et al. 2009). Usually, erythema migrans stays for several weeks and then disappears, and this does not mean the eradication of rashes (Flisiak and Pancewicz 2008). Numerous EM appear rarely, and is an indication for the dissemination of the infection. Erythema migrans skin changes might be accompanied by systemic signs such as fever, muscle and joint pains, headaches, meningeal signs and lymph nodes enlargement that may be treated as certificate of spirochetemia (Wormser et al. 2006).

Neuroborreliosis

B. burgdorferi can cause disseminated infection and the most popular and severe form is Neuroborreliosis. The cases of neuroborreliosis commonly found in the Europe. Neuroborrelisis usually involve central as well as peripheral nervous system. It might be caused by all three species of *B. burgdorferi*. *B. garinii* are mostly isolated from cerebrospinal

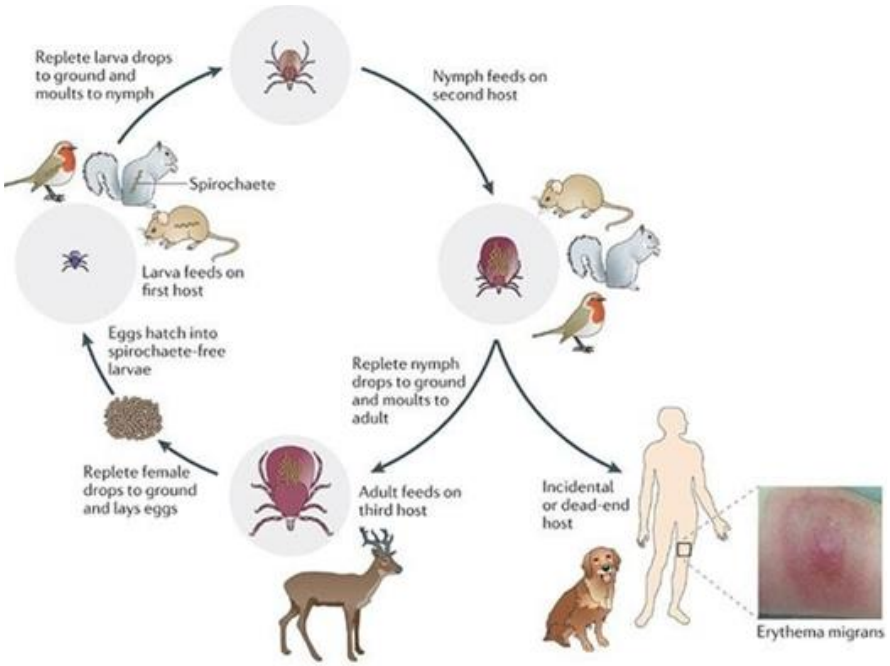

Fig. 4: Borrelia burgdorferi life cycle and transmission from tick to the final host (Radolf et al. 2012)

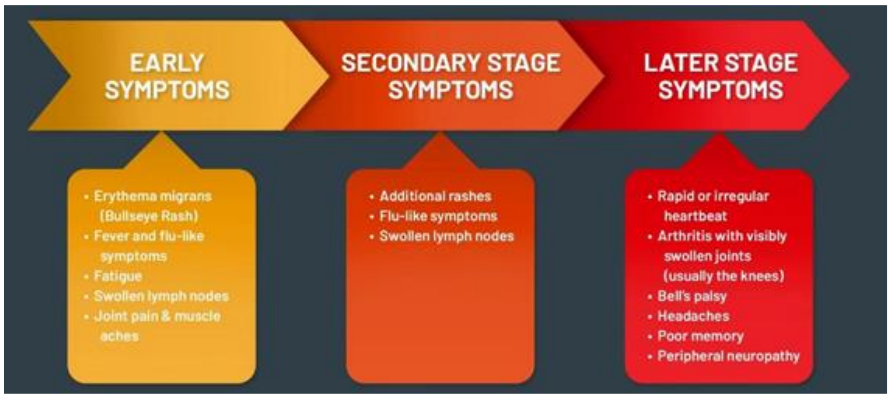

Fig. 5: 3 Stages of Lyme disease (Centers for Disease Control and Prevention 2019)

Fig. 6: Erythema migrans ("classic" Lyme disease rash) (Centers for Disease Control and Prevention2019)

fluid (CSF) than other species in Europe (Flisiak and Pancewicz 2008). In early stages of the disease, neuroborreliosis might proceed with cranial nerve paralysis, most frequently the paralysis of facial nerve. CSF inflammation and changes may cause paralysis. The early stages of neuroborreliosis might cause nerve roots or single peripheral nerves paralysis. Meningitis and encephalomyelitis may also occur. A slow course of encephalomyelitis might appear in the late stage of Lyme disease. It may also proceed to peripheral neuropathy as well as dysaphia and paresteses might appear during the chronic infection. Encephalopathy, dominating memory impairment and dizziness may also appear during the course of disease (Wormser et al. 2006).

Lyme Arthritis (LA)

One of the frequent manifestations of the *B. burgdorferi* infection is Lyme arthritis (LA). In both early and late stage of Lyme disease infection, Lyme arthritis appears. Nearly 10% of Lyme disease patients have persistent arthritis and show resistant to antibiotics, along with the remaining symptoms of disease, despite of using standard antibiotic (Aguero-Rosenfeld et al. 2005). Frequently administered antibiotic might not be effective, because spirochetes can persist in the joints during arthritis despite of elimination of the pathogens. Remains of spirochetes in the joints and arthritis without spirochetes can be differentiated by DNA detection of *B. burgdorferi* in synovial fluid or synovium (Stańczak et al. 1999). Lyme disease could exist with various clinical appearances, for example, muscle ache, arthralgia or periarthritis can persist for months or even years. Most cases of Lyme arthritis attack the knee joint, followed by the humorous and shoulder joints. The temporal and mandibular joints, small joints of hands and legs, elbow, wrist, hip and ankle joints are rarely infected. In rare cases of LA, it may cause permanent damages to the affected joints which are irreversible and also cause permanent immobilization of the joints (Kocbach-Przudzik 2019).

Lyme Carditis

Carditis may appear at the early stage of Lyme disease in 21 days after infection. However, this duration might last from 1 week to 7 months. In *B. burgdorferi* infection, heart problems might be appeared with other forms of Lyme disease such as EM or nervous systems (Afari et al. 2016). One of the features of heart infection in the course of *B. burgdorferi* is the acute onset and atrio-ventricular dissociation as partial or total atrio-ventricular block (Yeung and Baranchuk 2018). Myocarditis, pericarditis, benign cardiac insufficiency, and chronic hemostatic cardiomyopathy are fewer common complications of Lyme carditis. The persistence of *B. burgdorferi* in cardiac muscle during spirochetemia that appears during the early stage of disease, might be the cause of myocarditis (Patton and Phillips 2018). However, the chances of disease progression toward myocarditis and pericarditis are very rare (Shapiro and Wormser 2018).

Acrodermatitis Chronic Atrophicans

After several years of infection with Lyme disease, acrodermatitis chronic atrophicans might appear as red or blue-red stain occurring on the skin of the distal parts of the limbs (Fig. 7) (Stanek et al. 2012). It is a long standing, chronic, and progressive form of Lyme disease that appear more frequently in Europe than in the USA, affecting male patients with older ages. Acrodermatitis chronic atrophicans is mostly caused by *B. afzelii* (Bhate and Schwartz 2011).

Fig. 7: Acrodermatitis chronic atrophicans (ACA) is typically located on the extensor sites of extremities: (A) ulnar and hand lesions, (B) bluish-red lesion on the back of a patient's hand and waxy appearance of theskin of fingers, (C) lesions on a patient's left foot and lower leg (Stanek et al. 2012).

Diagnosis

The early diagnosis of Lyme disease associated with erythema migrans does not need any serological tests. Erythema migrans appears in between 2-30 days beyond the bite of the infected tick, while anti-Bb antibodies appear in around 2-4 weeks after the initial tick bite. The patients with EM might have negative results for serological test (Flisiak and Pancewicz 2008). In disseminated disease, the diagnosis becomes more difficult and based on careful epidemiological history to confirm any exposure to tick bites, typical clinical signs of the disease and test positivity for anti-Bb antibodies in the patient's serum (MSD veterinary manual).

Two-step diagnosis is needed to detect the pathogen including the first step is based on ELISA (enzyme- linked immunosorbent assay) and then the results must be confirmed by a more specific Western blot assay (Zajkowska et al. 2000). Humoral response starts with immunoglobulin M (IgM) antibodies that usually appear in about 2 to 4 weeks after infection. The level of IgM antibodies peaks 8 to 10 weeks post infection and starts to disappear gradually, which in some patients might remain for several years. Immunoglobulin G (IgG) antibodies can be detected in serum about 6 weeks post infection and reach the peak levels after 4 to 6 months. It can be detectable in serum for many years (Ross Russell et al. 2018). Anti - myelin antibodies are

Table 1: Treatment of Lyme disease (Mark and Klempner 2001).

Clinical picture	Drugs	Dosage	Administration	Duration[days]
	DoxycyclineAmoxicillin	100 mg bid	popo	14-21
	Cefuroxime	500 mg tid	po	14-21
EM		500 mg bid		14-21
	AmoxicillinDoxycycline	500-1000mg tid	popo	14-28
Lyme disease witharthritis	Cefuroxime	100 mg bid	po	14-28
		500 mg bid		14-28
Lyme disease with nervous system, heart, or recurrent joint involvement	Ceftriaxone Ceftriaxone Penicillin G	2000 mg q24h	iv iv iv	14-28
		2000 mg tid		14-28
		3-4 mu q4h		14-28
Acrodermatitis Chronic Atrophicans	Amoxicillin Doxycycline	500-1000mg tid	po po iv iv	14-28
	Ceftriaxone Ceftriaxone	100 mg bid	iv	14-28
	Penicillin G	2000 mg q24h		14-28
		2000 mg tid		14-28
		3-4 mu q4h		14-28

EM – erythema migrans, bid – twice a day, tid–3 times a day, po–per os (by mouth), iv– intravenously, q4h – in each 4hrs, q24h – in each 24 hrs.

detected in the serum and CSF in patients where central nervous system (CNS) borreliosis causes demyelination. The CSF cell count is increased to several dozens or several hundred in cases of meningitis, accompanied by a slight elevation of CSF protein level and specific intrathecal IgG or IgM antibody synthesis, which is detected using ELISA test. CSF abnormalities might be absent or minimal in early stages of the Lyme borreliosis, and limited to a slight increase in the protein levels (Murray and Shapiro 2010). There are difficulties in serological tests and it may be a result of differentiation within individual *Borrelia* species. It is impossible to obtain valid results of serological test by using diagnostic antigen derived from only one strain (Aguero-Rosenfeld et al. 2005). Variable major protein-like Sequence (VlsE) is a recently described marker which can improve the diagnosis of Lyme disease (Aberer 2007).

Treatment and Prevention

The main treatment for the Lyme disease is the use of antibiotics. For the selection of an appropriate treatment, the stage of Lyme disease and the duration of the treatment should be concerned. Antibiotic treatment for Lyme borreliosis lasts a minimum of 21 days (Dybowska 2006). First-line antibiotics used for the treatment purpose includes doxycycline, amoxicillin, ceftriaxone, cefotaxime and penicillin G. Azithromycin or clarithromycin might be used as an alternative for amoxicillin or doxycycline. The combination of antibiotics and long duration treatments with antibiotics is also not recommended (Bockenstedt et al. 2002; Wormser and Schwartz 2009). Table 1 shows treatment protocol of Lyme disease at various stages.

The best way to prevent the infection with *B. burgdorferi* is by prevention of infected tick bites. Removing the ticks as soon as possible after any exposure protects the host against infection with spirochetes. Ticks should be removed with proper care. The possibility of spirochete transmission to humans is increased while removing the ticks carelessly that might regurgitate the tick gut content. Removing of the ticks needs a single movement, and the site of the bite should immediately be cleaned and disinfected. The injured individual should be thoroughly observed for up to 30 days, looking for signs and symptoms of Lyme disease. Active prophylactics of Lyme borreliosis i.e., vaccination is not available. *B. burgdorferi* vaccine based on protein A of the external envelope of spirochete (OspA) was developed and registered in the USA, but the vaccine was removed during 2002 (Piesman and Eisen 2008, Richer et al. 2011).

Conclusion

Lyme borreliosis is the most wide-spread disease transmitted by ticks in Europe and the USA and creates many diagnostic and therapeutic problems. It can either be localized or systemic which can mostly be manifested in the skin as well as musculoskeletal signs. However, it can distribute to other body parts, specifically nervous system and heart. The disease is diagnosed on the basis of clinical signs and then confirmed through serological tests. It can be treated with antibiotic for a period of two to four weeks. The disease might be prolonged in the patients with delayed therapy and can lead to irreversible tissue damage.

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21

Hemoparasites Co-infections in Bovines in the Tropics

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INTRODUCTION

Tropical regions of the world are located between Tropic of Capricorn and Tropic of Cancer, among the territories of more than one hundred countries from America, Europe, Asia, Africa and Oceania (Fig. 1). Tropical regions represent only a 7% of terrestrial surface, but biological diversity found in these regions is the richest of all climates, containing more than 50% of world's species (Beck 2019). However, the optimal conditions for bovines also serve as the best conditions for a number of parasites and other microorganisms that have been adapting and evolving since hundreds of years ago (Rosenberg and Zilber-Rosenberg 2011; Cavicchioli et al. 2019).

Livestock production around the world is one of the most important source of food for world population, given the fact that bovines may be used for meat, milk or double purpose production in the developing countries located in tropical regions. Approximately 453 million bovines are just in the Sub-Saharan Africa and South Asia (Oosting et al. 2014). Meanwhile, according to FAO (http://www.fao.org/faostat/en/?#data/), tropical regions have provided more than 31 thousand million tonnes of world cattle meat and milk production in the last five years (Beck 2019).

At the same time, the economic effects of infectious diseases affecting livestock producers quality due to the losses by detriment in weight gain, daily milk production, reproductive capability, diagnosis and treatment expenses, and mortality. Unfortunately, very limited studies conducted yet to determine the real economic impact of hemoparasitic diseases in the world, however, some works obtain results on the little scale or with a short sample size that can underrepresent the real effects of these diseases. Bovine anaplasmosis, for example, has been estimated to produce an economic loss of more than $100 million dollars per year in the US only (Kocan et al. 2010). A recent study showed that expenditure due to theileriosis represents 13.83% of the farm costs of a dairy farm in Pakistan (Rashid et al. 2018). Economic losses due to babesiosis has also been estimated in thousands of million dollars per year (Ozubek et al. 2020). However, economic significance has to be evaluated considering the effects of both diseases and vectors in order to develop control strategies that allows the reduction of both factors to improve animal health and thereby to achieve One health (Rodríguez et al. 2009; Kocan et al. 2010).

Bovine Hemoparasites in the Tropics

The co-infections in bovines is not rare and the evolution has made parasites and host to adapt according to each other and to maintain certain steadiness, producing the enzootic stability of diseases (Esteve-Gasent et al. 2020). The problem comes when this stability breaks and negative effects show on the host species in the form of clinical signs of disease, reduction or lack of production, poor genetic improvement or economic losses due to treatment expenses, damages to production and death of the animals (Rodríguez et al. 2009). There are following hemoparasitic infections causing economic impact on bovines found in the tropical regions of the world.

a. *Anaplasma* spp

Anaplasma (*A.*) *marginale* is a gram-negative bacterium, belonging to the order Rickettsiales, which cause enzootic bovine anaplasmosis in Europe, Asia, Africa and Latin America (Kocan et al. 2010). *Anaplasmataceae* family contains other species of *Anaplasma* that infects cattle, such as *A. centrale* (*A. marginale* subsp. *centrale*) or *A. phagocytophilum* which has a wider range of hosts and is a zoonotic microorganism (Kocan et al. 2010).

In bovines, *A. marginale* is the most pathogenic species of the genus which parasitizes erythrocytes of cattle causing fever, jaundice, loss of appetite, weight loss, abortions, low milk production and death (Kocan et al. 2010). Transmission of *A. marginale* occurs through the ticks belonging to the genus *Rhipicephalus (Boophilus) microplus* and *Dermacentor* (*D.*) *andersoni, D. variabilis, D. albipictus, D. hunter* and *D. occidentalis* (Kocan et al. 2003, 2010; Ueti et al. 2007; Guzmán-Cornejo et al. 2016). Hematophagous insects (*Tabanus* spp., *Anopheles, Psorophora, Haematobia irritans* and *Stomoxys calcitrans*) can also transmit the microorganism mechanically (Ristic and Kreier 1984; Blouin and Kocan 1998; Bautista-Garfias et al. 2021).

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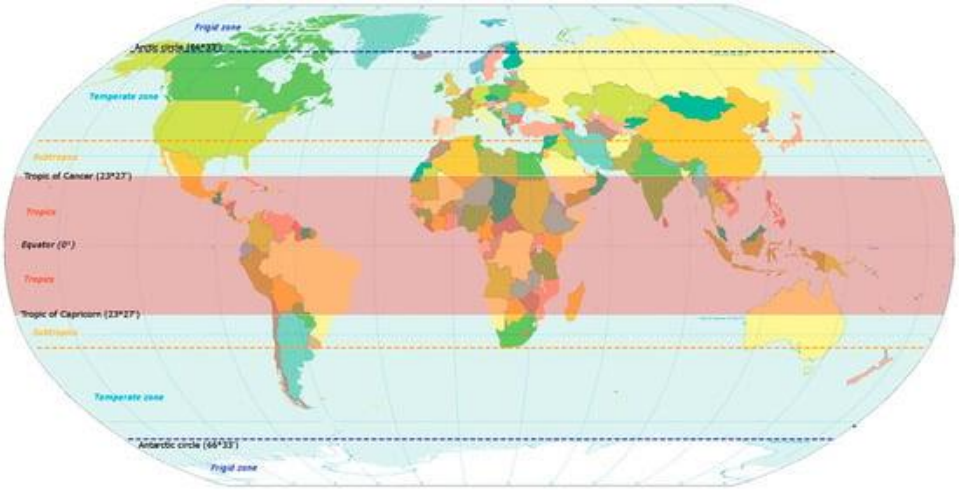

Fig. 1: Tropical regions of the world. Delimited by the Tropic of Cancer and Tropic of Capricorn, the region is characterized by the presence of a great diversity of species. (Mavridou et al. 2018).

Once recovered from acute initial infection, bovines become chronically infected presenting cycles of rickettsemia every 6 to 8 weeks. In these persistent cases, microscopic identification of pathogen will be difficult and serologic (indirect or competent ELISA) or molecular (PCR, qPCR, LAMP, RLBH) diagnosis will be more precise, sensitive and specific (Carelli et al. 2007; Wen et al. 2016; Paoletta et al. 2018; Salinas-Estrella et al. 2022a).

b. *Babesia* spp

Bovine babesiosis is an infectious disease caused by protozoan parasites *Babesia* (*B.*) *bovis* and *B. bigemina* which are considered as most pathogenic species for bovines (Henker et al. 2020), and are widely distributed around the world. Ticks are the biological vector of these pathogens including *Rhipicephalus* (*R.*) *microplus* and *R. annulatus* (Mosqueda et al. 2012; Esteve-Gasent et al. 2020). The infection produces fever, anemia, haemoglobinuria, apathy, anorexia, drop in productivity, and in some cases nervous movements and death (Bock et al. 2004). Severity of disease depends on bovine immune status, age, strain of the parasite and number of infecting microorganisms (El-Dakhly et al. 2020). Recovered animals become chronic asymptomatic carriers and a source of infection for non-infected animals (Chávez-Larrea et al. 2021). Conventional treatment of bovine babesiosis is based on imidocarb or diminazen azeturate. More recently many other drugs have been used to treat bovine babesiosis including triclosan, nerolidol, artesunate, epoxomicin, gossypol and atovaquone (Mosqueda et al. 2012). Control of this disease relies on tick control, surveillance diagnosis, and adequate nutrition to maintain an optimal immune status. Vaccines are yet to be developed against *Babesia* spp. (Esteve-Gasent et al. 2020). Several approaches in vaccine development include immunization with attenuated strains, cell culture and genome-based vaccinology (Mosqueda et al. 2012, 2017).

c. *Borrelia* spp

Borrelia spp. are gram-negative spirochaetes which are 5–20 µm long and up to 0.5 µm wide, and causes disease in humans and animals. Among these, *Borrelia* (*B.*) *theileri*, a causative agent of bovine borreliosis is well-known to infect cattle and other mammals (horse, sheep, goats and deer). This disease has already been diagnosed in the cattle of South Africa, Nigeria, Australia, Brazil, Mexico and Argentina (Yparraguirre et al. 2007; Cordeiro et al. 2018; Morel et al. 2019; Qiu et al. 2021). The infection begins by attachment and prolonged feeding of infected vectors i.e., *Rhipicephalus* ticks (*R. microplus*, *R. evertsi*, *R. annulatus*, and *R. decoloratus)* (Smith et al. 1978; Matton and Melckebeke 1990; Yparraguirre et al. 2007; Cordeiro et al. 2018; Qiu et al. 2021). Bovine borreliosis is a low pathogenicity disease; however, signs such as fever, hemoglobinuria, lethargy and anemia can be present in the infected animal (Callow 1967). This disease usually occurs associated with babesiosis and/or anaplasmosis worsen the hematological parameters of the animal, especially in splenectomized cattle (Smith et al. 1985). While, in Africa, borreliosis is associated with babesiosis, theileriosis, anaplasmosis and eperitrozoonosis (Koch et al. 1990).

B. burgdorefi sensulato- complex is the causal agent of Lyme disease in humans and can also infect cattle. The main vectors of *B. burgdorferi* in tropic regions (South America, Africa and Australia) belongs to the *Ixodes* (*I.*) *ricinus* species complex (*I. ricinus*, *I. scapularis*, *I. pacificus* and *I. persulcatus*). The clinical signs associated with the acute disease include fever, stiffness-swollen joints, lethargy, anemia, decreased milk production, erythematous rash, chronic weight loss, lameness and spontaneous abortions (Post et al. 1988; Parker and White 1992; Wells et al. 1993). *B. burgdorferi* persist in the nature within an enzootic cycle involving ticks and mammals and the geographical area can

Table 1: Main diagnosis test to *B. burgdorferi*

	Diagnosis test	Reference
Identification of the agent	Giemsa-stained blood smears	Matton and Melckebeke 1990
	Polymerase chain reaction (PCR)	Lebech 2002
	Dark-field immunofluorescence microscopy	Wittenbrink et al. 1994
	Bacterial culture	Zhioua et al. 1999
Serological	Indirect fluorescent antibody	Parker and White 1992; Burgess et al. 1993
	Antibody capture enzyme-linked immunosorbent assay (ELISA)	Burgess et al. 1993
	Serotyping using monoclonal antibodies against OspA and OspC bacterial surface proteins	Wagner et al. 2012

Table 2: Occurrence of Bovine theileriosis in tropics

Specie	Disease	Vector	Geographical Distribution	Symptoms	References
T. Parva	East Coast fever, corridor disease and Zimbabwean Theileriosis	*Rhipicephalus species, Appendiculatus (R. zambesiensis,* and *R. Duttoni)*	Eastern, central and southern Africa	High fever, swelling of the lymph nodes, dyspnea, and high mortality. (Death occurring approximately three weeks after infection).	Bishop et al. 2004; Kiara et al. 2018; Selim et al. 2022
T. Annulata	Tropical theileriosis	Hyalomma species, (*H. anatolicum, H. rufipes, H. impeltatum and H. dromedarii)*	Middle Eastern of Africa, and south Asia (india)	Swelling of the lymph nodes, pyrexia, anemia, dyspnea, emaciation, and diarrea. In chronic diseases some neurological and reproductive signs may develop.	Bishop et al. 2004; Kiara et al. 2018; Liu et al. 2022; Selim et al. 2022
T. Mutans	Benign bovine theileriosis,	*Amblyomma species,* (A. *variegatum)*	Caribbean islands, Western, Eastern, Central and Southern Africa and	Mild disease	Flanagan and Le Roux 1957; Kiara et al. 2018; Selim et al. 2022
T. Velifera	Benign bovine theileriosis, Benign bovine theileriosis	*Amblyomma* species, *(A. variegatum*	Western, Eastern, Central and Southern Africa	Mild disease	Kiara et al. 2018
T. Taurotragi		*Rhipicephalus* species	Eastern, Southern and Central Africa	Mild disease	Kiara et al. 2018
T. Orientalis, (Ikeda)		*Haemaphysalis species (H. longicornis)*	Australia	Anemia, pallor, lethargy, pyrexia, elevated heart rate, recumbency, weakness, and death in extreme cases.	Perera et al. 2014; Oakes et al. 2019; Marendy et al. 2020

be affected by climatic factors and host density. The life cycle of *B. burgdorefi* is complex and takes 2 to 6 years depending on the tick species. It begins when an infected tick feeds on its host releasing saliva or coxal secretions on the biting site. The bacteria need 48 h of tick attachment before enters the host and its transmission begins depending on the *Borrelia* specie (Gern 2009).

The recommended diagnosis test in cattle is a smear of peripheral blood, stained by Giemsa, nevertheless, a high number of spirochetes are required for proper diagnosis (Matton and Melckebeke 1990). Several other diagnostic test for *B. burgdorferi* has been enlisted in Table 1.

For treatment of cattle borreliosis, the recommended antibiotics include oxytetracycline and procaine penicillin. Oxytetracycline is commonly used for human borreliosis and is effective for treatment of bovine borreliosis, although with limited success, in treating a Bovine Lyme Borreliosis (Matton and Melckebeke 1990). Even though in 5 days of treatment a significant improvement is observed, it is important to finish the treatment for the clearance of spirochaetes from the blood circulation (Post et al. 1988).

d. *Theileria* spp

Theileriosis is a tick borne hemoparasitosis caused by the family Theileridae (order Piroplasmida, genus Theileria), which parasitize wild and domestic animals where the appropriate tick vectors are found (Bishop et al. 2004; Kiara et al. 2018).

Theileriosis is an economically important disease and range from mild (inapparent reactions) to fatal, therefore some species that infect cattle are relatively benign (asymptomatic) whereas others as *Theleiria* (*T.*) *parva* and *T. anulata* are responsible for a severe illness (Uilenberg 1981; Irvin and Morrison 1987; Mans et al. 2015; Lawrence and Mans 2017). African buffalo represent an important wildlife reservoir for cattle infection (Young et al. 1978). The pathological significance along with tick vector and geographical distribution of various species infecting cattle has been illustrated in Table 2.

Theileria life cycle involve intracellular stages in the vectors and the host. The protozoan infects and develop in the leukocytes (schizont) or erythrocytes (piroplasm) depending on the species (Ali et al. 2017): *T. parva* infect T and B lymphocytes, while *T. annulata* infect monocytes, dendritic

cells and B-lymphocytes (Baldwin et al. 1988; Spooner et al. 1989; Stephens and Howard 2002).

Infected tick feeding secretes sporozoites (infective form) from salivary glands into bovine blood, infecting leukocytes and multiply inside them by merogony. The schizont associates with the mitotic spindle during cell division, therefore, the parasites are able to divide synchronously with the bovine cells. It ensure infection remains in daughter cells, facilitating multiplication and differentiation to merozoites, which are released and invade erythrocytes forming piroplasms (Hulliger et al. 1964; Dobbelaere et al. 2003; Von et al. 2010; Torina et al. 2020). In susceptible bovine, infection usually results in death within 3 to 4 weeks approximately.

The immune response of the host acts against extracellular stages (sporozoites or merozoites), the antigen of the macroschizonts and piroplasmic stages on the surface of the invaded cells (Seifert 1996).

Treatment for the prevention and control of infection in the initial stage includes the anti-protozoal Buparvaquone, which is considered as an effective drug against theileriosis. However, even after preventive treatments of theilleriois, the disease still represents a serious threat to livestock. Currently, low-pathogenicity parasites derived from infected cells in vitro are used as vaccines in many countries (Liu et al. 2022).

e. *Trypanosoma* spp

Trypanosomosis is caused by a hemoprotozoan parasite *Trypanosoma*, which affects domestic, wild animals, and humans across the world. In Africa, Asia and South America, *Trypanosoma* (*T.*) *vivax, T. congolense, T. evansi, T. equiperdum, T. cruzi*, and *T. theileri* represent a potential risk for a cattle population of more than 500 million (Jones et al. 2001; Osório et al. 2008; Van den Bossche et al. 2010; Gelaye et al. 2020). In bovine trypanosomosis, *T. vivax* is the most pathogenic and important agent infecting cattle (Jones et al. 2001). However, the specie that causes American Trypanosomosis in humans is *T. cruzi*, and it has been found in many wild (rodents, bats and marsupials) and domestic animals such as dogs and pigs (Ramsey et al. 2012).

The transmission of *Trypanosoma* spp. from vector occurs either through stercorarian (infective stage developed in the digestive tube) or salivarian route (infective stage developed in salivary glands) (Haag et al. 1998). In this regard, parasites are usually transmitted by tsetse flies (in Africa) and mechanically by other blood-sucking arthropods, such as *Haematobia irritans*, *Stomoxys calcitrans*, and *Tabanus* spp. (Osório et al. 2008). Additionally, although less common, the *Trypanosoma* spp. is also transmitted by ticks, including: *Rhipicephalus microplus*, *Ixodes ricinus*, *Hyalomma anatolicum*, and *Amblyomma cajennense* (Latif et al. 2004; Krige et al. 2019; Zeb et al. 2019; Luu et al. 2020).

In Latin America, the infection is present in 10 out 13 countries of South America (Jones et al. 2001; Dagnachew et al. 2015). Molecular characterization of *Trypanosoma* parasites corroborates the West African origin of South American isolates, which were possibly introduced by cattle imported from Africa at the end of the 19th century; however, a genetic distance separated these parasites from the East African isolate (Hill et al. 2005). Additionally, morphometric studies suggest a difference in the surface antigens diversity and its inability to infect and grow in tsetse flies between the American *Trypanosoma* spp. and the African parasite through DNA labeling, and biochemical analysis of isoenzymes (Haag et al. 1998; Jones, 2001).

The severity and symptomatology of the infection depend on the host and the *Trypanosome* spp. Thus, the presence of a parasite in the blood results in anemia, causing progressive weight loss, anorexia, detrimental effects on fertility, reproduction, and economic losses in milk and meat production in infected bovines (Holmes et al. 2000). Hematological alterations observed in natural and experimental infections include the simultaneous development of leukopenia, lymphopenia, and neutropenia as well as variations in the concentration of total serum proteins. Currently, we know that bovine trypanosomiasis prevalence is related to host factors such as age, sex, breed, purpose, and abiotic factors such as management system, population density, extension of exploitation, presence and control of vectors, geographic regions, agroecological zones, climatic season and application of trypanocidal treatments (Holmes et al. 2000). In this regard, Anti-*Trypanosoma* drugs will continue to play a significant role in the bovine trypanosomosis integrated control. However, the inappropriate use of these chemical compounds results in the development of resistance, which represents a continuous threat to their sustainable use (Miruk et al. 2008). Finally, the advance in elucidating the mechanism involved in the pathogenic differences, drug resistance, and genetic composition could be an approach to diagnosis and control (Dagnachew et al. 2015; Gelaye et al. 2020).

Epidemiology of Most Common Hemoparasites Co-infections in Bovines

Recently, vector-borne diseases have shown a new geographic distribution worldwide. Many of these diseases are caused by hemoparasites. The critical point of this distribution relies on several conditions, including vector's adaptation to new climatic conditions and the migration and transportation of vectors and cattle, respectively. Therefore, the diseases and sick animals have begun to appear in geographical regions never reported before (Shope 1991; Gray et al. 2009; Chávez-Larrea et al. 2021). According to a study, *Babesia* spp. was reported in cattle in Quito, Ecuador at an altitude of 2469 meters above the sea level (m.a.s.l.), which is the highest altitude reported for babesiosis and the vector *R. microplus* (Chávez-Larrea et al. 2021).

The hemoparasites that affect red blood cells of bovines mainly belong to the genus *Anaplasma* spp., *Babesia* spp., and *Theileria* spp.; however, some other microorganisms have been described too, including species of genus

Ehrlichia, *Trypanosoma*, *Setaria*, and *Mycoplasma* (Kamyingkird et al. 2020, Ngasaman et al. 2021). Among the most representative species of each genus are: (bacteria) *Anaplasma marginale*; *Ehrlichia ruminantium*, *E. minasensis*; (protozoan) *Babesia bovis, B. bigemina, B. divergens, B. major, B. jakimovi, B. ovata, B. ocultans*; *Theileria orientalis* complex (*T. mutans, T. buffeli, and T. sergenti*), *T. annulata, T. parva, T. orientalis, T. taurotragi, T. velifera*; *Trypanosoma evansi*; (mycoplasmas) *Candidatus Mycoplasma haemobos* and *M. wenyonii* (Niethammer et al. 2018; El-Dakhly et al. 2020; Agina et al. 2021).

Age of the animal may be an associated risk factor regarding the clinical or subclinical presentation of disease, as adult cattle present serious clinical illness of bovine anaplasmosis as compared to calves (Kocan et al. 2003).

In many cases, only one type of hemoparasite is found in sick animals; however, in other cases, the animals are diagnosed with more than one hemoparasite, which exacerbates the clinical signs and, the health deteriorates rapidly (Tembo et al. 2018). Some of the most important co-infections reported worldwide in the years 2017-2022 are shown in Fig. 2. Table 3 shows a broader view of the prevalence of hemoparasites in cattle in recent years.

Many recent prevalence studies are based on PCR testing to detect the pathogenic DNA in the sample (Rodríguez et al. 2009; Mans et al. 2015). Unfortunately, several reports are based on opportunity, incidental finding or searching for specific pathogens and are not representative of the real prevalence of a country. There is a lack of comprehensive epidemiological studies worldwide to know the status of hemoparasite co-infections that are causing serious health problems and thus affecting cattle production (Cordeiro et al. 2018; Cavicchioli et al. 2019; El-Dakhly et al. 2020; Chávez-Larrea et al. 2021).

Diagnosis, Treatment and Control of Hemoparasite Coinfections in Bovines

The diagnosis of hemoparasitic infection is usually made on the basis of clinical signs but it give false negative results due to several factors such as lack of information on the clinical history, unspecificity of the clinical signs and non-declared management practices or treatment. So, laboratory tests are always needed to support the presumptive diagnosis. The most common, easy and inexpensive is observation of stained blood-smears with an optical microscopy using Giemsa staining (Al-Hosary et al. 2015), however, it may be more useful during the acute phase of disease when there is a high amount of circulating hemoparasites. In addition, microscopy allows to obtain more information about the general state of blood cells such as its shape, size and ratio of RBCs and WBCs which can help to confirm or discard a diagnosis. However, it requires an experienced microscopist to clearly identify the pathogens and species present in the slides (Mosqueda et al. 2012).

Serological tests (Complement fixation test, ELISA and its variants, IFA and immunochromatographic strips) are fast and effective for detection of antibodies in a herd, but it will be detected after the start of an immune response (Torioni de Echaide et al. 1998; Mosqueda et al. 2012; Vieira et al. 2017; Tayebwa et al. 2018; El-Sayed et al. 2019; Torina et al. 2020; Salinas-Estrella et al. 2022a). Hence, false negative results are the risk at the beginning of infection, whereas false positive results may present when there are cross-reactions of antibodies with its site of recognition (Rodríguez et al. 2009; Esteve-Gasent et al. 2020).

Molecular tests based on PCR or its variants are very useful in low parasitemia cases and facilitates sequencing and identification of pathogen species. Duplex or multiplex PCR or qPCR, and RLBH are examples of molecular tests that allows diagnosis of coinfections simultaneously which is ideal in those places where coinfections are a great problem (Ananyutthawongese et al. 1999; Decaro et al. 2008; Bilgiç et al. 2013; Paoletta et al. 2018).

Pathogens represent a major threat to cattle and one of the main constraints for the improvement of the livestock industry. Therefore, having a better control of diseases transmitted by cattle ticks and other vectors would greatly contribute to improve meat and milk production (Johansson et al. 2020). Development of resistance to acaricides and antibiotics leads to requirement of more sustainable and reliable measures for control of vector-borne diseases. For instance, wildlife management, alternative husbandry practices or a combination of strategic tick control and vaccination and preventive serological testing must be a part of production practices to complement the management (Jia et al. 2020; Johansson et al. 2020; Salinas-Estrella et al. 2022b).

Conclusion

Hemoparasites such a protozoan parasites (*Babesia*, *Teilleria and Trypanosoma* species) and intracellular-obligate bacteria (*Anaplasma* and *Borrelia* species) are some of the pathogens transmitted by arthropods, which have limited the production of livestock in tropical regions, since their climatic characteristics provide ecological niches, which are auspicious to the development of vectors (ticks and Tsetse fly).The economic losses caused by these diseases are mainly due to cattle death, drop of production, cost of treatment, preventive measures and vector control. Lack of commercial vaccination represents a serious problem in places with a high incidence. Antibiotic resistance in humans is a problem occurring worldwide and is still on the path of control. In this sense, indiscriminate use of antibiotics in animals contributes to the problem, but treatment of bovine infectious diseases relies on a few pharmacological compounds. In addition, there is a lack of comprehensive epidemiological studies worldwide to know the exact status of hemoparasite co-infections that are causing serious health problems and thus affecting cattle production. Use of diagnostic tests are a great

Table 3. Representative cases of prevalence of hemoparasites distributed worldwide.

Disease	Species	Prevalence	Method	Country	References
Anaplasmosis	*A. marginale*	n= 216 87.9%	Nested PCR	Pakistan	(Bisen et al. 2021)
		n=520 10.30 %	PCR	Thailand	(Junsiri et al. 2020)
		n= 104 21.15%	Semi-nested PCR	Bolivia	(Ogata et al. 2021)
		n= 223 95.5%	Quantitative PCR	Cuba	(Díaz-Sánchez et al. 2020)
		n= 650 20%	cELISA	Egypt	(Selim et al. 2021a)
		n= 200 77%	iELISA	Brazil	(Ramos et al. 2020)
		n=62 42%	Quantitative PCR	Russia	(Fedorina et al. 2019)
Babesiosis	*Babesia* spp.	n=264 18.93%	PCR	Ecuador	(Chávez-Larrea et al. 2021)
	B. bigemina	n= 150 19.33%	PCR	Egypt	(El-Dakhly et al. 2020)
	B. bovis	n=725 27.9%	PCR	Mongolia	(Otgonsuren et al. 2020)
	B. bigemina	23.6%			
	B. divergens	n=95 37.89%	Indirect Immunofluorescence	Germany	(Springer et al. 2020)
	B. bovis	n=40 7.5%	Multiplex PCR	Iran	(Rajabi et al 2017)
	B. bigemina	92.5%			
	B. bovis	n=487 69.8%	ELISA	Indonesia	(Guswanto et al. 2017)
	B. bigemina	27.5%			
	Babesia spp.	n=60 15%	PCR	Bolivia	(Ogata et al. 2021)
Theileriosis	*T. annulata*	n=96 54.16%	PCR	India	(Selim et al. 2021b)
	T. orientalis,	n=260 36.5%	PCR	China	(Wang et al. 2018)
	Theileria spp.	n=61 72.13%	PCR	Malaysia	(Agina et al. 2021)
	T. parva	n=479 22.7%	ELISA	Cameroon	(Silatsa et al. 2020)
	T. mutans	41.1%			
	T. velífera	n=392 13%	PCR/Reverse Line Blot (RLB)	Ethiopia	(Hailemariam et al. 2017)
	T. mutans	66.1%			
	T. orientalis	51.8%			
Trypanosomiasis	*Trypanosoma evansi*	n=61 4.92%	PCR	Malaysia	(Agina et al. 2021)
	Trypanosoma (Duttonella) vivax	n=15 80%	PCR	Brazil	(Vieira et al. 2017)
	T. vivax	n=45 35./%	PCR	Venezuela	(Eleizalde et al. 2021)
Ehrlichiosis	*Ehrlichia ruminantium*	n=182 6.6%	Semi-nested PCR	Cameroon	(Esemu et al. 2018)
	Ehrlichia ruminantium	n=392	PCR/Reverse Line Blot (RLB)	Ethiopia	(Hailemariam et al. 2017)
	E. minasensis	0.5% 0.26%			
Hemoplasmosis	Hemoplasmas	n=208	PCR	Uganda	(Byamukama et al. 2020)
	C. Mycoplasma haemobos and *Mycoplasma wenyonii*	32.2%			
	C. M. haemobos	n=400 9.5%	PCR	Japan	(Tatsukawa et al. 2021)
	M. wenyonii	40.3%			

C. M. haemobos	n=410 56.59%	PCR	Germany	(Niethammer et al. 2018)
M. wenyonii	8.54%			
C. M. haemobos	n=27 96%	PCR	Mexico	(Quiroz-Castañeda et al. 2019)
M. wenyonii	96.29%			

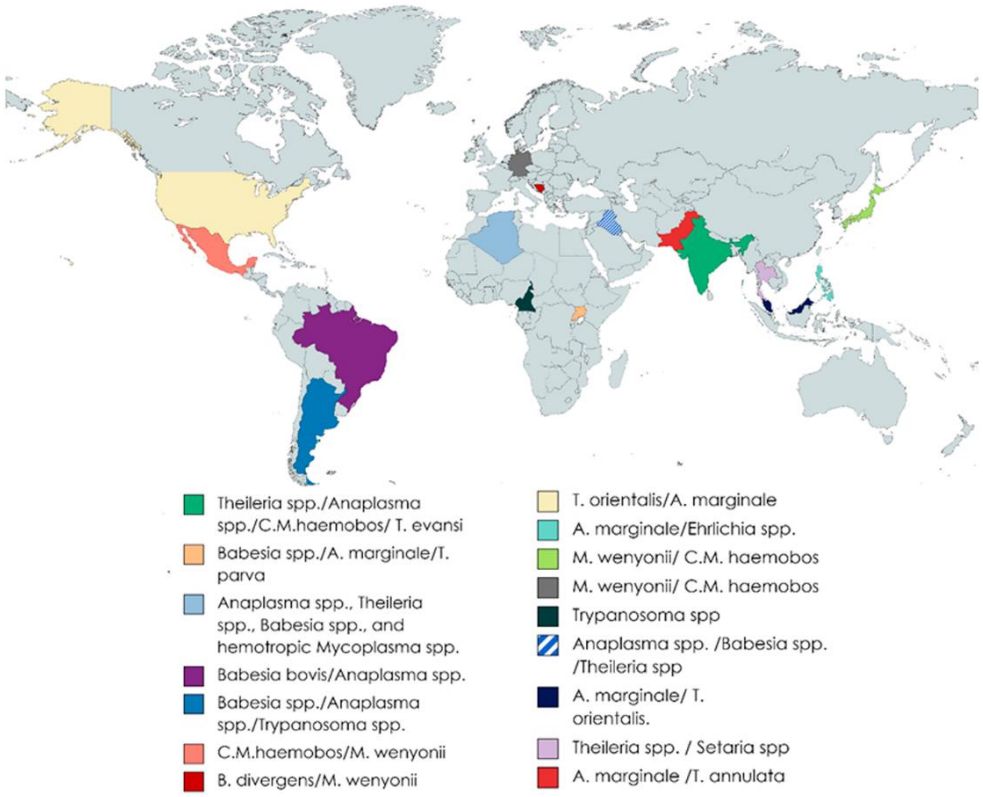

Fig. 2: Worldwide distribution of representative co-infections of hemoparasites that infect cattle in 2017-2022. Created with Mapchart.net (Vieira et al. 2017; Niethammer et al. 2018; Paoletta et al. 2018; Tayebwa et al. 2018; Quiroz-Castañeda et al. 2019; Paguem et al. 2019; Quiroz- Henker et al. 2020; Agina et al. 2021; Ngasaman et al. 2021)

tool for surveillance and prevention of outbreaks, contributing to avoid or at least control negative effects of diseases. Therefore, it is of great importance, to characterize infectious diseases of bovines in the tropics and to promote control strategies in order to mitigate the impact of those diseases on bovine production around the world.

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Amoebiasis in One Health Perspective

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INTRODUCTION

Amebiasis is an infection caused by the parasite *Entamoeba (E.) histolytica*, which is transmitted through contaminated food or water. Ingesting the parasite can lead to infection in the digestive tract. The parasite can also spread from person to person through contact with fecal matter, either directly or through contaminated objects (Kucik et al. 2004). People who have poor hygiene practices, those who live in areas with poor sanitation and those who travel to areas where amebiasis is common are at a higher risk of infection. There are two main types of amebiasis. First is Intestinal amebiasis which is the most common form of amebiasis and is characterized by symptoms such as diarrhea, abdominal pain, and weight loss. Second type is Extraintestinal amebiasis (Nasrallah et al. 2022). This occurs when the parasite spreads from the intestine to other parts of the body, such as the liver, lungs, or brain. It can cause symptoms such as fever, weight loss, and pain in the affected area. It's important to note that some individuals may be infected with the parasite but show no symptoms, making it possible to spread the infection to others without realizing it (Kantor et al. 2018).

Transmission

The most common mode of transmission for amebiasis is through the consumption of contaminated food or water. This is because the parasite can survive for several days outside of the human body, allowing it to persist in contaminated sources (Zulfiqar et al. 2018). Contaminated sources of food or water can include untreated water sources, such as lakes or rivers, as well as food been handled by infected individuals without proper hand washing (Uyttendaele et al. 2015). This can include raw fruits and vegetables that have not been properly washed or cooked meat that has not been fully cooked. In addition to food and water, amebiasis can also be transmitted through person-to-person contact (Agbalaka et al. 2018). This can occur through the direct exchange of fecal matter, such as through sexual contact, or through indirect contact with contaminated objects or surfaces. For example, an infected individual who does not wash their hands after using the bathroom can spread the parasite to others by touching contaminated surfaces or objects (Dayaram et al. 2021). People who are at a higher risk for amebiasis include those who live in areas with poor sanitation, those who travel to areas where the infection is more common, and those who have weakened immune systems. This includes individuals with HIV/AIDS, those undergoing chemotherapy, and individuals who have undergone organ transplantation. Additionally, people who have poor hygiene practices, such as not washing their hands regularly, are also at a higher risk of infection (Chappuis et al. 2004).

Epidemiology

The prevalence studies on human amoebiasis suggest that the disease is prevalent and endemic in developing countries including South America, Asia, and Africa. It is commonly found in those areas where nutrition, water quality and hygiene status are very poor (Ali et al. 2008; Ximénez et al. 2009). The worldwide molecular prevalence of the disease is estimated up to 3.6% (Cui et al. 2019). However, the highest seroprevalence noted in Pakistan was 73% having more infection in those individuals admitted in the hospitals (Samie et al. 2020). Table 1 enlist various waterborne outbreaks of amoebiasis occurred in different regions of world.

Life Cycle

The life cycle of the parasite responsible for amebiasis, *E. histolytica*, is relatively simple and involves three main stages: the cyst stage, the trophozoite stage, and the infective stage (Guillén 2023).

1. Cyst Stage: This stage is characterized by the formation of protective cysts that encapsulate the parasite and help it survive in the environment. The cysts are spherical structures with a tough outer layer that protects the parasite from harsh environmental conditions, such as changes in temperature, pH, and desiccation.

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Table 1: Outbreaks of human amoebiasis in various regions of the world

Country	Cases	Suspected sources	Reference
US, Chicago	1507	Leaked sewage which contaminated the water pipes of hotels	Markell 1986
Italy	17	Contaminated ice cream and raw fruit consumption	De Lalla et al. 1992
Taiwan (China)	730	Contaminated underground water supply	Kow-Tong et al. 2001
Taiwan (China)	140	Traveling to endemic areas	Lai et al. 2000
Georgia	177	Contaminated municipal water	Barwick et al. 2002
Japan	13	_	Abe et al. 1999

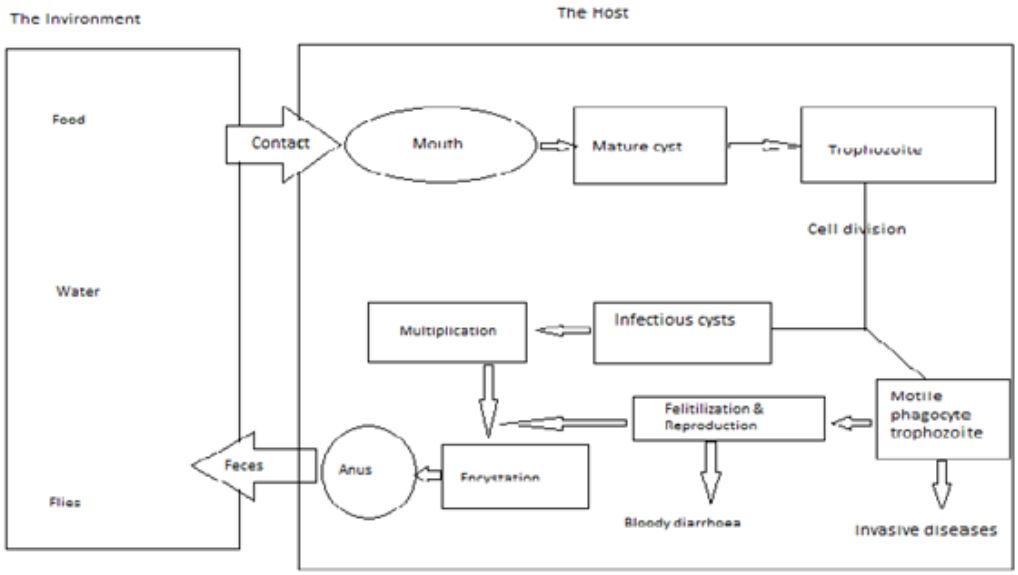

Fig. 1: Life cycle of *E. histolytica* in humans (Hategekimana et al. 2016).

2. Trophozoite Stage: The cysts are ingested by a host and then release the trophozoites, which are the actively growing and reproducing stage of the parasite.
3. The trophozoites invade the intestinal wall and cause tissue damage, leading to symptoms such as abdominal pain, diarrhea, and bloody stools. In severe cases, the parasite can invade the liver and cause liver abscesses(Assafa et al. 2006).
4. Infective Stage: The trophozoites can then re-encapsulate themselves into cysts, which are then excreted from the host in the feces. These excreted cysts can infect new hosts when they are ingested, completing the life cycle of the parasite (Mortelmans et al. 1997). Fig. 1 illustrate the different stages of *E. histolytica* life cycle.

Clinical Signs

Clinically, amoebiasis is of 2 types i.e., intestinal, and extra-intestinal amoebiasis. In majority of the infection (almost 90%) parasite colonizes in the large intestine of host leading to asymptomatic intestinal amoebiasis, while in others (10%) parasite may cross the intestinal barrier leading to amoebic abscesses and amoebic colitis (Kantor et al. 2018).

In asymptomatic infections, parasite colonizes in the colon, feeds on the commensal organisms and take nutrient from the host leading to the development of cyst that passes through the faeces and locate new host to continue its life cycle (Carrero et al. 2020). In case of pathogenic *E. histolytica*, the trophozoite form of parasite may become invasive in nature and start to destroy the intestinal epithelium which provokes the inflammatory process ultimately leading to amoebic colitis (Nagaraja and Ankri 2019). The symptoms of amebiasis can range from mild to severe and can include abdominal pain, diarrhea and bloody stools. In more severe cases, the parasite can invade the liver, causing liver abscesses, which can be life-threatening if left untreated (Li et al. 2021).

Diagnosis

Early diagnosis and treatment of amebiasis is important to prevent the progression of the infection and minimize the risk of complications. In this note, we will discuss the various methods of diagnosing amebiasis (Shirley and Moonah 2016).

The first step in diagnosing amebiasis is to take a thorough medical history and perform a physical examination. During this examination, the healthcare provider will ask about symptoms such as diarrhea, abdominal pain and weight loss, which are commonly associated with the infection. They may also ask about recent travel to areas where amebiasis is more common, as well as any risk factors for the infection. The most common diagnostic test for amebiasis is a stool sample

analysis. This test involves collecting a sample of stool and examining it for the presence of the parasite. This test is simple, non-invasive, and is often used as the first line of diagnosis for amebiasis. The healthcare provider may also perform a rectal swab test, which involves collecting a sample of the stool from the rectum (Tanyuksel and Petri Jr 2003).

Tests for Amebiasis

In addition to stool sample analysis, other diagnostic tests that may be used to diagnose amebiasis include 1- Blood tests: This can help detect antibodies produced by the body in response to the parasite. This test is particularly useful in diagnosing extraintestinal amebiasis, which occurs when the parasite spreads to other parts of the body. 2- Imaging tests: This may include an X-ray, CT scan, or MRI, which can help detect the presence of the parasite in other parts of the body, such as the liver or lungs. 3- Endoscopy: This procedure involves inserting a flexible tube with a camera attached into the digestive tract in order to examine the intestinal lining. This test can help diagnose the presence of the parasite in the intestines and can also be used to obtain a sample for further testing. Once the diagnosis of amebiasis has been confirmed, the healthcare provider will discuss the appropriate treatment options with the patient. Treatment options may include medication, such as metronidazole or tinidazole, which are effective in eliminating the parasite. In severe cases, surgical intervention may be necessary to remove the infected tissue (Haque et al. 2003).

Molecular Detection

Molecular detection methods are increasingly being used to diagnose amebiasis due to their high sensitivity and specificity compared to traditional diagnostic methods. Some of the most commonly used molecular detection methods for amebiasis include:

1. Polymerase Chain Reaction (PCR): PCR is a powerful technique that allows for the detection and amplification of specific DNA sequences. In the case of amebiasis, PCR can be used to detect the presence of the parasite's DNA in stool samples, providing a highly sensitive and specific diagnosis of the infection (Li et al. 2021).
2. Loop-Mediated Isothermal Amplification (LAMP): LAMP is a rapid, low-cost, and highly specific molecular detection method that is particularly useful for the detection of parasitic infections in resource-limited settings. LAMP can be used to detect the DNA of E. histolytica in stool samples, providing a rapid and accurate diagnosis of amebiasis (Uddin et al. 2021).
3. Real-Time PCR: Real-time PCR is a variation of PCR that allows for the simultaneous amplification and detection of DNA in real-time. This technique is highly sensitive and specific and can be used to detect the presence of the parasite's DNA in stool samples, providing a rapid and accurate diagnosis of amebiasis (Li et al. 2021).
4. Microarray: Microarray is a high-throughput molecular detection method that allows for the simultaneous analysis of multiple DNA sequences. In the case of amebiasis, microarray can be used to detect the presence of specific genetic markers associated with the parasite, providing a highly sensitive and specific diagnosis of the infection (Nagaraja and Ankri 2019).

It is important to note that molecular detection methods are not always readily available, particularly in resource-limited settings. Additionally, these methods may not be as accessible as traditional diagnostic methods, such as stool microscopy or antigen detection tests. Nevertheless, molecular detection methods have the potential to revolutionize the diagnosis and treatment of amebiasis and other parasitic infections, providing a rapid, accurate, and cost-effective means of detecting and managing these infections (Nagaraja and Ankri 2019).

Treatment

Early detection and treatment of amebiasis is crucial to prevent the progression of the infection and minimize the risk of complications. Diagnosis of amebiasis is often delayed due to the lack of noticeable symptoms in the early stages of the infection. The symptoms of amebiasis can be similar to those of other digestive tract infections, such as dysentery, and therefore a correct diagnosis is often not made until the condition has advanced. In some cases, amebiasis can cause serious complications, such as liver abscesses or perforations in the intestine, which can be life-threatening if not treated promptly. Early treatment of amebiasis is also important to prevent the spread of the infection to others. The parasite that causes amebiasis is highly contagious and is spread through contaminated food, water, and surfaces. In addition, individuals who have been infected with amebiasis are at risk of re-infection, especially if they do not practice good hygiene habits and follow proper food and water safety practices. Early detection and treatment of amebiasis is also important to minimize the impact on a person's quality of life. Individuals who have been infected with amebiasis may experience a range of symptoms, including diarrhea, abdominal pain, and weight loss, which can be distressing and can significantly affect a person's daily life. Early treatment can help to alleviate these symptoms and minimize the impact on a person's quality of life. In conclusion, early detection and treatment of amebiasis is crucial in order to prevent the progression of the infection and minimize the risk of complications. By working with a healthcare provider and following proper hygiene practices, individuals can reduce their risk of infection and ensure prompt and effective treatment if necessary. Early detection and treatment can also help to minimize the impact on a person's quality of life and prevent the spread of the infection to others. By understanding the importance of early detection and treatment, individuals can take steps to ensure their health

and well-being, and prevent the spread of amebiasis in their communities (Montaño et al. 2020).

Home Remedies for Amebiasis

Amebiasis is an infection caused by the parasite *E. histolytica* and is most commonly found in developing countries with poor sanitation conditions. While it is important to seek medical treatment for amebiasis, there are also several home remedies that can help to alleviate the symptoms and speed up the recovery process.

1. Garlic: Garlic has antimicrobial properties that can help kill the parasite causing amebiasis. Crush 2-3 cloves of garlic and mix with a glass of water. Drink this mixture 2-3 times a day.
2. Ginger: Ginger has anti-inflammatory and anti-parasitic properties, making it effective in treating amebiasis. Simply add 1-2 inches of fresh ginger to a cup of boiling water and let it steep for 10 minutes. Drink this tea 2-3 times a day.
3. Aloe Vera: Aloe vera has antimicrobial and anti-inflammatory properties, making it an effective home remedy for amebiasis. Mix 1 tablespoon of aloe vera juice with 1 glass of water and drink 2-3 times a day.
4. Turmeric: Turmeric has antimicrobial properties that can help kill the parasite causing amebiasis. Mix 1 teaspoon of turmeric with a glass of warm milk and drink twice a day.
5. Papaya: Papaya contains an enzyme called papain that helps break down proteins and has been found to be effective in treating amebiasis. Eat 1-2 slices of ripe papaya daily, or take papaya supplements as directed by a healthcare professional.
6. Yogurt: Yogurt contains beneficial bacteria that can help restore the balance of bacteria in the gut and prevent the growth of the parasite causing amebiasis. Eat plain, unsweetened yogurt daily.
7. Fennel seeds: Fennel seeds have antimicrobial and anti-inflammatory properties, making them effective in treating amebiasis. Drink fennel seed tea 2-3 times a day. Simply boil 1 teaspoon of fennel seeds in a cup of water for 10 minutes, strain, and drink.
8. Lemon: Lemon is high in vitamin C, which has been shown to have antimicrobial properties. Mix 1 tablespoon of lemon juice with a glass of warm water and drink 2-3 times a day.
9. Oregano: Oregano has antimicrobial properties that can help kill the parasite causing amebiasis. Add 1-2 drops of oregano oil to a glass of water and drink 2-3 times a day.
10. Pumpkin seeds: Pumpkin seeds are high in zinc, which has been shown to have antimicrobial properties. Eat a handful of pumpkin seeds daily or add them to your diet in the form of pumpkin seed oil or pumpkin seed supplements.

It is important to note that these home remedies should not be used as a substitute for medical treatment, but rather as a complementary therapy to help alleviate symptoms and speed up the recovery process. If patient is experiencing symptoms of amebiasis, it is important to seek medical treatment as soon as possible (Mishra 2020; Passos et al. 2021).

Preventive Measures

There are several prevention measures that can be taken to reduce the transmission of amebiasis. These include:

1. Proper hand washing: Regular hand washing with soap and water is essential in reducing the spread of the parasite. This is particularly important after using the bathroom and before handling food.
2. Safe food and water practices: This includes avoiding contaminated food and water sources, as well as properly washing fruits and vegetables and cooking meat to the appropriate temperature.
3. Safe sexual practices: This includes avoiding sexual contact with infected individuals and using protection during sexual activity.
4. Improved sanitation: Improving sanitation in areas with a high incidence of amebiasis can help reduce the spread of the parasite. This can include measures such as proper disposal of human waste, providing access to clean water, and increasing awareness about the importance of hygiene practices.
5. Vaccinations: Currently, there is no vaccine for amebiasis, but research is ongoing to develop a vaccine that can prevent the infection (Li et al. 2021).

Conclusion

Amebiasis is a serious infection that requires prompt medical treatment. With proper treatment, the infection can be effectively treated and prevented from spreading to others. However, it is important to seek early detection and treatment to minimize the risk of complications and prevent the recurrence of the infection.

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Rift Valley Fever

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INTRODUCTION

Rift Valley Fever (RVF) is an acute viral infection which is spread by arthropods mainly mosquitoes. The disease is of zoonotic importance as it can spread in domestic animals as well as in humans (Sissoko et al. 2009). The RVF virus can also spread through the direct contact with the infected organisms, but it is very rare that this virus spread directly from humans to humans (Seufi and Galal 2010). It can spread through exposure from tissues of infected animals, body fluids and viremic blood or by biting of the mosquitoes (Hassan et al. 2011). Signs and symptoms include fever, muscle aches, headaches, loss of vision, liver problems and encephalitis and may also cause abortions in females. People who work with butchers, deals with the raw meat having rift valley fever or infection have a greater chance to get the infection and the people who sleep outdoor or spend the night-time outside their homes are more exposed to the mosquitoes which may be infected with RVF virus, so more chance to cause infection. Lab workers, farmers, herdsmen, abattoir workers and veterinarians are also at risk (Hassan et al. 2011, Seufi and Galal 2010). It is considered among the transboundary animal diseases (Sindato et al. 2011). Initially, it was only present in Africa, but now it has spread to most of the world (Bell et al. 2018, Himeidan et al. 2014).

History and Background

RVFV was first observed in rift valley in Kenya in 1930 from sheep and then spread to other regions of the world through animal movement from one place to another place (Bashir and Hassan 2019, Hassan et al. 2011) like African countries, Republic of Comoros, Arabian Peninsula, Madagascar, Saudi Arabia, Yemen, Egypt (Sissoko et al. 2009). In the rift valley numerous newborn deaths of lambs and abortion in pregnant sheep was happened in 1930. High mortalities of sheep on the farm occurred that led to its investigation. Blood from the ill lamb was taken and to check for bacterial or viral infection, it passed through a porcelain filter and was then inoculated in the healthy lamb, the same clinical signs and symptoms were observed. Investigators came to the point that outbreak occurred during high mosquito activity, so to confirm this, they isolated the healthy sheep at high altitude under mosquito nets. This approach confirmed the mosquito involvement in the disease transmission that was later confirmed by the isolation of RVFV from different species of mosquitoes involving *Aedes* and *Culex* (Wright et al. 2019).

Importance

Host Spectrum

The susceptibility of host depends upon age and species (Sindato et al. 2011). The major amplifying hosts of RVFV include cattle, sheep, and goat, although it causes disease in many other species, including buffaloes, camels, and many mammals (Borrego et al. 2016). Humans are dead-end RVFV hosts (Borrego et al. 2016, Bird et al. 2009).

Epidemiology

The severe epidemics occurred due to climatic conditions including huge greenery and floods (Kwaśnik et al. 2021). Frequently, epidemics have occurred in Africa and other countries. Major epizootics of RVFV occurred in many countries, including Africa and Asia (Kimani et al. 2016; Métras et al. 2011).

Mortalities

RVF-infected patients have a greater risk of mortality in case of hemorrhagic fever, jaundice and neurologic disease (Atkins and Freiberg 2017). Naive communities are at greater risk of inducing large mortalities and morbidities (Grossi-Soyster and Labeaud 2020).

Status of Virulence

The major factors of virulence are viral NSs proteins that suppress the innate immune status of the host (Boshra et al. 2011).

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No	Region	Epidemic year	Disease burden	References
1:	Kenya and south Africa	1950-1951	100000 sheep died and abortions up to half million	(Wright et al. 2019)
2:	Horn of Africa	1961-1962		(Martin et al. 2008)
3:	Horn of Africa	1982-1983		(Martin et al. 2008)
4:	Horn of Africa	1989		(Martin et al. 2008)
5:	Horn of Africa	1997-1998		(Martin et al. 2008)
6:	Horn of Africa	2006-2007		(Martin et al. 2008)
7:	Sudan	1973		(Martin et al. 2008)
8:	South Africa	1974-1975	110 human cases and 7 deaths	(Martin et al. 2008)
9:	Egypt	1977-1979	200000 human cases and 598 deaths	(Wright et al. 2019)
10:	Mauritania	1987	Considerable human cases and 220 deaths	(Wright et al. 2019)
11:	West Africa	1987-1988		(Martin et al. 2008)
12:	Egypt	1993		(Martin et al. 2008)
13:	East Africa	1997-1998	89000 human cases and 478 deaths	(Martin et al. 2008, Wright et al. 2019)
	Saudi Arabia	2000	880 human cases and 123 deaths	(Wright et al. 2019)
14:	Kenya	2006-2007	US$66M losses, 684 cases and 155 deaths	(Martin et al. 2008)
15:	Somalia	2006-2007	US$471M losses	(Labeaud et al. 2010)
16:	Tanzania	2007	50000 mortalities in livestock	(Labeaud et al. 2010)
17:	Sudan	2007-2008	747 human cases and 230 deaths	(Labeaud et al. 2010, Baba et al. 2016)
18:	Mayotte	2007-2008		(Labeaud et al. 2010)
19:	Madagascar	2008		(Labeaud et al. 2010)
20:	Swaziland	2008		(Labeaud et al. 2010)
21:	South Africa	2008,2009,2010		(Labeaud et al. 2010)

Economic Effect

RVF poses severe economic effects and food insecurity (Bashir and Hassan 2019). It is a potential bioterrorism weapon. Due to deaths in livestock, losses in revenue generated. Quarantine procedures and disease burden on the animal cause less production of animal products. Moreover, continuous abortions in pregnant animals, result in the loss of progeny that induces financial burden (Grossi-Soyster and Labeaud 2020). It ~~is~~ greatly affects the agro industries and the worldwide trade (Muga et al. 2015; Peyre 2015).

Zoonosis

Most of the emerging infectious illnesses cause zoonosis. There is an increasingly high zoonosis in endemic areas (Seetah et al. 2020). It is declared as one of the threats by the US center for disease control, to the livestock industry (Seetah et al. 2020). Slaughterhouse workers, farmers, herders and veterinarians are at greater risk of acquiring infection (Paweska 2014).

Etiology

Whole Family

The etiological agent of rift valley fever is a virus named as RVFV or arbovirus, family; *bunyaviridae*, genus; *phlebovirus* (Flick and Bouloy 2005). RVFV is a single-stranded negative-sense, tripartite RNA virus. It can survive in both biotic and abiotic environment (Meegan and Bailey 2019).

Topographic Spread (genus, species)

The vectors of the RVFV are the mosquito species belonging to the genus *Culex*, *Aedes* and *Manzoni* (Atkins and Freiberg 2017).

Life Cycle

Basically, the genome of RVFV consists of three segments. These three segments are termed as S, M and L segments and these segments are RNA segments. Among the three, L and M have negative polarity (Paweska 2014). These three segments encode different genes. S segment encodes genes; N, NSs, M; Gn, Gc and NSm genes and the last segment L, encodes RNA polymerase L gene. The pH dependent RVFV virions bind primarily through endocytic pathway to cellular receptors. After entry into the cell, uncoating takes place and ribonucleocapsid principally comprised of genomic RNA segments and proteins, is released in cytoplasm. Within 40 minutes after viral infection, viral m RNA synthesis takes place by viral polymerase through transcription (Ikegami et al. 2009). After transcription, replication of viral RNA takes place within 1 to 2 hours then viral RNP and RNA segments packaging starts. Finally, RNP packaging leads to viral virions formation. The surface of RVFV virions is symmetrical icosahedral lattice. Nucleoproteins have no pathogenic significance (Boshra et al. 2011).

Animals

Pantropic hemorrhage and hepatic necrosis cause high mortalities in young animals and abortion in pregnant animals (Martin et al. 2008).

Sheep

The incubation period ranges between 24-36 hours including listlessness, bloody diarrhea and loss of appetite. Postmortem findings reveal mild splenomegaly and liver necrosis of multifocal nature (Faburay et al. 2016). In acute cases sheep have 100% mortality rate.

Goat

The pathogenesis of the disease is less severe in lambs of the same age. It does not result in febrile illness. In disease condition, necrotic hepatitis is followed by necrotic lesions that is focal in nature in adults (Wright et al. 2019).

Cattle

These are less susceptible as compared to goats and sheep. These develop an acute disease with a 0-5% mortality rate, but calves have 10% mortality rate. Viraemia and liver pathology can be seen (Wilson et al. 2016)

Camel

Camels are less susceptible than others; this includes foot lesions, hemorrhages, and abortions during epidemics (El Mamy et al. 2011).

Monkey

African green monkeys acquire the neurological disease that is similar to humans (Wonderlich et al. 2018).

Humans

Commonly RVFV cause hepatitis, hemorrhagic complications, and encephalitis (Martin et al. 2008).

Pathological Findings

Hepatitis/hemorrhagic Disease

Liver is the organ which effects severely and bear burden of RVFV in almost all species. Hemorrhagic disease and jaundice develop because of enlargement of the liver, which is the major site of replication of RVFV. The level of liver enzymes, including alanine transaminase and aspartate transaminase, increases (McElroy and Nichol 2012). Platelet count and hemoglobin level decrease, that ultimately increase clotting time. Thrombosis, scleral icterus and delirium may also be present. There is a high fatality rate among hemorrhagic fever patients (Ikegami and Makino 2011).

Ocular Disease

In 2 to 5% of patients, ocular manifestations are observed, that develop within 3 weeks after the start of the symptoms (Al-Hazmi et al. 2005). It causes uveitis, retinal hemorrhage, retinitis, and blind spots (McMillen and Hartman 2018).

Neurologic Disease

It includes encephalitis, hemiparesis, excessive salivation, vertigo, weakness, decerebrate posturing and pleocytosis (Ikegami and Makino 2011).

Abortions/Miscarriage

In 2006, there was a significant increase in abortion frequency, but more in animals than humans (Baudin et al. 2016).

Clinical Sign and Symptoms

Humans

In humans, diverse symptoms scale from headaches and photophobia to encephalitis and retinitis (Boshra et al. 2011; Flick and Bouloy 2005). The symptoms vary according to the severity to disease; likewise, the flu may be accompanied by nausea, headache, arthralgia, joint aching and myalgia. Moreover, diarrhea, fever, oliguria or anuria can also be seen. Some patients may have the symptoms like vomiting, loss of appetite, light sensitivity and stiffness of neck. Hemorrhagic or encephalitic disease conditions account for less than 1%. Meningoencephalitis state in humans develop within 1 to 4 weeks after the onset of the disease, its clinical features include memory loss, headache, confusions, hallucinations, vertigo, disorientation, lethargy, convulsions, and coma. After 2 to 4 days of the illness, hemorrhagic sign and symptoms appear that is evidenced by the bleeding from gums or nose, or from GIT, ecchymosis, petechiae, venipuncture sites, purpura, or menorrhagia. In this case, the fatality rate is up to 50%. Usually, death happens after 3 to 6 days of the symptoms. Further, in some patients, ocular lesions cause a severe form of disease. In this case, scotomas and central vision loss were observed, which lead to blindness in one or both eyes (Paweska 2014).

Animals

Severe clinical signs and symptoms are present in sheep, which include fever, abdominal pain, and disinclination to move (Martin et al. 2008). Goats develop less severe signs and symptoms than sheep. In cattle, the adults are mostly asymptomatic, although fever-like symptoms may be present. The signs and symptoms appear in camels include hemorrhages, ocular discharge, and foot lesions (Martin et al. 2008).

Treatment in Practice

Currently, we provide supportive care to cure RVF (Atkins and Freiberg 2017). For chemotherapy, kinase inhibitors alone or in combination can be used (Bell et al. 2018).

Vaccines

Treatment by vaccines started soon after the isolation of RVFV in 1931 (Bird et al. 2008). No vaccine schedule is present for humans and animals in non-endemic regions (Borrego and Brun 2021). Smithburn vaccine is commonly used in Africa, and it was established by Smithburn in 1949 and cause immunity for lifetime in vaccinated animals (Sindato et al. 2011). The soldiers of the United Nations who are staying in infected countries were administered by a vaccine that is formalin-inactivated mosquito-derived (Lancelot et al. 2019). MP-12 is conditionally approved by the FDA for administration in the USA (Lancelot et al. 2019); it was developed by the Egyptian virulent strain ZH548 but still has teratogenic effects in sheep (Boshra et al. 2011). Several inoculations are required in the case of DNA vaccines, as they are less immunogenic (Lancelot et al. 2019). Clone 13 does not cause abortion in ewes (Boshra et al. 2011). A human infected with 74HB59 is the source of collection of clone 13 vaccine (Wright et al. 2019).

Antiviral Therapeutics

Ribavirin is used against ZH501 strain, inhibit replication of virus (Atkins and Freiberg 2017). It enhances survival rate up to 100% at dose rate 18.8 mg/kg, subcutaneously. Ribavirin at the dose rate of 75 mg/kg can treat the peracute state of RVF disease in animals (Kimani et al. 2016). Favipiravir/ T-705/Avigan is a broad-spectrum antiviral agent. Its efficiency has been evaluated in hamsters against RVFV ZH501 strain. It is effective against different genera of bunyaviruses (Scharton et al. 2014). In mouse model, rapamycin, an FDA-approved drug, is used to control the pathogenesis of RVFV by decreasing N protein production (Bell et al. 2018).

Type 1 interferon α/β holds the key significance in treating RVFV as have great antiviral potential (Borrego et al. 2016). Argovit is a silver nanoparticle with 35 nm size approximately, is the commercial preparation used to treat RVFV in humans and animals (Borrego et al. 2016). Eryl methylidene, a Rhodanine derivative, is an effective drug against the rift valley fever virus as it has broad-spectrum activity and inhibits virus cell merging (Labeaud et al. 2010, Wolf et al. 2010). Bavituximab is another broad-spectrum antiviral agent; it shows its antiviral activity by targeting phosphatidylserine, which is visible on the plasma membranes of the infected cell and also of enveloped viruses (Labeaud et al. 2010, Soares et al. 2008). Other antiviral therapeutics include suramin, sorafanib and bortezomib etc. (Atkins and Freiberg 2017).

Insecticide Treatment

At mosquito breeding sites, larvicide treatment is useful; some of the common larvicides include Bacillus thuringiensis israeliensis and pyroxyprofene or methoprene (Lancelot et al. 2019). In the live bait trap technique, cattle are treated with remnant insecticide, which kills mosquitoes during feeding and stops transmission of RVFV (Lancelot et al. 2019, Poché et al. 2015).

Treatment Being Searched

Several vaccines against rift valley fever virus are in clinical trials (Grossi-Soyster and Labeaud 2020). ChAdOx1 is a human vaccine that is under processing (Stylianou et al. 2015). To fight with the RVFV, the kinases are being studied in translational pathway (Bell et al. 2018). For effective medication, we are searching for host-based therapeutics (Bell et al. 2018).

Control Measures

The proper prevention and control need well collaboration between entomologists, health and veterinary authorities, biologists and environmental specialists (Hassan et al. 2011). This one health approach will help us to eradicate RVFV from the world. Various control measures include larvicides for vectors, vaccines for animals, control of animal trade and proper training sessions for the awareness of public (Meegan and Bailey 2019). Vaccination is the best method in animals for the protection of human health. To control RVFV, the best way is to vaccinate both humans and animals (Atkins and Freiberg 2017).

Avoiding direct contact with the infected body tissues, fluids; mosquito evading, proper bed nets and proper use of mosquito repellent sprays, moreover restrict themselves to the houses during peak feeding hours of mosquitoes (dawn and dusk) (Grossi-Soyster and Labeaud 2020). Instant precaution includes the use of personnel repellents. We should be careful in dealing with the infected animals for examination, milking or during other nursing approaches and use personnel protection equipment (Lancelot et al. 2019).

In order to stop the outbreaks, we should use meat and milk after proper cooking and boiling and stop the consumption of non-inspected meat (Sindato et al. 2011). In endemic areas proper care can control RVFV that include proper pasteurization of the food; light color clothes that comprise of long-sleeved shirts and trousers are preferred (Paweska 2014).

Conclusion

Rift Valley fever (RVF) is an arthropod-borne viral disease of ruminants, camels, and humans. It is considered as a

significant zoonotic issue causing uncomplicated influenza-like illness but may also lead to hemorrhagic illness with liver involvement. The ocular or neurological lesions may also be present. In animals, RVF may be inapparent in non-pregnant adults, but outbreaks are characterized by the onset of abortions and high neonatal mortality. Jaundice hepatitis and death are seen in the older animals. Outbreaks are generally linked with heavy rainfall, producing high population of mosquitoes which act as a main vector. After virus amplification in vertebrates, mosquitoes act as secondary vectors to sustain the epidemic. The above discussion and the relationship of disease between animals and humans ensure the concept of one health triad and needs appropriate control measures to limit the spread of disease.

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Strategies for Malaria Prevention and Control

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INTRODUCTION

Globally, a handful of malaria vaccine publications have endeavoured to provide a detailed image of all clinical trials that have occurred in the past. Now, it is challenging to sum up, all projects in a single rave as the field has expanded at an unprecedented rate. WHO has compiled a "rainbow table" spreadsheet, an inclusive publicly available collation regarding global malaria vaccine projects published in the past years (Schwartz et al. 2012).

Malaria is a life-threatening disease affecting young children and pregnant women caused by parasites of the *Plasmodium* genus. With about half of the world's population on the verge of infection, it poses a significant health hazard. It is transmitted to the host when pathogen-ridden mosquitoes bite them. People from third-world countries are at a greater risk of getting the infection and are more susceptible to death, especially children below five years of age residing in sub-Saharan Africa (Laurens 2018).

The need to develop a vaccine against malaria has been stressed from the documentation of the parasite in 1897. In 1897, Ronald Ross discovered the mosquito (vectors) that transmit the disease. Moreover, the parasite can only be transmitted by the female *Anopheles* mosquito. The appearance of resistant parasites and vectors has triggered to focus on other control achievements, including a vaccine. (Mahmoudi and Keshavarz 2018). Malarial immunity through vaccination was established more than 30 years ago when individuals were immunized via continual bites of *Plasmodium falciparum*-infected mosquitoes, but irradiated mosquitoes still hold metabolic activity. (Arama et al. 2014). After being neglected for decades, attempts to cope with malaria have increased significantly with the international community's funding. An increase in funding has boosted the status of proceedings comprising control of malaria, such as the acquisition and dispersal of artemisinin-based combination therapy (ACT), the anti-malarial drug group of choice and insecticide-treated bed nets (ITNs) along with other mosquito vector control plans. These medications have been temporarily associated with the decline in the incidence of malaria of more than 50% in certain zones of Africa. Regrettably, the poor healthcare infrastructure of many malaria-endemic countries hinders the implementation of ACTs and ITNs. Moreover, it has been observed that the microorganism is developing resistance to anti-malarial drugs and rapidly spreading it. Even now, the opposition has been set in Asia to the artemisinin derivatives. So, an effective vaccine is needed to control, eliminate, or even eradicate malaria (Crompton et al. 2010).

Only two species of *Plasmodium* are in the run for vaccine development out of five species that cause malaria in humans. More than 90% of malaria-related deaths are attributed to *Plasmodium (P.) falciparum*, and there is a similar ascendency of *P. falciparum* projects in the malaria vaccine landscape (Schwartz et al. 2012). Unfortunately, to develop a fruitful vaccine for *falciparum* malaria, there are certain complications, such as the extreme intricacy of malarial parasite life, intricate and diverse parasite genomes, immune dodging, and the complex nature of the infectious cycle of the parasites (Mahmoudi and Keshavarz 2018).

Vaccines are at the top of the list in promoting both individual and public health, among all the highly effective tools. Vaccination against infectious diseases has made the most significant contributions to global public health compared to all other human interventions. Presently, no licensed or registered vaccine exists for malaria. Some experts deemed it necessary to eliminate malaria. The WHO published strategic goals to accredit malaria vaccines encountering *P. falciparum* and *P. vivax* with no less than 75% protective efficacy against clinical malaria and reducing spread to enable elimination (Laurens 2018).

There are quite a few malaria vaccine candidates who have undergone different phases of clinical trials; however, until now, there was not a good candidate with practical usefulness. Currently, the contenders are directed against those stages of the pathogen life cycle, which comprises humans and mosquitoes for a malaria vaccine. Still, up until now, for potential vaccine development, only some proteins have been considered (Crompton et al. 2010).

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Table 1: Discoveries regarding malaria by various scientists in the history (Cox et al. 2020)

Sr.no	Year	Discovery	Scientists
1	1880	Discovered parasites in blood as well as sexual stages of malaria in bloods were discovered	Alphonse laveran and William MacCallum
2	1897	Different phases of transformation cycle in culicine mosquitoes and birds infected with *Plasmodium relictum* were found.	Ronald Ross
3	1898	It was described after certain experiments and observations that plasmodium is spread by mosquitoes that act as their vectors	Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli
4	1948	It was reported and described that agents (parasite) responsible for malaria are grown in liver or hepatic system before they gain entry into the blood vessels	Henry
5	1982	The last stage in the life cycle that is the presence of inactive stages in the liver was completely described	Wojciech Krotoski

Malaria History

Malaria is an old Infectious disease. According to all proofs and experiments, malaria was first documented in China in about 2700 BC, in clay tablets in Mesopotamia in 2000 BC, in Egypt in 1570 and in Hindi textbooks in the sixth century. These historical records are kept with so many caries and precautions. Still, these are very important for studies when we move into the following centuries, and we get firm knowledge through these records. Greeks that contain Homer in 850 BC, Agrigentum having state Empidocal in 550 BC, and Hippocrates in 400 BC was well known in the documentary and different aspects of poor health, including fever and spleen enlargement, were observed in the people that were resident of dirty areas. Antoni van Leeuwenhoek found bacteria in the year 1676, and the formation of the germ theory of diseases by Louis Pasteur and Koch during 1878-1879 also made help in the discovery of malaria at an intensive degree (Cox et al. 2020). The general history of malaria was described in 1849 (Poser Charles and George 1999). In 1970, widespread resistance was developed to malaria, and there was no treatment for malaria (Butler et al. 2010). Malarial disease always has been a public problem. It impacts death and infection rates in underdeveloped countries. After that, a noticeable decrease in malarial cases was seen between 2000 to 2010, but it has always remained a challenge (Corine et al. 2020). Table 1 highlighted the various discoveries in the history regarding malaria.

Malaria Status in Pakistan

In Pakistan, each year, 3.5 million confirmed cases of malaria are reported. Between 2015 to 2018 there is regular increase in malaria cases. According to WHO, in 2017 and 2018, 60% of the people in Pakistan lived in the malaria-endemic region (Ali et al. 2010). Out of six countries in the Eastern Mediterranean region, Pakistan has the highest ratio of malaria transmission. The prevalence of malarial infection is different in different provinces and varies in different cities due to climate changes. The province wise prevalence of malarial infection in Pakistan in 2017 was 1.1% in Punjab, 26.5% in Sindh, 20.5% in Baluchistan, and 30% in Khyber Pakhtunkhwa (Ali et al. 2010).

Strategies about Prevention of Malaria

With the advancement in technology, various techniques have been developed to control malaria. Vector control and community mobilization are the effective methods which are described below;

Vector Control

The term "vector control" refers to a set of actions taken against a disease vector with the goal of protecting recognized disease transmission hotspots while limiting the disease vector's capacity to spread the disease. The capability of populations of local vector, or more specifically, the size of the population of the vector, human biting behaviours, and duration relative to the sporogony period determines the susceptibility to malaria. Climate, regional ecology, humans, and vector activity significantly impact each of these variables. To be as effective as possible, vector control strategies must be tailored to the local environment. The goal of vector control during an elimination phase is to lower the populations of local vector having capacity below the very critical level required to uphold transmission (Gueye et al. 2016).

Main Methods for Vector Control

Insecticide-treated Mosquito nets (ITNs)

Long-lasting insecticidal nets (LLINs), which have insecticide lasting up to 3 years, and conventionally treated nets, which have insecticide lasting up to 12 months, are both ITNs. WHO directed all health ministries as well as donor organizations to increase ITN distribution, focusing on populations of young children and expectant mothers since they are at high risk (WHO 2007). With periodic mass distribution campaigns, most national malaria control programs currently use ITN distribution to provide universal coverage.

Larval Source Management (LSM)

LSM is the control of aquatic/watery habitats that may serve as breeding grounds for the mosquito to halt the maturation

of immature stages. It is still neglected as a malaria control tool in Africa despite being one of the oldest weapons in the fight against the disease (Fillinger and Lindsay 2011). LSM got increasing attention as a result of the recent realization that outdoor biting plays a role in the transmission of malaria and offers benefits of lowering outdoor as well as indoor mosquito populations (Gies et al. 2009).

LSM can be Further Classified as

a. Habitat Modification

Landscaping, land reclamation, surface water drainage, and filling are all examples of permanent changes to land and water. It can be completed with basic tools and supplies in remote locations (Fillinger and Lindsay 2011; Tusting et al. 2013).

b. Larviciding

Mosquitoes can regularly be controlled by spraying biological insecticides or chemicals on water bodies. It works better in locations with few, stable, and easily identifiable habitats. The anopheline mosquito larvae control and the decreased numbers of adult mosquitoes have been demonstrated to be effective with microbial larvicides. They do not affect other aquatic species, which gives them a safety edge over chemical larvicides (Tusting et al. 2013).

c. Biological Control

Watery ecosystems are being invaded by natural enemies (e.g., invertebrates, parasites, predatory fish, and disease organisms) (Fillinger and Lindsay 2011; Tusting et al. 2013). To make this strategy work, a lot of resources, and better organization from professionals is needed.

d. Habitat Manipulation

By manipulating water levels, for example, actions like flushing, clearing drains, exposing, or shading, habitats are frequently taken to the sun. Habitat manipulation is more suited in environments with scarce resources, like habitat modification (Tusting et al. 2013).

Indoor Residual Spraying (IRS)

The main Global Malaria Eradication Campaign strategy is IRS. It contributed to the complete eradication of malaria in certain countries and considerably reduced its impact in others (WHO 2015, Global technical strategy for malaria 2016–2030). In 2015, the IRS provided protection to almost 106 million individuals. Its recent growth into areas with high transmission has prompted concerns about its long-term viability as it has traditionally concentrated on areas of low or seasonal transmission (WHO 2015, Global technical strategy for malaria 2016–2030). Several nations have employed IRS to eradicate malaria and manage epidemics.

Methods Under Development

Mass Drug Administration (MDA)

Using the curative drug dose to treat the whole population in a certain area without checking for infection and irrespective of the appearance of signs and symptoms is known as mass drug administration. Since the early 1930s, it has been used to manage malaria and in the 1950s (Poirot 2010), WHO promoted its elimination and eradication. MDA with antimalarials has proven to be effective when used in conjunction with other malaria prevention strategies. For instance, MDA with sulphadoxine-pyrimethamine and IRS achieved significant malaria control levels during the Garki Project in Northern Nigeria in 1969 (Molineaux and Gramiccia 1980). Primaquine and chloroquine were administered to almost 70% of the population of Nicaragua, preventing 9200 instances of malaria (Garfield and Vermund 1983).

According to current research, ivermectin mass medication administration is working well in controlling malaria, especially for residual malaria. An endectocide that has been approved for use in humans is ivermectin. It is a semi-synthetic derivative of Streptomyces avermectin fermentation products. Over one billion treatments have been administered for neglected tropical diseases such as lymphatic filariasis (Chaccour et al. 2013; Chaccour et al. 2015), onchocerciasis, and strongyloidiasis over the previous 25 years. The drug makes blood deadly to malaria mosquitoes after being ingested for around six days while it is still in the bloodstream. As a result, following a single conventional oral dose, fewer Anopheles mosquitoes survive to bite a person who has had ivermectin treatment (Chaccour et al. 2013; Chaccour et al. 2015).

House Improvement (HI)

Houses are the primary transmission habitat in many endemic regions (Huho 2013; Bayoh 2014; Barreaux 2017). In the past, it was believed that better housing was a factor in the malarial eradication in the USA and the decrease in disease incidence in Europe (Zhao 2016). Modern homes typically give protection against malaria that is comparable to ITNs and are preferable to older homes constructed of natural materials that have numerous openings for mosquitoes to enter. Comparing contemporary housing to traditional housing, data from demographic, health, and indicator surveys of malaria carried out in the 21 SSA nations between 2008-2015 demonstrate a decrease in malaria prevalence (Tusting 2015).

Swarm Sprays

The sites of mating swarms appear to be linked to swarm indicators on the ground (i.e., wood piles, walls, or the boundaries between grass and footpaths) which are consistent throughout the seasons (Diabaté et al. 2011).

Sugar Feeding

A novel vector control method, called attractive toxic sugar bait (ATSB), kills both female and male mosquitoes as they search for vital sources of sugar in the open air (Beier 2012). The ATSB method employs a fruit or floral aroma to draw mosquitoes in, a sugar solution to stimulate eating, and an oral toxin to kill the insects. The mosquitoes that consume the toxic ATSB solutions are destroyed. Either plants will be sprayed with the ATSB solutions, or they will be suspended in straightforward bait stations. Given its simplicity in terms of technology and operation, safety for the environment, and affordability, this intervention is great for reducing malaria in low- to middle-income nations. Spinosad and boric acid are the typical insecticides used by ATSBs; however, ivermectin has lately emerged as a viable option (Müller et al. 2010; Beier 2012).

Community Mobilization

All malaria preventive efforts must succeed in part due to community mobilization and methods for behaviour modification. This might take the shape of community-based initiatives, media, information, education, and communication (IEC) items used in public health communication. Communities can gain a better understanding of the disease by utilizing influential members of the community and teaching them about the advantages and proper application of malaria preventive methods. Misconceptions concerning the spread of malaria should be dispelled, as should the need for prevention and quick diagnosis and treatment when one suspects the disease (Ingabire et al. 2014).

Malaria Vaccine Development

Pre-erythrocytic Stage Vaccine (Live attenuated liver stage)

Live attenuated vaccine is still the most critical choice because it offers long-term sterile immunity to malaria transmission. The attenuation of irradiated sporozoites depends upon the random mutations that block the liver stage development. Therefore, immune individuals support attenuated heterogeneous populations, but the genetically dissipated sporozoites limit this study solely for experimental purposes (Silvie et al. 2002). Despite all limitations, sporozoites have proved helpful in providing long-term sterile immunity (Morrot and Zavala 2004). In humans and mice, experimental sporozoites have been shown to provide immunity against malaria transmission at the liver stage (Nussenzweig et al. 1967; Hoffman et al. 2002).

Moreover, genetically attenuated parasites (GAPs) are formed by transfection of the asexual blood stage. Therefore, it causes consistent and continual production of genetically stable attenuated sporozoites. Complete cessation of the hepatic stage demonstrates the production of GAPs even with weak preventive measures. Recently, a gene named USI3 has been identified in the parasite *Plasmodium berghei*. It is known that deletion in this gene results in the loss of a parasite's ability to mature in merozoites. Animals that were attenuated with the three consecutive doses of the removed USI3 gene demonstrated that animals had sterile immunity even for a more extended period. This experiment must be translated for *P. falciparum* (Mueller et al. 2005).

Blood Stage Vaccine

Immunity develops against individuals over time by naturally exposing people to the pathogenic agent, but sterile immunity can only be induced artificially. Over time, as children become sexually mature, they have also obtained the degree of semi-immunity that protects them against serious infections, but not against all infections. In passive transfer studies at the early stage, it came to know that when immunoglobin from semi-immune individuals acts against the blood stage, it cures the clinical complications in a person with no or low immunity (Cohen et al. 1961). It is also seen that children who live in endemic areas develop a degree of immunity against cerebral malaria in only one or two episodes that protect them against severe disease. Antigens that are present on the surface of infected RBCs and merozoites are erythrocytic malarial vaccine candidates which include merozoite surface proteins 1, 2 and 3 (MSP1, MSP2, MSP3); apical membrane antigen (AMA1); glutamate-rich protein (GLURP); ring-infected erythrocyte surface antigen (RESA); serine -repeat antigen and erythrocyte-binding antigen (Gupta et al. 1999).

Some studies in Gambia have shown that the protective effect of antibodies in a genetically diversified field of MSP3 is even stronger than in target-conserved areas (Polley et al. 2007). A vaccine trial was held in Papua New Guinea using a mixture of RESA protein, MSP1, and MSP2, which showed an increased number of infections from non-vaccine type parasites with MSP2 compared to those who received a controlled vaccine (Genton et al. 2002).

Merozoite Vaccine

The merozoite antibody-mediated vaccine can be obtained by targeting any of the merozoite surface proteins (MSP), peripheral surface proteins, and, to a lesser extent, secretory

organelle proteins (Siddiqui et al. 1987). In the recent advanced studies, clinical trials of first surface protein like MSP1 having AS02 adjuvant were recommended with 42k Da carboxyl protein fragment (Stoute et al. 2006). MSP1 is a major pleomorphic protein in two allelic forms; both are still being studied preclinically (Woehlbier et al. 2006). Recently, MSP3 phase 1 clinical trials, B and T cell epitopes along with aluminum adjuvant, showed high antibody levels in vaccinated organisms. When transferred to the mouse model, it was seen that antigen-specific antibodies could inhibit parasite growth in vitro along with clear parasitemia and monocytes (Druilhe et al. 2005). These are the evaluation in the efficacy trial and showed that the choice of functional antigen should not depend on the genes but should be based on the functional assay (Dorfman et al. 2005). A rodent malaria model system, explain why the antibody response is short-lived and it is because the parasites induce the deletion of antigen-specific memory B cells (Wykes et al. 2005).

Subunit Vaccine

The live attenuated or killed vaccine is not feasible in many diseases. In a subunit vaccine, an antigen or part of an antigen is identified from a pathogen that induces immunity against the whole pathogen on vaccination. The hepatitis B vaccine is an effective protein subunit vaccine. This vaccine was designed to give the maximum humoral immune response. Proteins have a significant variation in their immunogenicity. So, the protein subunit vaccine does not apply to many diseases (Courouce et al. 1981).

The latest generation of subunit vaccination is DNA-based (Ulmer et al. 1993: Li et al. 1993). The DNA sequence from *P. falciparum* was inserted into various recombinant DNA viruses forming recombinant viral vaccines or inserted into plasmid DNA molecules forming DNA vaccines (Schneider et al. 1998; Wang et al. 1998). DNA vaccines are taken up by the expressed host protein and form T cell epitopes that join with the HLA molecule which is prime naïve to T-cells and form the memory T-cells (Gurunathan et al. 2000). Viral vaccines also work similarly, but viruses infect the cells and express T-cells antigens before the start of infection (Miyahira et al. 1998). DNA or Viral vaccines induce a high T-cell response but not a good antibody response (Paoletti 1996; Gurunathan et al. 2000).

Whole Sporozoite Vaccines (WSV)

The work on WSV has been a challenge since the 1970s. It was thought that the idea of WSV was impractical because of the synthesis of irradiated sporozoite (Smith et al. 1991). In 2010, a company named Sanaria worked on harvesting PfSPZ from the salivary gland of a mosquito infected by laboratory parasites, followed by preservation, vialing, and cryopreservation in liquid nitrogen (Hoffman et al. 2010). The efficacy of WSV in humans is seen to be dependent on the dose (Seder et al. 2013; Mordmuller et al. 2017; Sissoko et al. 2017). The level and duration of protection in homologous or heterologous sporozoite in malaria-naive adults depend upon the dose and regime with either PfSPZ-CVac or PfSPZ vaccine that has achieved a high level of immunity (Epstein et al. 2011; Ishizuka et al. 2016; Epstein et al. 2017).

Placental Malarial Vaccine

The placental malaria vaccine targets the chondroitin sulfate A (CSA) that binds the parasites and sequesters them in the placenta. Other pre-erythrocytic and erythrocytic stage vaccines that protect the general population against malaria can also protect pregnant women. Naturally, antibodies to the CSA are present to protect against malaria after several pregnancies, as in endemic areas, mothers are resistant to placental malaria (Fried et al. 1998). Placental parasites express the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) which is the member of VAR2CSA that bind to the CSA binding site (Salanti et al. 2003). The antibodies induced by the VAR2CSA prohibit parasite binding to CSA (Fried and Duffy 2015). VAR2CSA is a complex target with an extracellular domain >300kd, six BDL domains, and some interdomain regions. Recently, in field cases, seven to eight BDL domains have been found (Doritchamou et al. 2019).

Conclusion

Malaria still poses a threat to public health, especially in Sub-Saharan Africa, where it is a major cause of morbidity and mortality, particularly among children. Significant strides have been made in reducing malaria-related morbidity and mortality over the past 10 years. The vector control strategy still needs to be rapidly developed to realise its full potential. ITNs and IRS are the main malaria prevention and control methods because of their proven efficacy in lowering disease load. However, setting goals for eradicating malaria in numerous nations is justified by scaling up the combination of vector control measures. The development of novel vector control techniques is essential for the eradication of malaria, however many of these techniques have limitations, particularly in terms of lowering the disease burden, necessitating more research. The cost is the biggest obstacle that makes IVM a missed prospect in many endemic nations. Antimalarial use for high-risk groups, including children and pregnant women, lowers the disease burden in endemic nations. NGOs, governments, scientists, and research institutions must work together to develop improved malaria prevention strategies. This would end the disease's needless deaths of children under five by 2030, as the Sustainable Development Goals mandated.

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Toxocariasis

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INTRODUCTION

Among the infectious diseases transmissible between animal and human populations (zoonoses), dogs and cats are considered as a major reservoir to spread infection to the public since they can harbor diverse pathogens such as helminths. Human Toxocariasis is caused by nematode larvae of the *Toxocara* genus, one of the most prevalent parasites in the world (Mangaval et al. 2001; Luna et al. 2018) including *Toxocara (T.) canis* in dogs, to a lesser extent *T. cati* in cats (Fan et al. 2013), and possibly *T. vitulorum* in cattle and buffalo, as well as *T. leonina* in a wide range of carnivores. It is considered as a major helminth infection in many countries, especially in tropical regions (Fan et al. 2015); however, it can be found in industrialized countries, mainly in rural areas (Duréault et al. 2017). This zoonosis arises from disparities in healthcare and is associated with conditions of poverty and poor hygiene measures (Walsh and Haseeb 2012). According to the Centers for Disease Control and Prevention (CDC), in the US, toxocariasis is listed as one of the top five neglected diseases (Tyungu et al. 2020).

Parasite Morphology

The *Toxocara* genus belongs to the class Nematoda, order Ascaroidea, superfamily Ascaridoidea, and comprises of 21 species. *T. canis* and *T. cati* are the species most commonly involved in human toxocariasis. Taking this into consideration, the description of the morphology and characteristics of *T. canis* is made as a reference for the etiological agent of the disease in humans (Okulewicz et al. 2012).

Adult parasites have a cylindrical shape, elongated, and is ivory white in color. It is important to highlight that, externally, there are irregular transverse striae with eminent cervical wings, which are longer than wide. In addition to this, there are lips surrounding an oral orifice that is continuous with the esophagus; these lips, in turn, form a bulb with two lateral lobes separated by a canaliculus (Bowman 2020).

The adult specimens exhibit sexual dimorphism. The males measure between 4 and 10 cm in length by 2.5 mm in diameter; at the caudal end, they display an elongated finger-like appendage without caudal wings, with two series of about 20 to 30 small preanal papillae and five postanal papillae on each side of the tail, and they do not have a gubernaculum. In the case of females, they are larger, measuring 5-18 cm in length by 2.5-3 mm in diameter; the genital organs develop on either side of the vulvar region, which is located anteriorly. Females can expel around 200,000 eggs/day that measure 85-90 µm by 75 µm, are ovoid, and have a thick cover with small depressions, which favors their viability in the external environment for long periods of time even under unfavorable environmental conditions (Holland and Hamilton 2006).

At the time of oviposition, eggs are yellow in color due to the bile pigments discharged into the host's digestive tract. This coloration is not observed when the eggs are obtained by hysterectomy from a gravid female. There are four larval stages (L1 to L4), with L3 being the one found inside the embryonated eggs and is considered to be infective stage (Bowman 2020). Regarding the size of larval stages, L1 can measure up to 0.5 µm, L2 up to 500 µm, L3 up to 1.5 mm, and L4 up to 20 mm. The cuticle, nervous system and ganglion nuclei, as well as the excretory and digestive systems, are formed during the L1 stage, with few changes observed in the L2 phase in which virtually an increase in size is perceived. During the third larval phase (L3), the differentiation of the digestive apparatus and the genital apparatus occurs with the appearance of the lips and the genital outline. Finally, in the fourth larval stage (L4), both the lips and the sex of the nematode are differentiated, culminating in the adult stage with sexual maturity, growth, and expansion of the cervical wings (Despomier 2003).

Characteristics of the biological cycle of *Toxocara*

The biological cycle of *Toxocara* can be direct when it takes place in only one host or indirect when there is the participation of more than one host (more than one species); this cycle is represented in Fig. 1.

Non-embryonated eggs are expelled in the feces of the definitive host (canids*: T. canis* and felids: *T. cati*) into the environment, where they embryonate, reaching the L3 stage

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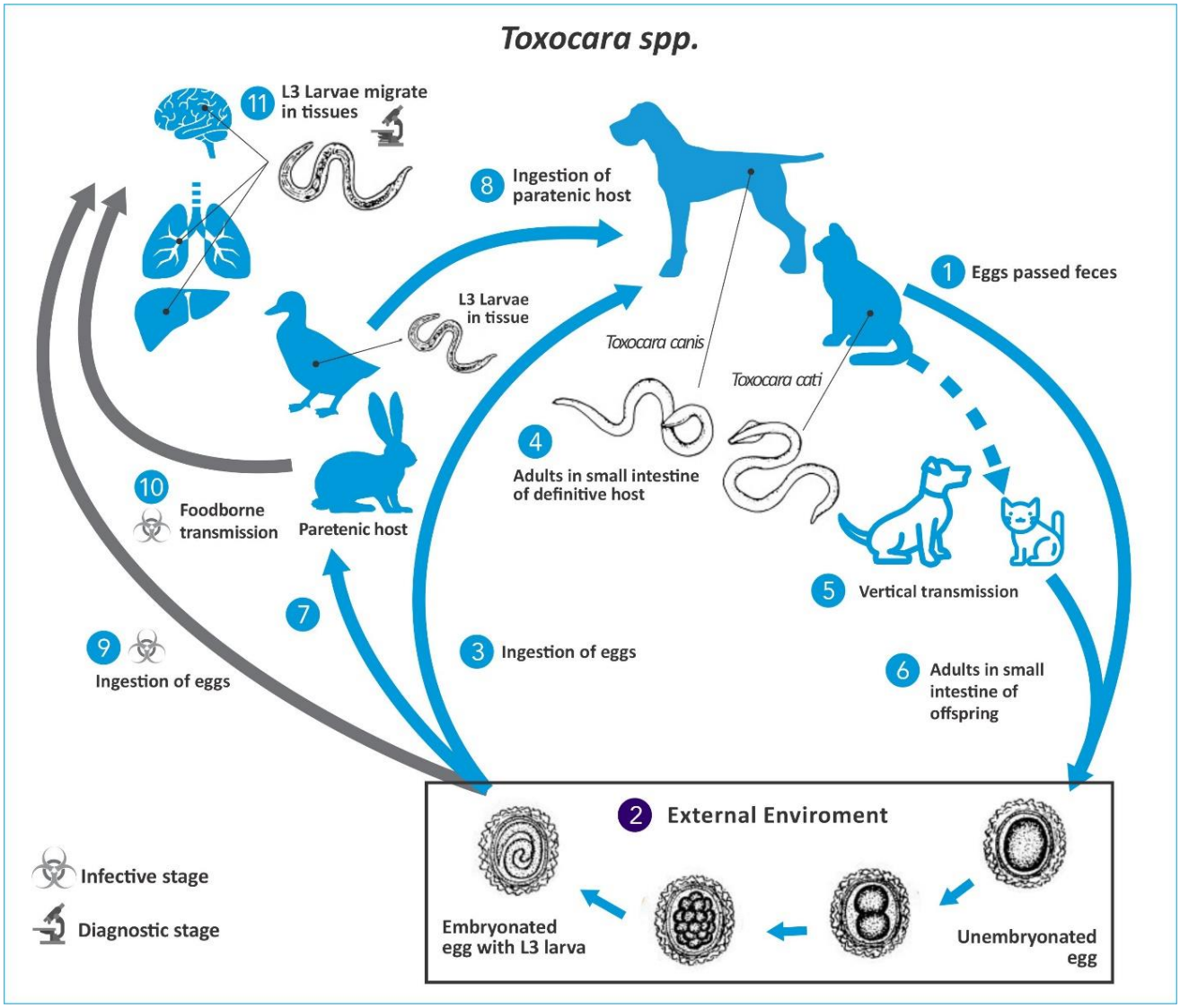

Fig. 1: Biological cycle of *Toxocara* spp.

embryo formation, the presence of O_2 and high relative humidity of 85-95% are essential requirements (Despommier 2003). It has been reported that at in a period of between one to four weeks, depending on environmental conditions. For an efficient process of temperatures between 12-18 °C, 54 days are required for the eggs to become infectious, while between 25-30 °C the time is reduced to 14 days. However, regardless of how long it takes for the eggs to embryonate and become infective, they can survive under optimal circumstances for at least one year (Chia-Kwung et al. 2003).

To complete the biological cycle, the definitive host must ingest the embryonated eggs, which hatch in the intestine, releasing the infective larvae that penetrate the intestinal wall. In the case of young animals, these larvae migrate and pass through different organs, reaching the lung via blood. When they reach the lumen of the bronchi, they are expelled with the secretion of mucus to the pharynx by coughing, and from this site, they are swallowed, passing through the gastrointestinal tract to settle definitively in the small intestine. In this organ, they mature, reach the adult stage, mate, and the females begin oviposition around 3 to 4 weeks after ingestion of the eggs. In adult dogs, infection by the oral route is also possible in the same way, culminating in the development of adult worms and the production of eggs; however, some L3 larvae remain encysted in the tissues, which justifies that in bitches with advanced gestation and due to hormonal influence these larvae are reactivated and migrate through the placenta (transplacental infection), reaching the fetal liver from where they pass to the heart through the suprahepatic vein and the vena cava, and from the heart through the pulmonary artery to the lungs. The pulmonary population of larvae is maximum between 3-5 days postinfection. Most of the larvae perforate the bronchial

wall, reaching the air space, and from there, they move through the trachea to the pharynx, where they are swallowed. For this reason, three weeks after birth, puppies can already harbor sexually mature worms in the small intestine, capable of releasing eggs into the external environment (Oge and Ozbakiş-Beceriklisoy 2019). On the other hand, although it is more frequent in female cats than in female dogs, another form of transmission is by the lactogen (transmammary) route, either by the reactivation of encysted larvae or by infection of the mother during the beginning of pregnancy; both transplacental and transmammary infection are considered as the mechanisms of vertical transmission of the parasite (Gates and Nolan 2009). It has been reported that 98.5% of infections in puppies are prenatal and 1.5% occur during lactation (Gates and Nolan 2009).

Another route of entry of the infective larvae to the definitive host is the ingestion of paratenic hosts, fundamentally rodents in which the larvae are encysted in different tissues. In this case, the parasite cycle is completed in less time because these larvae do not migrate through the animal's tissues. Molting to the adult stage begins earlier, and egg production and shedding take place after a short prepatent period. Embryonated eggs with infective L3 can also be ingested by paratenic hosts. Although these eggs lose their cover and the larvae are free and move through different organs, they do not mature in the paratenic hosts. Human is among this type of host, also called an aberrant host, and it is generally due to the fact that they maintains playful or professional contact with the definitive hosts. Accidental ingestion of these eggs causes human toxocariasis due to the presence of larvae of this parasite. Contact between *T. canis* and man can also begin by ingestion of eggs containing the L3 larva, hatching can take place both in the stomach and in the small intestine since the stimuli required are very diverse, this can partly explain the wide range of paratenic hosts (Jasim and Hadi 2021).

The fundamental place of penetration of the larvae is the small intestine, particularly the ileum. The eggs that reach the colon and have not hatched are eliminated for the most part. It has been determined that the exact site of penetration is the Liewerkühn crypts, possibly because these are the areas with less motility during ingestion. It has also been reported that Paneth cells degranulate at the time of larval penetration. From the intestine they spread to the liver mainly through the portal route, although there is some evidence of intraperitoneal dissemination or direct passage through the lymphatics to the lung and spread through the systemic circulation to all parts of the body (Chen et al. 2018).

Transmission Mechanisms

Toxocara uses various sources of infection, adult parasites can reside on a wide range of domestic and wild definitive hosts, as shown in Fig. 2 (Holland 2017). Humans are mainly infected by accidentally ingesting embryonated eggs of the nematodes *T. canis*, *T. cati* and / or congeners, which

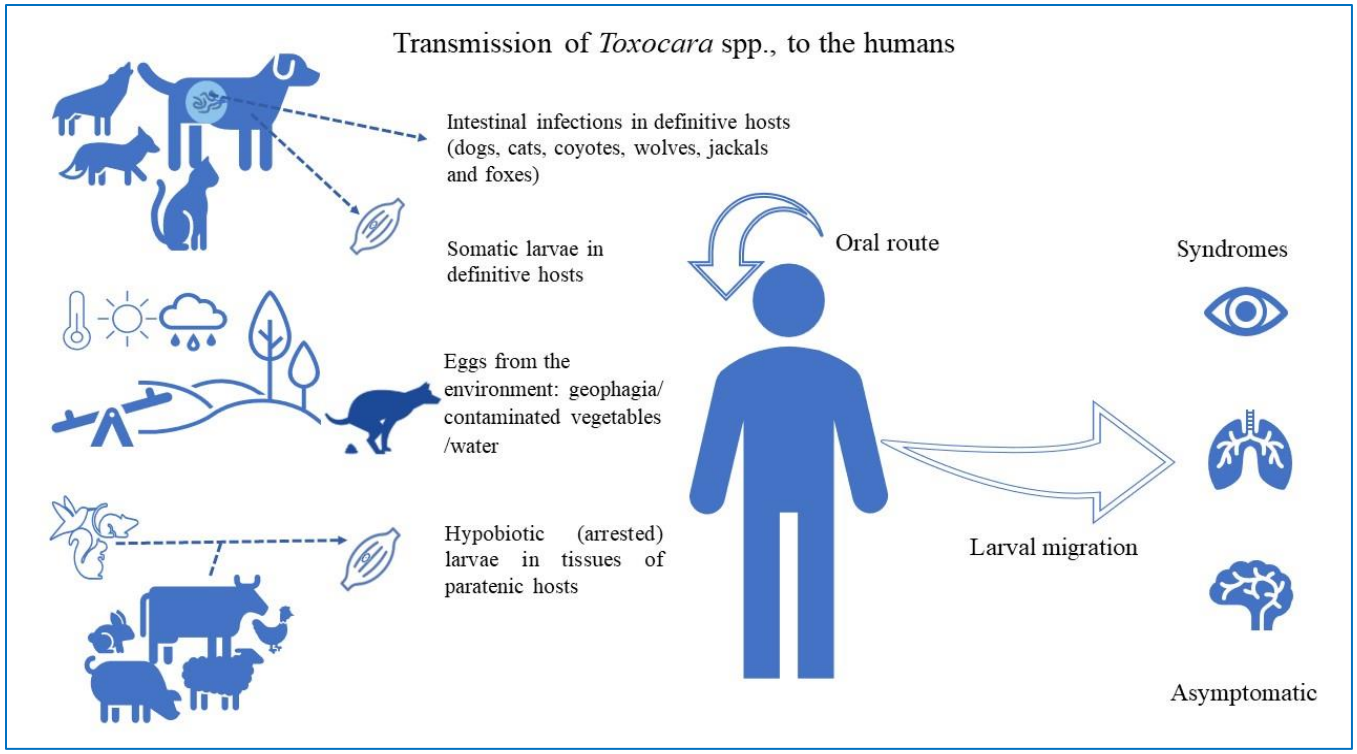

Fig. 2: Important epidemiological factors in the transmission of *Toxocara* spp., the four reservoirs of the parasite, important keys for its control.

contaminate raw vegetables and water, when carrying out recreational activities in parks, playgrounds, and sandboxes or through geophagy; to a lesser degree, by paratenesis, a type of transmission by the consumption of potentially infectious *Toxocara* larvae, encysted in the tissues of paratenic hosts, not sufficiently cooked; cattle, sheep, pigs, rabbits, chickens, and rodents serve as this type of host that can be food for both humans and definitive hosts and be reservoirs of *Toxocara* larvae (Alho et al. 2021). The transmission of *Toxocara* eggs present in the hair of dogs and, in some cases, of cats is an unlikely direct route of transmission to humans, as the eggs require an incubation period to become infective (Holland 2017; Maurelli et al. 2019).

In an extraordinary way, the infection of a patient after the ingestion of live slugs has been reported as an alternative therapy for esophageal reflux. In this particular case, the role of these as phoretic vectors has been hypothesized, transporting the infectious eggs in their mucus (Fellrath and Magnaval 2014).

Epidemiology

Dogs and wild canids, including foxes, coyotes, wolves, jackals, hyenas, and dingoes are the definitive hosts for *T. canis*, cats as definitive host for *T. cati* (Rostami et al. 2019a), buffalo (*Bubalus bubalis*), and cattle for *T. vitulorum* (Olmos et al. 2021). Puppies, kittens, and calves are the most important source of the adult parasite in the intestine, and therefore from very resistant eggs expelled to the outside that spread the infection; adult animals serve as reservoirs of the parasite, producing larvae that encyst in tissues, a role that should not be underestimated. Humans and other domestic and wild species serve as paratenic hosts; that is, species in which the biological cycle is not completed, however, serve the parasite to bridge an ecological gap in its life cycle. An infected mouse facilitates transmission of potentially infective larvae to dogs, cats, or foxes. In this regard, and despite the various investigations on this subject, the relative infective capacity of a variety of vertebrate and invertebrate hosts is unknown; it is very likely that they play a predominant role in disseminating infectious larval stages or helping the parasite to avoid unfavorable conditions in the absence of a definitive host (Holland and Hamilton 2006; Holland 2017; Olmos et al. 2021).

The prevalence of infection in dogs by *T. canis* shows wide variation worldwide, from 86 to 100% in puppies and from 1 to 45% in adult dogs; in the case of *T. cati*, 38.3% have been reported in Spanish feral cats, 79% in feral cats in Denmark, 91% in feral cats on farms in the United States, and 4.6% in the Netherlands (Fan et al. 2015). The presence of dogs and cats in urban public areas is common in many regions and contamination by their faeces significantly increases the risk of human infection by *Toxocara* (Traversa et al. 2014; Tyungo et al. 2020). The dissemination of these eggs in the environment depends on factors such as plant cover, wind, rain, displacement of definitive hosts and even the activities of birds, flies, beetles, earthworms, slugs, which indirectly determine the availability of eggs for susceptible hosts (Fan et al. 2015). Several investigations show that public spaces such as sandboxes and parks offer a continuous risk of acquiring toxocariasis. In Japan, in the city of Tokyo, 41.2% of sandboxes were found to be contaminated; in Kansas 6.6% and in Brazil 87.1%, in Portugal, 85.7% (Quattrocchi et al. 2012; Otero et al. 2018); in New York, from 29.6 to 66.7% (Tyungo et al. 2020). It is known that the viability of these eggs and their infectivity can be maintained for months and even years in adequate temperature and humidity conditions (Fan et al. 2013). The temperature, light, humidity, pH, the substrate and the vegetation can affect them; once these are eliminated in the faeces by the definitive hosts. These eggs are the main source of infection for humans due to contamination of water and food and possibly due to direct contact with dogs. In this regard, it has been reported through a systematic review and meta-analysis that eggs of *Toxocara* in different stages of development: non-viable (in all fur samples analyzed), viable/non-embryonated eggs (50.7 to 86%), embryonated (2 to 70.8%) and larvae (0.3 to 8.1%). These results suggest a low risk of infection by this route, in addition, emphasizing that these require adequate time and conditions to embryonate and reach the infectious larval stage 3 (Maurelli et al. 2019). Various studies worldwide have been carried out to find out the status of Toxocariasis in humans, despite this, it is not possible to compare the results because of different diagnostic tests, cut-off points, type of antigen, and type of population under study, in addition to this, diagnostic accuracy is significantly reduced due to cross-antigenicity, particularly in regions where polyparasitism is common (Smith et al. 2009).

Through a systematic review and meta-analysis of five international databases for the period from 1980 to 2019, Rostami et al. (2019a) determined that one-fifth of the world's population (1.4 billion individuals) is exposed to *Toxocara*, its prevalence varies depending on the country and region (Table 1). However, it is highly prevalent in developing countries, in comparison with developed countries, also highlighting the importance of the clinical sequelae of the syndromes that the parasite develops.

Toxocariasis in Humans

More than 70 years before, toxocariasis was described for the first time in 1950 and it was considered a rare disease that mainly affected children (Magnaval et al. 2001). Currently, extensive knowledge has been generated about this helminthzoonosis, now it is known that a variety of clinical syndromes can develop including Visceral Larva Migrans (VLM), Ocular Larva Migrans or Ocular Toxocariasis (OLM), Neurotoxocariasis (NT), and Covert and Cutaneous Toxocariasis (CT) (Jasim and Hadi 2021). In endemic areas with high prevalence, *Toxocara* larvae have a severe medical and social impact because these produce significant morbidity that can have debilitating and long-lasting effects

Table 1: Estimates of seroprevalence of toxocariasis in people for the period from 1980 to 2019, by Regions of the World Health Organization. Source: (Rostami et al. 2019[a])

Region	Percentage (%)
African	37.7
South East Asia	34.1
Western Pacific	24.2
American	22.8
European	10.5
Eastern Mediterranean	8.2
Global seroprevalence	19.0

This prevalence is related to several risk factors for this important helminthozoonosis, which are summarized in Table 2.

Table 2: Predisposition factors to infection by *Toxocara* spp. Source: (Quattrocchi et al. 2012; Fan et al. 2015; Kyei et al. 2015; Rostami et al. 2019a; Tyungo et al. 2020; Quintero-Cusguen et al., 2021).

Etiological agent	
Large egg production capacity	
High resistance of the infecting phase to adverse environmental conditions	
Use of vertebrate and invertebrate hosts to maintain and spread in the environment	
Various routes of transmission	
Little knowledge of its pathogenicity mechanisms, possibility that strains of *T. canis* have specific tropisms	
Human	
Genetic factors	Cultural/socioeconomic factors
Susceptibility or resistance to infection by immune response	Pet ownership/ mainly puppies
	Geophagia/ nail biting/ history of dirt play
Being Hispanic, Black non-Hispanic	Poor hygiene/ not washing hands with soap before eating.
	Being male
Age (early age)	Consume raw meat/ non-potable water.
	Have a lower income level/live in extreme poverty
	Having a low level of education/ a lower human development index
	Garbage collectors/ farmers
	Immunocompromised
Environmental/geographic	
Increase in untreated/uncontrolled definitive hosts	
Polluted environment	
Countries with tropical and subtropical climates/ higher humidity, temperature, and rainfall.	
Rural environment	
Unhygienic environment	

that impair productive capacity and children development (Walsh and Haseeb 2012; Tyungo et al. 2020). When humans accidentally consume the infective larvae, these cannot develop into the adult form, so these migrate through the bloodstream to different organs, mainly the liver, heart, kidneys, brain, eyes, and muscles. The clinical manifestations depend on the intensity of the infection, the greater the number of infective eggs ingested, the greater the number of migrating larvae, and the immune system will detect them and develop a more energetic defense response (Kyei et al. 2015). This larval migration can last for months or years, causing tissue damage and causing local or systemic inflammatory reactions as a result of the death of these larvae, as well as type IV hypersensitivity reactions, mediated by Th1 cells and the development of eosinophilic granulomas; and type I hypersensitivity, with IgE production, eosinophilia and increased expression of cytokines IL-13, IL-5 and IL-4, due to a Th2 reaction (Quintero-Cusguen et al. 2021); which will manifest different symptoms according to the affected organ (VLM), sometimes waves of migratory larvae can be generated in the viscera. On the other hand, the migratory larvae can damage the retina by inducing granulomatous reactions that are responsible for the decrease or loss of vision (OLM). The larvae can migrate to the brain and spinal cord with associated neurological compromise and produce neurotoxocariasis (NT), resulting in the presentation of epilepsy, eosinophilic meningoencephalitis, myelitis, cerebral vasculitis and neuropsychological deficits, which is very serious as toxocariasis has been associated to reduced cognitive function, producing debilitating effects, in children from socioeconomically disadvantaged populations. Finally, one less severe syndrome called covert toxocariasis or common toxocariasis has been described, with skin manifestations such as chronic urticaria, chronic pruritus, and miscellaneous eczema (Jasim and Hadi 2021; Quintero-Cusguen et al. 2021).

Diagnosis

In dogs and cats, the diagnosis is mainly carried out by coprological examination of eggs in faeces under the microscope (Gates and Nolan 2009; Okulewicz et al. 2012), by serological tests, such as ELISA (for antibody or antigen

detection) and Western blot (Noordin et al. 2020) or by molecular methods, for example, PCR (Khademvatan et al. 2013; Öge and Özbakiş-Beceriklisoy 2019; Phoosangwalthong et al. 2022), and loop-mediated isothermal amplification (LAMP) technique (Azimian et al. 2021). In the case of humans, the diagnosis of Toxocara larvae may be accomplished by the detection of specific IgG antibodies against the parasite using serological tests (Zhan et al. 2015; Rostami et al. 2019b; Noordin et al. 2020) or by the detection of Toxocara larvae antigens by molecular assays (Despommier 2003; De et al. 2013).

Advantages and Disadvantages of Conventional Control

The conventional control of toxocariasis disease in humans has been done for decades through anthelmintic products, such as: 1) albendazole, 2) mebendazole, 3) thiabendazole, and 4) other drugs such as anti-inflammatory deugs (Chen et al. 2018).

The control advantage of these compounds is their easy application, speed, and efficiency (approximately in a range of 45 to 70%) depending on the compound. However, these compounds have side effects such as nausea, abdominal pain, and the most worrying reversible effects including hepatotoxicity, leukopenia, and alopecia caused mainly by albendazole in a dose of 400 mg orally for 5 consecutive days (Satou et al. 2005; Frazier et al. 2009).

The drug products (albendazole, mebendazole, thiabendazole) bind to free β-tubulin, which is an essential protein-like component of microtubules in helminths. These drugs have a great affinity for said component, which induces the inhibition of tubulin polymerization and the periphery of cytoplasmic microtubules. Additionally, anthelmintic compounds and mainly benzimidazoles alter the glucose metabolism of helminths, regarding the thiabendazole compound, it targets NADH oxidase reductase in helminths (Magnaval et al. 2022).

The disadvantages of the use of the aforementioned anthelmintic products are mainly anthelmintic resistance; however, another significant factor is the damage to beneficial organisms such as dung beetles that help keep grasslands clean, likewise they are used as biological models of environmental changes and have also been used to evaluate anthropogenic impacts on biodiversity due to the response to different levels of forest conversion and the eco-relationship of the presence of mammals (Sánchez-Hernández et al. 2022). In this context, the use and abuse of these products (macrocyclic lactones: ivermectin) have decreased the populations of these organisms in the Mexican southeast (Basto-Estrella et al. 2014), and in the Amazon and Pantanal, two regions of Brazil, the populations of these dung beetles have decreased by up to 50% to 70% due to the use of ivermectin, altering ecological niches (Correa et al. 2022). For this reason, it is urgent to implement sustainable alternatives to control toxocariasis.

Sustainable alternatives for Controlling Toxocariasis

Nowadays, multiomics tools, specifically proteomics, have shown potential for the generation of somatic and excretory-secretory proteins with specific functions for the invasion of pathogens in relation to the evasion or modulation of the immune system for the development of new generation vaccines. These proteins activates the host immune system (Zheng et al. 2020).

Totomoch-Serra et al. (2021) report the consolidation of cutting-edge technologies such as single cell analysis, immune repertoire analysis, multiple phenotyping, and spatial transcriptomics, which help to determine immune function and involvement in various infections by parasites such as toxocariasis.

On the other hand, in a study reported by Zhen et al. (2020), they used omic techniques such as genomics and transcriptomics and identified a number of genes that participate in the development of *Toxocara* and the interaction of the parasites and their hosts, and made the prediction and function of unknown genes by the comparison of other species. Omic sciences contribute to the development of new drugs, vaccines, and diagnostic tools for the sustainable control of toxocariasis worldwide.

In Brazil, a study has been carried out on the *in vitro* evaluation of ovicidal fungi isolated from the soil (*Acremonium*, *Aspergillus*, *Bipolaris*, *Fusarium*, *Gliocadium*, *Mucor*, and *Trichoderma*) on *T. canis* eggs, obtaining promising results after 14 days post-confrontation (fungus-egg interaction) (De Souza Maia Filho et al. 2012). Another biocontrol agent that has been evaluated is the fungus *Trichoderma (T.) virens* on *T. canis* eggs. The results of the mentioned study showed that the number of larvae obtained in the different organs was lower in the group of animals that were infected with the embryonated eggs of *T. canis* exposed to the fungus *T. virens* compared to the group of animals that received embryonated eggs without exposure to the fungus *T. virens*. The fungus *T. virens* showed potential as a biocontrol agent on *T. canis* eggs (De Souza Maia Filho et al. 2016).

Some authors have suggested the immunological control of Toxocariasis as a possible alternative (Barriga 1988; Jaramillo-Hernández et al. 2020). It must be considered that *Toxocara* eggs contaminate a wide variety of food, so there must be strict control of aliments destinated to human consumption (Bolicar-Mejia et al. 2014; Chen et al. 2018; Healy et al. 2022). In this context, it has been indicated that contact of young people (under 18 years old) with dogs and cats is a significant risk factor for Toxocariasis (Fitz et al. 2022). The development of new molecular tools has been suggested to facilitate the diagnosis and new control approaches to Toxocariasis in humans (Guangxu et al. 2017, Azimian et al. 2021).

Conclusion

Toxocariasis is a worldwide zoonotic issue that is increasing year by year. The major reasons behind its spread include

climate change, people not following basic sanitary measures to dispose off dog feaces properly and ignorance of this issue by the health authorities. In the years to come, better control measures for toxocariasis must be implemented under the One Health scheme if success is pursued.

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Problems and Perspectives Related to Cystic Echinococcosis in Pakistan: Solutions in One Health Context

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INTRODUCTION

Pakistan is an endemic region for cystic echinococcosis (CE) which is a disease of economic and health concern for both animals and humans. Echinococcosis, also known as hydatidosis, is one of the major neglected tropical diseases (NTDs; WHO 2019) having endemicity to regions with prominent pastoral activities (Craig et al. 2015). NTDs impact lives of over 1 billion people in low- and middle-income countries having limited surveillance capacities (Rai 2022). Currently, Pakistan bears high global burdens for seven major NTDs (Herricks et al. 2017). CE is ranked as 4th most widespread helminth disease in Pakistan (IRD 2017) with 20,500 identified human cases (Herricks et al. 2017). Being an agricultural country and hosting a large rural population, 113 million people of Pakistan are at risk, and one of the largest agrarian communities in danger of getting CE and other infections (Zhang et al. 2015).

Life Cycle of *Echinococcus granulosus*

Cystic echinococcosis is caused by larval stages of a tapeworm species, *Echinococcus granulosus*, which has cyclozoonotic pattern between different intermediate (domestic animals and humans) and definitive hosts (dogs) (Thompson et al. 2017). The dogs take up the parasite while ingesting contaminated offal containing hydatid cysts with viable protoscoleces (PSCs). Upon reaching the digestive tract of the definitive host, PSCs evaginate in upper duodenum after exposure to high stomach temperature in presence of pepsin and bile salts. Each protoscolex has the ability to develop into a mature tapeworm. Mature tapeworms release the embryonated eggs which are either passed into feces separately or through disintegration of terminal proglottid from the tapeworm body (Craig et al. 2003). Eggs are ingested by a suitable intermediate host (sheep, goat, cattle, buffalo, camel, horse) which harbors the hydatid cysts/metacestodes (larval stage) developing in main visceral organs like liver and lungs (Romig 2003). Humans also become accidental hosts after ingesting eggs of *E. granulosus* via contaminated water or food (Ito et al. 2017). Fig. 1 outlines different life cycle stages of *E. granulosus* in the intermediate and definitive hosts.

Human Cystic Echinococcosis

Humans acquire the infection by accidental exposure to eggs of the parasite. Farming and nomadic communities, having close contact with dogs are at the highest risk of infection. Human CE is usually asymptomatic and does not cause major identifiable pathologies and remains unnoticed for years until the active cyst grows large enough to exert pressure on the adjacent tissues or induce other pathological events (Eckert et al. 2001). Clinical symptomatology is highly variable, with no disease specific symptoms, largely depending on size, number and location of cyst (Moro and Schantz 2009). Usually, 38 to 60% cases are asymptomatic and accidentally diagnosed during other medical examinations (Kern 2003). Generally, patients show fever, high abdominal pain and signs of allergic reactions (Budke et al. 2013). If the liver is affected, hydatid cyst can compress the bile duct resulting in obstructive jaundice, allergic manifestations and abdominal pain (Pakala et al. 2016). Clinical manifestations associated with pulmonary cysts include chronic cough, pleuritic chest pain, dyspnoea, haemoptysis and lung abscesses (Eckert et al. 2001; Kern et al. 2017). In pulmonary CE case patient may expel remnant of hyaline membrane of ruptured cyst (Ramos et al. 2001). Symptoms and signs in atypical sites are usually pain and tumor like growth (Eckert et al. 2001).

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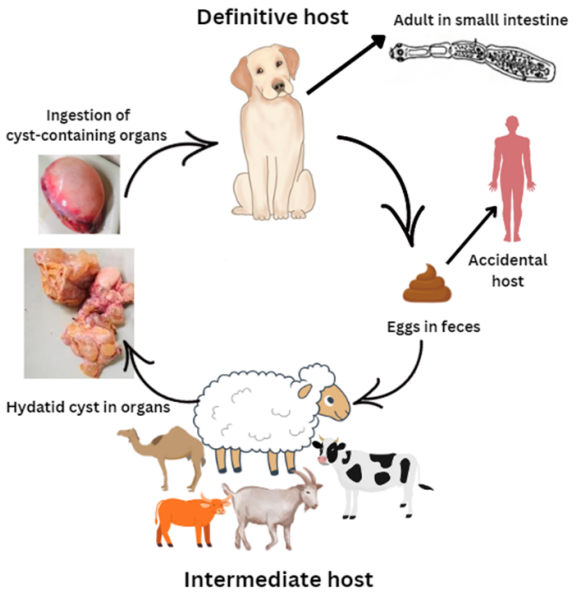

Fig. 1: Life cycle of *E. granulosus* responsible for causing cystic echinococcosis.

Cystic Echinococcosis in Livestock in Pakistan

Pakistan is the focal point for presence of *E. granulosus*, however, due to limited number of studies, the endemic situation is underestimated (Zhang et al. 2015). In Pakistan, large rural population is specially at risk due to multiple soil transmitted helminths (STHs) because of poverty, hygiene, illiteracy, poor knowledge about diseases, malnutrition, environmental degradation and security issues (WHO 2013; Blum et al. 2018). *E. granulosus* is spread all across Pakistan and in all livestock species. Apart from rural areas, urban and peri-urban localities are also at risk and there is an upward trajectory of CE in Pakistan (Haleem et al. 2018; Khan et al. 2018, 2020). Karachi and areas near Afghan border, Northern Punjab and Khyber Pakhtunkhaw (KP) are at the highest risk of contracting this disease (IRD 2017). Sindh province has also high burden of hydatidosis due to significant economic losses among livestock (Anwar 1994). Fig. 2 manifests the hydatid cysts (metacestodes) in livers and lungs of the livestock species.

CE was reported for the first time in Pakistan in 1953 by Lubinsky (1959) at Rawalpindi reporting high prevalence of 15.4%. During the subsequent years, several studies have been carried out reporting differential rates of prevalence of CE in the livestock of Pakistan (Khan et al. 2020) with highest number of studies from Punjab. Prevalence as high as 60.46% has been reported in buffaloes slaughtered at urban slaughterhouses of Punjab (Shahzad et al. 2014). A comparative picture on prevalence of CE in Pakistan among different domestic ungulates is depicted in Table 1. Lack of proper sanitation, health and education facilities also correspond to making livestock a suitable reservoir for *E. granulosus*. Moreover, economic and health conditions in the country are relatively poor compared to the developed economies of the world which further elevate the risk of hydatidosis transmission (Mehmood et al. 2020a).

Human Cystic Echinococcosis in Pakistan

The actual burden of human CE on economy is not estimated in Pakistan. The prevalence figures are inaccurate due to the lack of reporting and improper identification. Accurate estimate of incidence and prevalence of CE is always difficult because of asymptomatic nature of the disease. Additionally, access to medication and surgical interventions remains limited for most of the people in Pakistan. Rough estimate of the incidence can be given by hospital admissions/discharge data and number of cases tested at the reference laboratories (Muqaddas et al. 2019).

Risk Factors for Cystic Echinococcosis Prevalence

Frequency and intensity of CE is influenced by a number of factors operating at both the definitive host and intermediate host levels.

a- Risk factors for definitive hosts for cystic echinococcosis infection

There are around three million stray dogs in Pakistan which play a very crucial role in the continuation of life cycle of *E. granulosus* and high rate of infection among the domestic animals. Access of dogs to infected and uncooked offal, animal slaughtering locations, open butcher shops and extensive livestock farming are the major determinants favouring disease perpetuation in Pakistan (Mehmood et al. 2020a). Free roaming and stray dogs are at more risk of getting infected by *E. granulosus* eggs than to other types of dogs (Otero-Abad and Torgerson 2013). Similarly, high infection rates are reported for farm dogs living in close vicinity to the livestock (Pérez et al. 2006; Guzel et al. 2008). The dogs from rural areas have higher prevalence of *E. granulosus* (30%) than those from urban areas (18%) (Chaâbane-Banaoues et al. 2016). Younger age group and male gender of dogs are more prone to infection (Parada et al. 1995; Buishi et al. 2005). Socio-economic background of dog owners is also an infection determinant in definitive hosts as lack of knowledge on disease transmission and deficiency in deworming and anthelminthic treatment is related to high infection pressures in dogs (Buishi et al. 2005; Huang et al. 2008).

b- Risk factors for intermediate hosts for developing cystic echinococcosis infection

Epidemiology of animal echinococcosis relies primarily on the mode of transmission of the disease (Otero-Abad and Torgerson 2013). Predominantly extensive livestock

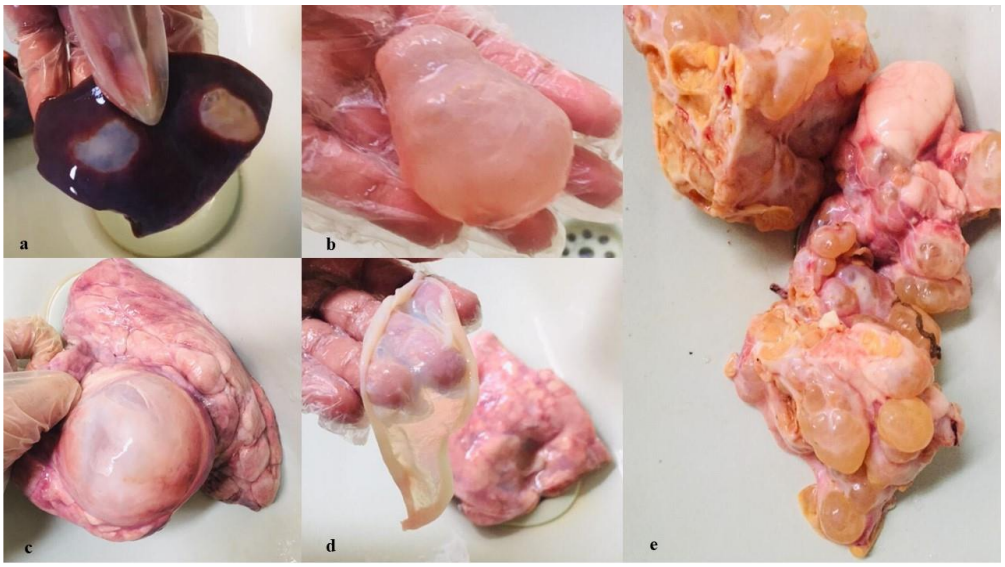

Fig. 2: Cystic echinococcosis in animals. The blocks show: a) hydatid cysts in the liver b) individual hydatid cyst c) hydatid cyst in the lungs d) germinal layer of hydatid cyst e) multiple hydatid cysts in the lungs.

Table 1: Disease prevalence among the livestock (intermediate hosts) from different geographical areas of Pakistan

Province	City	Prevalence (%) in domestic ungulates				Reference
		Sheep	Cattle	Buffalo	Goat	
Punjab	Rawalpindi	4.6	15.4	-	2.1	Lubinsky 1959
	Faisalabad	-	35	-	-	Anwar 1994
	Rawalpindi	-	38.90	33.06	-	Khan et al. 1990
	Lahore	-	6.43	-	-	Khan et al. 2010
	Chakwal	8.55	8.42	6.90	2.99	Khan et al. 2018
	Lahore	11.36	-	-	7.77	Iqbal et al. 2012
	Lahore	8.3	9.6	12.3	7.5	Khan and Haseeb 1984
	Lahore	-	27	35	-	Sheikh and Hussain 1968
	Lahore, Jhang, Okara	20	45.45	60.46	20	Shahzad et al. 2014
	Lahore, Gujranwala, Gujrat, Faisalabad, Sheikhupura, Pakpattan	7.52	5.18	7.19	5.48	Latif et al. 2010
	Sargodha	3.24	2.44	-	2.44	Mustafa et al. 2015
	Lahore, Rawalpindi, Multan, Sargodha	8.99	9.13	9.49	3.58	Mehmood et al. 2020a
Khyber Pakhtunkhaw	Peshawar, Swabi, Bannu, Charsadda, Mardan, Swat, Laki Marwat, Nowshera, Karak, Kohat	15.38	15.79	15.88	3.25	Haleem et al. 2018
Balochistan	Peshawar	21.73	11.39	19.07	3.57	Mehmood et al. 2020a
	Quetta	31.1	-	-	21.1	Ahmed et al. 2006
	Quetta	25.00	-	-	7.93	Mehmood et al. 2020a
Sindh	Larkana	-	-	24.4	-	Mirani et al. 2002
	Larkana	10.6	-	-	10.02	Surhio et al. 2011
	Hyderabad	-	-	13.46	-	Ehsan et al. 2017
	Larkana	16.66	6.05	24.40	3.27	Mehmood et al. 2020a

production systems, traditional animal husbandry practices, nomadism and uncontrolled animal movements favor the occurrence and endemicity of the disease (Dakkak 2010). Principal factors promoting disease among domestic animals are the extent of contamination in environment by parasitic eggs and age of the intermediate host (Otero-Abad and Torgerson 2013). More cyst abundance is observed in older farm animals (Tashani et al. 2002; Umur and Kaaden, 2003; Erbeto et al. 2010). Sheep and goats of 3 years or older are at 1.6 times more risk to CE infection than the younger animals (Marshet et al. 2011). Certain other factors complementing disease dispersal are gender (Daryani et al. 2007; Ibrahim 2010) and type of livestock species (Cardona and Carmena 2013). Females are at high risk of disease contraction due to slaughtering at old age which increases the exposure to parasitic infection (Pour et al. 2012; Otero-Abad and Torgerson 2013). Animal echinococcosis is more frequently seen in small ruminants which show higher infection rates compared to large animals. Sheep are more vulnerable to *E. granulosus* infection than goats and cattle (Erbeto et al. 2010; Marshet et al. 2011), however it is important to note that buffaloes and sheep are the key hosts in CE epidemiology in Pakistan (Mehmood et al. 2020b). Additionally, cattle could also be considered to have prominent role in disease spread as South Asian clime offers a suitable environment for development of *E. granulosus* besides harboring a large

Fig. 3: Risk factors for perpetuation of cystic echinococcosis in Pakistan.

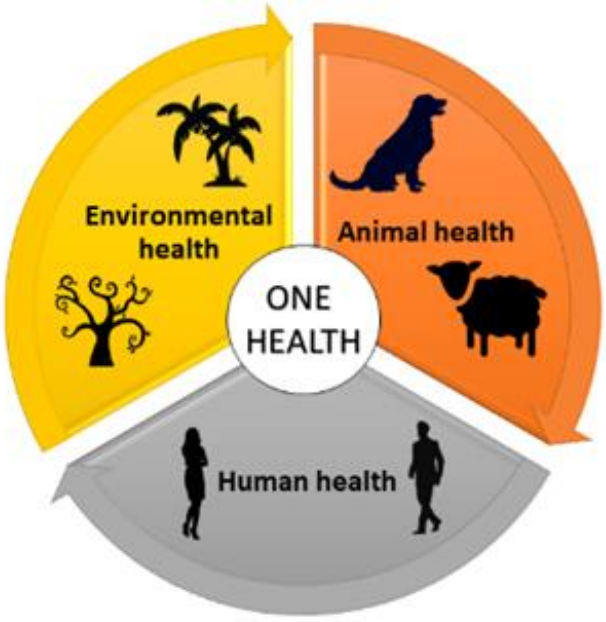

Fig. 4: One Health concept unifying human, animal and environmental health

population of buffaloes and cattle (Mehmood et al. 2022). Livestock infection is also modulated by meteorological conditions like humidity and environmental temperature, however, seasonal differences in prevalence (Mehmood et al. 2020a) are of negligible importance due to chronic nature of the disease (Otero-Abad and Torgerson 2013). Fig. 3 highlights the possible risk factors responsible for spread of cystic echinococcosis in Pakistan.

Risk Factors for Human Cystic Echinococcosis

A sound understanding of the risk factors associated with human CE is essential for reducing the disease incidence (Possenti et al. 2016). Chances of disease increase in pastoral and nomadic communities which live in close association with dogs having low socioeconomic status. Human hydatidosis is a public health problem of rural communities. A study from Sindh and Punjab concluded that people associated with farming and aging between 21-30 years were at more risk of contracting the disease (Muqaddas et al. 2020). Transhumant movement of people, along with their livestock can aid in the transfer of CE in both animals and humans (Eckert et al. 2001). Limited access to health care facilities and using contaminated water sources due to low socio-economic status results in high incidence of CE (Barnes et al. 2017). All these putative factors could play their role in transmission modalities of hydatidosis particularly in rural areas with limited resources. Due to lack of resources and poor infrastructure of slaughterhouses, eradication of zoonotic echinococcosis is extremely difficult to achieve (Maudlin et al. 2009).

Problems Linked to Diagnosis of Human Cystic Echinococcosis

Preoperative diagnosis of human CE is reliant on imaging techniques including ultrasound imaging (US), computed tomography (CT), magnetic resonance imaging (MRI) and radiography and serological methods including enzyme linked immunosorbent assay (ELISA), latex agglutination, direct heamagglutination and immune electrophoresis (Hernández-González et al. 2018) whereas, histopathological diagnosis confirms hydatidosis at the postoperative stage. IgG ELISA (anti-*Echinococcus* serum antibodies) is a readily available technique but often fails to diagnose CE as it does not have desired specificity and sensitivity (Craig et al. 2007). IgG antibodies detection may sometime give false-negative results as reported for 20% cases of hepatic cysts and 40% of the pulmonary cysts (Eckert et al. 2001). Calcified cyst or cysts from brain or eye usually give low or no antibody titre. Similarly, false positive results have been documented from individuals having other helminthic diseases (Eckert et al. 2001) due to cross reaction (Brunetti et al. 2010). Though imaging techniques are commonly the primary approach for CE diagnosis, but often lead to misdiagnosis or misjudgement, when hydatid cyst is localized at atypical sites or presents confusing lesion features (Shang et al. 2019). Due to misdiagnosis, relapse or metastasis of echinococcosis after the surgery is also documented (Kern et al. 2017).

Problems Linked to Treatment of Human Cystic Echinococcosis

Human CE is complicated to treat as in some cases cyst remains asymptomatic for over 10 years (Frider et al. 1999). WHO advocates stage based therapeutic approach based on cyst characteristics and available medical facilities. The method of treatment includes four approaches i-e

chemotherapy, percutaneous methods, surgery and wait and watch strategy (Brunetti et al. 2010). Despite the importance of surgery, medicosurgical approach is gaining wide spread acceptance (Craig et al. 2007). Chemotherapy involves treatment with benzimidazole carbamtes (mebendazole and most commonly used albendazole) which kills the whole metacestode stage whereas praziquantel has a substantial effect on protoscoleces (Kern 2003). Both anthelmintic drugs have broad spectrum action and show symptom alleviation. Chemotherapeutic treatment often reduces the internal pressure by softening the cysts which can be later removed/excised easily during surgery (Pawlowski et al. 2001). Patients receiving albendazole and praziquantel prior to surgery have reported nonviable protoscoleces in comparison to the patients receiving only albendazole. Albendazole interacts with eukaryotic β-tubulin (cytoskeleton protein) by inhibiting its polymerization to microtubules. Cyst glycogen reserves start to drain (as a secondary effect), bringing degenerative changes in mitochondria and endoplasmic reticulum of germinal layer of hydatid cyst which leads to cellular autolysis (Scholar and Pratt 2000). Commonly used surgical interventions are partial or total cystectomy and organ resection such as lobectomy depending upon the nature of cyst. There are 2-25% chances of relapse in postoperative cases (Eckert et al. 2001). Puncture-Aspiration-Injection-Re-aspiration (PAIR) technique is commonly used to aspire hydatid fluid (HF) form the cyst of CE patients (Smego et al. 2003). Clinical outcome can be improved by combination of chemotherapy and medical treatment (Kern et al. 2017).

Cystic Echinococcosis Associated Economic Losses and Socioeconomic Burden

According to an estimate, globally 1 million or more individuals are suffering from CE and livestock sector is facing annual loss of 2 billion US $ due to *E. granulosus* infection (Torgerson and Macpherson 2011). Hydatidosis has become serious economic burden for resource-poor low-income countries like Pakistan. Public health spending (US $ 36.2 per capita) in Pakistan is even below the WHO low-income countries bench mark of 86 US $ (PES 2017-2018). Clinical diagnoses, surgical operation, long-term chemotherapy by albendazole along with chronic impairment of patients' quality of life are the main factors for the socioeconomic cost of the disease. CE is not only a significant burden for family of infected individual, but also for the community as a whole (Torgerson 2003).

Health surveys are important to assess the mental and physical health state of CE infected person in comparison with a control population. Surgically treated patients for CE report significant decrease in their quality of life (Torgerson and Dowling 2001), along with considerably higher unemployment rate (Torgerson 2003). CE burden on human population is estimated by calculating monetary losses and disability adjusted life years (DALYs). Direct monetary losses due to CE include costs of diagnostic tests, surgery and postsurgical care and treatment (Mastrandrea et al. 2012; Kern et al. 2017) whereas indirect costs are related to lost wages as a result of reduced competence to work during and after hospitalization (Harandi et al. 2012). Human CE is responsible for 19, 300 deaths worldwide and around 8,71,000 disability adjusted life years per annum where one DALY can be thought of as one lost year of healthy life (WHO 2019).

Due to asymptomatic nature of CE (Brandt et al. 2003) its economic impact is substantially underestimated (Budke et al. 2006). However, in Pakistan the estimation of actual losses is difficult because of lack of identification and under-reporting in both humans and livestock. Incidence rates are difficult to determine because of large number of asymptomatic cases which go unnoticed providing only rough estimates based on information from testing laboratories and hospital admissions data (Muqaddas et al. 2019). Lost wages, treatment costs and production losses in livestock (condemnation of viscera and significant decrease in fecundity, milk production, hide value and carcass weight) are a few major economic losses associated with cystic echinococcosis (Torgerson 2003).

Prevention and Control Strategies for Cystic Echinococcosis

Control of CE requires targeted control at three levels including human, livestock and dogs. Without appropriate surveillance, impact of prevention and control programs becomes difficult to measure. The control approaches are given below:

i) Control of hydatid cysts needs regular dosing/deworming of dogs with praziquantel (PZQ), which will reduce *Echinococcus* worm burden in the definitive hosts (Lembo et al. 2013). PZQ is a highly effective anthelmintic drug to date, with limited toxicity (Macpherson and Craig 2000). The prepatent period of *E. granulosus* is approximately six weeks, therefore, dosing dogs at frequent intervals is the most effective and quickest control measure for reducing both dog-livestock transmission and dog-human transmission, decreasing egg production and infection pressure (Torgerson and Budke 2003). Managing dog populations to reduce their numbers could help to reduce transmission, especially in conjunction with other measures such as dosing dogs and stricter livestock slaughter practices.

ii) Vaccine (EG95) against ovine echinococcosis has been recommended to control infection in livestock (Lightowlers et al. 1999). This vaccine is not uniformly effective for *E. granulosus* intraspecific variants or genotypes. Additionally, there is no vaccine available for the definitive host (Craig et al. 2017).

iii) Vaccination in conjunction with other measures such as the inspection of animals at the slaughterhouse, improving hygiene practices and husbandry, regulated slaughtering at

abattoirs and proper dumping of offal are useful in the control of hydatid cyst (Craig et al. 2017).

iv) Health education of the general public and rural communities by increasing their awareness about the disease, efforts to change social practices including fencing of vegetable gardens to prevent access by dogs, avoiding use of raw vegetables without washing, improved sanitary conditions in slaughterhouses and preventing access of dogs to raw viscera would help to control the disease (Craig et al. 2007).

v) Modelling for CE

Quantitative and qualitative forms of mathematical models provide a straightforward means to estimating the infection pressure to animals and humans (Torgerson and Heath 2003).

vi) Surveillance studies describing the rates of infection and molecular investigations determining etiological agents at particular geographical areas are of primary importance while implementing a control program. Data sources from epidemiological surveillance reporting livestock infections and hospital admission data are of central importance to initiate a control program (Craig et al. 2017).

vii) An **integrated approach** combining vaccination of intermediate hosts and anthelmintic treatment of dogs is by far the most effective intervention to control CE (Craig et al. 2007).

One Health Action and Implementation Measures

One Health is a unifying concept which aims at achieving sustainable balance between animals, humans and the environment (ecosystems), signifying integration of these elements and that the human health is dependent upon ecosystem health (Fig. 4).

A complete control initiative taken under the umbrella of One Health requires reduced disease transmission and infection risks and complementing the regional chemotherapeutic campaigns for disease prevention and subsequent control. Unfortunately, no measures have been taken on any scale for disease prevention from the relevant authorities in Pakistan. Despite endemicity and considerable hospital records and animal infections, nothing has been done so far on any interventional front described by WHO to tackle NTDs including i) preventive chemotherapy ii) innovative and intensified disease management iii) water, sanitation and hygiene (WASH) and iv) veterinary public health services. One important aspect to be specifically focused is to break the transmission cycle of CE by identification of main reservoir species, climatic factors, areas with higher prevalence, routes to human infection and sociocultural practices involved in disease dissemination. Once these factors are taken into account, targeted control programs based upon approaches given above can be designed to mitigate the risk of disease in endemic foci. Ideally, search for new drugs and vaccine targets must also be carried out since current anthelmintic drugs are losing their efficacy due to development of resistance among the parasites. Additionally, trainings and workshops must be conducted for the healthcare professionals/workers to enhance their skills for disease management (NTDs are barely given consideration during routine medical examination in Pakistan). Improving basic sanitation, provision of clean water, management of slaughtered animals' waste, regulated animal slaughtering at fixed areas, regular deworming of dogs and health education of agrarian communities regarding disease and dog-contact can substantially reduce disease burdens and may result in sustainable elimination of CE (Mehmood et al. 2020a).

Conclusion

Cystic echinococcosis is endemic to Pakistan and no effective surveillance programs have been implemented to monitor yearly disease prevalence in animals and humans. Due to the infectious nature of disease and adaptability to domestic herbivores, CE control would require years of consistent efforts and commitments for complete elimination. Understanding the disease distribution, economic impacts, and risk factors is critical while developing a control program. Identification of research gaps and definition of priorities within the contextual framework of health preparedness in Pakistan would be a suitable approach for these poverty-associated diseases. Following the WHO roadmap guidelines for elimination of NTDs and optimizing strategies for long term eradication programs must be prioritized by the health authorities. In the absence of sustainable efforts, it is highly probable that CE will remain in steady equilibrium in host animals maintaining its life cycle between dogs and domestic herbivores and remain a nuisance for human population.

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27

Parasitic Diseases of Fish

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INTRODUCTION

The word 'parasite' comes from the Greek word's '*para*' meaning 'beside' and '*sitos*' meaning 'food'. The organisms known as parasites prey on and, in turn, injure their hosts after getting food and shelter from them. The parasites live in obligate association and get benefits such as nutrition at the host's expense, mostly without killing them. They use the energy that is otherwise required by the host for growth, development, maintenance, and reproduction and ultimately affect hosts survival (Overstreet 2021).

Fish Parasites

Fish parasites belongs to various classes and comprised of protozoans, pentastomes, trematodes, turbellarians, nematodes, cestodes, leeches, acanthocephalans, monogeneans, isopods, copepods, crustaceans, and lice. Their life cycles range from simple, needing no intermediate host, to complex and indirect, requiring one or more intermediate hosts. Fish can act as primary, paratenic, or intermediate host in the life cycle of parasites. Taking the life cycles of the identified parasites under inspection is very important for effective treatment. For instance, merely the theronts that is free-swimming developing life stage of the ciliated ectoparasite, *Ichthyophthirius multifiliis* are targeted at and affected by chemical treatments (Hoffman 1999, Roberts et al. 2001).

Diagnosis of Parasites in Fish

All infected fish must be tested or diagnosed using appropriate data which comprises an explanation of the fish's background from the owner of the fish, an assessment of water quality, an inspection of clinical indications, a physical test, an analysis of wet-mount cytology of skin scrapes, biopsy of gills and gathering the fecal samples (Reavill and Roberts 2007).

No specific indications of parasitic diseases in fish are seen but a group of symptoms may be observed. The general signs of parasitic infection include flashing behavior (scratching of body on the bottom of the tank or pond), sluggishness, skin bruises along with loss of scale, sores, formation of mucus, fast opercular motions, gasping, decrease in body weight, osmoregulatory disturbances, and morbidities (Roberts et al. 2007). External parasites may be seen clearly on gill cytology preparation and wet-mount skin of tranquillized fish. Internal infestations of parasites can be diagnosed by creating a wet mount of fresh fecal samples, gross visualization of the parasite at the outlet, evaluation of blood smears, histopathology, and necropsy inspection (Roberts et al. 2001, Roberts et al. 2007).

Protozoa

Ciliated Protozoans

White Spot Illness

Ichthyophthirius (multifiliis, sometimes known as "white spot illness" or "ich," is a parasitic disease that influences the fish living in freshwater across the world (Hoffman 1999, Baker et al. 2007, Noga 1996). The fish without scales i.e., catfish, is specifically in danger, because this parasite can live in a variety of temperatures and hosts. The systems that show overcrowding and bad status of water, causes more tension and decreased immune functioning in fish, which in turns raises the fatality rate. *I. multifiliis* can cause acute disease which may lead to 100% death rate (Noga 1996, Hadfield et al. 2007). The marine complement is *Cryptocaryon irritans*, both similar clinical symptoms (Roberts et al. 2009).

Life Cycle

The two parasites have a direct life cycle characterized by a free-swimming infective stage (theront) which is sensitive to the treatment. The feeding stage is enclosed within a sac called Trophonts like white nodes. These Trophonts burst out from the epithelium and turn into encysted tomonts having outer sticky capsules that attach to lifeless substrate in the environment, including gravel stones, nets, plants, and many more (Baker et al. 2007, Longshaw and Feist 2001). These tomonts split, generating tomites that breach the nodule's wall

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to release moving and disease-carrying theronts. The disease-causing theronts takes 48 hours to locate a new host at 25°C (Stoskopf et al. 1993, Noga 1996, Longshaw and Feist 2001). The thereont crosses the epithelium after obtaining a host and transforms into a ciliated trophont. *Ichthyophthirius* transmission is through the aerosol scattering of infective stage (Wooster et al. 2003). Ich has a temperature-dependent life cycle. At 25°C, it seems to last in 3 to 6 days, while at 15°C, it lasts about 10 days. At temperatures between 15 and 25°C, disease occurrence is most prevalent. Compared to *Ichthyophthirius*, *Cryptocaryon* has a longer life cycle, hence needed prolonged therapy (Roberts et al.2009).

Clinical Signs

Clinical signs include white, raised nodules up to 1mm (0.5mm for Cryptocaryon) on the skin and gills (Fig. 2), flashing, formation of mucus, sluggishness, shortness of breath, secondary bacterial or fungal diseases, and osmoregulatory disturbances due to the epithelial and gill damage. Upon examination of gills under the microscope, hyperplasia, more mucus, and tissue damage may be noticed (Reavill and Roberts, 2007).

Diagnosis

A wet-mount cytology of the skin or gills is inspected to confirm the diagnosis. Ich is a large sized parasite that is entirely covered in cilia, moves slowly and comprises of a nucleus that has the shape of alphabet C- or horseshoe (Fig. 3) (Noga 1996).

Chilodonella

Chilodonella is the condensed, ciliated parasite with a heart- or onion-shaped morphology. Striations that are evident on the parasite's length confirmed the existence of cilia. *Chilodonella* can flourish in brackish water and an array of temperatures. Its marine equivalent name is *Brooklynella hostilis*, which was found in the Brooklyn Aquarium. Both parasites can cause extreme tissue damage and serious sickness (Stoskopf et al. 1993; Noga 1996; Baker et al. 2007).

Clinical Signs

Clenched fins, mottled skin, enhanced formation of mucus, secondary skin ulcers, proliferation and merging of the lamellae, respiratory instability (gapping, piping, opercular flaring, augmented gilling), hypertrophy, and high fatality rates are the clinical indications (Palmeiro et al. 2009). Brooklynelliosis is a fetal disease that is cause by the ciliated protozoan *Brooklynella hostilis.* The afflicted fish use things to scratch their bodies. This parasite harms the skin and causes skin bleeding due to its adherence to the skin and gills (Fig. 4) (Cruz-Lacierda et al. 2004).

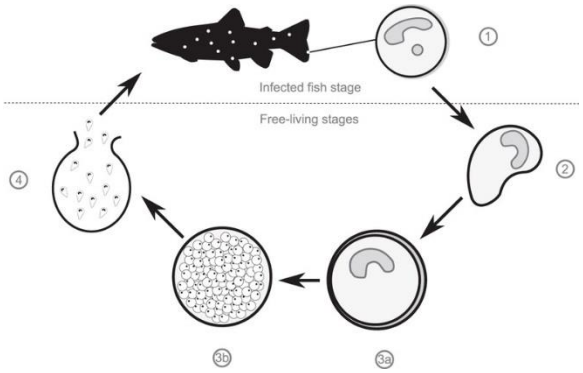

Fig. 1: Life cycle of the endoparasite *Ichthyophthirius multifiliis*

Fig. 2: White spot disease due to the protozoan *Ichthyophthirius multifiliis*

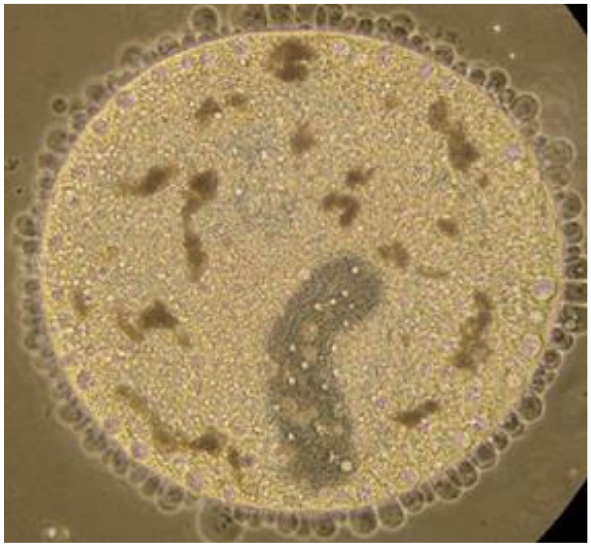

Fig. 3: *Ichthyophthirius multifiliis* (a wet-mount observation)

Diagnosis

The examination of the wet mount prepared from skin and gills enables the parasite identification. On wet-mounting, *Chilodonella* demonstrates a gliding or circling motion (Longshaw and Feist 2001; Weber and Govett 2009).

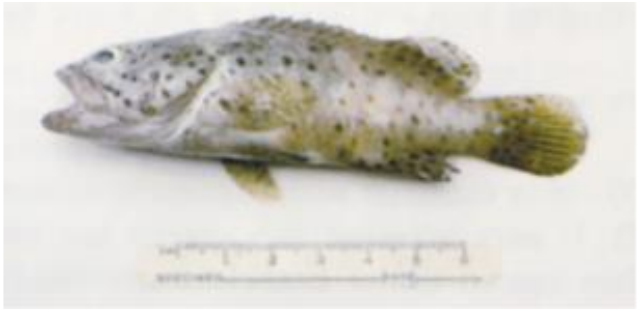

Fig. 4: Brooklynelliosis in *Epinephelus tauvina* showing excessive disruption and bleeding skin

Fig. 5: Chilodonelladiasis

Tetrahymena and *Uronema*

Tetrahymena which is parasitic species of freshwater and *Uronema* which is the marine species, are ciliated parasites that are the causative agents of visible gill and skin lacerations and systematic infections internally (Stoskopf et al. 1993; Noga 1996; Longshaw and Feist 2001; Weber and Govett 2009).

Clinical Signs

Tiny white spots on the skin, sloughing, skin contusions, malformations in the gills, and atrophy are all indications of infection (Fig. 5). Fish that suffer from systemic illnesses might exhibit nonspecific symptoms including anorexia nervosa and sluggish behaviour. After the onset of the infection, the fish may die instantly (Stoskopf et al. 1993; Noga 1996).

Tetrahymena, sometimes referred to as "guppy killer" or "guppy sickness," is a pathogen that primarily affects cichlids, guppies, and other livebearers. It has also been reported that this parasite lives in aquatic organic waste (Stoskopf et al. 1993; Noga 1996).

The *Tetrahymena's* clinical signs are similar to *Uronema* infection. *Tetrahymena* infection can also cause muscular edema and periocular lacerations. Due to the intimate relationship between the skin and the cornea, keratitis may also be caused by these protozoa and other parasites (*Cryptocaryon*, *Ichthyophthirius*, *Henneguya*, and *Glugea*) (Williams and Whitaker 1997).

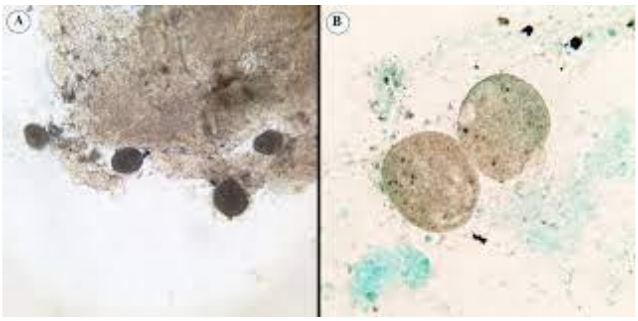

Fig. 6: *Cryptocaryon irritans*

Fig. 7: White spots on body surface of fish infected with *Cryptocaryon irritans*

Diagnosis

Wet-mount examination or immunohistochemistry of the skin and gill tissue are used to find parasites. In the event of deep or systemic infestations, immunohistochemistry of the affected organ or tissue will be required (Palmeiro et al. 2009).

Cryptocaryonosis

As sick fish exhibit few to many whitish or grey dots on their outer surface and gills, cryptocaryonosis is sometimes known as "white spot sickness." Cryptocaryonosis is brought on by an attack of *Cryptocaryon irritans* (Fig. 6) (Nagasawa and Cruz-Lacierda 2004).

Clinical Signs

Whitish or gray marks appear on the body and gills (Fig. 7). Anorexia nervosa, lethargy with abnormal swimming pattern, dark body, bleeding, and protruded eyes are the indications of infection. Excess mucus is formed and fish scratches its body with objects (Nagasawa and Cruz-Lacierda 2004).

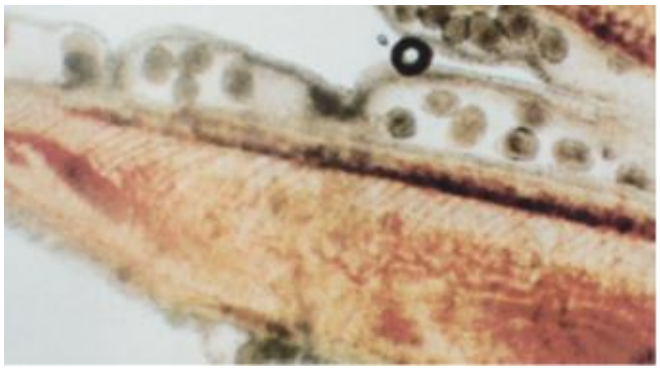

Fig. 8: *Cryptocaryon irritans* on gills of *Cromileptes altivelis*. Fresh mount

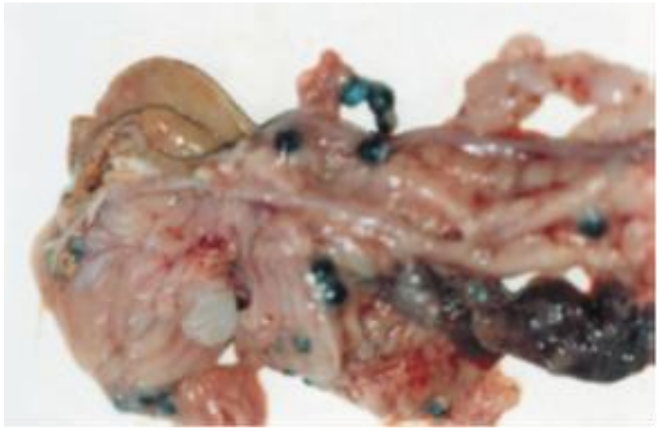

Fig. 9: Brownish-black cysts (arrows) on parenchyma of digestive organs of *Epinephelus tauvina*

Diagnosis

Under a microscope, spherical parasites moving inside the host and mucus on the surface of the body could be seen (Fig. 8) (Nagasawa and Cruz-Lacierda 2004).

Prevention Techniques

Fish should be treated with 0.5 ppm copper sulphate (CuSO4) for 5-7 days with vigorous aeration while being maintained in freshwater. Every day, freshwater that's being used for treatment needs to be replaced (Nagasawa and Cruz-Lacierda 2004).

Microsporidiosis

Microsporidiosis is brought on by a microsporidian infection of fish. Microsporidia are protozoa and endoparasites that have been detected in China and India, including *Epinephelus tauvina* and *Epinephelus* species. Spores in the form of pear are housed in minute nodes that sprout on the sick tissue (Nagasawa and Cruz-Lacierda 2004).

Clinical Symptoms

Fish with illness have enlarged bellies. Various-sized brown to black nodules might be detected in internal organs and adipose tissue (Fig. 9) (Cruz-Lacierda et al. 2004).

2.1.2- Sedentary or Sessile Ciliates

Koi, catfish, and goldfish are among the fish raised in ponds that commonly reveal sedentary or sessile ciliates in water that is rich in organic trash and dissolved solids (Stoskopf et al. 1993, Noga 1996). In addition of being primary invaders on skin ulcers, several parasites can cause epithelial damage in some species of pet fish. *Epistylis* (previously known as *Heteropolaria*), *Capriniana piscium* (previously called *Trichophyra*), *Apiosoma* (previously known as *Glossatella*), and *Ambiphyra* (called *Scyphidia* in past) are among the species that are often sighted (Noga 1996).
Epistylis leads to white, fluffy bruises on the borders of the fins and tail opercula, mouth and throat. Due to their similar indications, these bruises may be mistaken for fungus or columnaris sickness. *Capriniana* prefers gill tissue in particular and causes severe respiratory impairment in sick fish through mechanical obstruction (Noga 1996; Longshaw and Feist 2001; Reavill and Roberts 2007).

Diagnosis

The methods adopted for detection of sessile ciliate infestations are wet-mount cytometry (Fig. 10) and immunohistochemistry of infested tissues (Noga 1996).

Trichodina and *Trichodinella*

Trichodina and *Trichodinella* species are two prominent ciliated parasites that may be encountered on aquarium fish kept in both freshwater and saltwater. Although some of these parasites will parasitize the urinary bladder or oviduct, most of these parasite strains have a unique propensity for illness in skin and gill epithelium. Malnutrition, overpopulation, excessive organic litter in the water, and recent poor state of water are the factors that are frequently linked to these parasites. The parasites are usually seen among pool fish like goldfish and koi (Stoskopf et al. 1993; Baker et al. 2007; Weber and Govett 2009).

Life Cycle

Like in many other protozoan parasites, the life cycle is direct, and reproduction takes place by binary fission. Fomites and live plants added to ponds and tanks result in the introduction of *Trichodinids* into water (Noga 1996; Baker et al. 2007; Reavill and Roberts 2007; Weber and Govett 2009).

Fig. 10: *Apiosoma spp.* (Wet-mount examination).

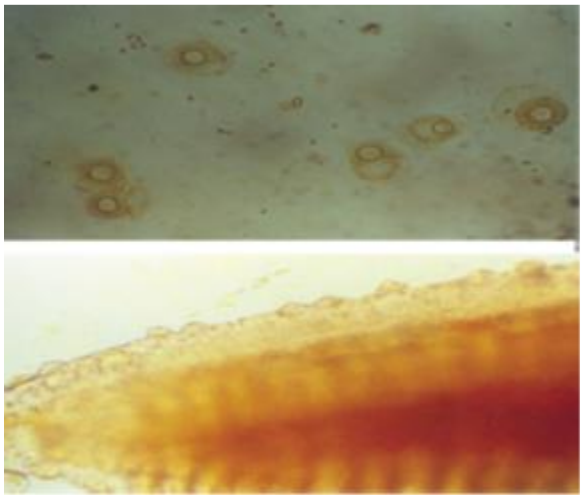

Fig. 11: *Trichodina* sp. from *Epinephelus coioides*: a) On body surface b) On gill filaments.

Clinical Signs

Flashing, murky skin because of increased secretion of mucus, dermatological haematuria, frayed fins and tail, sluggishness, and persistent fatality rates are all common manifestations of extreme branchial infections. The parasite has been portrayed as a "scrubbing bubble" or flying saucer (Fig. 11) (Noga 1996; Baker et al. 2007; Reavill and Roberts 2007; Weber and Govett 2009).

Flagellated Protozoans

Amyloodinium ocellatum and *Piscioodinium*

In marine and freshwater tropical fish, parasitic dinoflagellates (*Amyloodinium (A.) ocellatum* and *Piscioodinium*) can be encountered. These two parasites resemble *Ichthyophthirius* in terms of their life cycles, outward characteristics, and reactivity to temperature. Only the dinospore that is free-living is impacted by therapy. Elasmobranchs and teleosts can also get sick from *A. ocellatum* (Noga 1996). It has been seen to be transmitted up to three meters in active airflow systems, like *Ichthyophthirius*, through aerosol scattering of water drops. (Roberts-Thomson et al. 2006).

Clinical Signs

The epidermis and gills are the more likely or inclined sites for invasion, and a large infection can result in edoema, enlargement, infection-related redness, bleeding, issues with osmoregulatory function, and necrosis in the gill filaments. It is also referred as Amyloodioniosis, which is caused by *A. ocellatum*. Other pathological changes, in combination with respiratory disruption, can be seen as a darkish, gold look on the skin. That's why disease is also called so the named as "velvet sickness," "gold dust illness," and "rust disease" (Fig. 12) (Reavill and Roberts 2007).

Diagnosis

The process of identifying the disease from its symptoms is done by wet-mount cytometry or immunohistochemistry of the skin and gills (Fig. 13) (Baker et al. 2007).

Ichthyobodo

It was previously referred as *Costia* and is a microscopic, flagellated parasite of freshwater fish that is found worldwide in a diverse range of species. It is not larger than the red blood cell. The parasite may survive in a wide temperature range of 2–30°C (Reavill and Roberts 2007).

Clinical Signs

Acute lung trouble, lethargy, sadness, flashes, anorexia, epithelial inflammation and excessive mucus secretion are few of the clinical symptoms leading to fatalities. Death may occur prior to any clinical symptoms (Reavill and Roberts 2007).

Diagnosis

The diagnosis is made based on wet-mount cytometry. The organism's motion has been compared to that of a candle that is "twitching" or to uncontrolled spirals (Palmeiro et al. 2009).

MYXOZOA

Myxosporea (myxosporidiosis)

There are several families and subspecies in the class Myxosporea belonging to the phylum Myxozoa and the majority of which are fish parasites. Some types are well-known freshwater fish infections. Myxosporea infections in

Fig. 12: Adult Siamese fighting fish (*Betta splendens*) with velvet disease

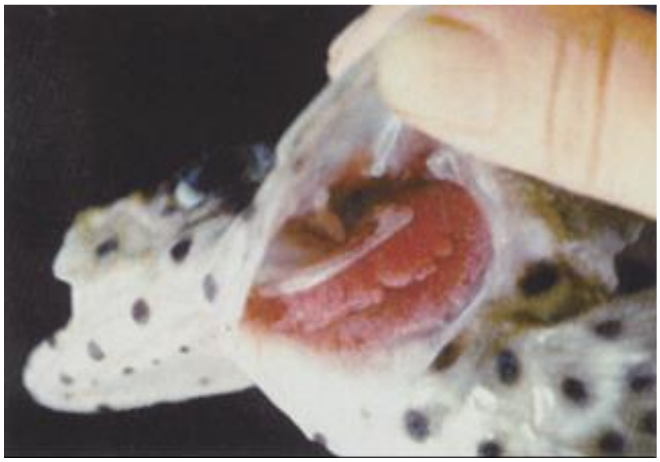

Fig. 13: *Cromileptes altivelis* having yellow gills due to *Amyloodinium ocellatum*

farm marine fish have been encountered more often in recent years. One or more disease spreading sporoplasms, one or more closures, and one or more bipolar capsules seem to have an internal polar filament helix. Whirling disease, PKD, sphaerosporosis, and ceratomyxosis are the four deadliest illnesses that affect freshwater fish. Whirling disease is caused by *Myxobolus cerebralis* (Alvarez-Pellitero and Sitjá-Bobodilla 1993).

Life Cycle

It was proven 18 years ago that the myxosporean's life cycle involves an intermediate oligochaete host. This information has made it easier to take care of the environment, such as using ceramic or plastic pools or tanks and regularly sanitizing them to stop the growth of oligochaetes and the subsequent spread of illness. Consideration of the finite effectiveness of current therapies like fumagillin and toltrazuril for myxosporea and other species is very crucial (Alvarez-Pellitero 2004).

Clinical Signs

Pathological changes include spine bending, darkening of the hind portion of the body and irregular swirl swimming. The vulnerability of illness is variable depending on the species, but all salmonid species may be diseased (Fig. 14) (Alvarez-Pellitero 2004).

Diagnosis

The histological examination of the skull cartilage or their enzymatic digestion proceeded by a microscopical study of the characteristic spores serves as the cornerstone for the diagnosis. Additionally, a PCR test can also be performed (Alvarez-Pellitero 2004).

Proliferative kidney disease (PKD)

Tetracapsuloides bryosalmonae, originally referred as PKX, has recently been recognized as the causal culprit. Although this myxosporean generates spores in a bryozoan host, but phases of this parasite that are without spores are found in the kidneys of several salmonid fish. A death rate of 30-50% occurs because this highly disease-spreading parasite can cause harsh sickness in rainbow trout (Canning et al. 1999).

Clinical Indications

Visible clinical symptoms are belly enlargement, hyperpigmentation and bulging eyes (Fig. 15). Internal indications comprise the fact that one can see enlarged kidneys and in more severe instances, cirrhosis. Immunohistochemistry of the kidney reveals interstitial proliferation together with tubular degeneration and persistent systemic inflammatory interstitial nephropathy (Fig. 16). This parasite also has the side effects of poor dietary metabolism and depressed immune system (Canning et al. 1999).

Diagnosis

The macroscopical identification is based on the complete observation of increased size of kidney. Confirmation is attained by seeing the parasitic stages in histological sections or squash preparations by skilled examiners (Canning et al. 1999).

Spaherospora renicola

Massive populations of *Spaherospora (renicola* are seen in intense cultivation of cyprinids, primarily *Cyprinus carpio*. While spores and their sporognic states are found in the renal tubules, prolific phases can travel via the circulation of blood and inflame the swim bladder (Sitja-Bobadilla and Alvarez-Pellitero 1992).

Fig. 14: Whirling disease

Fig. 15: Polycystic kidney disease

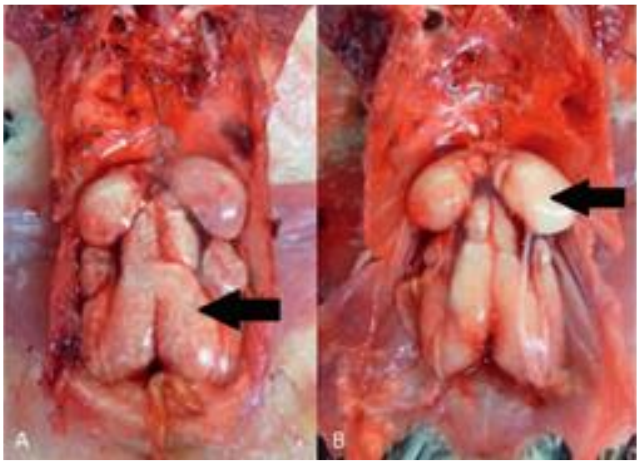

Fig. 16: Polycystic kidney disease in Rainbow lorikeets

Clinical Signs

While spores and their spore forming stages are found in the renal tubules, prolific phases can travel via the circulation of blood and inflame the swim bladder. The parasite *S. renicola* might be very dangerous. It causes ballooning, degeneration, and epithelial deterioration in the renal tubules, which compromises functional status of kidney (Fig. 17). Junior carps have swim bladder soreness as their swim bladder phases mature. Fish can also exhibit certain pathological symptoms, such as abnormal movements and swimming in ring patterns (Alvarez-Pellitero 2004).

Ceratomyxa shasta

On the west coast of North America, *Ceratomyxa shasta* is a significant disease-causing agent that has led to significant losses in salmonid communities, both in the wild and in captivity (Alvarez-Pellitero 2004).

Life Cycle

This Myxosporean's life cycle has been shown to involve an intermediate host that is a polychaete. For diagnosis, a PCR test can be performed (Alvarez-Pellitero 2004).

Clinical Signs

The main organ infected is the intestine, where parasites can be detected in the epithelium, causing tissue damage, hypertrophy, and lymphatic invasion. Severe complications of the illness result in the transmission of the parasites to certain other organs, anorexia, sluggishness, abdominal enlargement, ascites, and bulging eyes in the fish. According to fish species, there may be considerable fatality rates since vulnerability varies (Alvarez-Pellitero 2004).

Enteromyxum spp.

Two species of this genus infect the digestive tract of ill fish and are of pathological concern for marine fish with significant economic value. The myxosporean originally referred as *Myxidium leei* but because of phenotypic and genetic research, it was renamed as *Enteromyxum leei*. It produces the most important myxosporidiosis of cultivated sparids in the Mediterranean Sea, that is now called enteromyxiosis. The vulnerability extent of the fish is significantly broad: seabass, mullets, *Sciaenops ocellatus* and several marine aquarium fish, related to 25 species are infected (Branson et al. 1999).

Clinical Signs

Malnourishment and fatality are the two main outcomes of this parasite's assault on the gastrointestinal system, which causes severe enteritis with permanent repercussions. Therefore, the most extreme degree of slenderness, referred as "knife-fish," fundamentally constitutes the clinical manifestations. Some stock losses, particularly in *Diplodus puntazzo*might reach as high as 80% (Alvarez-Pellitero 2004).

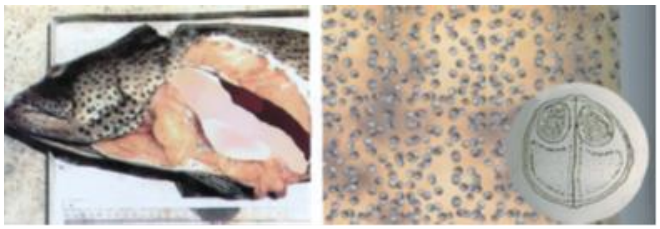

Fig. 17: Myxosporeans in kidney of *Epinephelus malabaricus*

Diagnosis

Immunohistochemical analysis of the intestine and recognition of parasitic phases are done for diagnosis of the parasite. So, the parasitic stages may also be observed in newly made smears by skilled examiner (Alvarez-Pellitero 2004).

E. scophtahlmi

Enteromyxum (E.) scophthalmi is the parasite belongs to the genus *Enteromyxum* and is mainly detected in turbot *Scophthalmus maximus*. *E. scophtahlmi* is a significant parasite for turbot farms because it can cause 100% tank or population mortality, which has a negative influence on the economy **(**Redondo et al. 2002).

Clinical Signs

Anorexia nervosa, caquexia, droopy eyelids and a distinctive pronounced bony hump on the head are the exterior pathological symptoms of disease. At site of tissue damage, further findings include the accumulation of fluids in the colon, intestinal bleeding, and internal organ pallor **(**Redondo et al. 2002).

Diagnosis

The recognition of the parasite is specifically done by microscopic examination of fresh smears and histopathology. The use of PCR technique is limited (Alvarez-Pellitero 2004).

CESTODA

Biology and Taxonomy

Tapeworms are the endoparasites which are found globally. The body of mature cestodes is flat made up of sticky scolex at the apex, the part capable of growing called neck, and the strobilus having different number of androgynous proglottids (Barber and Huntingford 1995; Barber et al. 1995).

Life Cycle

The life cycle of cestodes always need a certain host and one or more intermediate hosts. Fish may be an intermediate host for variable larval stages of parasites or as a main host. When fish is secondary intermediate host the larva of various tapeworms may have various tissue tropisms, but when fish is the main host, the cestodes that attain the mature state produced eggs in the gut of fish (Barber and Huntingford 1995; Barber et al. 2008) (Fig. 18).

Clinical Signs

Clinical indications extend from no symptoms to sluggishness, persistent loss of appetite, decreased weight, long term intestinal swelling, intestinal blockage, and harsh damage of mucosa. The traditional zoonotic infections caused by fish tapeworms are diplogonoporiasis and diphyllobothriasis, also called as 'tapeworm pernicious anemia'. Diphyllobothriasis is a situation which involves megaloblastic, macrocytic anemia along with thrombocytopenia and leukopenia due to lack of vitamin B12. This shortage is a consequence of more need of vitamin B12 in the ATP formation reactions in *Diphyllobothrium latum* and *D. dendriticum*. It is also demonstrated in the larval tapeworms having the capacity to produce anaphylactic reactions in animals that feed on contaminated fish meat. Similarly, the hypersensitivity reactions in humans have also been suggested (Paladini et al. 2017).

Diagnosis

Cestodes may be separated from the fish, cleaned and washed in water and then fixation in formalin or70–99% ethanol is done. At this stage, the cestodes can be kept preserved for a long time. To examine the main properties of internal structure of proglottids and for attaining a better perception of any disease caused by the parasite, histology of the mature tapeworms is beneficial. Identification can also be done by visual examination and wet mount preparation from feces (Paladini et al. 2017).

Treatment

To treat the infection praziquantel is given orally at 50 mg/kg for one dose, or 5 to 12 gm/kg of feed every 24h for 2 to 3 days. Treatment should be provided in an isolated tank so that the eggs of died cestodes may not scatter in the tank (Paladini et al. 2017).

ACANTHOCEPHALA

Biology

Acanthocephalans are distinguished by an invertible proboscis that is differently equipped with a sequence of hooks, the number and arrangement of which have phylogenetical significance. They are also described as "thorny headed" or "spiny headed" worms. A junctional skin

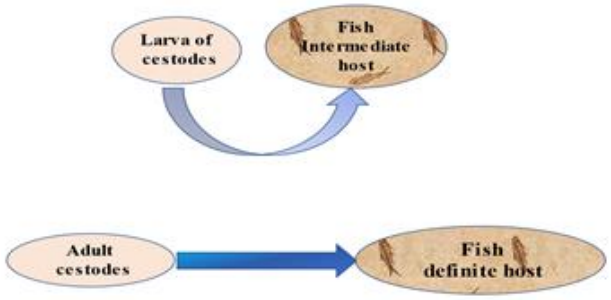

Fig. 18: Life cycle of cestodes

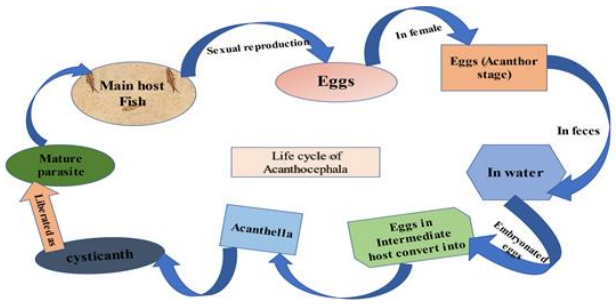

Fig. 19: Life cycle of *Acanthocephala*

serves as the body's boundary and because acanthocephalans are without a digestive membrane, they obtain their nutrition through the cuticular surface. They are hermaphroditic; males have different numbers of testicles, a copulatory bursa and "cement glands" that are responsible for closing the female's uterus after fertilization, in accordance with the species (Kennedy 2006).

Life Cycle

The mature parasite specifically dwells the gut of the main host, in which they undergo sexual reproduction. In the female the impregnated eggs grow to the acanthor stage and then eggs are secreted in the feces of the host in water. When taken in by intermediate host, the embryonated eggs emerge into an acanthella that afterwards enclose in a cyst in the tissue of host till a larval cystacanth is liberated (Fig. 19). Fish may behave like a paratenic host, for species like *Acanthocephalus anguillae*. Fish can also act as main host for some species like *Pomphorhynchus laevis* in trout, *A. anguillae* in European eels, *Anguilla anguilla*, and *A. lucii* in northern pike, but other piscine species may become a postcyclic host. For instance, parasites are capable to live in the predatory host if they ingest a host infected with mature parasite (Kennedy 1999).

Significant Pathogens within the Group

Considerable pathogens in this group include *Acanthocephalus* spp., *Bolbosoma* spp., *Echinorhynchus* spp. and *Pomphorhynchus* spp. (Paladini et al. 2017).

Clinical Signs

The clinical effect of the parasites does not depend on the number of parasites in the fish instead on the number of parasites to body size of the eel. The surface area of gills enhances significantly with the increasing body length (Hughes 1966) and the area of adherence also enhances with the size of host (Buchmann et al. 1989b). So, there will be serious effect of only few parasites in glass eels and young fish that will result in no troublesome in large sized eels. More infestations cause the eels to become sluggish and anorectic. The primary symptom is reduced feeding process and the obvious mark of gill-disease is that the fish look for the surface of water because diseased gills get in less oxygen. When reach the utmost point eel rotate its upper side down and finally die (Woo and Buchmann 2012).

The fish farms that provide uninterrupted flow of water in the tanks and biofilters, the diseased eels in them are incapable to be at their upper position in tanks and flow with water streams. This cause capturing of affected eels at the outlet (Buchmann et al. 1988b). More mucus is produced due to the hyperplasia of mucous cells which causes bashing or cudgeling of basic gill filaments and attachment of gill lamellae with each other and with neighboring filaments. Bleeding can also appear due to feeding of parasite and injection of hooks telangiectasis are found in highly affected eels (Woo and Buchmann 2012).

Diagnosis

The gathered parasites are particularly freed from the tissues of the host. These parasites are adhered to the tissue of host by piercing needles. After removing from the tissue of host, these are preserved in 70–95% ethanol for morphological and molecular-based studies. Brown et al. (1986) gave an explained method of collection, fixation, preservation, and examination of acanthocephalan. Alcohol-fixed specimens are cleansed with glycerol or stained with Mayer's acid carmine, for studying internal anatomy of these helminths. SEM may help in mapping and analyzing the framework of the proboscis and spines on body, but histology works for exploring host–parasite relationships and the pathogenicity (Paladini et al. 2017, Austin and Newaj-Fyzul et al. 2017).

TREMATODA (DIGENEA)

Morphology

These endoparasites belongs to the phylum platyhelminths and have complicated life cycles. All of them are androgynous besides some types living in blood (*Schistosomatidae*) and some tissue attackers found in marine fishes (*Didymozoidae*). They are known as "flatworms", but all species are not dorso-ventrally flattened (Thatcher 2006).

Life Cycle

The mature trematodes live in the digestive tract, blood circulation or hypodermic connective tissue of vertebrates. The whole process of mating and egg production take place in the hosts. The eggs are taken to the outside with host's feces or urine which burst in water after a short time. The primary larval phase of trematodes is miracidium that is ciliated and floats looking for a proper species of snail. After attaining snail, the miracidium enters into the body wall with the aid of its frontal penetration glands and reach the hepatopancreas. There it turns into a sac-like sporocyst after removing its cilia. The third stage of larva called rediae is formed in sporocyst, that ruptures in the snail's digestive gland. Cercariae are then produced from rediae that liberate from birth pores and leave the snail for finding an intermediate host or make encyst on vegetation. The tail of cercariae is removed during encystation and the resultant body is now a metacercaria. Sometimes, the main host is inhabited by cercariae directly. Species infecting the blood (*Sanguinicolidae*) and tissue forms (*Didymozoidae*) follow this direct way, while other fish trematodes get into the host in the form of metacercariae (Fig. 20) (Thatcher 2006).

Diseases

1. **"Black-spot disease"** is the disorder produced when cercariae attack the skin and form encystation there. This encystation is viewable to naked eye when host fish accumulates pigment cells around. The metacercariae in the skin does not destroy the health of the fish. Sometimes these black spots are too much that they turn the fish unlikeable to the consumer (Thatcher 2006).
2. **"Yellow-spot disease"** is the resembling situation. The metarcercariae of the family *Clinostomidae* cause this disease because of their yellow color (Thatcher 2006).
3. **"Eye fluke disease"** is the disorder due to the larval trematodes across the world. The larvae are observed moving around and in the eye of infected fish. No usual controversial reaction occurs, but the worms interrupt the sight of fish. Fish can become blind and is preyed by piscivorous birds (Ashton et al. 1969). Thatcher (2006) discovered that in Amazonian fish (*Chaetobranchus semifasciatus*), larval trematodes can cause branchial carcinoma.

Prevention and Treatment

Snails and plants in the environment of fish must be removed for a good and healthy aquarium.
There is no feasible treatment for encysted metacercaria. The mature trematodes may be vanished from the intestinal tracts of fish by using Di-N-Butyl Tin Oxide that is combined with the ratio of 0.3 % with respect to the body mass and weight and is given for one to five days. (Thatcher 2006).

Monogenetic Trematodes

The parasitic flatworms or flukes called monogenetic trematodes often reside on the skin of their fish hosts. The loose end browsing behavior of mouth and puncture of their adhesion organ both harm the host. They are known to be a significant fish disease in aquaculture. *Gyrodactylus* "skin flukes" and *Dactylogyrus* "Gill fluke" are considered as the most prevalent members of this group (Ernst et al. 2002; Ogawa 2002; Grau et al. 2003).

NEMATODES

Nematodes or roundworms are endoparasites having unsegmented bodies. The mature nematodes are visible to the naked eye. Nematodes affect various species of fish including *Epinephelu coioides, E. malabaricus, Cromileptes altivelis* and *Plectropomus leopardus* mainly prevalent in Indonesia, Malaysia, and Thailand. Common disease-causing agents of nematods are *Philometra* sp., *Anisakis* sp. and *Raphidascaris* sp. They can infect the growing or fingerling stages (Nagasawa and Cruz-Lacierda 2004).

Clinical symptoms

Dark red or black roundworms (without segments) are adherent to the parenchyma tissue of the digestive organs, muscles, fins, branchial chamber, and gonads of the ill fish (Fig. 21). Highly infected fish has faded and lean body (Nagasawa and Cruz-Lacierda 2004).
Parasite disturbs feeding which causes less growth and body becomes lean. Muscular destruction of infected gonads causes sterility (Nagasawa and Cruz-Lacierda 2004).

Transmission

Fish is considered as the main host for nematode parasites. Mature nematode produces egg, which burst into free-swimming larva, that is ingested by an invertebrate intermediate host (Nagasawa and Cruz-Lacierda 2004).

Diagnosis

The parasites are examined by microscopic examinations. The parasites are seen by operating the affected tissues. A mature *Philometra* sp. may grow up to 20 cm in length (Nagasawa and Cruz-Lacierda 2004).

COPEPODS

The copepods are ectoparasites of skin having sectioned shelly bodies (clearly divided bodies protected by shells) with segmented appendages. Copepods affect various species of fish including *coioides, E. fuscoguttatus, E. malabaricus,*

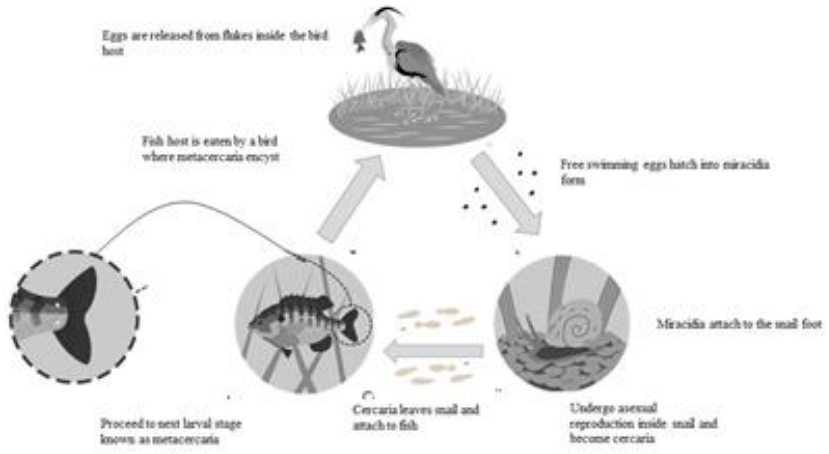

Fig. 20: Life cycle of the parasitic fluke *Clinostomum marginatum*, the yellow grub

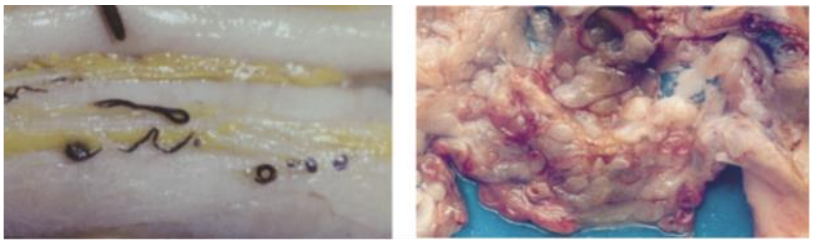

Fig. 21: Nematodes on tissue of digestive organ: a) *Plectropomus leopardus* b) *Epinephelus coioides*

Cromileptes altivelis and *Plectropomus leopardus*. Common pathogens of the group are *Caligus epidemicus*, *Caligus* sp. and *Lepeophtheirus* sp. They infect the small fingerling stages (Nagasawa and Cruz-Lacierda 2004).

Clinical Signs

These parasites are translucent and don't remain adhered to the body and fins of fish. They can be seen as white patches (Fig. 22). The affected places lack scales and have bleeding ulcers. Clumsy body, sluggishness, fish come to the surface to get oxygen, anorexia nervosa and excess mucus formation are the clinical indications. Highly infested fish may become lean (Nagasawa and Cruz-Lacierda 2004).

Prevention

Water should be changed to impede diseases. Freshwater washing of 10-15 minutes, or chemical washing by 150 ppm hydrogen peroxide for 30 mins should be established. Vigorous aeration should be given to the under-treatment fish (Nagasawa and Cruz-Lacierda 2004).

ISOPODS

Isopods have 10-50 mm sized body with short sections and two eyes. The parasite is seen in *Epinephelus (coioides* and *E. malabaricus. Rhexanella* sp. is seen in *E. coioides*. Isopods infect the fingerling stages of fish (Nagasawa and Cruz-Lacierda 2004).

Clinical Signs

The parasite adheres the body surface, mouth, nasal and opercular area (Fig. 23). Fish shows less opercular motion, becomes anorexic, body becomes thin, less growth, and it scratches its body with aquatic objects. The stress of parasites' body weight damage the fish tissue, skin layer and filaments of gill destroy. Small fish having high infestation die in 1-2 days (Nagasawa and Cruz-Lacierda 2004).

Prevention

Physically, parasite can be taken off and smashed. Washing by 200 ppm formalin for 30-60 mins is recommended. The aeration should be done and under treatment fish should remain in clean water (Nagasawa and Cruz-Lacierda 2004).

LEECHES

Leeches are ectoparasites having striped bodies, and pair of suckers that help in feeding and motion. The parasite causes sickness in *Epinephelus bleekeri*, *E. coioides*, *E. fuscoguttatus*, *E. lanceolatus*, *E. malabaricus* and *Cromileptes altivelis* etc. *Zeylanicobdella arugamensis* causes infection in *E. coioides*. Small growing fishes are heavily infested (Nagasawa and Cruz-Lacierda 2004).

Clinical Signs

The black and brownish parasites adhere in small blotches on infected locations like the body, fins, eyes, brachial and mouth spaces (Fig. 24). Diseased fish have ragged fins,

Fig. 22: Caligid copepods like white blotches on *Cromileptes altivelis*

Fig. 23: Isopod adhered on *Epinephelus coioides*

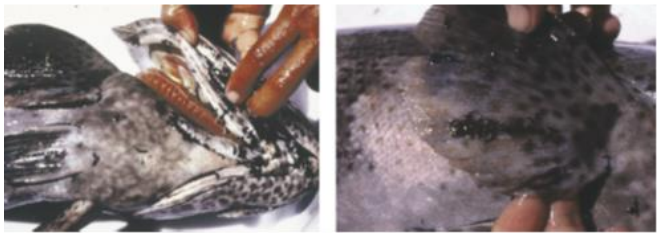

Fig. 24: *Zeylanicobdella arugamensis* on opercular space and pectoral fin of *Epinephelus coioides* broodstock

Fig. 25: Physical removal of leeches attached to *Epinephelus coioides* using a wet cloth

bleeding and irritation on adhering and feeding places of parasites, anorexia, anemia, sluggish and slow motion and fish come to surface for aeration. Highly infested fish exhibit high fatality rate (Nagasawa and Cruz-Lacierda 2004).

Life Cycle

Pre-disposing factors are poor maintenance of facilities and poor water state. Transmission is from one fish to other. Adult leeches release from fish and put their cocoons on rocks, shells or vegetation. A cocoon has one egg that burst into a young piscicolid leech, which then adheres to a host to become adult. After putting cocoons, adult leeches die (Nagasawa and Cruz-Lacierda 2004).

Prevention

From the water used for cultivation of fish, leeches can be eliminated by filtration. Physically, moist piece of cloth is used to clean blotches of the parasite (Fig. 25). Washing with formalin for an hour and powerful aeration will remove parasite. The post treatment fish are transmitted to clean water. Accessories used in cultivation must be cleaned with chlorine and placed in sunlight (Cruz-Lacierda et al. 2004).

1- Amoebae

Some naturally occurring amoebae have the potential to alter their behaviour and cause harm. Salmonid gill illness has been linked to several forms of amoeba (Nagasawa and Cruz-Lacierda 2004).

Amoebic Gill Disease (AGD)

AGD is a condition brought on by the commensal, free-living amoeba. A significant issue in marine salmon farming is *Paramoeba perurans*, which causes gill deterioration and death in infected fish. It has been viewed as the deadliest contagious disease and has become a critical challenge for sea-caged Atlantic salmon and rainbow trout in Tasmania (Roubal et al. 1989; Munday et al. 1990; Bryant et al. 1995; Findlay et al. 1995). There have also been reports of gill amoebic illnesses in fish apart from salmonids, such as European catfish (Dykova et al. 1998; Paniagua et al. 1998). AGD most frequently manifests at water temperatures between 10 and 20°C, while it can occasionally happen in temperatures above average. Raised, multifocal, white mucoid patches on the gills of sick fish are signs of severe disease and are sites of primary and secondary laminae epithelial proliferation. Desquamation of the epithelium, localized problems with blood flow, and increasing alterations symptomized by irritation before this step (Dykova et al. 1995; Adams and Nowak 2003). The gill respiratory surface area is reduced or destroyed because of all

the aforementioned alterations. Fish with AGD would experience severe cardiovascular abnormalities and acid-base imbalances that would lead to abrupt cardiac dysfunction and death (Powell et al. 2002).

Conclusion

There are numerous parasitic diseases around the world. Parasitic diseases are common in fish, and they can cost a lot of money to the fish farmer. As some pathogens are zoonotic in nature, so aqua farmers, fish technicians and processors must practice good hygiene. Many diseases can be avoided with proper management and vaccinations.

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28

Use and Abuse of Sorghum and Jequirity Plants in Cattle

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INTRODUCTION

Sorghum was first domesticated in Africa region and is now considered as an important cereal crop of dry land grown for animal feed, human food, and various other purposes on six continents. Protein makes up 6–25% of sorghum's composition, along with oil (3.4–3.5%), ash (1.2–18%), carbohydrates (70-80.7 percent), and fiber (2.3–2.7%). One of the earliest green forages for animals who are breastfeeding is sorghum. Sorghum that has been steam-flaked digests Organic Matter (11%), Nitrogen (10%), and Starch (25%) more quickly during post ruminal digestion (Zinn et al. 2008) It is a climbing deciduous plant that can get as tall as 6 meters. Although the seeds are deadly, there are several medical benefits of using it. These are used as an emetic, irritant, abortifacient, and contraceptive in medicine. Cattle eyes are treated with crushed Abrus roots when these get white. Alkaloids, phenolic, and flavonoid components extracted by using ethanol, chloroform, and petroleum ether have been found to have antidiarrheal and anti-fertility properties. The potentially lethal neurological condition of tetanus (*Clostridium (C.) tetani* infection) can affect cattle. Deep, necrotic wounds and postpartum metritis are the most typical symptoms of *C. tetani* infection in cattle. The clinical symptoms that precede death include a rapid, irregular heartbeat, breathing problems, restlessness, and anxiety since the heart and brain are the first organs to be impacted by oxygen deprivation.

The fodder provided to cattle can be poisonous too if the fodder is deficient in water or faced drought conditions. This may lead to toxicity to livestock followed by mortality. Sudan grass and sorghum are two separate groups of plants that produce cyanide, which can be toxic and poisonous to livestock under certain conditions i.e., water scarcity or drought. The plants which produce cyanogenetic glucosides are also called cyanogenetic plants during their growing phase (Albright and Clive 1997). Glucosides are glucose molecules that hydrolyze and decompose into glucose sugars, when water is added. During the decomposition process of cyanogenetic plants, the cyanide breaks and loose from its chemical link and transforms into the poisonous hydrocyanic acid, often known as prussic acid and denoted by HCN. The previously existent glucosides and undamaged, still-bonded cyanide are not very poisonous, nonetheless, if specific enzymes are present. These are very harmful to both animals and humans if plant's hydrolysis or chemical degradation enzymes are found together. It might be obtained from various sources. Digestive juices for example the HCl which is already present in the gut may cause hydrolysis to occur (Nath and Sharanya 2021).

Livestock toxicity by plants is often correlated to problems of management and field conditions. Animals typically graze excessively when they are hungry. Overgrazing, corralling, trucking, trailing, or introducing animals to a different range result in behavior changes leading to voracious eating of what they are offered which may lead to toxicity. For instance, animals occasionally ingest plants like greasewood and lupine, and these are poisonous for animals if they eat too much of these very quickly (Forero L and Nader G 2011).

Sorghum Plants

One of the most important cereal crops in the world, sorghum (Sorghum bicolor *L. Moench*) provides feed, food fuel, fiber, and biofuel/chemical feedstocks in a variety of environments and systems of production. Sorghum is the fifth-largest cereal crop in the world (Kerosvich et al. 2005). Sorghum is (a C4 plant) frequently grown in the semi-arid tropics, which are prone to drought. Based on their capacity to produce high yields in the field during drought circumstances, many sorghum cultivars that are drought-tolerant have been discovered (Jagtap et al.1998).

Sorghum is a grain and staple meal that can withstand droughts and is a good source of more than 20 different minerals and protein. Due to environmental and genotypic influences or interactions, the concentration of the protein and mineral elements content in sorghum varies (Gerrano et al. 2016). Sorghum contains ash (1.2-1.8%), protein (6-25%), oil (3.4-3.5%), carbohydrate (71.4-80.7%), and fiber (2.3-2.7%) with 89.2 to 95.3% dry matter based on the type of sorghum cultivated (Gerrano et al. 2016).

Africans first developed sorghum, which is now a significant dry land grain crop used on six different continents for animal feed, human food, and other purposes. Sorghum can withstand heat and drought pretty well (Lacy et al. 2006).

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An ideal system of cropping should be able to rapidly remove massive volumes of nitrogen and allows countless biosolid applications throughout the season of growing. Crops that are produced for fodder, such as sorghum or Sudan grass × sorghum hybrids, are likely to achieve these goals. Sorghum has an extensive root pattern that is highly efficient at scavenging nitrogen out of the soil and a very high capacity for absorbing nitrogen (Pedersen et al.1995).

Sorghum is a high-standard genome reference and genetic model species of the PACCAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Arristidoideae, and Danthonioideae) for C4 grasses because it has diversely variable germplasm, standard genomics, and genetic platform, a wide range of adaptations, and utilization as a forage, grain, bioenergy crop, and sugar (~65M hectares) on a global scale. Sorghum is primarily self-pollinated, and its tractable genetics make hybrid/ inbred breeding, quantitative genetic study, and population development possible (Mullet et al. 2014).

Sorghum, the perfect crop for dry locations, is regaining popularity due to its drought resilience and potential biofuels production (SORGHUM: A grain of hope, 2011).

Concentration Table

Sorghum fodder's nutritional analysis has shown that it is an excellent source of nutrients with modestly high protein content and can be used as a viable green fodder to feed animals in regions with insufficient rainfall (Ramana DB et al. 2018). The nutritional analysis of sorghum has been mentioned in Table 1.

Uses of Sorghum

Sorghum is a multipurpose crop in a true sense as it has the potential to satisfy diverse human needs as well as animal dietary needs (Iqbal 2015). Sorghum is an excellent feed for livestock and companion animals (Rooney et al. 1982). Nowadays, livestock consumes around 48% of sorghum grains produced across the world (Dowling et al. 2002). The stem can be utilized as a source of fuel, fiber, and most recently as feedstock for cellulosic ethanol. The grain can be used as food or as animal feed (Wang, Upadhyaya and Dweikat 2016).

Sorghum is either utilized whole or as distillers dried grains in animal feed (Ronda et al. 2019). For dairy cattle in lactation, distillers' grains provide a useful source of energy and protein (Schingoethe et al. 2009).

The feeding of sorghum grain seems to consistently boost starch utilization and milk protein and milk output (Theurer et al. 1999). The more starch is degraded in the rumen, the more ammonia is incorporated into microbial cells during protein synthesis (Leng and Nolan 1984). Dairy cows fed SF sorghum can produce more milk by increasing the fraction of microbial nitrogen and total nitrogen flow to the duodenum (Theurer et al. 1999). Sorghum is one of the oldest cultivated green forages for lactating animals (Iqbal 2015).

To improve the nutrition, health, and performance benefits of foods and create functional foods, sorghum is utilized as a food ingredient and added to other foods (Khalid et al. 2022). The post ruminal digestion of Organic Matter (11%), Nitrogen (10%), and starch (25%), as well as the total tract digestion of Organic Matter (8.3%), Nitrogen (8.2%), and starch (8.9%), were all increased (Phosphorus < 0.01) by steam-flaking sorghum (Zinn et al. 2008). For a cow (450 kg, 5 kg milk/day) suckling a calf, sorghum bran fed at 1 kg per kg milk with a base diet of sorghum stover is sufficient (Mahabile et al. 2000).

Phenolic compounds and fat-soluble compounds (polycosanols) extracted from sorghum are beneficial for the gut microbiota and parameters associated to obesity, inflammation, oxidative stress, dyslipidemia, diabetes, hypertension, and cancer (de Morais Cardoso et al. 2015).

Sorghum meal is also a preferred feed ingredient by newly weaned dairy calves and is considered highly palatable (Miller-Cushon et al. 2014).

It is an important source of B-complex and fat-soluble vitamins (Waniska et al. 2004). It is also a source of a few minerals, proteins (kafirins, which have high levels of polymerization, extensive disulfide bridges, and strong interactions with tannins and starch, rendering proteins resistant to enzymatic breakdown in the digestive tract), lipids, vitamins, and phenolic compounds (Birhanu 2021).

Jequirity Plants

The plant *Abrus (A.) precatorius* is a member of the Fabaceae family, also known as the jequirity or rosary pea Chanoti, chirmu, ratti, cham-l-kharosh, gunchi and rosary bean are the common names used in various regions of Pakistan (Oladimeji and Valan 2020). It is a deciduous climbing plant that can grow up to 6 meters, and occasionally up to 9 meters, in length. In order to support themselves, these stems wriggle over the ground and twine with other surrounding plants. To build necklaces and rosaries, the vibrant seeds are frequently utilized as beads. These are poisonous, yet have a lot of medical uses. The extremely toxic chemical abrin, indole alkaloids, and anthocyanins are only a few of the medicinally beneficial compounds found in the seeds. Despite being exceedingly poisonous, these are employed in medicine as an emetic, irritant, abortifacient, and contraceptive. The seeds are also bitter, diaphoretic, expectorant, aphrodisiac, purgative, antiperiodic, and emetic. In many parts of the world, these have been crucial in the conjunctivitis treatment process. Glycyrrhizin and trace levels of the toxin abrin are present in the roots and leaves. These are anti-allergic, anti-inflammatory, and expectorant, in addition to have calming effects (Dp et al. 2021). The nutritional analysis of jequirity plant has been mentioned in Table 2, proximate and mineral composition of leaves in Table 3 and 4 and proximate composition of seeds in Table 5, respectively.

Table 1: Nutrition analysis of Sorghum Plant (Ramana DB et al. 2018)

Parameter	Mean ± SE	Range
Hemicellulose	8.20± 0.79	28.03
ADL	8.39± 0.58	20.85
Silica	3.10± 0.21	7.99
Crude protein	12.42± 0.47	15.95
ADF	68.78± 0.86	30.99
Dry matter	26.30± 0.50	26.26
NDF	76.99± 0.41	12.06
Total Ash	9.18± 0.21	6.93
Cellulose	33.23± 0.71	31.72

Table 2: Concentration Table (Garaniya and Bapodra 2014)

Parameter	Percentage
Crude Protein	8± 0.00
Crude Fiber	2.00 ± 0.00
Crude Fat	6.50 ± 2.12
Ash	7.00 ± 1.41
Moisture	11.00 ± 0.00
Total carbohydrate	65.50 ± 3.12

Table 3: Proximate Composition of Leaves (Paul et al. 2013)

Element	Concentration (mg/100 g)
Calcium (Ca)	231.83 ± 0.204
Iron (Fe)	24.14 ± 0.002
Potassium(K)	246.94 ± 0.252
Magnesium (Mg)	25.66 ± 0.012
Sodium (Na)	94.10 ± 0.145
Zinc (Zn)	6.09 ± 0.020
Copper (Cu)	0.07 ± 0.004

Table 4: Mineral composition of Leaves (Paul et al. 2013)

Parameter	Percentage
carbohydrate	42.42%
Crude Protein	39.20 %
Nitrogen	6.272%
Crude Fiber	9.08%

Table 5: Proximate Composition of seeds (Das et al. 2016)

Element	Concentration (mg/kg)
Magnesium (Mg)	1046
Calcium (Ca)	975
Iron (Fe)	213
Potassium(K)	11132
Zinc (Zn)	48
Phosphorus (P)	2302
Sulfur (S)	1841
Rubidium (Rb)	4.0
Strontium (Sr)	1.0
Manganese (Mn)	25
Copper (Cu)	13
Molybdenum (Mo)	1.0
Lead (Pb)	1.0

Ethnoveterinary Uses of Jequirity Plant

Jequirity Plant has been traditionally used for treatment of different diseases in cattle. On affected areas with swelling, pasted leaves of *Abrus (A.) precatorius* are applied until healing (Ishika 2015). Leaf of *A. precatorius* are also used to treat salivation from the mouth as traditional medication (Usha et al. 2016). Seeds of *A. precatorius* soaked in water for overnight and its paste give instant cure for any urinary trouble and in indigestion in cows (Bharali et al., 2015). The roots of *A. precatorius* has been used to treat blood dysentery. Paste of root along with boiled rice is given to treat dysentery (Deepa et al., 2014). Use of *A. precatorius* in cattle for the expulsion of placenta have been reported (Takhar and Chaudhary 2004). Crushed seed of Abrus given with jaggery in retention of the placenta. 12 to 15 seeds should be fed for placental expulsion (Bhatt et al. 2019). *A. precatorius* have been found useful in curing eye diseases of cattle (Abhijit and De 2010). When cattle's eyes become white, the crushed roots of Abrus are applied. (Shivakoti 2011). Alkaloids, phenolic and flavonoid constituents obtained by ethanol, chloroform, petroleum ether extraction are found useful as antidiarrheal and antifertility agent (Janakiraman et al. 2012). For the treatment of mastitis in milk cow, roots of *A. precatorius* and *Leonotis (L.) nepetifolia* (1:2) is made into a paste and applied as a poultice on the mammary gland twice a day for 3 days (Mandal and Habibur Rahaman 2022). Seeds of *A. precatorius* are also used to treat cattle Helminthiasis. For this purpose, pound seeds, or grinded leaves (0.4kg) mix with 2 L of water, or boiled. Method of preparation is maceration, decoction and then powder extract of seed. Dosage for adult animals is 4-5 mature seeds, 2 seeds for calves or drench 200-300 ml to 50- 70 calves and adults (Matovu et al. 2020). It is also used for treatment of fractures in animals (Garaniya and Bapodra 2014). Seeds of *A. precatorius* are used by Malayali tribes for neck infection (Selvaraju et al. 2011). For the treatment of anthrax, the stem bark of *A. precatorius* is pounded and boiled in water with tubers of *Curculigo (C.) orchioides*, leaves of *Vitex (V.) negundo* (each 50 g), garlic, and pepper (15 g). The resulting decoction is then administered orally once a day for a week (Narayana and Narasimharao 2015). Seed extracts of *A. precatorius* (20 mg) dissolved in drinking water is given once daily for 4 days for the treatment of trypanosomiasis (Pragada and Rao 2012). Leaves of *A. precatorius* is used traditionally for wound healing (Sehgal and Sood 2013). Crushed roots of *A. precatorius* are used to treat cough, cold and pneumonia and seeds are used against constipation (Patil et al. 2015). Cows with an appetite loss are treated with a drink made from *A. precatorius* seed and coconut oil (Guruprasad and Prasad 2019). Regularly, seeds of *A. precatorius* are crushed, soaked in water over night, and administered to the animals in the morning through oral route for three days for the treatment of lack of estrus (Meena and Kumar 2015). For three to five days, young leaves of *A. precatorius* are administered to grazing animals to treat mouth ulcers caused by feeding on very rough leaves (Joseph et al. 2013). To get rid of body lice, cows are fed with the crushed roots of *A. precatorius* (Ahmmed et al. 2010). Leaves of *A. precatorius* are also used for the of Foot and Mouth Disease (FMD) treatment (Nair et al. 2017).

Activity against Pathogens

Methanolic extracts of *A. precatorius* have good activity against veterinary pathogens i.e., *Clostridium (C.) septicum*, *Aspergillus (A.) fumigatus*, *Brucella (B.) abortus*, *Salmonella (S.) enterica* and *Candida (C.) albicans* (Sandhya Deepika D 2021).

Tuberculosis

Bovine tuberculosis (TB) is a condition marked by the progressive growth of certain granulomatous lesions or tubercles in the lymph nodes, lung tissue, or other organs. The cause of the illness is *Mycobacterium (M). bovis*. Nearly all warm-blooded animals, including bison and buffalo, are susceptible to the disease, which can also affect other bovine species. Not all species are equally prone to the illness; some serve as spillover (end) hosts while others serve as maintenance hosts. Seeds of Abrus are used for the treatment of tuberculosis (Garaniya and Bapodra 2014).

Tetanus

Cattle are susceptible to the potentially fatal neurologic disease known as tetanus *(C. tetani* infection). Tetanus's clinical symptoms typically go unnoticed until the disease has progressed to an advanced stage, at which point it is difficult to treat and manage infected animals, and their prognosis is generally not good. Primary clinical signs included erect tail, stiff gait, and prolapsed nictitating membranes also called prolapse of the third eyelid. Gram-positive bacillus (*C. tetani*) produces exotoxins which are the cause of the neuroparalytic syndrome of tetanus. The most common infection sites in cattle for *C. tetani* include deep, necrotic wounds, either surgical or traumatic, necrotic lesions of the vagina or vulva following dystocia, and severe postpartum metritis (Garber and Smith 2011). *A. precatorius* leaves are traditionally used to treat tetanus (Garaniya and Bapodra 2014)

Muscle Relaxant

To treat swollen joints and stiff muscles, mustard oil and crushed leaves of *A. precatorius* are combined and applied topically or wrapped around as a poultice (DeFilipps and Krupnick 2018). Activities of various parts of jequirity plant is mentioned in Table 6.

Sorghum Toxicity

Sorghum toxicity has been seen commonly in Pakistan. The syndrome is reported in horses, sheep and cattle. Atherogenic nitriles such as beta-cyan alanine, cyanogenic glycosides, and nitrates have been suggested as basic causative agents (Francis and Charles Kenworthy 1915). The syndrome commonly develops in horses when they have grazed hybrid Sudan pastures for a long time ranging from weeks to months and causes axonal degeneration and myelomalacia in the spinal cord and cerebellum. Sorghum toxicity is characterized by incoordination, cystitis, urinary retention and alopecia on the hind legs due to urine scald (Doggett and Hugh 1970). The urinary bladder dysfunction is related to the spinal cord damage. The incoordination may progress to paralysis. Deformities occur in the fetal musculoskeletal system and abortion may happen in the late pregnancy (Blaney et al. 2010). Although fetal toxicity is not mostly observed in horses, the impact on reproduction is the primary concern. Dietary supplements containing sulfur may be beneficial (Boyd et al. 1938). Pyelonephritis commonly results in death in affected horses. Antibiotic therapy is an option, but if ataxia has started in, a full recovery is rare (Geor RJ 2007).

Mechanism

When first cutting of sorghum is done, and plants are getting ready for next growth, farmers usually do not water it, due to which the wilted, young or stunned plants that develop large quantities of prussic acid (i.e., hydrogen cyanite), which is dangerous for animals and may cause paralysis and death. The overconsumption of sorghum is resulted in to the toxicity (Subrahmanyan D et al. 2008).

Prevention: Sulphur (0.26-0.4%) is given in daily doses for cattle and sheep when climate conditions are favorable for wilting after a prolonged drought (Whitmore JS 2000).

Immediate Treatment

Sodium nitrate 20% solution is given and immediately followed by sodium thiosulphate 20% solution as for cattle and half for sheep. This treatment is only beneficial if given on immediate basis (Subrahmanyan D et al. 2008).

Hydrocyanic Acid/Prussic Acid Toxicity

Hydrogen cyanide (HCN), also known as prussic acid, is an organic compound. Plants normally don't produce it. However, cyanogenetic glycoside can be stored in significant amounts in a number of common plants. In plants, cyanide is present in two forms:

- Free form (hydrocyanic acid)
- Bound form (cyanogenic glycoside)

Cyanogenic glycosides are accumulated in significant amounts. The following plant species frequently result in the prussic acid toxicity in livestock:

- *Acacia*
- *Sudan grass*
- *Sorghum–Sudan grass hybrids*
- *Sorghum halepense* (Johnson grass)
- *Sorghum spp* (Brimer L 2007).

Table 6: Different types of Extracts of the parts of jequirity plants and their activity (Das et al. 2016)

Part of the plant	Type of Extract	Activity
Leaves	water extract	Anti-inflammatory activity
Shoot	methanol	larvicidal activity
Leaves	methanol	bronchodilator activity
Roots, seeds and leaves	Methanol and petroleum ether	Antibacterial Activity
Leaves	ethyl acetate	Antiserotonergic Activity
Seeds	Petroleum ether	Anticancer activity
Leaves	ethyl acetate	Antiserotonergic Activity
Seeds	aqueous extract	Nephroprotective activity
Seeds	ethanol	Anti-oxidant activity
Seeds	ethanolic extract aqueous extract	Anti-fertility activity
Red & white seeds	Ethanol	Anti-arthritic activity
Seeds	hexane, chloroform, methanol and water	Anti-microbial activity
Leaves	chloroform and ethanol	Cytotoxic property

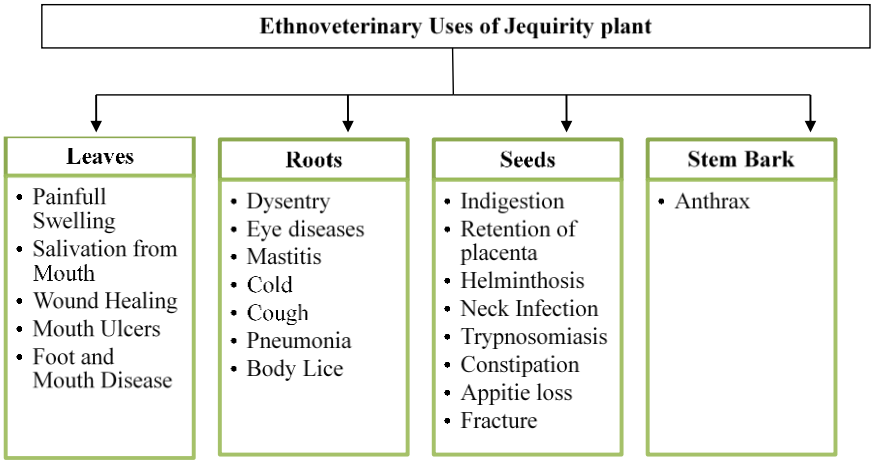

Fig 1: Ethnoveterinary uses of Jequirity Plant.

Toxicity symptoms typically appear 15 to 20 minutes after ingesting the poison. Death happens quickly, in acute cases within 1-2 hours after the clinical manifestations become apparent, or in around 2-3 minutes (Robson and Sarah 2007). Animals are frequently discovered dead with no obvious clinical symptoms or signs. Because the heart and brain are the first organs to suffer from hypoxemia, clinical symptoms that appear before death include breathing problems, a rapid, weak, irregular heartbeat, restlessness, anxiety, and depression. Other symptoms include salivation, bloating, terminal convulsions, and bright red mucous membranes (Carrigan and Gardner 1982).

On the basis of clinical and/or post-mortem findings, prussic acid poisoning is diagnosed. Additionally, the blood may appear bright red and poorly clot during post-mortem inspection. A few hours after death, the blood will turn dark again, the muscles may be dark, and there may be hemorrhages on the surface of the heart as well as in the trachea and lungs (Muwel et al. 2018). A brick red color on filter paper indicates the cyanide. The glycoside breaks down to create free HCN when wilting, frosting, or stunting damages the plant cells. Physical disruption (i.e., mastication) may also release HCN (Beasley DM ad Glass WI 1998).

Pathophysiology

HCN/Cyanide containing compound → impairs oxidation (cytochrome oxidase enzyme system) → histotoxic anoxia → dyspnea, convulsions, tremors and finally death (Beasley DM and Glass WI 1998.)

Treatment

- Establish oxygen transport at the cellular level
- Sodium nitrite @ 20mg/kg body weight IV (10g/100ml of distilled water or normal saline)
- Carefully repeated @ 10mg/kg every 2 to 4 hours
- Sodium thiosulfate @ 500mg/kg body weight IV
- Sodium thiosulfate @ 30g/cow/buffalo PO (to detoxify remaining HCN in rumen)
- Methylene blue @ 4-22 mg/kg IV (if doubt about nitrate poisoning) (Subrahmanyan D et al. 2008)

Seeds of Jequirity (*A. precatorius*)

A. precatorius can be found all over the tropical region. It is a perennial vine with trailing twine that bears oval, glossy

red and black seeds along with yellow or red blooms. Jewelry and necklaces feature seeds as beads. Seeds of *A. precatorius* possess a powerful phytotoxin known as abrin. It is largely used by chammers or leather workers for cattle malicious toxicity. The decorated seeds are soaked in water and ground into a mass, which is made into small, sharp pointed spikes (hind. *Sui* or *sutli* in local language) and hardened in the sun. For use, two of the spikes are edged in a brick and inserted by their base into two holes at the edge of a wooden handle. A forcible blow is then stuck with the handle, driving the protruding spikes into the animal flesh, where they are left, causing death in 18-24 hours. The site of insertion is ingeniously selected so that symptoms are selected as according to the disease prevailing that time. For example, the cheeks are selected when there is outbreak of hemorrhagic septicemia, in the hind quarter when black quarter is prevailing. Sometimes, other poisons like madar, arsenic, latex, aconite, etc. are added to *sui* to augment its destruction action (Van Kampen 1970). In horse oral toxicity is characterized by; inappetence, violent purging, lassitude, shivering, in coordination and paralysis. In *sui* toxicity, there is local edema, anorexia, fever, later sub normal temperature, convulsions, coma and death (Soldán et al. 2001) In cattle, salivation, nasal discharge, nausea, vomiting, profuse hemorrhagic diarrhea with watery feces, occasionally ulcerative lesions in mouth and dehydration, stiffness of muscle, incoordination, muscular spasms, ataxia, convulsions, trembling, paralysis, coma and death is common (Ballantyne et al. 1972).

Emergency Treatment

Gastric lavage is used to remove the toxin from the stomach as soon as possible. Then, activated charcoal, demulcents, and saline purgatives are used. In case of toxicity, symptomatic and supportive treatment is given to the animal. Anti-abrin serum, papain and HCL by mouth, saline purges and are choline. Remains of *sui* should be removed and wound should be washed with $KMNO_4$ lotion. It is considered as the chemical toxicity. No specific antidote is available (Egekeze et al. 1980).

Aversion (Training livestock to avoid eating toxic plants)

It's crucial to comprehend the factors at play when beneficial forage turns into a toxic plant in order to prevent toxicity. It might be challenging to make a definitive diagnosis of potential plant poisoning. It's crucial to be aware of the poisonous plants that are present wherever you go and to know exactly which circumstances they can harm animals. When assessing disease and lost production in cattle, toxic plants are one of the major contributors to financial loss for the animals (Rohila N et al. 2018). Toxic plants can affect animals in many ways, including death, chronic illness, debilitation, birth defects, decreased weight gain, increased parturition interval, abortion and photosensitization. Other factors to take into account include forage loss, additional fencing, higher labor and administration costs, and frequent interference with the proper forage collection, in addition to more evident losses. By giving animals a certain food and then giving them emetics to make them feel nauseous, it is possible to train animals to avoid eating that food. The animal avoids eating the food since it tastes bad because of the disease it has been given. A proposed technique to stop livestock poisoning from attractive and widespread hazardous plants is conditioned food aversion (CFA). Cattle have been trained to avoid eating tall larkspur (*Delphinium barbeyi L. Huth*), a particularly troublesome toxic plant. However, in field grazing situations, a number of factors affect the development and maintenance of dietary aversions. Animals' capacities to acquire and maintain aversions may vary depending on their age and gender (Rosenberger et al. 1979). Strength of the aversion depends on the novelty of the plant and the severity of the produced illness. Animals are motivated to try the avoided meal by social pressure or peer pressure, and the aversion will vanish if it is not reinforced. For certain animals, it may be challenging to transfer the aversion they developed in a controlled environment like a pen to a complex vegetation community in the wild. If these challenges can be resolved, CFA might be a useful technique for lowering the risk of poisoning on rangelands with toxic plant infestations (Kellerman et al. 2005). In Pakistan, the majority of rangelands are heavily populated with toxic plants. Naturally, the majority of wild and domestic animals that graze on rangelands do not die suddenly after ingesting harmful plants. Grazing animals employ a variety of physiological or behavioral adaptations that are interconnected to lower the danger of poisoning (Durrani MJ et al. 2010.). Control strategies are based on:

(1) Changing diet to avoid or reduce toxicity ingestion (learning behavior)
(2) Dilute the toxin by selecting a mixed diet (hunger reduce)
(3) Allowing cyclical or intermittent consumption of a toxin (boost immunity)

Aversive conditioning and random consequences, in which animals learn from the positive or bad effects of consuming different forages, are essential tenets of these three techniques (Church and David 1991). Losses of domestic livestock prove that learning is not a perfect preventive strategy. However, with knowledge, the majority of livestock can graze on ranges with hazardous plants and thrive (Yousef and Mohamed 1985).

Conclusion

The domestic livestock are more frequently affected by toxic plants than wild ungulates are likely due to human management mistakes that often outweigh coping

mechanisms. Additionally, compared to cattle, wildlife probably has a higher tolerance and ability to detoxify poisons. Better is to do not let the livestock to graze plants that are wilted, immature, drought-stressed, or injured by frost. Never let livestock graze sorghum that is only 50 cm tall. Before letting livestock graze, feed them hay to satisfy their hunger. Poisoning risk will be decreased if the material is fed as silage. Correct ensilage for three weeks reduces toxin levels by about 50%. Some of the poison will be expelled as gas when feeding out. It is still advised to test this feed before using it. In order to get rid of any free prussic acid, linseed gruel needs to be properly cooked. Sulfur supplements can be added to feed as a preventative measure.

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Hip Dysplasia in Large Breed of Dogs

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INTRODUCTION

Hip dysplasia is a condition which includes the anomalous change of the hip joint. Previously this matter were recognized in human being by the father of medicine and Anatomy, Hippocrates about 400 years BC. Hip dysplasia in the canine species were earliest termed by Schnelle in 1935 (Schnelle 1935; BUIE 1947). More lately, this deformity has rarely been seen and detected in other animals, including bovine, equine and feline species and also in the rabbits and dingoes (Owiny et al. 2001). Hip dysplasia in dogs is caused by a number of causes, starting with genetics. Larger dogs, like the Great Dane, Saint Bernard, Labrador Retriever, and German Shepherd Dog, are more prone to hip dysplasia than smaller dogs. This hereditary propensity can be exacerbated by elements like an excessive growth rate, certain types of exercise, an incorrect weight, and an imbalanced diet. Some dogs start to exhibit symptoms of hip dysplasia as early as four months of age. Others have it concurrently with osteoarthritis as they get older. There are a few indications that owners should be aware of in both situations. Depending on the disease's severity, the amount of inflammation, the amount of joint looseness, and how long the symptoms have been present, these symptoms may vary. Hip dysplasia has been a problem for the dog. And these signs included a decline in activity, reduction in range of motion, a challenge or resistance climbing, leaping, running, or stair climbing, Laziness in the tail and "Bunny hopping," swaying gait. When moving, there is grating in the joint, reduction in thigh muscle mass The shoulders' muscles have a noticeable hypertrophy as a result of compensating for the back. Pain rigidity or limping, more than fifty years ago, Schnelle detected the problem of hip dysplasia in the United States of America, however few cares were rewarded by the breeders and researchers through the first twenty-five years to solve this problem. Meanwhile, the problem has changed into very widespread issue in a large number of strains. This reality requisitioned for firm procedures of dominance this issue (Comhaire 2008).

Pathogenesis and Symptoms

Hip dysplasia is considered as a growth of a movable, ill-fitting coxa-femoral articulation. In most of the cases, joint laxity affects both limbs and infrequently one is included. The constancy of the hip joint is usually certain by the lax tissue that is the association between the coxa acetabulum and the femoral head, like thigh muscles, joint capsule, and the major attachment ligaments (Karsli et al. 2021). Furthermore, the steadying consequence for the hip joint is occurred by a synovial fluid and the joint capsule, producing a vacuum-like space (Sevil-Kilimci and Kara 2016).

Scanty constancy permits an extra or fewer acute displacement of the head of femur bone out of the acetabular cavity, mostly in the dorsolateral view, inducing a partial luxation of different degrees until the total dislocation is happened for the joint. Joint dislocation make the body incapable of bearing the physiological weight as well the movement function within the articulation and overloading other certain parts in the body (Nakahara et al. 2014).

This stimulates lesions and erosion of numerous functional anatomical structures, like the articulation cartilages, attachments ligaments, bones, and articulation capsule. As a result, infection and deterioration of these tissues consequence in the arthritis and osteoporosis, finally leading to chronic osteopathy. Thickness of joint capsule and the sever rupture of the round ligament are seen, in addition to the loosening of the natural color of articular cartilage specially in the over loaded areas which display caps, destruction, and eburnation of subchondral bone in sever conditions (Lievense et al. 2004).

All these signs cannot be noticed only by the anatomical dissection of the bone specimens but the x-ray of affected bone is also needed for revealing the partial dislocation of hip joint. The partial dislocation affects the marginal edges of the acetabular cavity particularly during the abnormal weight-bearing. All the fibrocartilages marginal edges became flattened and micro cracks can be observed in addition to forming of exostoses and osteophytes which is consider as the final frame of the coxa arthrosis. Leading to obvious loss of acetabulum depth, lately formation of fibrous tissue consider a main reason for making the joint cup very shallow (Lewis et al. 2013).

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The mushroom-shaped deformation of the femoral head is a clear signs of hip dysplasia and the head of the femur loses its spherical well-rounded surface. With aging, the abnormality of the hip joint could be without any signs appear and this malformation start as thickness of the femur neck due to the remodeling that occur and result in the adaptation of the acetabular cavity surface and the femoral head to improve articular contact within the joint (Gulanber et al. 2006).

One of the dominant sign is the acute pain that produced by the irritation of extra movement and exercises. Cold, wet environmental conditions may worsen the pain. Other possible symptoms of hip dysplasia in dogs are lameness during walking and trolling after the rest time (Anderson 2011).

Other than the stiffness of the limbs, the dog faces swinging of pelvis and disability to complete lifting of the hind limb from the ground. Animal also feel difficulties in climbing stairs, jumping over barriers and partial atrophy of thigh muscles, as well as the restriction in the range of motion (ROM) in the hip joint (Mabuchi et al. 2006) . Even still under anesthesia, the adduction, abduction and rotation movement is mostly painful and all these symptoms shown occasionally, but frequently turn out to be continuous at later stage of the case. The researchers proved that there is no linear relationship that links the three conditions i.e., degree of pain, movement troubles, and the degree of morphological variations in the joint and this is what clearly noticed in the chronic degenerative arthropathies (Todhunter et al. 1999).

Diagnosis

Mostly used technique to diagnose hip dysplasia in canine species is by X-ray examination. Nevertheless, further procedures for diagnosis such as stretching, flexion and extension of the limb are recommended to diagnose hip dysplasia and the degree of joint laxity, pain, twinge, and deficiency of joint laxity consider as first signs of joint dysplasia and obviously seen in new born puppy especially when the osseous structure is not adequately developed for radiography test. Lifting the femur bone in a dog can estimate the degree of dislocation of the femur head and also provide good knowledge about joint development (Cargill and Thorpe-Vargas 1995; Kohn 2007).

Various studies measured a total of 786, dogs' average age was 6-8 weeks in both sexes, via palpation procedure under anesthesia and re-examined again after 10 months by radiography. The findings referred that approximately in 90% of the dogs at 8 weeks of age, the kinetic ability for femur head cannot exceed further than 2 mm, and they will have mild or acute hip dysplasia at age of one year. Conversely, rather tight joints with a lifting possibility of 2 mm or less, showed dysplasia later in about 40% of dogs. It is also noticed that most results of hip dysplasia diagnosis in both young and adult dogs through the palpation technique have a high rate of error, hence it should be confirmed with the x-ray examination (Samuelson 1972 ; Evans 1989 ; Zhang et al. 2009).

The examination main problem is in the diagnosis of different degree of joint laxation particularly the minor degrees in the maturation stage which can't be examined in growing dogs, and become possible only when the skeletal system reaches the total growth phase, thus the first stages of joint luxation and subluxation is difficult to discover. Hence the x-ray is the better way for early diagnosis (Poy et al. 2000).

Then, the suitable size of the X-ray film used is 25 cm x 35 cm and should be a high-quality film with optimum density, contrast and acuity as well to pay attention to the condition of yielding the radiographic film through revealing and extending the legs, pelvis bones, and femurs, with the knee joint. The “frog-leg” like position is a radiographic position described as the pelvis should held in symmetrically projection (Fries and Remedios 1995).

The femurs should be stretched caudally and parallel that makes the knee to rotate in the medial direction, thus that the knee cap manifest centrally in the trochlear grooves. Highly quality X-ray films are of the greatest significance to evade false positive or false negative explanations. Therefore, correct standing position is essential for finding any small variation from the anatomical deviations considered within the normal limits for breed and age. The better positioning of animal makes a resting of skeletal muscles and helps the detection of joint laxity. It require anesthesia eithergeneral anesthesia or epidural anesthesia for the radiography of the pelvic joints (Peterson 1992).

In 1954 Schnelle announced about a classification scheme suggesting a new classification system which provide a variation of pathological results regarding the variety of hip dysplasia severity. The Orthopedic Foundation for Animals (OFA) in the United State of America published three significantly schemes giving the breed a number for evaluating the normal cases with following grades i.e., ‘Excellent’; ‘Good’ and ‘Fair’ for conformation of the pelvic joints (Todhunter et al. 1999; Fordyce 2002).

The grades ‘Borderline Conformation’; ‘Mild’; ‘Moderate’ and ‘Severe’ Hip Dysplasia are not recommended for breeding. The Scientific Committee of the Federation Cynologique International (FCI), in an effort to establish an International Certificate, compared the classification systems of their breeders’ organizations and proposed a standardized grading system (Brass and Paatsama 1983; Peterson 1992).

A scheme was explained in Britain just to restrict the hip dysplasia in German shepherd breed about ten years ago and particularly in 1983 this scheme extended to involve other types of dog breeds. The scheme is classified and evaluated nine different aspects of hip dysplasia numbered from “0” which means (normal) and “6” and that means (extremely abnormal). With aging, the osseous deformity occur as an expression for the hip dysplasia. The researchers (Freudiger et al. 1973; Bartolomé et al. 2015) confirmed that the age of one year consider as the typical age for radiological diagnosis in Germany and Britain. The Scientific Committee of the FCI recommended that the suitable age for the large breed dogs is about one and half year and further that their

taxonomy scheme valid to dogs between one and two years, may be adopted for older dogs but the secondary arthritis variation have to evaluated according to the age of dogs (Stock et al. 2011).

The age of 24 months has been established as the minimum age for evaluation the dogs by the Orthopedic Foundation for Animals. Some dogs look as semi-normal in the age of 6 to 18 months but later they will show other signs of hip dysplasia during the radiographic examination. These signs is consider as an appearance of minor arthritic modifications and it is easy to identify than insignificant grades of laxity, but degenerative arthropathy can sometimes have other ancestries (Bouw 1982).

The period between 2-6 years old is the best time for the final estimation announced by the Orthopedic Foundation for Animals (Morgan and Stephens 1985). Henrigson et al. (1966) found that variation due to age might invalidly be recognized to pelvic dysplasia after six years. In contrast, if the X-ray image is not considered as an acceptable standard , then it may reduce the diagnosis of few cases of moderate partial luxation in dogs one year old (Genevois et al. 2020).

The long period of waiting delays the vital decisions about breeding and selection of dogs for controlling of pelvic dysplasia, hence, the dogs should be marked by a clear sign on the pinna that might be a letters or numbers in addition to marking the radiographic film. (Ohlerth et al. 2019).

Genetic Evidence

Such as color and height and most traits that involved under the Mendelian laws of dominant and recessive genes. Hip dislocation progresses under a group of composite genes action and it is difficult to know their heredity transmission and understand it according to these laws. Presently it is hard to figure out how many genes that involved in hip dysplasia, nonetheless there are large number of genes (Zhang et al. 2009 ; Alsada et al. 2020).

Thus, pelvic dysplasia is considered as a quantitative character with constant diversity between the sound joint and the worst alteration, that of lasting dislocation. Hip dysplasia is confirmed by many statistical methods of population genetics as a polygenetic trait (Bartolomé et al. 2015). Polygenetic difference is specifically determined both by the addition or the combination of genetic factors. There are two heredity traits i.e., additive and non- additive heredity and the additive heredity traits turn out to be more obvious according to the number of current genes (King 2017).

Meanwhile, non-additive heredity doesn't depend on the number of genes very much but depend on group of gene combinations. The last revealed gene grouping cannot be affected by selection; only genes are inherited, not a set of them (Ohlerth et al. 2019).

The selection process will be successful only when the dissimilarity of the features rely on the additive action of genes. The genetic pattern for the parents can be proven only by statistical methods that might represent a sufficient number of descendants. The additive gene inheritance showed that the significant role that played, and that what obtained through the selection in contradiction of hip dysplasia (Stock et al. 2011; Gaspar et al. 2016).

The progeny can be enhanced if the canine breeds that have an average small figure of genes for positive traits are bred with alike small transporters of these genetic factors. After long period of selection in the large breed dogs such as German shepherd dogs, it is be expected that heritability process will be reduced by decreasing the additive gene variation (Guo et al. 2011).

In addition to that some impacts of non-additive gene combination also exists, revealing that the approximation of hip dysplasia heritability in German shepherd dogs fluctuated from 20-60% (Henrigson et al. 1966 ; Leighton et al. 1977 ; Hedhammar et al. 1979; Stock et al. 2011).

The variation be determined by the dog groups examined and the procedures that applied. It was proven that some large breed dogs which rarely suffered from hip dysplasia, may also have a small ratio of additive genetic variation and lesser heritability (Henricson and Olsson 1959; Hedhammar et al. 1979; Van Der Velden and Brooymans-Schallenberg 1983).

The basic reasons that have been suggested for the wide spreading of hip joint dysplasia could be chosen for other characteristics with potential heredity links to hip dysplasia. The extreme angulation for the hind limb, oblique croup, defective character, and oddity, have been conferred nonetheless need a confirmed research (Kaman and Gossling 1967; Hedhammar et al. 1979 ; Steiger 2007; Guo et al. 2011; Anderson 2011).

The characteristic traits of hip dysplasia which is considered as one of Quantitative hereditary characteristics, are affected by different grades through environmental factors (Gustafsson et al. 1975; Belfield 1976; Hedhammar et al. 1979). Example include obesity, extra protein and calcium intake and high energy diet, rapid growth rate and excessive exercising (Bouw 1982;Fries and Remedios 1995).

Many theories revealed that the environment and climate condition, style of life and diet might influence on the hip dysplasia disorder but in contrast other theories contested these factors but without genetic predisposition for some breeds of dogs, environmental stimuli alone will not form the hip dysplasia in these breeds (Hedhammar 2007; Peterson 2017).

The continuous selection process for different large breeds of dogs e.g. Boxer, Rottweiler, Hovawart, Golden Retriever, Dobermann, Newfoundland, Great Dane, leonberger, German wirehaired pointer and German shepherd dogs in Germany proven the success of individual genotyping however it was not determined easily (Adams et al. 2000; Alsada Alwaeily et al. 2020).

Although from some dog's breeds that appears to be free from the hip dysplasia, the polygenetic mode of inheritance makes it understandable. Within ten years the hip dysplasia in dogs that were free from this disorder, raised from 10% to 25% and continuously rising (Muller and Saar 1972; Van Der Velden and Brooymans-Schallenberg 1983; Lattimer 1995).

The results of strict selection led to the strains with an inferior rate of the dominance of hip dysplasia, which was mating only with dogs free of hip dysplasia showing normal and better shape. One of the most important breeds that were selected from among other different breeds that was evaluated where all of their radiographs for the hip dysplasia that was taken from strict program of the German Shepherd Dog Club (SV) since 1967 (Miqueleto et al. 2013).

It showed a largest number of radiographic images that examined are154, 774 until the end of 1987. In 1976, the dogs categorized as “normal”, “near normal” (marginal), “still accepted” (moderate-dysplasia) and this German Shepherd breed have been yearly evaluated regarding to this category (Runge et al. 2010).

The category that named “A” stamp, this German Shepherd breed classified as an official breed without restricted rules. Just dogs that suffer from extreme hip dysplasia were be disqualified from reproduction. Annually the percentage of mating is decreased for the puppies that suffer from mild hip dysplasia which is forbidden to any breed of dogs not carrying the "A" mark, and that make most of breeders to be interested to choose the individuals with normal or semi normal condition, according to the available rich genetic information. Add on to easily accession and know all the history of strains (Genevois et al. 2020).

In 1967 the scheme has been shown to progress and achieved the rate of dogs lacking symptoms of hip dislocation raised up to 20% over ten years. As well as a minor increase was noticed in the semi-normal type besides and a decrease in the simple and mild types. Some certain radiographs with the features of mild to moderate hip dysplasia, already identified by a specialist veterinarian, were not sent for authorized estimation and major documentation (Henrigson et al. 1966). An article shows that this ratio might be 15%. The point that the cases of mild hip dysplasia illness dropped meaningfully, display the bearing to a significant upgrading in pelvis joint modulation in the species. Hence, subsequently, it is not, complemented that the recurrence revealed is demonstrative of the whole German shepherd dog population in West Germany (Brass 1989; Miqueleto et al. 2013). Similar direction was noticed in German shepherd dogs in Paris, Finland and Switzerland all used the same agenda that recommend by the FCI (Brass 1989 ; Genevois et al. 2020).

To acquire the whole removal of pelvis dysplasia means an uphill struggle. Constant choosing of the perfect constitution makes the genetic will at a low level, postponing the realization of this goal. The suitable pelvic position of offspring copulation, parents, and ancestors is a hopeful onset and the descendants analysis is extremely suggested. Hedhammar et al. (1979) recommended the calculation of at least thirty dogs that were selected randomly from groups of the progenies of five to 10 litter.

However, Freudiger et al. (1973) measured the estimation of the pelvic joints of at least eighteen canine from three or more litters, for the testing of sires. Hip dysplasia can be transmitted from progenitor to the offspring depending on the male and female genetic map.

The progeny program taking a long period of time as well high budget for the selection of high-quality breed. In addition to this process consuming time exceeded of two diagnosis result, heritability and selection factors are related because phenotypes with higher heritability will cause faster change when the same selection pressure is placed. Hence, the males dogs (sires) that own high awards clearly have a higher impact on the breed more than the females (dam). With over half the population of domesticated dog breeds being affected by hip dysplasia, new methods for abating this disorder need to be done. Carbohydrate sulfotransferase 3, fibronectin 1, and fibrillin 2 are three very recently mutated genes that showed in a modern researches about pelvic dysplasia (Reagan 2017).

It will be very helpful if each owner support the breed control programs, because of the high percentage of wrong and negative data that obtained during the procedure of hip palpation in dogs suffer from mild or extreme hip joint dislocation, thus the researchers recommendation are to use a plans for a future genetic alignment which depend on major documentation of all observed individuals of a dog breed beside of using radiographic assessment to adequately evaluate hip condition and electronic data-processing may offer indispensable information (Ginja et al. 2010).

Conclusion

Hip dysplasia in canine species consider as a one of the most painful, polygenic and heritable disease. Its symptoms are clearly obvious and showed as an anomalies in hip joint due to wrong position of acetabular cavity and the head of femur bone. The period of this disease starts at the third week in predisposed dogs. Thus, in conclusion, by looking at the genetic components of hip dysplasia, most newly articles and researches proved that there are three main mutated genes responsible about the appearance of hip dysplasia. So the researchers can possibly discover some modern methods to fix these mutations that could occur. For the owners and breeders, DNA tests are obtainable to hypothetically recognize the mutated genes before breeding and the environmental factors also affect canine hip dysplasia, so dog owners need to have the proper knowledge about their canine(s) to ensure that they are feeding them a proper diet and giving them the appropriate amount of exercise.as well as using radiography method will improve and help the breeder during the pedigree selection. Estimation process for hip joints will be very beneficial method and economical for breeders and owners of dogs, in addition to provide a healthy life to the dog by reducing the stress of more severe and risky operations.

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My Talk with the Speechless

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INTRODUCTION

A common word used to describe the types of social attachments that frequently develop between humans and their pets is "human-animal bond". Many animals, for instance, lab rodents, working horses, dogs, and dairy goats are not usually recognized as pets because they are raised for a purpose i.e., the goats are used for meat and milk production. It is not comfortable for humans both morally and psychologically to avoid the beneficial aspect of these animals (Davis and Balfour 1992; Serpell 1996).

Human-pet bonds are quite common and favoured. Various estimates show that Americans own about 80 million cats and 75 million dogs as pets, in addition to millions of birds, reptiles, amphibians, and fish. According to a 2012 survey, approximately 63 percent of US families have at least one pet and 45 percent have more than one pets. The figures in the European Union are likewise showing about 60 million dogs and 80 million cats. According to the 2014 Euromonitor International, the number of pets in developing countries such as Brazil, Thailand, and Turkey are rapidly increasing. Although pet ownership is probably more popular today than it has ever been, this fascinating human behaviour is neither modern nor limited to more affluent, "westernized" societies (FEDIAF 2014).

Historical Perspective

In the excavation at a pre-Natufian cemetery in Jordan, almost 14 to 17 thousand years old pet fox were found buried with a human body. This archaeological proof provides information on emotional relations between humans and animals from a long time ago (Maher et al. 2011). This archaeological evidence is also found around the world, for example, a 12–14-thousand-year-old human and dog remains were found in Israel and Germany, and a fossil of a 9,500-year-old cat and human was discovered in the Mediterranean island of Cyprus (Davis and Valla 1978; Vigne et al. 2004; Morey 2006).

Cultural Perspective

Pet keeping among hunters and horticulturists is a routine matter rather than an exception, as per many explorers and anthropologists. Hunters typically capture these pets as young animals, which are then cared for by their entire families. Animals, especially companion ones, are the source of emotional support for their humans. The emotionally attached person to his pet gives all the facilities to the pet. The owner also gives a proper burial when the pets die. And this time is also very tough for the owner to get over it. There are also some social taboos against some animals kept as a pet. There are also some strict rules against the unethical killing of animals for the social well-being of man. The eating of animals that are selected as a pet is also prohibited even if their meat is normally consumed (Simoons and Baldwin 1982; Serpell 1989; Erikson 2000).

Animal domestication began because of the widespread practice of keeping pets in pre-agrarian communities, according to several writers (Galton 1883; Sauer 1952). Most forms of physical intimacy between humans and animals appear to have been morally dubious throughout the medieval and early modern periods, and the habit of keeping pets only as companions was also often prohibited (Salisbury 1994; Serpell 2005). In some situations, engaging in human-animal relationships could lead to charges of witchcraft (Serpell 2002).

Pet ownership was not a piece of cake. Before the modern period, only the elite and upper classes could afford the ownership, but after the modern period, the urban middle class of Western society have their pets which resulted in the spread of pets into various sectors of society. This shift in

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thoughts and conduct toward animals can be partially linked to the constant influx of Europeans and Americans into urban areas from remote regions at this time. There is no longer a need for value systems that categorize humans and non-humans into distinct moral spheres because of the urban migration that tends to exclude larger segments of the population from actual participation in the consumption of animals (Serpell and Paul 1994).

Pets are also used for many therapeutic purposes as they are a tool to test the efficiency of a drug. The York Retreat was the first metal institute in England that use animals as a tool in the eighteenth century. In the Victorian era, pets became a more pronounced subject to be used in British mental institutions. John Locke (1632-1704) was an English philosopher who use animals to develop a sense of empathy and responsibility in children by allotting a pet to everyone (Serpell 2011).

Moral Code of the Animal-Human Bond

The evolutionary history shows that the use of animals as pets and companion animals is not so common (Serpell and Paul 2011). A recent study shows the estimate of expenses to have a pet dog in America is almost $17,500 to $93,500 which costs daycare, medicine, and dog walkers. And to have a cat as a companion animal, the owner has to pay almost $17,000 for its care throughout its life. On average Americans spend $50 billion a year on the health and well-being of their dogs, yet it can be challenging to find or quantify any clear benefits (Forbes Magazine 2014).

Human-pet ties seem to be common, regardless of the fact that whether our attention is on hunters or on homeless persons leading hard lives on the streets (Rew 2000; Taylor et al. 2004). Darwin's theory explains natural selection based on the maintenance and spread of the behaviour of humans. This theory focuses on the survival of the fittest, hence the lack of utility which explains pet ownership is a big challenge to biologists and psychologists (Hamilton 1966; Williams 1966).

'Bond' or 'Bondage'

One of the major challenges faced by the concept of the animal-human bond is the living cost of the pet in the owner's pocket with no vivid benefits and some potential harm to the owner or his family members. This theory states that pets like dogs, cats, and many other companion animals are like social parasites which take too many advantages from the owner in the form of shelter, food, and medication (Fig. 1), but don't comply in returning the favour to that extent. Also cited as proof of selection for phenotypic qualities that improve these animals' capacity to elicit human parental responses are the tiny size, neotenic face features, and infantilized behaviour of many canine breeds (Archer 1997).

The social parasitism hypothesis, though challenging to disprove, assumes that pet owners must either be at a competitive disadvantage with non-owners or that the fitness costs of pet ownership are insignificant in comparison to the risks of being overly selective when it comes to potential parental care recipients. The relationship between two individuals in which both get the benefits is called mutualistic interaction between them i.e., the coral reef fish and tiny cleaner wrasse. The *Labroides dimidiatus*, a wrasse, has an association with larger fish where the wrasse gets food, and in return, they remove the dead tissue and the ectoparasites from the mouth and gills of the larger fishes. During this period, wrasse remains unharmed and performs their work without any kind of hindrance (Herre et al. 1999; Johnstone and Bshary 2002).

Fig. 1: Pet owner caring attitude

Merits of the Animal-Human Bond

A significant surge in scientific interest in the potential health advantages of the human-animal link occurred in the late 1970s, thanks to the findings of a Ph.D. dissertation from the University of Maryland (Friedmann et al. 1980). The risks of cardiovascular disease are much lower among pet owners compared to non-owners, according to other research that looked at risk variables for the disease in

sizable population samples, such as blood triglycerides and cholesterol (Allen et al. 1991; Anderson et al. 1996; Friedmann et al. 2000; Wells 2009).

The purchase of a new pet has been linked to increases in owners' mental and physical health as well as to sustained decreases in their propensity to overreact in stressful situations and stimuli (Allen et al. 2001; Serpell 1991). Additionally, pet owners seem to be more robust in the face of difficult life circumstances, which leads to fewer health issues and fewer trips to the clinic for treatment (Siegel 1990). Significantly, pet owners who are very devoted to their animals tend to gain more from pet ownership than those who are less attached, and dog owners generally fare better than cat owners, possibly because the bond between dogs and their owners is, on an average, greater (Fig. 2) (Friedmann and Thomas 1995; Ory and Goldberg 1983).

Fig. 2: Pet and owner loving bond.

In comparison to non-dog owners, dog owners have been found to engage in more walking and general physical activity and some studies have indicated a strong link between dog walking and lower body weight as well as lowered risks of diabetes, hypertension, hypercholesterolemia and depression (Cutt et al. 2007; Coleman et al. 2008; Hoerster et al. 2011; Lentino et al. 2012).

Companion animals are also a source of healthy interaction and help in improving social behaviour within a society. It has been examined through many research studies that people having pets are socially more popular in a community even old persons and individuals with any kind of physical disability (Mader et al. 1988; McNicholas and Collis 2000; Wells 2004; Guéguen and Ciccotti 2008).

Pet ownership is favourably correlated with feelings of neighbourhood friendliness and social interaction between neighbours, according to community-based surveys. After correcting for demographic variables, pet owners also frequently do better than non-owners on tests of "social capital" and civic participation (Wood et al. 2005).

The Therapeutic Perspective of 'The Bond'

The great advancement in the work related to the use of a pet dog as a therapeutic agent was done by an American child psychotherapist named Boris Levinson in the 1960s and 1970s. He used to bring his dog named Jingles during the session with patients as they feel more comfortable in the presence of his dog. He says that pets help to deal with many psychological issues and physical disabilities of children. He used to say pets as “co-therapists” while dealing with patients (Levinson 1969).

The first researchers to empirically examine Levinson's theories were a husband and wife team of psychiatrists at Ohio State University named Samuel and Elizabeth Corson. Within the psychiatric hospital where they worked, in the 1970s, they established what they dubbed a "pet-facilitated psychotherapy" (PFP) program and chose 47 withdrawn and uncommunicative patients, the majority of whom had not responded well to more traditional treatment approaches. The next step was to involve every patient in the daily upkeep and exercise of a colony of laboratory dogs that resided close to the hospital. Even though they only published information about five subjects—all of whom had significantly improved—the Corsons reported "some improvement" in all of the patients at the end of the trial (Corson and Corson 1980).

In the late 1970s and early 1980s, a surge of research in Europe and North America was spurred by the Corson study with the aim of identifying and evaluating the advantages of AAIs (Animal-assisted interventions) in a variety of patient populations and therapeutic contexts. Unfortunately, a lot of these early studies had a number of design issues. Only six controlled experimental trials of animals' therapeutic value were found in an extensive assessment of the literature on

AAIs conducted in 1984; all of these studies targeted adult or elderly individuals. The studies indicated either "no impact" or "very small treatment benefits," according to the authors' analysis (Beck and Katcher 1984).

Only nine research (six comprising control groups and three pre-/post-treatment designs) that supplied sufficient statistical data to allow the computation of effect sizes were found in a meta-analysis of 112 pertinent studies conducted 19 years later, in 2003. Following the initial 1984 evaluation, all nine studies were released, and they were all done on adult and/or senior populations. The meta-analysis discovered an average effect size of 0.76, which is widely regarded as large, in contrast to the earlier evaluation that these therapies had only marginal therapeutic efficacy (LaJoie 2003).

Investigations into the potential processes underpinning the positive benefits of AAIs are still ongoing, however, the social-bonding hormone oxytocin has been linked to the phenomenon. Our knowledge of these mechanisms, as well as the specific ways in which they affect various subject (patient) groups in various treatment contexts, will continue to be improved by future research (Kruger and Serpell 2006; Moberg et al. 2011).

Non-Human yet Humane companions

The idea that pets might act as forms of non-human social support is congruent with the apparent connections between pet ownership and human health (Collis and McNicholas 1998; Garrity and Stallones 1998; Ortega and Casal 2006). A theoretical concept known as social support measures how socially integrated people are and how closely they feel a responsibility and obligation to one another (Eriksen 1994; Schwarzer and Knoll 2007).

An increasing corpus of research has demonstrated a strong correlation between social support and improved human health and survival (House et al. 1988; Glaser and Newton 2001; Lim and Young 2006; Lunstad et al. 2010). For instance, it has been demonstrated that social support components can guard against depression, schizophrenia, and suicide as well as the cancers, rheumatoid arthritis, diabetes, nephritis, and pneumonia (Sherbourne et al. 1992; Esterling et al. 1994; Kikusui et al. 2006; Uchino 2006).

Once more, the neuropeptide hormones oxytocin and arginine-vasopressin, which are also essential in the regulation of attachment behaviour and social bonding in mammals, appear to be mediating some of these advantageous benefits of social support (Donaldson and Young 2008). Furthermore, the hypothalamic-pituitary-adrenal (HPA) axis, which controls the stress response, is downregulated by the release of oxytocin associated with enjoyable social interactions (Heinrichs et al. 2003).

The emotional attachments of humans and animals have also been described as this relationship results in a positive impact on society. Four major studies reveal that the hormonal status (oxytocin) of pet owners shows fluctuations when they are having quality time with their dogs (Odendaal and Meintjes 2003; Miller et al. 2009; Handlin et al. 2011; Handlin et al. 2012).

Another study found that owners of dogs who received more visual attention (gaze) from their dogs during an experimental trial had considerably higher levels of oxytocin metabolites in their urine. These owners also admitted to having greater bonds with their more attentive dogs when questioned (Nagasawa et al. 2009).

The study of human behaviour shows that the isolation of an individual has a negative impact on society. According to the natural selection theory, primates usually lived in groups and form a community where they support each other. An isolated person living in such a supportive community receives a welcome from the people whenever they decided to go out (Silk et al. 2009; Silk et al. 2010).

Ethical Perspective of the Animal-Human Bond

The extremely huge number of animals that now live alongside people can have a damaging effect on the ecosystem. There are many obvious instances, such as the depletion of wildlife resources for the exotic pet trade, the effect of stray cats on wild bird species, and the contamination of parks and natural places with animal excrement (Coppinger and Coppinger 2001; Rosen and Smith 2010; Loss et al. 2012; Bush et al. 2014). Even meeting the nutritional needs of dogs can have a huge negative impact on the environment. A medium-sized family dog consumes about 360 pounds of meat and 210 pounds of cereal per year, according to one calculation. However, another estimate contends that America's 75 million domestic dogs may consume as many calories as about 35 million people. This much food would take the equivalent of about 20,000 square kilometers of agriculture to produce (Vale and Vale 2009).

While it is undeniable that species like dogs and cats have increased in number because of living alongside people, many individual animals pay a high price in terms of deteriorated health and welfare. Each year, millions of pets are abandoned, given to shelters, or put to death too soon as a result of broken human-animal ties. Thousands more are abused, neglected, or mistreated by their owners for a variety of reasons, from ignorance to wilful cruelty (Clancy and Rowan 2003; Arluke 2006).

Due to inbreeding, line breeding, or selection for extremely high physical conformation requirements, several purebred dog breeds suffer from painful and crippling health issues (Asher et al. 2009; Summers et al. 2010). The demand for some pets is outpacing the supply, which has led to an increase in commercial pet "farming," while the trade in exotic pets kills and causes great suffering to wild animals during their capture, transport, and subsequent acquisition by owners who are unaware of proper husbandry and care (McClennan 2012). Even the strongest and most loving of human-animal relationships can result in needless suffering

for animals, as when an overly attached owner insists on pointless veterinary procedures to prolong the life of a terminally sick pet at all costs (Beck and Katcher 1996). When comparing the perceived costs and benefits of our relationships with companion animals, all these negative features of the human-animal link create significant ethical issues (Beck and Katcher 1996).

Conclusion

Animal-Human bond has been maintained since the beginning of the dawn either through the food chain or by the touch of companion animals. Pet lovers across the world spend billions of dollars yearly on these creatures' called pets. All sorts of animals are kept as pets these days, no matter what species they belong to. In the modernized era, almost every household in western countries owns at least one pet dog or cat. Excavations at different regions across the globe provided us with evidence of people keeping pets centuries ago. The cultural perspective can be taken from the fact that it's prohibited to consume a pet even if it belongs to an edible meat-holding species. No matter how much the expense, pet lovers bear it happily and bring forth their love to their companion animals by providing them with the basic needs of life. There's a rise in businesses comprising pet toys, pet foods, and medicines used for their treatment as well. Companion animals bring joy to the colourless life of many people who suffer from social anxiety. Therapeutic experiments have shown the tremendous importance of keeping pets close to depressed patients as they love their owners and give them a reason to live life happily.

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Botanical Control of Parasites in Veterinary Medicine

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INTRODUCTION

Phytotherapy may be defined as the use of plants for the treatment of ailments and those represent a practice that dates since ancient times (Borges and Borges 2016). It refers to the use of whole plants, their parts such as flowers, leaves, roots and seeds as well as substances extracted from them (plant extracts and essential oils) for treating various diseases (Stanković et al. 2020). It also may imply their use to support traditional treatment with commercial drugs (Russo et al. 2009). Plants and their extracts are an important part of pharmacopoeia in less developed parts of the world, but more recently in the advancement societies (Russo et al. 2009; Borges and Borges 2016). However, plant-based products may also be used for the treatment of diseases in animals (Ul Abidin et al. 2021), prevalently in livestock (Calzetta et al. 2020). Ethnopharmacology may be implied in veterinary medicine due to the potential therapeutic efficacy, reduced susceptibility to microbial and parasitic resistance, as well as lowered risk of adverse effects and decreased residues in animal products and environment in comparison with chemotherapeutic agents (Calzetta et al. 2020). Moreover, botanical control of various diseases in animals can also be sustainable from the financial point of view (Prakash et al. 2021).

Therefore, medicinal plants are a valuable part of the field of drug discovery and represent an important source of new drugs and drug leads (Liu et al. 2020). In this regard, antiparasitic properties are a common point of focus in studies aimed to validate the pharmacological effects of herbal products. A huge number of such plants and their products are considered suitable for the treatment of almost every parasitic disease in livestock (Athanasiadou et al. 2007). In pets, there are also an increasing number of such studies in dogs and cats, whereby plants product were proven to be effective against various parasites (Štrbac et al., 2021a).

Resistance in Parasites as the Main Problem and Novel Strategies

The resistance in different parasitic species nowadays represents a worldwide problem due to decreasing efficacy of commercial drugs and consequent economic losses. Antiparasitic resistance (AR) may be defined as the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage (Geary et al. 2012). Although it is considered that AR is a natural and heritable process which will develop anyway for a certain time, the role of humans in its occurrence refers to the rate and speed of its development (Shalaby 2013). The main factors that may promote AR development are treatment frequency (especially overfrequent treatments), miss-dosing (especially underdosing), prophylactic mass treatments, continuous use of a single drug without combination or rotation and poor pasture management in the case of livestock (Shalaby 2013; Mphahehlele et al. 2019).

In the case of protozoan parasites, it was reported that the effectiveness of antiprotozoal drugs is being decreased (Capela et al. 2019). The especial problem represents protozoan infections for which usually very few treatment options exist such as trypanosomiasis (including durina), babesiosis, theileriosis and leishmaniosis, whereby continuous use of these drugs predictably leads to drug resistance (de Koning 2017). In terms of liver flukes, the main concern is with triclabendazole whereby its success in the treatment of these infections has led to over-reliance on this drug and the emergence of resistance, although

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resistance in *Fasciola hepatica* to albendazole is also reported (Fiarweather et al. 2020). The problem of AR of cestodes such as *Echinoccocus* (*E.*) *granulosus* to synthetic drugs is also present (Pensel et al. 2014). However, it is said that the problem of AR is far more severe in small ruminants, which requests dramatic changes in approaches to nematode control for decades (Kaplan 2004). In the case of gastrointestinal nematodes such as *Haemonchus* (*H.*) *contortus*, single and multi-resistant strains of various species to all groups of anthelmintic drugs that are used in practice (benzimidazoles, macrocyclic lactones, imidazothiazoles and even to newly developed drugs such as monepantel) are already reported, whereby the estimated time of the development of AR to a new drug is now less than 10 years after introduction to the market (Fissiha and Kinde 2021). The annual cost of anthelmintic resistance only in Europe is recently estimated at €38 million with a tendency to increase in the future, which in turn endangers the sustainability of livestock (Vinner et al. 2020) and world's food supply. In the end, AR is already present in many ectoparasites, whereby a range of pesticide drugs such as organophosphates, organochlorides and synthetic pyrethroids are no longer that effective due to their intensive use, which makes the control of ectoparasites very difficult (McNair 2015).

Not only from the aspect of resistance, but the use of only commercial antiparasitics is no longer sustainable because of the price of these drugs that continues to rise (Prakash et al. 2021). For example, the mean wholesale price of multiantiparasitic drugs albendazole and mebendazole increased between 2010. and 2019. from $3.16 to $582 and from $32 to $2853, respectively (Junsoo Lee et al. 2021). Finally, adverse effects on the host animals, residues in different animal products such as meat and milk as well as in the ecosystem and biodiversity represent a serious problem with many chemotherapeutic drugs which are currently available (Veerakumari 2015). All of this requires searching for novel strategies for the control of parasites in veterinary medicine, which according to Hoste et al. (2014) should be based on (i) developing new concepts of the use of chemical antiparasitic drugs (eg. target selective treatments); (ii) rational management of pastures; (iii) stimulating the host immune response (eg. development of vaccines) and (iv) investigating the efficacy of new drugs (phytotherapy, homeopathy and nutraceuticals). Some other alternatives are also suggested such as genetic selection of naturally resistant animals to parasites, biological control (the use of fungi, bacteria and even other parasites) (Maqbool et al. 2017), and in the case of ectoparasites, insect growth regulators (McNair 2015).

Plant Formulations and Advantages of their use

Plant products that are exhibiting pharmacological properties are often called plant secondary metabolites (PSM) and they make plants competitive in their own environment (Teoh et al. 2015). One of the most commonly examined plant products against parasites of veterinary importance are essential oils (EOs). They may be defined as aromatic, concentrated and complex mixtures of volatile nonpolar compounds extracted from plant material (Nehme et al. 2021; Štrbac et al. 2021b; Štrbac et al. 2022a) and represent a part of a plant immune system (Butnariu and Sarac 2018). EOs possess a wide number, varying from 20-80, of bioactive compounds that made up their composition, Ellse and Wall 2014). These compounds are belonging to various chemical groups including terpenes, terpenoids and phenylpropanoid derivates (Morsy 2017). However, the efficacy of EOs is often attributed to their major component(s), although the presence of other compounds may be important for synergistic effects (Ellse and Wall 2014). The other form of herbal products is plant extracts that are also complex mixtures containing a wide variety of secondary metabolites in different concentration ranges (Borges and Borges 2016), whereby the main difference between them and EOs is by obtaining process. Namely, while EOs are usually obtained for various commercial uses by various forms of distillation (mostly steam), plant extracts are mostly obtained by various forms of solvent extraction such as maceration and enfleurage (George et al. 2014). The most commonly examined types of plant extracts are aqueous and alcoholic (ethanolic and methanolic). Both EOs and extracts may be obtained from different parts of the plants such as leaves, flowers, seeds, wood and bark and even roots (Butnariu and Sarac 2018). Anyhow, plant extracts and EOs are extensively used in the control of parasitic, as well as bacterial and fungal diseases in animals (Abbas et al. 2018).

Interestingly, the principle of self-medication in animals is also well-known, whereby the infected grazing animals with various endoparasites on the field are searching for plants with higher amounts of bioactive compounds (Torres-Acosta et al. 2012), suggesting that the whole plants may also be used. In most cases, plant products with proven effects against endoparasites were applied perorally, for example, in animal feed or as a supplement, although there are some notes that rectal or injectional applications may also be used (Katiki et al. 2019). Oral application may be single, or consecutively during a couple of days, whereby single or a combination of plant products may be used. In the case of ectoparasites, usually different formulations of a few products or their bioactive ingredients are examined, which were applied topically or orally. In those cases, plant-based products especially EOs may also be used as repellents to protect animals against various ticks or insects such as flies (Lachance and Grange 2014).

Plant-based drugs have already shown the effect against various parasites (and their different life stages) of veterinary and medical importance (Bauri et al. 2015). Their pharmacological effects including antiparasitic derive from numerous types of bioactive compounds belonging to various chemical groups with a possible different

mechanism of action (Butnariu and Sarac 2018), as noted earlier, whereby some of them exhibit strong activity against various parasites. The presence of various compounds and their synergism is often associated with lower susceptibility to the development of resistance in comparison with synthetic drugs (Bauri et al. 2015; Borges and Borges 2016), consisting mainly of one active substance. Also, it is important to note that in the cases where the efficacy of plants is not enough to control parasites alone, they can be used along with other alternatives and rationally used commercial drugs in a form of integrated control. Next, plant formulations offer a possibility to reduce a problem with side effects and residual amounts in animal products, given to their natural origin which is often associated with lower toxicity to host animals and their raise free from chemical inputs. Environmental aspects including soil properties also favor plant drugs due to their biodegradability (Veerakumari 2015). In the end, due to mentioned financial aspect of the use of only commercial drugs and their increased price, the incorporation of botanical drugs and formulations into veterinary medicine is justified since these are much cheaper (Ul Abidin et al. 2021).

Studies that Examined the Antiparasitic Efficacy of Plant Products

Due to the urgent need for new drug sources against parasites in animals, the number of such studies is increasing in the last two decades, especially in recent times. Scientific validation of the sustainable use of plant products is needed prior to their approval for parasites control. Within that perspective, the potent efficacy and selectivity of many new plant products against various groups of parasites (protozoa, helminths and arthropods) have been revealed (Abo-El-Saboud et al. 2018; Calzetta et al. 2020). Some examples are given bellow.

Protozoa - *Babesia spp.*, a tick born protozoan parasites, are one of the major pathogens that infect erythrocytes in a wide range of animals and may cause several clinical signs. Nerolidol, a sesquiterpene compound present in EOs of many plants and approved by the U.S. FDA as a food flavoring agent, caused the significant *in vitro* growth inhibition of four *Babesia* species with IC_{50} values of 21, 23.1, 26.9 and 29.6 µM for *Babesia* (*B.*). *bovis*, *B. caballi*, *B. ovis* and *B. bigemina*, respectively at growth inhibition assay (Aboulaila et al. 2010). In a study of Aboulaila et al. (2018), *in vitro* inhibition assay of several plant-based decoctions including green tea, hibiscus, cinnamon and peppermint against *Babesia* and *Theileria* species was examined. The most successfully were green tea and cinnamon with IC_{50} values of 3.83, 6.25, 2,2 and 5,3% (v/v) as well as 7.83, 19, 5.9, 12.1, and 6% (v/v) against *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *T. equi*, respectively. In a study of Guz et al. (2020), EOs of *Achillea millefolium*, *Eugenia caryophyllus* and *Citrus grandis* were the most active against *B. canis* on anti-babesial assay with IC_{50} values of 51.0, 60.3 and 61.3 µg/mL, respectively.

Several trypanosomes may cause diseases that affect humans or animals, mostly in the region of Africa. EOs of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Eucalyptus camaldulensis*, and *Citrus sinensis* were found to possess *in vitro* dose-dependent activity against *Trypanosoma* (*T.*) *brucei brucei* and *T. evansi*, whereby all oils decreased the number of parasites over time at doses of 0.4, 0.2 and 0.1 g/mL (Habila et al. 2010). On the other hand, crude extract of *Cymbopogon citratus* leafs and *Lepidium sativum* seeds, administrated to mice at different doses ranged 100-400 mg/kG, significantly reduced the parasite load of *T. congolense*, but at the same time decreased lymphocytosis and increased neutrophil counts and, in the case of *Cymbopogon citratus*, significantly improved bodyweight of tested animals (Emiru et al. 2021). In a study of Azeredo et al. (2014), EOs of several plants were *in vitro* evaluated for inhibition activity against *T. cruzi*, the causative agent of Chagas disease, whereby *Cinnamomum verum* was the most effective against epimastigote form of parasites (IC_{50}/24h was 24.13 µg/mL).

Plant-based formulations, given in the food or water to the poultry, are examined in several studies for their effects against *Eimeria* spp., and are often considered very promising anticoccidial agents. Thus, broiler chicks supplemented with a premix (1 g/kG feed) containing the oregano (50 g/kG premix) and garlic (5 g/kg premix) EOs had improved final body weight, feed conversion ratio and reduced faecal oocyst excretion of *Eimeria tenella* (Sidiropoulou et al. 2020). Also, extract of several plants given to broilers in diets contained 30 mg/kG of extract, especially *Nectaroscordum tripedale*, were effective in the control of the same agent by reducing oocyst count per gram of faeces and improving previously mentioned parameters of performance in tested animals (Habibi et al. 2016). Natural formulations based on encapsulated thymol and carvacrol (active compounds of some EOs) at doses of 60 and 120 mg/kG given to the broilers in a corn or soybean meal-based diet have led to the reduction of side effects in broilers vaccinated (in doses 25 times higher than recommended) against coccidiosis (Lee et al. 2020).

Giardia (*G.*) *duodenalis* is the most prevalent flagellate protozoan infecting humans worldwide, but also dogs. On the other hand, other animals may act as reservoirs for this parasite and be related to zoonotic transmission. EO of *Citrus* × *aurantifolia* exhibited *in vitro* activity against *G. duodenalis* at antigiardial assay with an IC_{50} value of 6.96 µg/mL, whereby for example EOs from other plants were less or not effective in the same study (Popruk et al. 2017). The study of Moon et al. (2006) demonstrated that low concentrations (<1%) of EOs of *Lavandula angustifolia* and *L. x intermedia* during *in vitro* trial may completely eliminate *G. duodenalis*, *Trichomonas* (*T.*) *vaginalis* and *Hexamita* (*H.*) *inflate*. Hydroalcoholic extract of *Tanacetum vulgare*, applied *in vivo* to mice at a dose of 0.2 mL per day, significantly

reduced giardia trophozoites count in the small intestine of animals 5 days after treatment (Muresan et al. 2021).

Trematodes and cestodes - Fasciolosis caused by *Fasciola* (*F.*) *hepatica* is considered as the most important hepatic disease in veterinary medicine that causes major economic losses in the livestock industry. *In vitro* anthelmintic effect of fifteen tropical plant extracts on the activity of excysted flukes of *F. hepatica* was evaluated by Alvarez-Mercado et al. (2015), whereby the most potent was *Artemisia Mexicana* with an IC_{50} value of 92.85 mg/L, and which along with *Bocconia frutescens*, had a 100% efficacy at the lowest dose tested. Abbas et al (2020) evaluated the *in vivo* anthelmintic effect of the herbal mixture that includes 17 plants against *F. hepatica* in goats, which were administrated at dose rates of 1400, 1200 and 1000 mg/kg at an interval of 7 days for four weeks, whereby it reduced the number of eggs per gram in faeces for 25-52.94% and 29.55-82.35% depending on the dose, on Day 15 and 30, respectively.

The other herbs that showed promising effects against *F. hepatica* and *F. gigantica* were *Allium sativum*, *Lawsonia inermis*, *Opuntia ficus*, *Lantana camara*, *Bocconia frutescens*, *Piper auritum*, *Artemisia mexicana* and *Cajanus cajan*. The effect of these plants was on the inhibition of adult fluke motility as well as the induction of the rupturing of internal organs such as the uterus and caeca (Nwofor et al. 2019). The activity of six natural compounds (quercetin, silymarin, naringenin, flavone, resveratrol and betamide) were evaluated against *Opistorchis* (*O.*) *felineus* by using motility and mortality assays, whereby the most effective substance on the motility of adult flukes was quercetin with an IC_{50} value of 5.1 µM. On the other hand, a concentration of 10 µM flavone led to a mortality of 22-35% by day 15, which was significantly higher than that of untreated worms (Mordvinov et al. 2021).

Echinococcosis represents a tapeworm cosmopolitan zoonotic disease where dogs are definitive hosts of the parasite (adult worms), while livestock and humans are intermediate hosts (larval, cystic form). The *in vitro* effect of *Thymus vulgaris* and *Origanum vulgare* EOs against *Echinococcus* (*E.*) *granulosus* protoscoleces and cysts was evaluated by Pensel et al. (2014). The effect was based on the loss of protoscolex viability and loss of cyst mass, which was also confirmed at the ultrastructural level. Interestingly, isolated thymol, the main compound of *T. vulgaris* EO, had a considerably greater effect than that observed with EOs, which was explained by the antagonistic effect between components of EOs. In a similar study, thymol at a concentration of 5 µg/mL, as well as EOs of *Rosmarinus officinalis*, *Mentha piperita* and *Mentha pulegium* at 10 µg/mL showed *in vitro* effect on the proliferation of *E. granulosus* larval cells with a reduction of protoscolex viability as follows: *M. pulegium* 82%, *M. piperita* 77%, *R. officinalis* 71% and thymol 63%. (Albani et al. 2014). The *in vitro* effect of thymol was also demonstrated against *Mesocestoides* (*M.*) *corti* adult worms as well as on tetrathyridia, for which mainly changes were observed in its morphology (lower concentrations) and surface alterations and damage (higher concentrations) (Maggiore and Elissondo, 2014).

Nematodes - The widest number of research aimed to evaluate the anthelmintic effect of plants was conducted against gastrointestinal nematodes in ruminants, especially sheep, which is understandable due to the emergence of resistance of these parasites. In most cases, the effect was proved against blood-sucking nematode *Haemonchus* (*H.*) *contortus*, whereby different *in vitro* (egg hatch test, larval development test, larval and adult motility tests etc.) as well as *in vivo* tests (faecal egg count reduction test, the controlled efficacy test) were used. The EOs so far proven for efficacy against GINs in sheep were listed by André et al. (2018) and Štrbac et al. (2022b), whereby the effect of EOs such as *Origanum vulgare*, *Thymus vulgars*, *Coriander sativum*, *Lavandula officinalis*, *Citrus sinensis*, *Cinnamomum verum*, different *Mentha* spp., *Cymbopogon* spp., *Eucalyptus* spp. as well as their isolated bioactive compounds such as carvacrol, thymol, anethole, cinnamaldehyde, eugenol, carvone, eucalyptol should be emphasized. These compounds may affect the nematode reproductive system causing their lower fertility or may induce different neurological and structural changes leading to nematode paralysis and death (Štrbac et al. 2022b).

In some cases such as in the study of Katiki et al. (2019), toxicity studies were also performed, where the safety of the application of EO formulations was proved, at least from the aspect of physical examination, blood count and the function of liver and kidney. The main problem with most of these studies was the *in vivo* efficacy of these formulations, which was usually lower in comparison with their *in vitro* activity, and the efficacy of commercial drugs as well, due to anatomical-physiological specificities of the ruminant gastrointestinal tract and the instability nature of EOs on the other hand. However, this problem may be overcome by the use of the encapsulation technique in the preparation of these formulations or with a possible different way of use instead of peroral administration (Štrbac et al. 2022b; Štrbac et al., 2022c).

In the case of extracts, the list is also wide and in most of these studies a wider number of plants or the mix of different extracts was examined, whereby their effect was usually attributed to larval inhibition and increased adult mortality of sheep GINs (Jayanegara et al. 2022). On the other hand, the *in vivo* effect of herbal-based dewormer containing 17 plants was also demonstrated against *H. contortus* in goats, with a total reduction of EPGs in animal faeces from 33.33-61.76% and 40-91.18% on days 15 and 30, respectively, depending on the dose used. An increase in erythrocyte count, packed cell volume and haemoglobin concentration was also recorded, suggesting the role of examined herbal dewormer in reducing the signs of anaemia caused by blood-sucking *H. contortus* and *F. hepatica* as well (Abbas et al. 2020). In the end, several EOs were tested

for activity against the mix of different GINs isolated from faecal samples of cattle, whereby *Cymbopogon citratus* had the lowest larval and migration inhibition concentration (IC_{50}) values of 3.89 and 7.19 mg/ml, respectively (Saha and Lachance 2019).

Ascaris (*A.*) *suum* represents one of the most prevalent nematode parasites in pigs that also causes significant economic losses. A wide range of condensed tannins from diverse plant sources showed effect against this nematode, related to the reduced migratory ability of newly hatched L_3, as well as the reduced motility and survival of L_4 recovered from pigs. On an ultrastructure level, it was shown that tannins cause significant damage to the cuticle and digestive apparatus of the larvae (Wiliams et al. 2014). Microencapsulated, plant-based mixed functional food composed of several compounds isolated from EOs, given perorally to the pigs daily in a dose of 1.0 mg/kg after fourteen days significantly reduced worm counts (76.8%), female worm counts (75.5%), FEC (68.6%), and worm volume (62.9%) (Kaplan et al. 2014). In a study of Rakhshandehroo et al. (2017), methanolic extracts of *Artemisia dracunculus* and *Mentha pulegium* at all tested concentrations (50, 75, 100 and 125 mg/mL) had significant lethal effects on larvae of *Parascaris (P.) equorum*, which is common causative agent of disease in equids, especially young horses.

Botanical anthelmintics have also shown an effect against various nematodes in dogs and cats. In a study of Sinott et al. (2019), a concentration of 0.6 mg/ml of EO of Brazilian red propolis (the source of plant *Dalbergia ecastophyllum*) showed 100% larvicidal activity against *Toxocara* (*T.*) *cati* after exposure for 48 h, while 300 µg/mL represented the IC_{50}. *Ancylostoma* (*A.*) *caninum*, one of the most important hookworms in dogs, is the most tested parasites in such studies, with 12 plants showed *in vitro* anthelmintic effect on eggs, larvae and adult worms, and 6 plants showed *in vivo* efficacy (Ekawardhani et al. 2021). For example, 500 mg/ml of extract of *Euphorbia hirta* obtained from the leaf of the plant, given three days in a row in 2 stages per 2 weeks intramuscularly and perorally to dogs, reduced 100% FEC at the second stage (Adedapo et al. 2005). In another study, the combination of plant extract obtained from the seed of *Citrus aurantiifolia* (40 mg/kg) given with mebendazole (50 mg/kg) per day for two weeks to dogs also reduced 100% FEC at the end of the experiment (Hassanain et al. 2015).

Plants were effective not only against gastrointestinal nematodes, but also against heartworms (*Dirofillaria* (*D.*) *immitis*) and lungworms (*Dictyocaulus* (*D.*) *viviparous*). Thus, several plant extracts showed microfilaricidal effects against *D. immitis* in a study of Merawin et al. (2010), whereby *Zingiber officinale* exhibited the strongest activity given that its concentrations of 100 µg/ml µg/ml, 10 µg/ml and 1 µg/ml effectively reduce the relative movability to 93.72, 88.12 and 87.95%, respectively after 24 h. Using the larval migration inhibition assay, the effect of condensed tannins, as well as an extract containing crude sesquiterpene lactones, from *Cichorium intybus* on the motility of L_1 and L_3 larvae of *D. viviparous* was demonstrated (Molan et al. 2003).

Ectoparasites - Plant extracts and especially EOs are increasingly used in the controlling of diseases caused by ectoparasites in animals. Their effect is often related to a harmmful effect on the nervous system of ectoparasites, which may be due to the inhibition of releasing of acetylcholinesterase important for their activity and synaptic transmission, or due to the act on Octopamine whose disruption results in complete breakdown of the nervous system (Abbas et al. 2018). Botanical antiectoparasitic agents may be used for acaricidal and insecticidal purposes or for repelling them (Adenubi et al. 2018).

Among ectoparasites, tickborne infections are considered the most devastating due to causing major economic losses and their role in transmission of many serious pathogens (protozoa and bacteria). Thus, EO of *Tagetes minuta* showed dose-dependent efficacy against four species of ticks (*Rhipicephalus* (*Boophilus*) *microplus*, *Rhipicephalus* (*R.*) *sanguineus*, *Amblyomma cajennense* and *Argas miniatus*) on an adult immersion test (AIT) and the larval packet test (LPT), with a more than 95% efficacy at the concentration of 20% (Garcia et al. 2012). The same EO used at the same concentration also promoted the significant effects on all biological indicators analyzed for *R. microplus* (number of ticks, the average weight of the ticks, the average egg weight per engorged female and larval viability), since it showed 99.98% efficacy compared to the control group (Andreotti et al. 2013). EO of *Ocimum gratissimum* exhibited great larvicidal activity against different ticks (*R. microplus*, *Amblyomma sculptum* and *R. sanguineus*) with a IC_{50} values of 2.0 mg/mL, 5.5 mg/mL and 6.0 mg/mL, respectively (Ferreira et al. 2019).

The EOs of *Rosmarinus officinalis*, *Mentha spicata* and *Origanum majorana* showed strong repellency of 100, 93.2 and 84.3%, respectively against the tick *Ixodes* (*I.*) *ricinius* nymphs (El-Seedi et al. 2012). On the other hand, EOs of *Syzygium aromaticum*, *Thymus serpyllum* and *Thymus vulgaris* were the most effective in a study of Štefanidesová et al. (2017), since they repelled 83, 82 and 68% of tick *Dermacentor* (*D.*) *reticulatus*, respectively, at a concentration of 3%. However, the mixture of *Thymus serpyllum* (1.5%) and *Cymbopogon winterianus* (1.5%), showed higher repellency (91%) than individual oils. An orally applied formulation consisting of garlic oil (2.5%), allicin (0.05%) and rapeseed oil (8%) to the dogs infested by various ticks (*Ixodes* spp. and *R. sanguineus*) at the dose of 0.25 ml/kg for 3 successive days has led to the decrease in a tick number for 100% starting from 12 hours from the 3rd dose up to 28 days. Moreover, treatment with this mixture improved the health condition of tested animals since all haematological and biochemical parameters returned to normal values after the treatment (Amer and Amer 2020).

Infestation with *Dermanyssus* (*D.*) *gallinae*, a blood-feeding mite, represents a major problem in the poultry industry in recent years. EO of *Coriander sativum* at a concentration of 0.4 mg/cm^2 as well as *Ocimum basilicum*, *Mentha x piperita* and *Satureja hortensis* at concentration of 0.6 mg/cm^2 have led to >90% mortality of the mites after 24h of exposure using the *in vitro* direct contact method (Magdaş et al. 2010). The impact of the spraying of surfaces of hennery with the garlic extract on the number of *D. gallinae* was evaluated by Gorji et al. (2014), whereby two successive sprays 8 days apart reduced their number by 96%. In a study of Andriantsoanirina et al. (2022) several tens of EOs were *in vitro* evaluated against *Sarcoptes* (*S.*) *scabiei*, causative agent of sarcoptic mange in animals, whereby *Cinnamomum zeylanicum* and *Ocimum sanctum* oils were the most active in contact and fumigation bioassays, as well as in ovicidal activity. In this study, all mites were killed within one hour with these oils diluted at 1%. The ethanolic extract of *Ligularia virgaurea* at a concentration of 2 g/ml also exhibited strong acaricidal activity against *S. scabiei* since it killed all mites within 2 h (Luo et al. 2015).

Rabbits infested with *Psoroptes* (*P.*) *cuniculi* were topically treated two times at seven days interval with two ml of the EO of *Cinnamomum zeylanicun* leaves, whereby concentrations between 0.16 and 10% were effective as a drug and cured all animals (Fichi et al. 2007). The *in vivo* effect of EOs of *Allium sativum*, *Origanum majorana* and ozonated olive oil against the important ear ectoparasite *Otodectes* (*O.*) *cynotis* in cats was evaluated by Yipel et al. (2016), whereby practically all oils led to the elimination of parasites 30 days after treatment. The best results were shown by garlic EO along with permethrin 10 days after treatment. Several plant EOs were tested against *Demodex* (*D.*) *canis*, a dog mite with zoonotic potential, whereby *Melaleuca alternifolia* oil showed a faster and stronger effect compared to amitraz since it required less time to eliminate the parasites (8.100-100.67 minutes in comparison with 333.33 minutes) (Neves et al. 2020).

Finally, aqueous extract of *Azadirachta indica* was tested against sheep bot fly larvae (*Oestrus* (*O.*) *ovis*), whereby at different concentrations showed a significant, dose-dependent effect on time to L_1 mortality in an *in vitro* test, and interfered with larval development in an *in vivo* test (Cepeda-Palacios et al. 2014). Neem extract (A. indica) is also known for its wideuse (Ascher et al., 2000). *Lavandula officinalis* EO and camphor at 32% concentration were found to have a larvicidal effect against sheep blowfly, *Lucilia* (*L.*) *serrata*, since they cased the mortality of larva by 100 and 93.33%, respectively (Shalaby et al. 2016). EOs and extracts have also showed efficacy against many other ectoparasites including *Ctenocephalides* (*C.*) *felis* (cat flea), *Bovicola* (*B.*) *ocellatus* (chewing louse), *Haematopinus* (*H.*) *tuberculatus* and *Hippobosca* (*H.*) *equina* etc. (Abbas et al. 2018).

Conclusion

Antiparasitic resistance represents an urgent problem in veterinary medicine due to economic losses. In addition to the problem of residues in animal products and the environment, as well as the problem of rising drug prices, this interferes with the use of commercial chemotherapeutic agents. As a source of a wide number of bioactive compounds of natural origin, herbal medicines are marked as a promising alternative. The effect of plant products shown against various parasites may be utilized to reduce the use of commercial drugs, which may lead to slowing down the spread of resistance and solving other mentioned problems. Therefore, along with other alternatives and strategies for rational use of drugs, botanical anthelmintics offers a possibility for sustainable, integrated control of parasites of veterinary importance in future treatment approaches.

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Ethno-medicinal Approach to Cure Animal Diseases

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INTRODUCTION

People have used traditional medicines, primarily those with a herbal base to treat illnesses. Finding natural remedies for early humans and animals to protect them from different poisonous diseases was quite difficult. Early animals and humans also probably eaten poisonous plants frequently in pursuit of sustenance, yet they were still able to learn about natural remedies (Yuan et al. 2016). Traditional medicine is linked to extensive indigenous knowledge in many nations that dates back to ancient times. Indigenous traditional knowledge has been used to generate a number of commonly used items, including herbal treatments for human and animal health (Farnsworth 2007). The ability of plants to treat a variety of illnesses has been established. Ethno-veterinary medicines is a term which is used to refer to traditional knowledge, beliefs, practises, and cures for numerous disorders in rural areas. Due to the discovery of certain useful ethno-veterinary products over the past ten years, these practises have grown significantly. The use of conventional treatments offers a more affordable, practical and long-lasting substitute for synthetic medications and pharmaceuticals (Dilshad et al. 2010). In some studies, roughly 30–35% of losses in the animal breeding industry occur owing to improper animal husbandry techniques particularly in developing nations, where rural residents are strongly dependent on livestock farming for their livelihood activities (Abbasi et al. 2013).

Across the world, medicinal plants (MP) are crucial for the survival of underdeveloped populations. Flowers make up most of the medicinal plants. More than 10% of the approximately 32000 species of higher plants (Prance 2021) are utilised medicinally. By 2050, it is predicted that the global market for medicinal plants would grow to $5 trillion (US). Other animals also employ plants to self-medicate; this practise is known as zoopharmacognosy and is not limited to humans. Such ethnobotanical information was gathered by research on animal behaviour, especially that of sick animals, and through interviews with indigenous groups. These indigenous people learned this information from their elders as well. Therefore, the authenticity of such knowledge may be constrained (Shinwari 2010).

Previously survey was conducted to collect the information about the people who keep the animals for business purpose or for domestic purpose. They may have some knowledge and awareness about the use of medicinal plants for the cure of the diseased animals. The percentages of the concerned people have been shared with the Table 1. According to field studies, both wild and domesticated herbs are still used in many villages, where old individuals are frequently the repository of such knowledge. These people closely guard the plant-use information that has been passed down to them through many generations. The rediscovery of such information would make it possible, for instance, to link the traditional uses of plants with the creation of novel phytopharmaceuticals in order to support regional biology and protect ethno-biodiversity (Menale and Muoio 2014).

It is known that plants can fight a variety of diseases. The livestock industry, as a subsector, accounts for roughly 56% of the value added in the agricultural sector and 11% of the GDP (GDP). The livestock subsector employs about 30 million people who reside in rural areas of the nation. Thus, methods for reducing poverty benefit significantly from cattle raising. The national herd of Pakistan consists of 53.82 million goats, 26.99 million buffalos, 1.0 million camels, and 29.6 million cattle, 26.7 million sheep, according to the Economic Survey of Pakistan report (ESP 2010). People who live in distant places use medicinal herbs to maintain the health of their cattle. It is particularly challenging for pastoralists and nomads to access veterinary care because to their traditional way of life. Collectors of herbal medicines are inexperienced, and over half of the material they gather is discarded. Finding sustainable methods to gather therapeutic plants from the wild is so necessary. This entails educating local hunters about proper hunting methods, teaching people how to grow therapeutic herbs, and getting rid of some of the intermediaries in the

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Table 1: Characteristics of Respondants by Demographics

Sr. No.	Character (Demography)	Quantity	Percentage
1	Sex		
	Male From Community	90.0	60%
	Female From Community	60.0	40%
2	Age		
	19 – 39	42	28%
	40 – 59	67	44.67%
	60 or above	41	27.33
3	Educational Status		
	Primary	66	44%
	Elementary	39	26%
	Higher Secondary	24	16%
	Graduate	21	14%
4	Occupational Status		
	Farmer	55	36.67%
	Businessman	35	23.33%
	Employee	47	31.33%
	Jobless	13	8.67

supply chain. The majority of people live below the poverty line and indiscriminately take natural resources to supplement their inadequate earnings, which is one of the main causes of the loss of biodiversity. Due to its distinctive geology, which includes the Hindu-kush Himalayas and the Karakorum, Pakistan has an altitude range of 0 to 8611 m, resulting in a variety of climatic regions and a rich floral biodiversity. More than 6,000 kinds of higher plants can be found in Pakistan. The medicinal value of the local flora is at least 12%, and numerous plants are exported. A sizable market system for crude drugs called "Pansara" is solely dependent on uncultivated plant species. Ailments in both people and animals are treated with medicinal herbs. Most of the time, some plant species are thought to be specifically effective against a certain disease, but occasionally these have dual applications (Ali and Qaiser 2009). Fig. 1 demonstrate the herbaria distribution in Pakistan.

In Pakistan the collection of dried plants is managed in different areas of the country. The largest herbaria is arranged in the Islamabad and Karachi in the territory of the university which comprises of almost 175000 dried plants while, more than 90000 dried plants are managed at the NARC Islamabad. These are managed as because of use as the medicinal purpose (Ali 2008).

Hemorrhagic Septicemia

Hemorrhagic septicaemia (HS) and mastitis are also significant problems. There is use of many locally produced combination vaccines against hemorrhagic septicemia (HS) and mastitis whose formation is plants based. Some studies have shown that certain plant extracts have antimicrobial properties and can be effective in treating HS. These plant extracts include garlic, ginger, turmeric, neem and echinacea. However, it is important to note that more research is needed to fully understand the efficacy and safety of using plant extracts to treat HS (Kuralkar and Kuralkar 2021). The bacterium *Pasteurella multocida* is a facultative anaerobic Gram-negative which was (size: 0.20-0.40 0.6-2.5 m), non-motile, non-spore-forming, capsuled short rod or coccobacillus. It has been labelled as an opportunistic pathogen that causes a number of illnesses, including enzootic pneumonia in sheep and goats, purulent rhinitis in rabbits, atrophic rhinitis in pigs, and hemorrhagic septicemia (HS) in cattle and buffaloes (Reuben et al. 2021). Fig. 2 shows the distribution of Hemorrhagic Septicemia across different regions of world, Asia and Africa.

The leaves and whole plant are the two most common plant parts used in the preparation of traditional phyto-remedies, followed by different parts of plants. Due to their ease of access and collection compared to other plant parts like the root and stem, leaves were chosen over all other plant components. Additionally, leaves serve as the primary repository for a number of secondary metabolites that are concentrated there. Due to their rich interpenes, roots were chosen after leaves (Silva et al. 2021).

Foot and Mouth Disease

This is extremely contagious and results in significant economic losses in susceptible animals with cloven hooves, such as cattle, sheep, goats, swine and many types of wildlife. The virus that causes the vesicular sores on the foot, oral mucosa and mammary glands belongs to the family Picornaviridae and genus Aphthovirus. The FMD virus (FMDV) has seven antigenic groups, or sero types: O, A, C, SAT (1 – 3) and Asia1 (Di Nardo et al. 2015). Although there is no cross-protection between serotypes, but there is a significant amount of serological cross-reaction. The genetic diversity among FMDV serotypes is evidence that various genotypic groups, or "pools," have independently evolved and circulated viral strains (Estevez et al. 2022). In both domesticated and wild ruminants, as well as pigs, it is a highly contagious viral disease that results in significant economic losses due to morbidity, mortality, and trade restrictions. Despite of the fact that the illness is widespread in Pakistan, seasonal outbreaks happen every year. Some studies have suggested that certain plant extracts may have antiviral properties and could be useful in treating FMD. For example, research found that an extract of the plant *Echinacea purpurea* reduced the viral load in cell cultures infected with FMD virus (Yasmin et al. 2020). Similarly, another study found that an extract of the plant *Andrographis paniculata* reduced the replication of FMD virus in cell cultures (Hossain et al. 2021).

There are different plants which are used for the treatment of various diseases. The specific portion of the plants are involved in the cure of the specific disease (Table 2) (Dseva et al. 2022).

Black Quarter

Black quarter (BQ) is an acute, contagious illness brought on by the gram-positive, anaerobic bacteria *Clostridium chauvoei.*

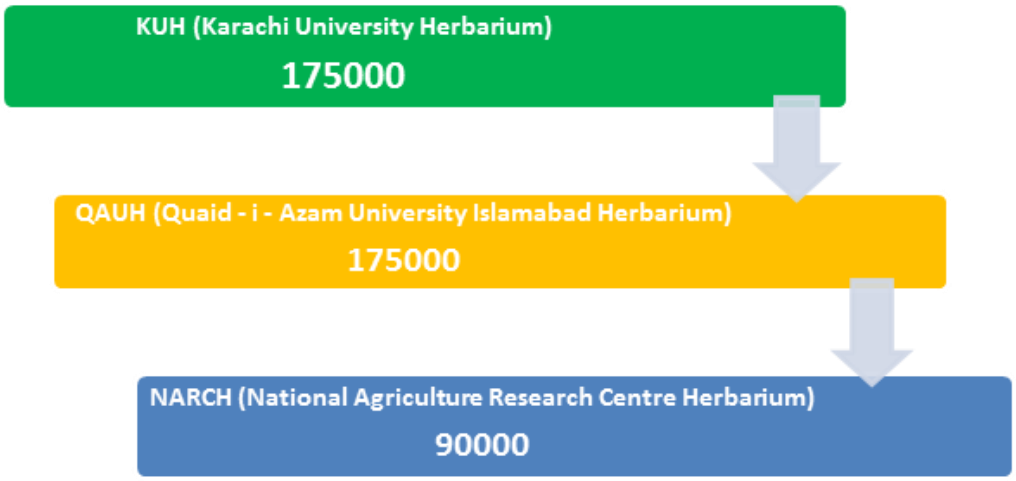

Fig. 1: Herbaria Distribution in Pakistan.

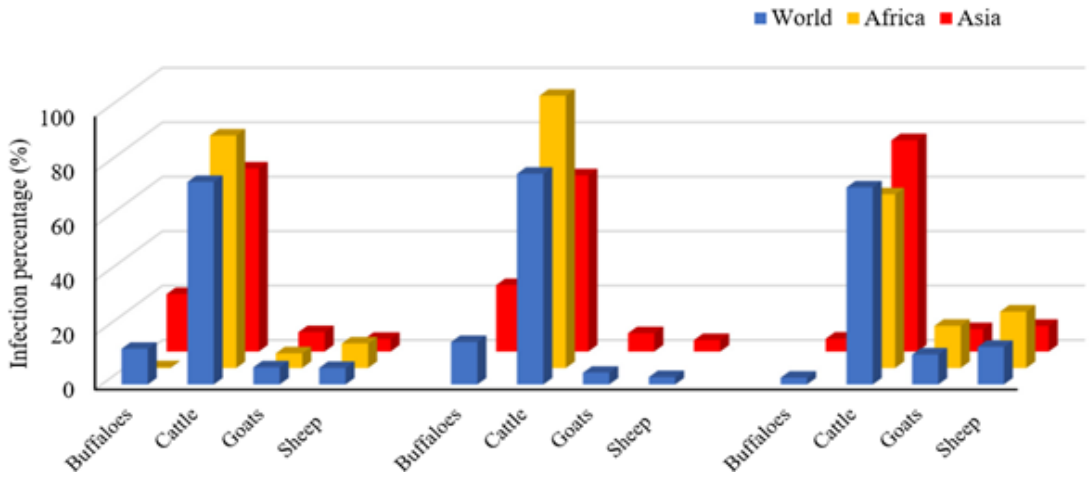

Fig. 2: Distribution of Hemorrhagic Septicemia among different animals in world, Africa and Asia

Table 2: List of Plants and their medicinal uses for different animal diseases

Sr. No.	Name of Plant	Used Portion	Advantage
1	*Abrus precatorious*	Seed	Strengthening the Placenta
2	*Caesalpinia bonnducella*	Seed	Timpani Production
3	*Calotropis gigantia*	Latex	Treatment of FMD-(foot and mouth disease)
4	*Momordica chanantia*	Leaf	FMD's Treatment
5	*Semecarpus anacardium*	Seed	FMD's Treatment
6	*Ficus racemosa*	Latex	Treatment of Bone Fracture
7	*Opuntia elatior*	Leaf	Treatment of Wound
8	*Tribulus terrestris*	Leaf	Treatment of Mouth ulcer
9	*Tamarindus indica*	Leaf and fruits	Treatment of foot disease
10	*Jatropha curcas*	Leaf and seeds	Treatment of Mouth disease, digestion

Inflammation, severe toxaemia, and gaseous oedema of the skeletal muscle are the hallmarks of this illness. Blackleg is a severe, often fatal condition that affects sheep and cattle that is also brought on by *Clostridium chauvoei*. Characteristic emphysematous swelling of the muscle lesions in cattle can appear without a prior history of wounds. As a rare form of the disease, cardiac blackleg has been observed in ruminants; nevertheless, the pathophysiology of this condition is not well known. In a study, the research on cardiac blackleg was conducted and reported two cases in 12–15-month-old Argentine feedlot steers. Over the course of 10 days, 14 out of 1,190 steers unexpectedly passed away. The animal's skeletal muscles were free of any detectable gross lesions. Two of the steers had underwent histology (Morrell et al. 2022).

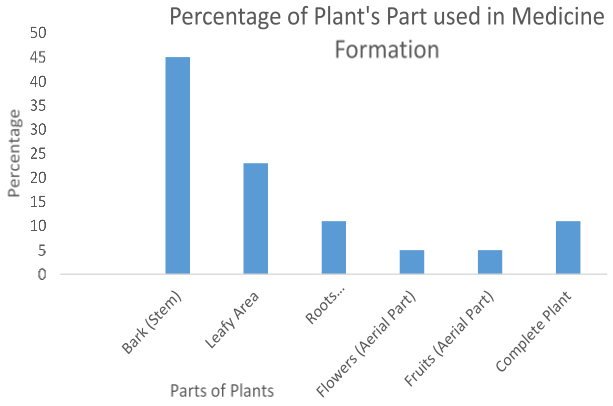

Fig. 3: Percentage of Plant's Part used in Medicine Formation.

Anthrax

Bacillus (B.) anthracis, a common zoonotic pathogen, frequently manifests as an unusual occurrence in world. Humans can become infected with anthrax through abraded skin, the respiratory tract, or the digestive tract after coming into direct or indirect contact with animals that have the disease. (Olani et al. 2020) The host becomes infected with *B. anthracis* after coming into contact with an infected animal (Savransky et al. 2020). Increased E-selectin production, which is a symptom of endothelial dysfunction, can result from excessive ROS generation (Doganay and Demiraslan 2015). The skin, lung, kidney, and liver may experience apoptosis, which is characterised by an increase in Caspase-3 and Multi Organ Dysfunction Syndrome (MODS). There was fewer animal than human reports, at a coarser spatial scale, but in places where there were clusters of human cases. Human incidence was lower when cattle vaccination rates were high (>25%), with the opposite trend occurring when vaccination rates fell. This suggests that livestock vaccination programmes reduce the prevalence of anthrax in both humans and cattle in Vietnam, however immediate improvement in livestock surveillance is required (Tan et al. 2022).

There is limited scientific research on the use of plant extracts to treat anthrax, and currently, there is no plant extract that has been proven to be effective against it. However, some plant extracts have been studied for their potential antimicrobial properties, which could potentially be useful in treating bacterial infections like anthrax (Dassanayake et al. 2021). *Aloe vera* extract has been shown to have antimicrobial properties against bacteria (Salama et al. 2022). Neem extract, which is derived from the leaves of the neem tree, has been studied for its potential use as an antiseptic and antimicrobial agent (Faujdar et al. 2020). Turmeric extract, which contains the active ingredient curcumin, has been shown to have antioxidant and anti-inflammatory properties, as well as the ability to inhibit the growth of certain bacteria (Abd El-Hack et al. 2021). Percentage of different parts of the plants which were used to produce medicines is different. Maximum medicine production occurs from the stem of the plants (Fig. 3).

Brucellosis

Brucellosis is the one of most prevalent infectious and transmissible zoonotic illnesses and has substantial morbidity and lifetime sterility rates. Intra/interspecific infection rates have dramatically increased in recent years as a result of inadequate management and scarce resources, particularly in developing nations. In cattle, poor milk production and a high body temperature are the main symptoms of abortion in the last trimester, whereas in humans, undulant fever, and arthritis are the main symptoms (Khan and Zahoor 2018). In recent years, both adults and children have used medicinal plants more frequently, to the point that 4 out of every 10 Americans now use these (Clarke et al. 2015) as an alternative therapy. Plants are used to make more than one-third of chemical medications, and there is a great deal of room for improvement in this area. A variety of ailments, including cancer, depression, bacterial diseases, rheumatic disorders, and acquired immune deficiency syndrome, are treated with medicinal plants. A native of Australia, the evergreen *Eucalyptus globulus* tree is also extensively distributed in Spain, Portugal, Italy, and India. It is used in traditional medicine to treat common infections (Asadi-Samani et al. 2016).

Mastitis

The most significant illness affecting dairy herds globally is bovine mastitis, which has a direct influence on farm profitability and food safety concerns. Antimicrobials are particularly effective in the prevention and treatment of this pathology, although the growing antimicrobial resistance of the organisms that cause this disease may reduce the effectiveness of traditional medications. Additionally, antibiotic residues in milk and the environment pose a risk to people's health. As a result, using plant extracts and essential oils as mastitis treatments for cattle may prove to be a viable option. Plant extracts and essential oils are frequently regarded as being safe for use by humans, animals, and the environment due to the well-described antimicrobial qualities that many plants possess (Lopes and Fontoura 2020).

Sunder (2013) examined the impact of *Morinda citrifolia* fruit juice on milk qualities of 13 healthy and 12 mastitis-affected dairy cows while evaluating these effects. Additionally, it was observed that consuming the fruit juice led to a significant reduction in the overall bacterial count in milk from cows infected with mastitis. The healthy animals in the treatment group showed no discernible change in these parameters. Although neither of the treatment groups' milk production levels considerably altered, the mastitis-affected animals did produce somewhat more milk after being given fruit juice.

Table 3: Different Kinds of Aflatoxins from Edible Oils

Sr. No.	Type	Melting Point	Boiling Point	Chemical Formula	Fluores cence
1	Aflatoxin-Type B_1	268.0^{0}C	528.16^{0}C	$C_{17}H_{12}O_6$	425nm
2	Aflatoxin-Type B_2	305.0 ºC	521.0^{0}C	$C_{17}H_{12}O_6$	425nm
3	Aflatoxin-Type B_3 /G_1	245.0 °C	612.1 °C	$C_{17}H_{12}O_7$	450nm
4	Aflatoxin-Type B_4/G_2	240.0 ºC	306.0^{0}C	$C_{17}H_{12}O_7$	450nm

Aflatoxicosis

Aspergillus (A.) flavus and *A. parasiticus* are the principal producers of aflatoxin, which is a form of mycotoxin. It has a significant negative impact on both human and animal health and is to blame for the loss of billions of dollars to the global economy by polluting various crops like cotton, peanuts, maize, and chilies. Aflatoxin types B1, B2, G1, and G2 are the most common and fatal of the more than eighteen distinct types that have been identified so far. Aflatoxin contamination can be controlled to a large extent by early fungal infection diagnosis. As a result, several techniques, such as chromatographic methods, molecular assays, and culture, are employed to identify aflatoxin contamination in crops and food products (Shabeer et al. 2022). The development and integrity of the plant can be harmed by *A. flavus* infection of vegetative tissues, which also offers serious dangers to the health of people and animals. As a result, methods that are secure and simple to use are used to stop *A. flavus* proliferation. In order to do this, *A. fumigatus*, a fungal endophyte, was employed as a secure biocontrol agent to inhibit the growth of *A. flavus* and its infection in maize seedlings. It's interesting to note that *A. fumigatus*, a harmless endophyte, displayed antifungal efficacy (such as 77% growth suppression) against *A. flavus*. Aflatoxin production was also decreased, particularly that of aflatoxin B1 (AFB1, 90.9%). Estimates were made of maize seedling growth, leaf and root morphology, and redox status changes at the plant level. *A. fumigatus* treatment of infected seeds markedly increased the rate of germination by almost 90% (Abdelaziz et al. 2022). Table 3 shows different kind of Aflatoxins from edible oils.

There are some aflatoxins which are naturally found in the edible oil. The major of four different kinds of these aflatoxins are present in edible oils and may also be produced naturally by many of the bacterial reactions. According to the latest classification of aflatoxigenic fungi, 18 out of the 33 species in the *Aspergillus* section Flavi produce aflatoxins naturally. The four major aflatoxin types, aflatoxins-AFB1, aflatoxins-AFB2, aflatoxins-AFG1, and aflatoxins-AFG2, can be produced by 16 of those 18 species, whereas the remaining two are synthesised from either AFB1 alone or from both AFB1 and AFB2. Most frequently polluted with AFB1, AFB2, AFG1, and AFG2 are oil seeds, particularly those from cotton, rape, sunflower, and coconut. The four main aflatoxin types identified in edible oils exhibit striking differences in their key physiochemical characteristics (Wanniarachchi et al. 2023). Nutrient infusion in utero can alter the embryo's physiological reactions. The physiological reactions of the embryo to aflatoxin B1 (AFB1) embryotoxicity can be modified by in ovo nutrition infusion (Elwan et al. 2022).

Avian influenza

Two diseases i.e., Avian-influenza and Newcastle-disease are major causes of morbidity and mortality in poultry. There are a number of reasons for this, including vaccination costs that may be unaffordable, the impossibility of storing attenuated live viral vaccines in a cold chain, and the potential ineffectiveness of commercial vaccines to defend against regionally developing strains. In comparison, vaccines made from plants are stable and safe (Nurzijah et al. 2022). The creation of transient gene expression systems in plants offers a flexible and reliable method for producing large quantities of recombinant proteins quickly and efficiently. VLPs may provide advantages such as considerable decreases in viral shedding and the capacity to distinguish between infected birds (Boskovic et al. 2015).

A key public health issue in recent years has been the animal infection with the avian influenza virus due to the possibility of a pandemic spreading throughout society. Additionally, a rise in drug-resistant influenza A virus cases has highlighted the urgent need for additional and widely accessible anti-influenza medications. It has been demonstrated for the first time that the crude ethanol and water extracts of five Asian medicinal plants, including *Andrographis paniculate, Curcuma Longa, Gynostemma pentaphyllum, Kaempferia parviflora, and Psidium guajava* have antiviral properties against H5N1 influenza virus infection in vitro and may be used as alternative antiviral compounds to treat H5N1 influenza virus infection (Chen and Guan 2015).

It is possible to produce H5N1 HA antigen in plants without modifying them genetically, as this enables quick scaling up to high-volume manufacturing. The absence of genetic modification is significant because, despite the efficient production of vaccine antigens by transgenic plants (which can take months to years, depending on the species), such methods would be impractical in emergency situations where large quantities of antigen would be needed within a few weeks of a reported outbreak. Using plant virus vectors modified to produce foreign genes is an alternate strategy. This strategy shortens development time by allowing the use of healthy, non-transgenic plants as a production system, but it depends on how well viruses replicate (Shoji et al. 2009).

Conclusion

The use of traditional medicinal practices, also known as an ethno-medicinal approach, has proven to be an effective method in treating various animal diseases. It is important to continue the research and incorporate these methods in conjunction with modern techniques to provide the best possible care for our animals. It is also very important to consider the safety and efficacy of these traditional methods before implementation. By combining the knowledge of traditional practices with modern scientific methods, we can improve the health and well-being of animals worldwide.

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Transmission Dynamics of Water-borne Protozoa: An Insight into Current Challenges and Control Measures in Developing Countries

AUTHORS DETAIL

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INTRODUCTION

Water-borne parasitic infections are one of the main health related problems of developing countries. This is because of the reason that there is no proper sewage and drinking water supply system for their community. These parasites are the main cause of diarrhea, dysentery, fever, malabsorption, lymphadenopathy, hepatitis, lactose intolerance, enteritis, and peritonitis in both humans as well as in animals. It has been reported that till 2007, 325 outbreaks of water-borne parasitic diseases occurred worldwide. Among these, 93% outbreaks were documented from North America and Europe. According to a study, during the period of 1948-2012, round about 537 outbreaks of water related protozoa have been reported (Khan et al. 2019).

Water-borne GIT Protozoa

Most of the etiological agents for gastrointestinal infections are protozoa and belong to the phylum Apicomplexa which includes mainly *Giardia* spp., *Isospora* spp., *Sarcocystis* spp., *Cyclospora* spp., *Entamoeba histolytica*, *Cryptosporidium* spp., *Balantidium coli*, *Toxoplasma gondii* and *Acanthamoeba*, in exception of a spore forming unicellular parasite i.e. *Enterocytozoon bieneusi* (Microsporidia) (Schets et al. 2008). In this study, *Cryptosporidium* spp. and *Giardia* spp. were dominant pathogenic protozoa (Kumar et al. 2014).

Life cycle of these protozoa is very simple and usually need only single host for their multiplication. Transmission mostly occurs by feco-oral route and they multiply within the host asexually and thousands of protozoa in the form of cyst or oocyst excrete out with feces. These oocysts are their infective stage and can survive in harsh conditions like temperature, chemicals, enzymes and chlorine treatment. This simple lifecycle of protozoa makes water very favorable for their transmission. Interestingly, they are very small in size and can easily passthrough physical barriers during filtration, making difficult to purify water from these pathogens. The outbreaks by these parasites occur when water bodies like lakes, dug wells and canals got polluted with the rainfall and overflow of the sewage system. Divers and other people particularly in summer season jump fall and get pushed in the canals and ultimately got exposed to canal water. Sometime accidental ingestion of canal water also occurs (Schets et al. 2008). In urban areas of Pakistan, the drainage of sewage water in canals is a common practice. In peri-urban and rural areas, this situation is worst because of non-availability of proper municipal supply for drinking purpose and people in these areas are dependent on the use of dug wells and canal water for drinking. Moreover, this contaminated canal water is used for irrigation purpose by which our vegetables and fodders got contaminated. Humans and animals got infected when they eat them in raw form (Mumtaz et al. 2010; Alam et al. 2014).

Among these protozoa, most pathogenic are *Cryptosporidium* spp., *Giardia* spp., and *Entamoeba* spp. In acute infections, most common conditions are enteritis, diarrhea and dysentery but in chronic cases, peritonitis, enteritis, hepatitis and lymphadenopathy mostly seen. Approximately 500 million people are suffering from amoebic dysentery per year. Out of theses, 0.1 million people die every year (Ananthakrishnan and Xavier 2020). Chances of *Cryptosporidium* and *Giardia* infections are mostly seen in children and immune-compromised patients, such as AIDS patients. These protozoa in these patients cause abdominal distension, malnutrition, fever, vomiting and diarrhea. *Toxoplasma gondii* and *Sarcosystis* also have a public health concern. Except of intestinal disturbances, these parasites also cause muscle fatigue, eosinophilia and neurological disorders in humans and animals. Sporulated oocysts are their infective stage which is ingested by the humans and animals by drinking improperly purified water. *T. gondii* is an opportunistic parasite of humans and cause neurological

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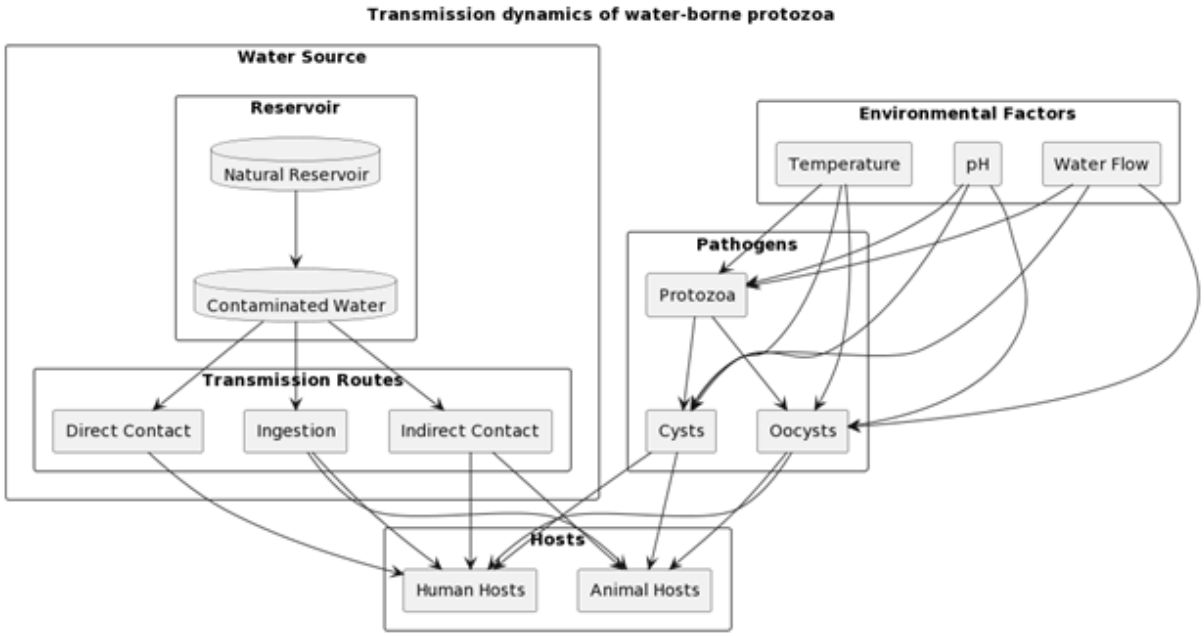

Fig. 1: Water-borne Protozoa Transmission to Hosts

diseases in newborn and abortion in adults (Squire and Ryan 2017). The Fig. 1 provides an overview of the transmission dynamics of water-borne protozoa to human and animal hosts, and the environmental factors that affect their spread.

Several methods are used for the detection and diagnosis of these parasites. Most commonly, conventional method is used in which protozoal cysts and sporulated oocysts are detected microscopically from water samples. Some serological tests like ELISA, CFT are also common in practice for parasitic detection with more sensitivity. However, this method can only detect these parasites at genus level. Molecular methods like PCR have the advantage of diagnosing these parasites at species level. Moreover, these advanced techniques have comparatively much more sensitivity and specificity (Slater et al. 2022).

Global Distribution of Water-borne Protozoa

Water borne protozoan infection is a global issue and is reported from a number of countries including Australia (Ma et al. 2022), Africa (Abuseir 2023), Bangladesh (Alam et al. 2014), Brazil (Taverne 2002), Bulgeria (Sotiriadou and Karanis 2008), Canada (Herwaldt 2000; Wallis et al. 2001; Ho et al. 2002; Murrow et al. 2002; Hopkins et al. 2013), China (Zhang et al. 2011; Lv et al. 2013; Liu et al. 2014), Ethiopia (Ayalew et al. 2011), France (Dalle et al. 2003; Villena et al. 2004; Aubert and Villena 2009), Germany (Gornik et al. 2000; Gallas-Lindemann et al. 2013), Iraq (Raza and Sami 2009), Iran (Mahmoudi et al. 2015), Ireland (Glaberman et al. 2002; Jennings and Rhatigan 2002), India (Kiran et al. 2014; Jain and Nahri 2015), Japan (Uga et al. 2005; Kourenti and Karanis 2006), Korea (Cheun et al. 2013; Moon et al. 2013), Malaysia (Mahsol et al. 2008; Kumar et al. 2014), Nepal (Sah et al. 2013), Netherland (Schets et al. 2008), New Zealand (Webber 2002), Pakistan (Ahsan-ul-Wadood et al. 2005; Mumtaz et al. 2010; Chaudhary and Chandra 2012; Khan et al. 2013; Masood et al. 2013; Alam et al. 2014), Poland (Sroka et al. 2006), Portugal (Lobo et al. 2012), Philippines (Baldo et al. 2004; Al-Hindi and El-Kichaoi 2008; Onichandran et al. 2014), Russia (Sotiriadou and Karanis 2008), Scotland (Wells et al. 2015), Spain (Perez et al. 2000), Sweden (Widerstr€om et al. 2014; Rehn et al. 2015), Taiwan (Chen et al. 2001), Thailand (Kumar et al. 2013), Turkey (Koloren and Demirel 2013; Demirel et al. 2014), Uganda (Tumwine et al. 2002),USA (Barwick et al. 2000; Ho et al. 2002; Lee et al. 2002; Murrow et al. 2002; Cope et al. 2015; Bedard et al. 2016; DeSilva et al. 2016), United Kingdom (Puleston et al. 2014; McCann et al. 2014).

Prevalence

Many research have been carried out to study the water borne protozoa due to their public health significance. According to a literature, 524 outbreaks have been documented till 2010 and most of their prevalence was found in America, Europe and Australia. In Asia, their prevalence is also significant (Karanis et al. 2007). Moreover, their prevalence is very high in peri-urban and rural areas of developing countries where people tend to use contaminated municipal water, dug well water and unfiltered canal water (Mumtaz et al. 2010; Baldursson and Karanis 2011; Masood et al. 2013; Alam et al. 2014; Kumar et al. 2016). Prevalence of various water-borne zoonotic protozoa in different countries from year 2000-2018 has been listed in Table 1.

Table 1: Worldwide prevalence of different water borne protozoa

Year	Country	Est. Cases/% Prevalence	References
		Giardia lamblia	
2009	USA	36	Bedard et al. 2016
2010	Korea	25	Cheun et al. 2013
	Bangladesh	>37%	Alam et al. 2014
	Ethiopia	41.9%	Ayalew et al. 2011
	India	55%	Jain and Nahri 2015
		Cryptosporidium Spp.	
2008	UK	422	Puleston et al. 2014
2010	Wales, UK	48	McCann et al. 2014
2010	Sweden	27,000	Widerström et al. 2014
2010	Canada	12	Hopkins et al. 2013
2011	Sweden	20,000	Rehn et al. 2015
2012	Korea	126	Moon et al. 2013
2013	USA	2780	DeSilva et al. 2016
		Entamoeba histolytica	
2009	Tajikstan	25.9%	Matthys et al. 2011
2011-2012	Nepal	6.1%	Sah et al. 2013
2013	India	25.4%	Kiran et al. 2014
2013-2014	Pakistan	5.9%	Chaudhary and Chandra 2012
		Toxoplasma Gondii	
2009-2010	Iran	5.9%	Mahmoudi et al. 2015
2012	Pakistan	7%	Khan et al. 2013
	Turkey	51.6%	Koloren and Demirel, 2013
2013	Turkey	13.2%	Demirel et al. 2014
2013	Scotland	8.8%	Wells et al. 2015
2015	Colombia	76.9%	Triviño-Valencia et al. 2016
		Enterocytozoon bieneusi	
-	China	9	Zhang et al. 2011
-	Portugal	54	Lobo et al. 2012

Transmission of GIT Protozoa through Water

Water is a necessity for almost all living beings. But it also provides a suitable and favorable route for the transmission of gastrointestinal protozoa. Once an animal or human got infected by any of the protozoa, it starts shedding a massive amount of infected cyst/oocyst in the environment. Due to close interaction of animals and humans with the natural sources of water, there are greater chances of infecting these sources (Bozorg-Haddad et al. 2021). Additionally, these water-borne protozoa may reach to ground water by infiltration of contaminated surface waters. Most reported concentrations of infected cyst/oocyst in water are up to 150/liter of water. However, greater concentrations have also been reported from different lakes, ponds, rivers, canals, furrows, sewage systems, municipal water and even in mineral water. *Giardia* and *Cryptosporidium* have been reported as the most frequently associated water-borne pathogens. Most deadly episode of *Cryptosporidium* outbreak was occurred in 1993 in USA when 0.4 million people got hospitalized causing an estimated economic loss of $96.2 million (Lee 2019). Several outbreaks of other water-borne protozoa have also been documented in different regions of the world (Mchardy et al. 2014).

The most common cause of diarrhea is protozoan infections in humans as well as in animals. *Cryptosporidium* spp., *Giardia* spp., *Enterocytozoon* and *Cyclospora* spp. are the main GIT protozoa causing diarrhea. This is the conclusion of a research done in China during 2012-2013. Fecal samples of 252 diarrheal patients had been collected and examined with nested PCR. Out of these 252, 76 samples were positive for any one of these four parasites (Liu et al. 2014). A study was conducted in Philippines for the awareness of water contamination with protozoa most likely *Cryptosporidium* spp., *Giardia* spp., *Acanthamoeba* and *Naegleria*. 33 samples from rivers, lakes, ponds, swimming pools and drinking water of peri-urban and rural areas were collected, and tests were positive for *Cryptosporidium* spp. and *Giardia* spp. by counting oocysts/liter. And PCR test for *Acanthamoeba* were also positive as well (Onichandran et al. 2014).

In France, a case was presented by in a hospital with severe peritonitis and severe abdominal pain. The patient was a butcher and was addicted to alcohol. When the case was studied, they found that he was suffering from *Balantidium coli*. This parasite is very common in wild animals and pork. This parasite can easily be transmitted by ingestion of food and drinking contaminated water. For this patient, specific antibiotic with metronidazole was given for peritonitis and to stop bloody diarrhea (Ananthakrishnan and Xavier 2020). A comprehensive study was performed on outbreaks of water-borne protozoan infections during the period of 2004-2010. A total of 199 outbreaks were reported during this time. These outbreaks occurred in Australia, South America, and Europe. Prevalence of *Cryptosporidium* spp., *Giardia lambia*, *Toxoplasma gondii*, *Cyclospora cayetanensis* and *Acanthamoeba* was reported as 60.3%, 35.2%, 2%, 1.5% and 1%, respectively (Baldursson and Karanis 2011).

Pregnant women were found the most susceptible host for the opportunistic parasites and these parasites were found to be very dangerous for not only the mother but also for the new borne babies. *Toxoplasma gondii* is found to be very prevalent in many European countries such as Belgium with 48.75% prevalence (Gebremedhin 2019) in pregnant women or those which were just given birth to babies. Similarly, 25.4% (Glynou et al. 2005), 21.2% (Kansouzidou et al. 2008) in Greece, 24.6% in Ireland (Ferguson et al. 2008) and 19.8% (Masini et al. 2008) prevalence was recorded in Italy. This parasite was found to cause neurological disorders in new borne babies and children of young ones.

Another study was conducted to check the prevalence of *Cryptosporidium parvum* and *Giardia lambia* in water samples from different countries of Southeast Asia. Total 221 samples of size 10 litter each from Malaysia, Thailand, Philippines and Vietnam were collected. Theses water samples were examined with respect to the methods of United states Environmental Protection Agency microscopically observed and subsequently screened using RT-PCR assays. From treated water samples *Cryptosporidium* oocysts were detected at the rate of 0.06 ± 0.19 oocyst/Liter concentrations while from non-treated water samples at the range of 0.13 ±

0.18 to 0.57 ± 1.41 oocyst/Liter concentrations. Similarly, *Giardial* cysts which were detected in treated water of Philippines at concentration of 0.02 ± 0.06 cyst/L while from untreated water samples at concentration of 0.12 ± 0.3 to 8.90 ± 19.65 cyst/L. This study revealed the potential risk to human population of these countries (Kumar et al. 2016).

Toxoplasmosis is a worldwide problem now a days and it is most common in females. Situation is worst in pregnant females around the globe. Toxoplasmosis was found in different states of America. Prevalence of *Toxoplasma gondii*, in Brazil was recorded 51.2% (Avelino et al. 2004), 60% (Olbrich-Neto and Meira, 2004), 70.6% (Leao et al. 2004), 77.5% (Porto et al. 2008), 61.2% (Carellos et al. 2008) and 48.7% (Rosso et al. 2008) was documented in Columbia. In Pakistan, a few years ago, drinking and surface waters have been examined and the occurrence of *Cryptosporidium* spp. and *Giardia lamblia* in these samples has been associated with diarrhea in animal and human population. In recent, samples of tap water, pond water, dug well, bore well water, hand pump water from KPK were examined and the prevalence of *Cryptosporidium parvum* and *Giardia lamblia* has been documented 36% (Alam et al. 2014).

Similarly, samples were taken from patients who were suffering from diarrhea with acute abdominal pain, and they were found positive for *Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum.* It has been observed that these people had poor socio-economic status and lack of facilities for purified or drinking water and they were tending to use contaminated water. Another study was conducted in Pakistan and the water samples were examined for the prevalence of water borne parasites. It has been observed that the prevalence of *Cryptosporidium parvum* and *Giardia lamblia* was highest in humans as well as in animals causing a huge economic loss (Masood et al. 2013). *Toxoplasma gondii* is an important zoonotic and opportunistic parasite. Basically, it is transmitted by several routes and water is also a source for its transmission. To study the sero-prevalence of this parasite in human population, studies conducted in different countries in last decade were compiled just to overview the worldwide occurrence of this parasite (Pappas et al. 2009).

Toxoplasma gondii is an opportunistic parasite of human. It infects men, women and even children. Having an opportunistic property, this parasite was found to be very prevalent in Immuno-compromised people such as HIV/AIDS patients. Because of the Immune deficiency of such people, this parasite attacked the central nervous system and causes nervous disorders and histopathology of the samples collected from their brain tissues, showed numerous lesions in the brain cells of *Toxoplasma* infected patients (Lago et al. 2009).

In 2006, two lakes and three rivers were suspected to have contamination with water borne protozoa. So, total 57 samples were collected from these natural sources and examined with molecular methods such as Immunofluorescence (IMS-IF) for *Cryptosporidium* and *Giardia* followed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and it has been observed that out of 57 samples, *Giardia* and *Cryptosporidium* cyst were detected at the rate of 165cyst/10L. Meanwhile, from these samples *Enterocytozoon bieneusi* was also found in 2 river samples. No respective co-relation was found in prevalence of bacteria and protozoa (Coupe et al. 2006).

Suitability of Protozoan Parasites to Waterborne Transmission

Many of the protozoan parasites have common physical and biochemical features which make them resistant to ecological stresses and help in successful dispersal in the aquatic environment. Following are some characteristic features which make these parasites to survive in the aquatic environment:

Shedding of Cysts/oocysts in Huge Amount

One of the characteristic features of these parasites is the asexual reproduction in which one cyst/oocyst can produce thousands of protozoa within the infective host. It enhances the probability of survival and transmission of these parasites in the environment. For example, infected cattle with *Cryptosporidium* shed 10^{6-8} oocysts/g of feces for 3-12 days which clearly indicates the huge impact of cattle in transmitting infective *Cryptosporidium* to the environment. Similarly, humans also play a significant role in spreading these parasites to the environment and contamination of different water bodies and recreational water sources. A clean example of contribution to contamination is that infected humans can shed 10^9 cysts of *Giardia* every day (Savioli et al. 2006).

Persistence in the Aquatic Environment

Protozoan parasites especially, *Giardia lamblia*, *Cryptosporidium parvum*, *Toxoplasma gondii*, *Entamoeba histolytica* and *Balantidium coli* are highly resistant to the harsh environmental conditions. They can survive for months due to their outer protective shell. However, in the aquatic environment, their survival is significantly affected by increase in temperature. Most of these parasites usually survive for 45 days at 30^0C. But their survival goes on decreasing with increase in temperature and at 22^0C, they can only survive for 45 days. Similarly, very low temperature also affects the viability of oocysts of these parasites. For example, cysts/oocysts of these parasites can live only for 24 hours at -20°C. The viability and infectivity of cysts/oocysts of these parasites is also affected by solar radiation, freeze-thaw cycles, and desiccation (Smith et al. 2006).

Smaller Size of their Cysts/oocysts

Most of the protozoa have a very smaller size ranging from 1µm to 50µm. However, Balantidium coli is about 150µm long. Due to their smaller size, they have a very low specific gravity due to which they continue floating in the water. Some researchers stated that sedimentation rate is higher regarding the occurrence of these parasites in water due to attachment of their cysts/oocysts with suspended particles. However, other researchers stated controversially and stated that they live freely which makes them more consistent and facilitates their transport to other water bodies. Due to this characteristic feature, they can pass any physical barrier like filtration process. Even, these parasites can also pass-through well-designed treatment systems which allow these parasites to expose the public communities (Savioli et al. 2006).

Resistance to Chemical Disinfectants

Protozoan parasites are highly resistant to chlorine-based disinfectants at optimum concentrations and exposure times which are commonly used practices in water filtration industries. Even, if the chlorine concentration is increased which might help in killing these parasites, it may lead to increased concentration of toxic byproducts within the water such as halomethanes. It illustrated the failure of the disinfection method used for cleaning the water. The best method to disinfect the drinking water is by using absolute-sized filtration paper (smaller pour size than parasitic cyst/oocyst) and appropriate disinfectant under optimum conditions (Betancourt and Rose 2004).

High Infectivity Rate

Generally, the infection after exposure to these parasites depends upon immune status of the host, number of cysts/oocysts ingested and associated risk factors. In any case and condition, a very few cysts/oocysts (5-40) are enough to cause infection in the host. For example, 10-30 oocysts of *Cryptosporidium parvum* are enough of cause infection in any kind of host including animals and humans. Similarly, 25-100 cysts are enough to cause medium infection in humans. Nevertheless, it is even unclear how many cysts and oocysts of parasites are present in the drinking water, but they do cause infection after ingestion. The reason behind this infection by a single cyst/oocyst is the asexual reproduction by which they can multiply in hundreds and thousands (Smith et al. 2006).

Surveillance and Control Measures

Due to high public health concern, water-borne parasites have become a major challenge for the sewage disposal and water industry which is responsible for providing safe drinking water to the world population. In this regard, different developed countries like USA, New Zealand, Australia and Canada have established some standards and regulation to their water industries including turbidity monitoring, removal of cyst/oocyst through proper filtration process and inactivation of detected water-borne pathogens. Unfortunately, none of these authorities made a standard for cyst/oocyst monitoring of water-borne protozoa. Moreover, these authorities were also unable to provide information regarding the protozoon species as well as their infectivity to the human population. In contrast, monitoring of cyst/oocyst in the drinking water is compulsory on regular basis in England, Ireland and wales. These countries have made a standard of existing less than one cyst/oocyst $10L^{-1}$ in drinking water provided by the water industry regardless of their viability and infectivity to humans. Regardless of their public health concern, presence of more than one cyst/oocyst $10L^{-1}$ in the water has been considered a critical question on the quality and standards of water-providing company in these countries (Carmena 2010).

Based on epidemiological studies of water-borne parasites and their worldwide outbreaks, scientists have made an action threshold level for the presence of cysts/oocysts in the water. It means that if the concentration of cyst/oocyst exceeds 3-30 cysts/oocysts $100L^{-1}$ of provided water, immediate action should be taken for the detection of these cysts/oocysts through most appropriate method to get the information regarding the infectivity as well as exact concentration of cyst/oocyst in the provide water. Mathematical and statistical methods have been a useful tool for checking the probability of outbreaks associated with water-borne protozoa (Casman et al. 2000; Pouillot et al. 2004).

Conclusion

Water is a main source for the transmission of gastrointestinal parasites. Most important gastrointestinal parasites are *Giardia* spp., *Isospora* spp., *Sarcocystis* spp., *Cyclospora* spp., *Entamoeba histolytica*, *Cryptosporidium* spp., *Balantidium coli*, *Toxoplasma gondii* and *Acanthamoeba* and *Enterocytozoon bieneusi*. These parasites have a cosmopolitan distribution and cause huge morbidity and mortality. These parasites mostly cause diarrhea and dysentery. The situation of illness is worse in young children and immunocompromised patients. Due to some characteristic features like smaller size, resistance to chemicals, high reproductivity and infectivity rate, they are suitable for water-borne transmission. There is no appropriate method for the removal and inactivation of cyst/oocyst in the water. However, surveillance and control measures are the only options to control the parasitic transmission through water. Exposure of animals to the natural sources of water should be stopped or minimized. Sewage water should be properly disposed of and irrigation of agricultural land with the sewage water should be stopped. There should be two-way treatment of water before use. Firstly, proper filtration and secondly should be treated with UV light, ozonization

and again membrane filtration. By using such preventive and treatment measures, water-borne transmission of gastrointestinal parasites could be stopped or minimized.

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Cryptosporidiosis and Giardiasis: Two Common Foodborne Parasitic Infections

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INTRODUCTION

Recent times have seen a lot of interest in infections caused by food and water. A group of disorders commonly known as "foodborne disease" arise as a result of eating food that has been tainted by chemicals or microorganisms. The sickness can be spread even by tainted water, utensils, and users' hands. Third-world countries have a greater frequency of food-related problems than developed countries. In rural regions, households still use untreated water for drinking, cooking, washing fruits, bathing, and swimming, exposing residents to diseases other than protozoan parasites. The majority of people in the globe still lack access to clean water and sanitary facilities. (WHO 2014; Javed 2016). As a result, millions of people in developing nations face a major risk from the possibility for protozoan infections being introduced into their water supply. However, this does not imply that these illnesses do not exist in any part of the world. While there are several early warning signs of food-related disorders, gastrointestinal dysfunction is frequently employed to make the diagnosis.

Acute, recurring, and impairing disorders can all be brought on by parasites (Alvi et al. 2020; Štrbac et al. 2020; Kandeel et al. 2022; Mahmood et al. 2022). Almost everywhere in nature, parasitic protozoa may be found. They bear responsibility for epidemics and persistent poverty in both developed and underdeveloped countries (Al-Malki 2021). Since that certain parasites are zoonotic in origin and hence live in animals, their dominance in food and water should be considered to be a public health issue (Thompson 2013). A number of illness outbreaks that have been connected to parasites in the past have caused a rise in the incidence of water- and food-borne parasites throughout time. In 2014, the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) issued their global risk assessment of foodborne parasites (FBPs) (WHO). Although being accepted as substantial foodborne pathogens, parasites are still undervalued when compared to bacterial and viral foodborne pathogens (Torgerson et al. 2015). It was followed in 2015 as a worldwide burden associated with foodborne pathogens (Trevisan et al. 2019). *Cryptosporidium spp* and *Giardiaspp* are the important protozoans causing diseases both in livestock and humans (Leung et al. 2019; Gorcea et al. 2020) Across the world these parasites have posed a serious threat. Despite the standard test for the diagnosis of these parasites and different treatment methods, the spread of these parasites is uncontrollable due to other managemental disorders (Siwila et al. 2020). In this study, we summarize etiopathogenesis, epidemiology and preventive measures for zoonotic cryptosporidiosis and giardiasis.

Cryptosporidiosis

Abdominal discomfort, vomiting, and diarrhea are the hallmarks of cryptosporidiosis, a zoonotic protozoan disease caused by the widely distributed *Cryptosporidium* (Dillingham et al. 2002). After consuming food or drink tainted with oocyst-containing feces, this parasite can spread through the faecal-oral route (Tzipori 2000; Tzipori and Ward 2000). About the pathogen's natural reservoir hosts, there is currently no accurate information (Khalil et al.2018).

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Tyzzer discovered *Cryptosporidium* during the first decade of the 20th century (Tzipori and Widmer 2008), but it wasn't until 1976 that it was revealed to be an opportunistic parasite of humans (Meisel et al.1976; Nime et al.1976). It was discovered in 1982 that *Cryptosporidium* can cause self-limiting diarrhea in people and, in those with impaired immune systems, can even be fatal (Fayer and Ungar, 1986; Majeed et al. 2022). The parasite can complete its life cycle with asexual and sexual reproductive stages on just one host (Tzipori 2002; Tzipori and Widmer 2008). There are currently at least 30 species in the genus *Cryptosporidium*, but from the perspective of zoonotic transmission, *Cryptosporidium parvum* and *Cryptosporidium hominis* are the most significant (Ryan et al. 2014; Thomson et al. 2017). A few members of the *Cryptosporidium* genus are known to infect several species, including mammals, birds, and reptiles (Leitch and He, 2012; Zahedi et al. 2016). Members of the genus are extremely particular to their hosts. The phylum Apicomplexa contains the internal parasite *Cryptosporidium*, which is important for both humans and animals (Suarez et al. 2017). *Cryptosporidium* species cannot be grown in vitro, in contrast to other Protozoa members (Karanis 2018). The only way to reduce the spread of this parasite is to take preventive measures, as there is no commercially licensed vaccination to prevent Cryptosporidium infections and high contagiousness (Thomson et al. 2017).

Life Cycle of Cryptosporidium

Each *Cryptosporidium* oocyst releases four sporozoites into the host's intestine (Tzipori 2000; Tzipori and Ward 2000). After excystation, sporozoites invade a host membrane that has been modified and is now isolated from the cytoplasm. This invasion causes the formation of a parasitophorous vacuole, where schizogony/asexual reproduction takes place, producing 8 merozoites (Bouzid et al. 2013). The infection spreads to additional places in the intestines through the ability of the generated merozoites to penetrate the neighboring epithelial cells. The merozoites go through two distinct cycles after that: an asexual stage during which they reproduce and create thin-walled oocysts that can infect the host on their own, and/or a sexual stage during which type II meront are produced and differentiate into microgametocytes and macrogametocytes. As a result of the union of these microgametocytes and macrogametocytes, a diploid zygote is created, which goes through sporogony to produce four sporozoites inside thick or thin-walled oocysts (Tzipori 2002). The thick-walled oocysts, which are ready to infect a new person, are released in the feces (Bouzid et al. 2013; Jenkins et al. 2010).

Transmission of Cryptosporidiosis

Nearly every region in the world has documented cases of Cryptosporidiosis, however, outbreaks are primarily linked to drinking contaminated water or using unsanitary swimming pools (Fayer et al. 1997 Fayer et al. 2000). The prevalence is probably substantially greater than the number of recorded cases because of the sharp rise in cryptosporidiosis incidence over the world over the past few years, which is well-depicted by clinical signs (Shrivastava et al. 2017). The difference in the prevalence of *Cryptosporidium* in developed and developing nations can be related to the latter's residents' continued lack of access to clean drinking water and adequate sanitary facilities (Bouzid et al. 2018; Shoultz et al. 2016).
It has been determined that at least 30 distinct species of *Cryptosporidium* can cause sickness in both people and animals. The most typical species that harm humans are *C. hominis*, *C. parvum*, *C. canis*, *C. felis*, and *C. meleagridis* (Šlapeta 2013; Ryan et al. 2014; Ayinmode et al. 2018). parvum is the most frequently discovered to be connected to intestinal *Cryptosporidium* infections in people out of these 5 species. Humans and ruminants serve as *C. parvum* hosts, hence it primarily affects people who frequently interact with ruminants (Dixon et al. 2011; Hunter and Thompson 2005). Animals can transmit *Cryptosporidium* to people, however, such cases are extremely rare. According to reports, rats, horses, sheep, goats, and goats are the main sources of human cryptosporidiosis (Hunter and Thompson, 2005; Ehsan et al. 2015; Thomson et al. 2017). When it comes to *C. canis* and *C. felis*, dogs and cats, respectively, carry these parasites without displaying any symptoms of illness (Ehsan et al. 2015; Shrivastava et al. 2017). However, these house pets pose a threat to the spread of *Cryptosporidium* to People (Leitch and He 2011; Ryan et al. 2014).

Clinical Picture of Cryptosporidiosis

An episode of self-limiting watery diarrhea is brought on by gastroenteritis brought on by a Cryptosporidium infection (Bouzid et al. 2013; Shoultz et al. 2016; Adler et al. 2017; Khalil et al. 2018). Even people who have never previously had contact with animals run the risk of contracting the disease if they mistakenly consume pool water that contains oocysts or drink untreated water (Fayer et al. 1997; Fayer et al. 2000; Bouzid et al. 2018). In people with poor health or impaired immune systems, the condition may progress severely (Bouzid et al. 2013; Florescu et al. 2016; Wang et al. 2018a). According to careful calculations, cryptosporidiosis kills more than 50,000 people per year (Shirley et al. 2012; Wang et al. 2018). Following oocyst consumption and infection, *Cryptosporidium* damages the intestinal membrane, causing increased permeability, decreased absorption, and increased fluid and electrolyte output (Petry et al. 2010; Kumar et al. 2018). The oocysts are particularly resistant to chlorine, chloramines, and chlorine dioxide, which allows them to survive for a very long time in the environment (Shrivastava et al. 2017). Humans can become infected with *Cryptosporidium* by touching objects that have come into contact with contaminated feces. Ingestion of oocysts found in contaminated food, water, or air

is the most typical method of transmission (Petry et al. 2010; Shrivastava et al. 2017). Recent data have demonstrated that respiratory secretions can also transfer cryptosporidiosis and cause pulmonary infections (Sponseller et al. 2014). Cryptosporidiosis is more likely to affect hosts with compromised immune systems than immunocompetent individuals. In immunocompromised HIV/AIDS patients, cryptosporidiosis can result in severe outcomes, including death (Samie et al. 2014; Wang et al. 2018). In addition to causing fever and poor food absorption, *Cryptosporidium* causes pancreatitis, sclerosing cholangitis, and biliary tract blockage (Wang et al. 2018).

Diagnosis Tools for Cryptosporidiosis

The primary diagnostic methods used all around the world involve detecting DNA in fecal samples or *Cryptosporidium* oocysts in feces by microscopy. Diarrhea associated with cryptosporidiosis is watery, which is often a symptom of many other illnesses. As a result, infections with rotaviruses, coronaviruses, Salmonella spp., and Escherichia coli are included in the differential diagnosis for *Cryptosporidium* (Mehta 2002 Khurana and Chaudhary, 2008). The diameter of a *Cryptosporidium* oocyst ranges from 4 to 6 µm (Khurana and Chaudhary, 2008; Ahmed and Karanis 2018). Three fecal samples collected on different days should be examined microscopically in order to rule out a *Cryptosporidium* infection in people with severe diarrhea because the detection of *Cryptosporidium* oocysts in fecal challenging (Omoruyi et al. 2014; Khurana and Chaudhary, 2008). Additionally, the fecal sample needs to be concentrated with formalin-ether to increase the likelihood that an oocyst will be seen under a microscope (Pacheco et al 2013).

The Ziehl-Neelsen method and phenol-auramine staining are further options for staining the oocysts. Oocysts are colored red or bright yellow by the stains, respectively (Omoruyi et al. 2014; Khurana and Chaudhary, 2008). Despite being the most often used diagnostic tool and being simple to use and inexpensive, the microscopic diagnosis of Cryptosporidia oocysts has a low sensitivity (up to 30%). Furthermore, accurate diagnosis by microscopy heavily depends on the microscopist's training. According to some reports, staining oocysts with a modified acid-fast stain can boost sensitivity by up to 55%. The two highly sensitive and specific procedures to diagnose Cryptosporidiosis are the immuno-chromatographic test and enzyme-linked immunosorbent assay (ELISA) (Agnamey et al. 2011; Hawash 2014). Additionally, these antigen/antibody-based detection techniques are thought to be ineffective in patients whose oocyst load is below the cutoff (Hawash 2014). Additionally, these techniques are costlier than polymerase chain reaction (PCR), the industry-standard method for finding *Cryptosporidium* in stool samples. Microscopy, ELISA, and immunochromatographic tests have been found in earlier studies to be inferior to PCR in terms of sensitivity, specificity, and cost (Autier et al. 2018; Friesen et al. 2018). Along with being superior to other oocyst detection techniques, PCR is not always available in all laboratories. Additionally, this technology cannot be used in developing nations due to issues like cost and the requirement for technical skills.

Giardiasis

Food-borne giardiasis is a disease caused by the ingestion of food or water contaminated with the *Giardia* spp (Mozer et al. 2022). *Giardia* species have a typical life cycle that consists of two active trophozoite and cystic forms. By directly or indirectly ingesting infected cysts, this parasite spreads through the fecal-oral pathway. After eating cysts, the incubation period lasts somewhere between 9 and 15 days. The symptoms of this illness include diarrhea, abdominal pain, nausea, and vomiting, which can last for several days (Linscott 2011). In some cases, the symptoms may persist for several weeks, leading to severe dehydration and weight loss. According to Rendtorff (1954), the infective dosage might be as little as 10 cysts, making host-to-host transmission easier. *Giardias pp* spreads to new hosts via the faecal-oral pathway, which involves oral contact with cyst-containing food or drink or direct contact with human or animal feces. *Giardia is* not considered as an opportunistic infection that causes persistent symptoms and enteritis in immunocompromised persons. Giardiasis symptoms in HIV-positive people are comparable to those in HIV-negative people.

Life Cycle of Giardia

Giardiasis is an intestinal infection caused by the protozoan parasite, *Giardia spp* (Einarsson et al. 2016). The life cycle of *Giardia* involves two stages: the cyst and the trophozoite (Bernander et al. 2011). The cyst is the infectious stage of the parasite. It is a hardy, environmentally-resistant form that is shed in the feces of infected animals and humans (Gerba 2009). The cyst is capable of surviving outside of a host for several months, making it highly transmissible through contaminated food and water sources. Once the cyst is ingested by a host, it transforms into the trophozoite stage (Evans-Osses et al. 2017). The trophozoite is the active, motile form of the parasite. It attaches to the lining of the small intestine and begins to reproduce by binary fission (Ikbal et al. 2022). The trophozoite stage is responsible for the symptoms of giardiasis, which include diarrhea, abdominal pain, and bloating. After several days in the host's small intestine, the trophozoites undergo a process called encystation (Mendoza Cavazos et al. 2023). During this process, the trophozoites transform back into cysts, which are then passed out of the host in the feces (Smoguła et al. 2023). The cysts are shed into the environment through the feces of infected hosts. They can survive in water, soil, and on surfaces for several months, allowing for transmission to new hosts through contaminated food and water sources (Carmena 2010). Once ingested by a new host, the cysts

transform back into the active trophozoite form, continuing the life cycle of the parasite. Overall, the life cycle of *Giardia spp* is characterized by the alternation between the cyst and trophozoite stages, which allows the parasite to survive in a range of environments and infect new hosts (Ehrenkaufer et al. 2018).

Transmission of Giardiasis

Giardiasis is an intestinal infection caused by a microscopic parasite called *Giardiaspp*. This infection is usually transmitted through contaminated water, food, or surfaces (Balderrama-Carmona et al. 2017). The most common source of transmission of giardiasis is through the ingestion of water that has been contaminated with *Giardia*cysts (Daly et al. 2010). The cysts can survive in water for weeks, making it possible for people to become infected by drinking water from contaminated sources such as streams, lakes, or poorly maintained water systems (Karanis et al. 2007). People can also become infected by consuming food that has been contaminated with Giardia, such as raw or undercooked meat, fruits, or vegetables that have been washed with contaminated water (Moreira et al. 2005). Giardiasis can also be transmitted from person to person through the fecal-oral route (Bui et al. 2016). This means that people can become infected by coming into contact with the feces of an infected person, such as when caring for someone who is sick or changing the diaper of an infected child. People can also become infected by touching surfaces that have been contaminated with *Giardia*and then touching their mouths or face (De France et al. 2022). It's important to practice good hygiene, such as washing your hands regularly and thoroughly, avoiding drinking untreated water from natural sources, and properly preparing and cooking food, to reduce the risk of contracting giardiasis (Yakubovna et al. 2022).

Clinical Picture of Giardiasis

The clinical picture of Giardiasis can vary widely, with some people experiencing no symptoms, while others may have severe symptoms (Choutka et al. 2022). The symptoms of Giardiasis usually appear 1-3 weeks after infection and can last for several weeks to months. The most common symptoms of Giardiasis include Diarrhea - which can be watery or greasy, abdominal cramps and bloating, nausea and vomiting, loss of appetite and weight loss, fatigue, Excessive gas or flatulence, foul-smelling stools that may be pale or greasy, fever (low grade) (Sengupta and Chakraborty, 2023). In severe cases, symptoms can include, dehydration, anemia, malnutrition, and chronic diarrhea. In some cases, people with Giardiasis may experience recurring symptoms even after the infection has been treated (Beiting and John 2022). It is important to note that not everyone infected with *Giardia*will have symptoms, but they can still spread the infection to others. The risk of death from giardiasis is generally low, but it can occur in severe cases. The parasite can cause dehydration and malnutrition, which can be life-threatening if not treated promptly (Weil et al. 2020). Additionally, in rare cases, the parasite can cause complications such as pancreatitis or a bowel obstruction, which can also be life-threatening. Foodborne giardiasis can result in significant economic losses due to its impact on human health and productivity (Mateusa et al. 2023). The direct costs of giardiasis can include medical treatment, hospitalization, and lost productivity due to illness. Indirect costs can include lost income and decreased economic activity due to decreased productivity (Collier et al. 2012). In addition, outbreaks of foodborne giardiasis can have a significant impact on the food industry, resulting in decreased consumer confidence and reduced demand for affected products (Slifko et al. 2000). This can lead to decreased sales and revenue for food producers and retailers. Overall, the production losses caused by foodborne giardiasis can be significant and can have both short- and long-term impacts on individuals, businesses, and the economy as a whole (Daniel et al. 2020).

Diagnostic Tools for Giardiasis

The diagnosis of foodborne giardiasis can be made through a combination of clinical symptoms, laboratory tests, and epidemiological investigations (Smith et al. 2007). Some of the diagnostic tools used to identify giardiasis include, the use of stool examination to identify the presence of Giardia cysts or trophozoites. It is the most commonly used diagnostic tool for giardiasis and has high sensitivity and specificity Goka et al. 1990; Hooshyar et al. 2019). Antigen detection tests are also used to detect the presence of Giardia antigens in stool samples using immunological methods. They are typically used when the microscopic examination is inconclusive or when there is a need for rapid diagnosis (Gonçalves et al. 2002). Other techniques include PCR which is a molecular diagnostic tool that can detect the presence of Giardia DNA in stool samples. PCR has high sensitivity and specificity and can detect the parasite even in low concentrations. However, it is more expensive and requires specialized laboratory equipment (Stark et al. 2011; Laude et al. 2016). Serological tests detect the presence of antibodies against Giardia in blood samples (Gilpin et al. 2022). In addition to these diagnostic tests, epidemiological investigations can help identify the source of the outbreak and the food or waterborne transmission of the disease. This may involve interviewing patients and collecting information about their recent food and water consumption.

Prevention of Giardiasis

Prevention is key in controlling the spread of food-borne giardiasis (Hosseinian 2022). Proper food handling, preparation, and storage practices can help to prevent the

contamination of food with the *Giardia*parasite. Here are some prevention strategies that can be employed. Cleanliness practices such as hand washing and cleaning of surfaces used in food preparation can prevent contamination (Osafo et al. 2022). Safe food handling e.g Foods should be cooked at the appropriate temperature, refrigerated promptly, and reheated properly to avoid the growth of bacteria. Water purification like Drinking water should be treated, boiled or filtered to remove parasites and bacteria (Malan and Sharma 2023). Proper sewage disposal systems and regulations can reduce the risk of contamination of water sources. Safe agricultural practices in which the use of clean water for irrigation and the use of appropriate pesticides and herbicides can reduce contamination. Overall, food-borne giardiasis can be prevented through proper food handling, water purification, and safe agricultural practices (Desalegn et al. 2022). Awareness campaigns and education can also play a significant role in preventing the spread of this disease. By practicing good hygiene and following proper food handling practices, we can help to reduce the incidence of food-borne giardiasis and promote good health in our communities (Agbalaka et al. 2019).

Conclusion

Giardia and *Cryptosporidium* are two parasites that frequently go unnoticed and undiagnosed yet represent serious problems for public health globally. Despite the widespread occurrence and severe effects of these parasitic diseases, which are mostly seen in immunocompromised patients, there are significant flaws in the present control programs, particularly with regard to the diagnostic resources available. The majority of diagnostic procedures also frequently misdiagnose the illness in endemic regions. In order to more accurately detect infections and outbreaks and lessen the burden that these parasites place on the public health system, more evidence-based advancements in the diagnosis and treatment of giardiasis and cryptosporidiosis are necessary. It is necessary to create molecular approaches that are sensitive, specific, straightforward to use, affordable, and high throughput because early detection is the most effective way to combat the illness.

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Scabies

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INTRODUCTION

Sarcoptic scabies (animal scabies, pseudo-scabies, canine scabies) is a contagious skin disease that affects both humans and wild and domestic animals. It is caused by the mite *Sarcoptes* (S.) *scabiei* (Bandi and Saikumar 2013; Chandler and Fuller 2019; Rowe et al. 2019; Turchetto et al. 2020; Moroni et al. 2022). It is transmitted to humans through contact with other infected humans or animals (Bandi and Saikumar 2013; Moroni et al. 2022). Scabies affects more than 150 mammalian species worldwide (Moroni et al. 2022). It is regarded as a permanent parasite, with a short life cycle. Diagnosis is confirmed by observing its presence in multiple superficial skin scrapings (Moroni et al. 2022).

The history of scabies was described by Dr. Reuben Friedman in the first half of the 20th century. In the Old Testament, "*zaraath"* is the term used for scabies. Aristotle and Galen noted the contagious nature of scabies, and the former used the term 'mite'. Celsus described sheep scabies and its treatment. Avenzoar described mites as small flesh worms that crawl under the skin and cause water-filled pustules. In the 13th-16th centuries, the presence of mites was observed in scabies lesions, but the causal link was not established. In the 17th century, Hauptman sketched imperfect mites. Giovanni Cosimo and Diacinto Cestoni studied the disease in sailors and produced a drawing of the mite in 1687 (Currier et al. 2011). In 1746, Linnaeus classified the mite as *Acarus humanus-subcutaneous*. The first accurate illustration of the parasite was sketched by DeGeer. Simon Françoís Renucci obtained a mite specimen from a young girl suffering from "the itch" on August 13, 1834. In the late 19th and early 20th centuries, Ferdinand Ritter von Hebra described the life cycle and stages of infection. Kenneth Mellanby described measures for scabies environmental disinfection during World War II (Currier et al. 2011). Scabies was listed by the World Health Organization as a neglected tropical disease in 2017 (Moroni et al. 2022).

Etiology

Scabies is caused by *Sarcoptes* (*S.*) *scabiei*. The name of the parasite comes from the Greek word *sarx*, meaning 'flesh', and *koptein*, meaning 'to cut', plus the Latin word *scabere*, meaning 'to scratch' (Hicks and Elston 2009). It is an arthropod of the class Arachnida, subclass Acari, order Astigmata, suborder Acaridida (Astigmata)—because it has no detectable spiracles or tracheal system— and family Sarcoptidae. Several, host-specific varieties have been described in the genus *Sarcoptes* (*S. scabiei* var. *canis*, *S. scabiei* var. *bovis*, *S. scabiei* var. *suis*, *S. scabiei* var. *equi*, *S. scabiei* var. *aucheniae*, *S. scabiei* var. *cuniculi*, *S. scabiei* var. *ovis* and *S. scabiei* var. *caprae*, which parasitize dogs, cattle, pigs, horses, llamas and alpacas, rabbits or goats, respectively). The subspecies infecting humans is *S. scabiei* var. *hominis*, which is distinct from that affecting animals (Burgess 1994; Chosidow 2006; Hicks and Elston 2009; Aydıngöz and Mansur 2011; Agusti et al. 2012; Gallegos et al. 2014).

The female mite is 300–500 µm long, 230–420 µm wide, and the male is 213–285 µm long, 162–210 µm wide (Burgess 1994). *S. scabiei* has a thin cuticle without heavily sclerotized scutes, a head with brown sclerotized mouthparts, and no division between abdomen and cephalothorax (Hicks and Elston 2009; Gallegos et al. 2014). It is pearly white or creamy white in color, translucent, small, oval, and flattened in shape, with eight legs attached to the ventral surface of the cephalothorax. The first two pairs of horny legs bear two claws (Chouela et al. 2002).

Epidemiology

Scabies is a globally distributed disease; however, it is most common in developing countries. It affects various domestic and wild species, as well as humans, as *Sarcoptes* variants have evolved to infest specific host species. Transmission occurs primarily through direct contact, which is favored by crowding or when animals are kept together in confined areas (Foreyt 2001).

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The exact number of infected cases are still unknown, however, over 300 million people are estimated to be affected. It is considered endemic mainly in tropical regions, with a variable prevalence, which in certain regions can be 5–10% (Hay et al. 2012). In a study conducted in Brazil in 2005, a frequency of 8.8% was determined in poor neighborhoods, versus 3.8% in a fishing community. However, this study found no variation in frequency in different seasons of the year (Heukelbach et al. 2005) in contrast to the findings of Mimouni et al. (2003), who reported a higher frequency in winter, probably favored by overcrowding during colder months.

Historically, younger age groups have been more vulnerable, and the frequency decreases in adults, while increasing again in the elder people. This distribution is consistent with the findings of Lapeere et al. (2008) in Belgium, where the incidence was higher in the elderly. In a study conducted in Cuba in children aged 0–14 years diagnosed with scabies, 69% were younger than 1 year. No difference was found with respect to gender. Interestingly, 45% of cases were in the poor socioeconomic segment (Saldaña 2020), in agreement with the findings reported in Brazil by Feldmeier et al. (2008) in which in addition to poverty, a low educational level was mentioned as a risk factor, possibly due to poverty conditions as a base element.

In the United Kingdom, Lassa et al. (2011) conducted a study and found a higher frequency in females than in males, with a relative risk of 1.24, along with a higher frequency in people aged 10–19 years. The authors identified an epidemic cycle of 15–17 years.

Pathogenesis

Scabies occurs mainly in immunocompromised patients, whether due to HIV infection, steroid treatment (systemic or topical), transplant surgery, or hematological malignancies (Remartínez et al. 2009). It is also prevalent in patients with physical or mental disability, including paralysis, sensory neuropathy, leprosy, or Down syndrome (DS). Subjects with an inability to perceive pruritus or those who are incapable of scratching are also susceptible (Singh et al. 2011; Roldán-Franco et al. 2019).

The biological cycle of the mite starts when female and male mate on the skin surface. A single copulation is sufficient for a lifetime of egg-laying. After mating, the male dies and the female digs shallow passages called burrows, where she lays eggs (Gallegos et al. 2014). The gravid female reaches the stratum corneum using her jaws and cutting claws. As she advances, she sucks tissue fluids to feed, leaving feces behind as she continues to burrow (Hicks and Elston 2009). Saliva and feces provoke a hypersensitivity reaction, causing widespread inflammatory responses in the skin (Currie and McCarthy 2010).

The female lays 2–3 eggs per day, which hatch after approximately 55 hours to produce nymphs that resemble the adult mite but are smaller and only have three leg pairs. The nymphs leave the burrow one day later and move to the skin surface. The adult female dies after 5 weeks. During this time, she will spread the burrow at a speed of 0.5–5 mm per day. She can survive for 24–36 hours at room temperature (Chouela et al. 2002; Hicks and Elston 2009).

The mite population can increase to 25 adult females after 50 days, and up to 500 mites by 100 days, producing the cutaneous eruption characteristic of classical scabies. This is caused by both infestation and hypersensitivity reaction (Chosidow 2006; Hicks and Elston 2009) and is observed as intensely pruritic erythematous furrows, vesicles, crusts, and papules resulting from a type IV delayed hypersensitivity reaction. Scabies can also occur as a psoriasiform dermatosis, affecting hands and feet (Gallegos et al. 2014; Harris and Vincek 2017). Lesions are most common on hand interdigital membranes and periungual areas, flexor surfaces of the wrists, the scalp, face and back (Currie and McCarthy 2010; Palaniappan et al. 2021).

Transmission

Scabies is transmitted primarily by close contact with infected individuals and, less frequently, by sharing clothing, sheets, or towels (Aussy et al. 2019). Although few mite subspecies can infect humans, there is a great variety of subspecies that can infect animals (de Gentile and Carsuzaa 2013). In animal-human transmission, companion animals are the main transmitting agents, followed by production animals such as rabbits, cattle (bovines and buffaloes), llamas, and pigs, and to a lesser extent, wild species such as gazelles and monkeys, due to the unusual contact between these species and humans (Moroni et al. 2022).

Clinical Variants

Three clinical variants of scabies are recognized. According to the symptoms, it can be classic/simple, crusted/profuse, or nodular (Plascencia et al. 2013).

Classic scabies. The main signs are pruritus and the occurrence of furrows and vesicles. Intense pruritus is an effect of mite burrowing, and in generalized cases it is related to an allergic reaction, with a typical increase in immunoglobulin E (IgE) levels. Furrows and vesicles are often found in the interdigital spaces of the hands and in the folds of the anterior aspect of the wrist, while the axillary folds, mammary papillae, umbilicus, genital organs, back, scalp, and face are less frequently affected; however, the entire body may be involved (de Gentile and Carsuzaa 2013). Pruritus begins 4–6 weeks after infection. In cases of reinfestation within 6 months after initial infection, it will develop within hours or days. In very severe cases, disseminated erythematous papules, excoriations, hemorrhagic crusts, linear scrapes (dermatitis), vesicles, and often pustules are found due to secondary bacterial infection.

Bruising problem which is secondary to rubbing and scratching is also common. The severity of signs varies from person to person (Richards 2020).

Crusted scabies. It occurs in immunocompromised patients. Its main trait is the absence of pruritus, which makes it difficult to diagnose. Lesions and desquamation products carry abundant parasites, which increases its infectivity (Barrutia 2021). It presents with marked hyperkeratosis involving the limbs, including subungual areas, although lesions may be generalized. Peripheral eosinophilia is usually the main sign in patients with keratinization disorders (Galiana et al. 2003). Compared to classical scabies, crusted scabies presents with localized keratotic plaques on the limbs, trunk, pinnae, and eyelids (Tirado-Sánchez et al. 2016).

Nodular scabies. It presents with erythematous nodules up to 2-cm in diameter. It is the least common variant (7%), and it mainly involves the buttocks, genitals, groin, or armpits. These lesions may be the result of a hypersensitivity reaction to the mite's secretion products (Plascencia et al. 2013).

Diagnosis

Definitive diagnosis requires microscopic detection of the mite, its feces, or eggs. However, in classical scabies the number of mites is scarce, so this method is limited, and a negative examination does not rule out the diagnosis. Therefore, physical examination and a compatible history allow establishing a diagnosis of suspicion and initiating treatment (Barrutia 2021). There are several diagnostic methods, including the following:

Müller's test: It consists of a cutaneous scraping, applying 1–2 drops of mineral oil or petroleum jelly on the lesion, which is scraped with a scalpel blade to extract the upper part of the tunnels. The sample is placed and spread on a slide, covered with a coverslip, and observed under a microscope (Morgado-Carrasco et al. 2021).

Burrow ink test: It consists of the direct application of blue-black ink on suspicious lesions, cleaning the surface with alcohol afterwards. The ink penetrates in the epidermal tunnels excavated by mites, facilitating the visualization of the furrow and, thus, differential diagnosis is possible with other pruritic dermatoses (Silvestre et al. 2020).

Dermatoscopy or epiluminescence microscopy: With this technique, the parasite can be observed in situ at 10X magnification. The sensitivity of this technique is 91%, and its specificity is 86% (de Gentile and Carsuzaa 2021). Small triangular structures can be observed, which correspond to the pigmented anterior section of the mite, and a linear segment behind the triangle, which contains small air bubbles, corresponds to the tunnels, eggs, and feces of the parasite (Morales and Matute 2008).

***In vivo* reflectance confocal microscopy (RCM):** Its usefulness has been reported to diagnose scabies and other parasites (Morgado-Carrasco et al. 2021). This technique allows a rapid and non-invasive confirmatory diagnosis. In scabies, it allows real-time observation of mites, eggs, and scybala. This technique also allows to monitor the response to treatment, as indicators of active infection can be observed, such as the presence of eggs in the furrows (Fusta et al. 2019).

Polymerase chain reaction (PCR): A PCR assay has been used recently to demonstrate scabies in patients presenting with clinically atypical eczema. In these cases, epidermal scales are usually PCR-positive for *S. scabiei* DNA before treatment and negative two weeks after treatment (Morales and Matute 2008).

Treatment

Various alternatives are available to treat scabies. The choice will depend on the clinical presentation and the patient's resources. In addition to medication, hygienic measures are required for a successful treatment, such as thorough cleaning of bedding and contaminated clothing, as well as the disposal of fomites that have had contact with companion animals or production animals, if these were the transmitters of the parasite (FitzGerald et al. 2014).

Cleaning measures are accompanied by the application of specific chemical agents against scabies; the best known are benzyl benzoate, lindane (1%), esdepallethrine/piperonyl butoxide, pyrethroids, macrocyclic lactones (de Gentile and Carsuzaa 2021), crotamiton, methotrexate, and sulfur (Plascencia et al. 2013). Other compounds have also been reported as active against the parasite, such as beuvericin, which at a concentration of 0.5% was effective in eliminating both adult parasites and eggs (AlKhoury et al. 2020).

Alternative treatments with plant extracts to eliminate the parasite have also been reported (Nakamura et al. 2022). A recent study lists about 28 plants, including fruit trees such as papaya (*Carica papaya*), where the whole plant can be used for treatment with effective results (Akram et al. 2020). The efficacy of some plants in eliminating the parasite can be due to their content of active compounds like alkaloids, tannins, flavonoids, and coumarin derivatives (Altaf et al. 2018).

Prevention

The disease can be prevented in humans by considering some risk factors, particularly avoiding overcrowding, which can be especially difficult in vulnerable groups because it involves changing the economic conditions in a household. In places such as hospitals, nursing homes for the elderly or schools, where outbreaks occur with some frequency, early detection and effective treatment are important (Jadraque et al. 2010). Likewise, health promotion activities should be carried out in endemic sites to enable the population and animal owners to recognize the routes of transmission of the disease and identify the problem at an early stage (Peraza 2021).

Those individuals who having close contact with animals or people infected with scabies should wear gloves, especially

when a person or animal is suspected of being infected (Jadraque et al. 2010).

Zoonosis

Scabies is a zoonotic disease that affects humans and a wide range of domestic and wild animals (Aydıngöz and Mansur 2011). It has been reported that the mite is not species-specific, but can temporarily live on other species, giving rise to cross-infection (Aydıngöz and Mansur 2011; Gallegos et al. 2014). Moroni et al. (2022) conducted a literature review on the zoonotic transmission of this parasite, focusing on outbreak sources, transmission, and diagnosis of strains involved in human cases, as well as on the treatments applied. Among the nine species of companion animals identified, dogs, cats, and goats accounted for the highest number of transmission cases, while miniature pigs, horses, rabbits, water buffaloes, llamas, and cattle were identified in a smaller proportion as transmission sources for their owners. Other domestic animals, and wild species (foxes, wombats, gazelles, chamois, and monkeys) may also serve as an occupational source for spread of disease to human.

Epidemiology: Parasitic diseases are very frequent, particularly, scabies due to *S. scabiei* var. *canis* shows a high zoonotic potential, accounting for 2–4% of all dermatological cases. It is noteworthy that there is not much information on prevalence indicators in animal populations, probably because when a case is identified, it is treated on a casuistic basis, with no records on its incidence or prevalence (Gakuya et al. 2012).

Pigs

Several works have reported the presence of scabies in pigs. The transmission from adults to young pigs is important, especially during lactation. Cordero et al. (2001) and Pedroso-de-Paiva et al. (2003) found that the key risk factors associated with the presence of disease are that pigs inhabit an area of less than 0.85 m^2/pig and have an air volume of less than 3.0 m^3/animal.

Dogs

Some authors report that it affects dogs of any breed, sex, or age, and can sometimes occur sub-clinically (Corrales et al. 2001), although others point out that it mainly affects young and short-haired animals (Quintero 2006).

Wild Species

Bornstein et al. (2001) reported the presence of scabies in six primate species, 11 canids, nine felids, six mustelids, two procyonids, and a wide variety of artiodactyls, as well as in rodents, lagomorphs, marsupials, and insectivores. Therefore, it is likely to affect more species than reported in the literature. Gakuya et al. (2012) reported this parasitic disease in African lions, gazelles, wildebeests, and cheetahs, with infection frequencies up to 12.7% in the latter species, and marginal frequencies of less than 1% in the others. It is noteworthy that the highest frequency was found in areas of coexistence with domestic species in the Masai Mara region, in Kenya.

Rasero et al. (2010) conducted a study in three European countries, using ten specific markers for *Sarcoptes* and determining the genotype through microsatellites. Variations in genotype were observed according to geographical segregation, with three major groups according to the host: herbivorous, carnivorous, and omnivorous. Segregation has generated new mite subpopulations, indicating host-specific adaptation of the parasites, as Walton et al. (2004) previously described in Australia.

Sheep and Goats

In sheep and goats, the *Sarcoptes* mite causes skin thickening, scabs, and alopecia around the mouth, in addition to erythematous papules around the eyes, ears, and legs, resulting in great economic losses for owners (Lastuti et al. 2018). Unshorn sheep are more affected, as humidity and dirt favor the perpetuation of the parasite life cycle. In Indonesia, prevalence rates of 5–100% were reported in goat herds, and mortality could be high in young animals, increasing the production costs for these animals (Lastuti et al. 2018).

Transmission: It occurs by direct contact, but cases of transmission by contaminated objects have been reported, as these parasites can survive for some time outside the body of the animals. Clothing, cleaning utensils, bedding, harnesses, and blankets can be sources of contamination. In pigs and other animals, viable parasites have been found on the walls and bars of pens. Although each animal species is a reservoir of the mite for its conspecifics, cross-transmission between different species has also occurred (Aussy et al. 2019).

Recent studies in Japan have found other routes of animal-to-animal transmission, such as hunter-prey interaction. This is not limited to wild species, but it can also be observed in the interaction of feral dogs and cats with their prey (Matsuyama et al. 2019).

Signs: Similar lesions occur in all animal species affected by this parasitosis. They begin or end at the point where the mite first enters the skin. Initially, small red papules and erythema of the skin may be observed, showing the entry site of the mite, as well as a local serous exudation that transforms into a superficial wet coagulum, with intense pruritus. Continued irritation gives rise to a subacute dermatitis with active parakeratotic proliferation and the formation of thin crusts, which eventually thicken and desiccate due to the large numbers of bacteria growing on them (Jubb et al. 2007); thus, secondary bacterial infections are frequent (Gakuya et al. 2012). Hairs are lost in these areas, and the skin thickens and shows discoloration (Quintero 2006).

Two clinical presentations can be found in pigs: the first one resembles an allergy, and it is common in young animals and piglets and after 2–10-week incubation, numerous red spots are observed all over the body of the animals. The second one, which manifests as hyperkeratosis, is usual in adult and old animals; the main sign is pruritus; additionally, the skin of the tail, snout, legs, and the inner side of ears in these animals shows abundant scabs (Fernández et al. 2018).

Treatment: Various topical scabicide products are available in the market. Sulfur formulations are less used nowadays as these may cause dermatitis. Benzyl benzoate, lindane, and crotamiton are commonly used. Topical acaricides available include permethrin 5%, deltamethrin 0.02%, lindane 1%, sulfur petrolatum 6–10%, crotamiton 10%, and in refractory cases oral ivermectin is recommended at a dose of 200 µg/kg once, repeated after two weeks (Osman et al. 2006).

Prevention: In animals, primary prevention requires adequate environmental sanitation, including washing and disinfection of areas where affected animals are kept, as well as avoiding overcrowding (Valdés 1997; Pedroso-de-Pavia et al. 2003). In dogs, it is common to apply secondary prevention, through early diagnosis and timely treatment. In that sense, it is advantageous that the disease is visible and can be diagnosed in early stages. For slaughter animals, it is important to separate the affected animal(s) to avoid transmission to the rest of the animals, and to apply the appropriate treatment. An integrated health approach is required to prevent the infection and, if necessary, control scabies outbreaks in places where cases have been observed, and where coexistence between humans, domestic, and wild animals is common (Rubini 2021).

Conclusion

Scabies is a neglected parasitic disease that is a major public health problem in many resource-poor regions. It causes substantial morbidity from secondary infections and post-infective complications. It is a disease of zoonotic importance, which affects different species and man, causing great economic losses. So, it is important to maintain a prevention and control system, especially in those species that are in close contact with each other and with humans.

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