# Coproantigen as a Tool for Monitoring Echinococcus Granulosus Infection in Definitive Host

28

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## ABSTRACT

This chapter delves into the utilization of Coproantigen as a diagnostic tool for *Echinococcus granulosus* infection in definitive hosts, primarily focusing on canine echinococcosis. *Echinococcus granulosus*, the causative agent of cystic echinococcosis, poses a significant public health challenge due to its zoonotic nature. The traditional diagnostic methods face limitations in sensitivity and specificity, highlighting the need for more efficient techniques. The chapter extensively explores the morphology, life cycle, and transmission dynamics of E. granulosus, emphasizing its diverse impact on both definitive and intermediate hosts. Significant attention is devoted to the Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test, a groundbreaking advancement in the field of veterinary parasitology. This test exhibits high specificity and sensitivity, enabling early detection of infections in dogs, even before the presence of eggs in faeces. The chapter evaluates the advantages, limitations, and practical applications of the Coproantigen test, including its role in active surveillance, monitoring treatment efficacy, and implementation in endemic regions. The comprehensive analysis underscores the necessity of incorporating the Coproantigen test into broader echinococcosis control programs. Combining this diagnostic tool with other methods and enhancing its accessibility in resource-limited settings are crucial steps towards effective management of the disease. This chapter contributes significantly to our understanding of canine echinococcosis and presents viable strategies for mitigating its zoonotic threat to public health.

## CITATION

Mohammed TA, 2023. Coproantigen as a tool for monitoring *Echinococcus granulosus* infection in definitive host. In: Abbas RZ, Hassan MF, Khan A and Mohsin M (eds), Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan, Vol 2: 346-360. https://doi.org/10.47278/book.zoon/2023.75

**CHAPTER HISTORY** Received: 23-March-2023 Revised: 20-April-2023 Accepted: 19-July-2023

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## 1. INTRODUCTION

Echinococcosis, caused by the dwarf dog tapeworm, is a highly endemic and significant parasitic zoonotic disease worldwide (Borji et al. 2013; Ananda et al. 2015). Its historical impact on human health is long-standing, with references to the disease found in ancient texts of Judaism, such as the Talmud, indicating the recognition of echinococcosis by Jewish people since ancient times (OIE 2022).

The scientific understanding of echinococcosis began to emerge in the 17th century when it was discovered that hydatid cysts found in infected humans were of animal origin. In 1766, Pierre Simon Pallas correctly predicted that the hydatid cysts found in humans were actually larval stages of tapeworms (Howorth 1945). Further progress in understanding the disease occurred in the 18th and 19th centuries. In 1782, the cyst and head of the tapeworm were described by Goeze, and in 1786, *Echinococcus (E.) granulosus* was accurately described by Batsch (Tappe et al. 2008). During the mid-19th century, Carl von Siebold conducted experiments that confirmed *E. granulosus* was causing the hydatid cysts in dogs. In the following decades, more details about *E. granulosus* and its life cycle, as well as its role in causing the disease, were elucidated (Howorth 1945).

### 1.1 TAXONOMY OF *ECHINOCOCCUS GRANULOSUS*

The taxonomy of the genus Echinococcus has undergone various reviews over the years, with earlier classifications based on morphological and biological observations of natural and experimental infections (Kumaratilake and Thompson 1982; Thompson 2017). The current arrangement of the genus Echinococcus within the family Taeniidae is shown in Table 1.

Within the genus Echinococcus, there are nine recognized species that affect humans and animals, as shown in Table 2. Among these, *E. granulosus sensu lato* is a complex of ten genotypes (G1-G10) that includes four distinct species*: E. granulosus sensu stricto* (G1-G3), *E. equinus* (G4*), E. ortleppi* (G5), and *E. canadensis* (G6-G10). Each species exhibits unique morphological, epidemiological, and transmission dynamics characteristic (Torgerson et al. 2006; Nakao et al. 2007; Hüttner et al. 2008; OIE 2022).

Advancements in genetic studies have confirmed the high intra-specific variation within Echinococcus species, leading to the recognition of the *E. granulosus* sensu lato complex with its ten genotypes. These genotypes differ in various aspects, shaping the epidemiology and transmission patterns of the disease (OIE 2022).

While significant progress has been made in understanding echinococcosis, challenges remain in the effective monitoring and control of the disease. Its complex life cycle, varying genotypes, and different transmission dynamics call for robust diagnostic methods, like the Coproantigen ELISA test, to detect and monitor infections in dogs accurately. Addressing the limitations of diagnostic tools and incorporating comprehensive surveillance and control strategies are crucial to combat this zoonotic disease effectively and protect both human and animal populations from its impact (Borji et al. 2013; Ananda et al. 2015).

### 1.2 MORPHOLOGY OF *ECHINOCOCCUS GRANULOSUS*

The morphology of *E. granulosus* involves distinct life stages, each with unique characteristics.

#### 1.2.1 EGG

The eggs of *E. granulosus* are spherical or ovoid in shape and measure about 24-40 µm in diameter (Fig. 1). They consist of two layers; an outer thin wall or shell, and an inner embryophore with radial striations. The embryophore contains a developing oncosphere, which is the first larval stage of the parasite. The oncosphere has three pairs of hooklets that are used for penetration into the host's tissues (Soulsby 1982; Zhang et al. 2021; OIE 2022; Teroj 2022).

Echinococcus eggs are very resistant to environmental conditions and can remain infective for up to a year in soil (Lahmar et al. 2007).They can also survive in water and damp sand for several weeks at specific temperatures. However, they are sensitive to direct sunlight, dry heat, freezing, and chemical disinfectants (OIE 2022). The eggs are passed in the feces of the definitive host (usually dogs or other canines) and are ingested by intermediate hosts (such as sheep, cattle, or humans). The peak period of egg production by the adult tapeworm occurs 40 to 80 days after infection (Soulsby 1982).

**Table 1:** Taxonomy of the genus Echinococcus

| Phylum | Class | Subclass | Order | Family |
|---|---|---|---|---|
| Platyhelminths | Cestoda | Eucestoda | Cyclophyllidea | Taeniid |

**Table 2:** Classification of the genus Echinococcus

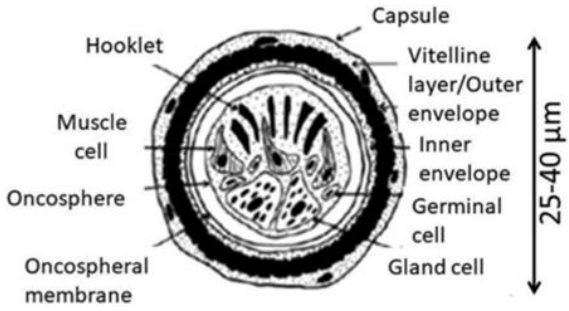
| Genus | Note |
|---|---|
| *E. granulosus sensu stricto* (G1-G3) | This is the most common and widespread species that causes cystic echinococcosis in humans and animals. It mainly infects dogs as definitive hosts and sheep, goats, cattle, camels, and pigs as intermediate hosts. It has a cosmopolitan distribution and is endemic in many regions of Africa, Asia, Europe, Oceania, and South America. |
| *E. equinus* (G4) | This species mainly infects horses as intermediate hosts and dogs as definitive hosts. It has a restricted geographic distribution and is mainly found in Europe, Africa, and Asia. It causes cystic echinococcosis in humans but at a lower frequency than *E. granulosus sensu stricto.* |
| *E. ortleppi* (G5) | This species mainly infects cattle as intermediate hosts and dogs as definitive hosts. It has a worldwide distribution but is more prevalent in Africa and South America. It causes cystic echinococcosis in humans but at a lower frequency than *E. granulosus sensu stricto* (Torgerson et al. 2006; Davidson et al. 2012). |
| *E. canadensis* (G6-G10) | This species comprises five genotypes that infect different intermediate hosts such as camels, cervids, pigs, rodents, and lagomorphs. It has a worldwide distribution but is more prevalent in North America, Asia, and Africa. It causes cystic echinococcosis in humans but at a lower frequency than *E. granulosus sensu stricto*. |
| *E. multiloculari s* | This is the most pathogenic species that causes alveolar echinococcosis in humans and animals. It mainly infects foxes as definitive hosts and rodents as intermediate hosts. It has a circumpolar distribution and is endemic in many regions of Asia, Europe, North America, and Alaska. |
| *E. oligarthrus* | This species mainly infects felids as definitive hosts and rodents as intermediate hosts. It has a neotropical distribution and is mainly found in Central and South America. It causes polycystic echinococcosis in humans but at a very low frequency. |
| *E. vogeli* | This species mainly infects bush dogs as definitive hosts and rodents as intermediate hosts. It has a neotropical distribution and is mainly found in Central and South America. It causes polycystic echinococcosis in humans but at a very low frequency. |
| *E. shiquicus* | This species mainly infects Tibetan foxes as definitive hosts and plateau pikas as intermediate hosts. It has a restricted geographic distribution and is only found in the Tibetan plateau of China. It causes alveolar echinococcosis in humans but at a very low frequency. |
| *E. felidis* | This species mainly infects lions as definitive hosts and antelopes as intermediate hosts. It has a restricted geographic distribution and is only found in the Serengeti National Park of Tanzania. It causes cystic echinococcosis in humans but at a very low frequency. |

### 1.2.2 ADULT STAGE

The adult stage of *E. granulosus* is a tapeworm that resides in the small intestine of the definitive host. It is typically less than a centimeter long (2-7 mm) and consists of 3-5 proglottids (Fig. 2), occasionally up to 6 proglottids (Soulsby 1982; Muller et al. 2002). Each proglottid has a single genital opening on the lateral margin that alternates irregularly from side to side. The first two proglottids are immature, While the penultimate proglottid is sexually mature and contains male and female organs. The last proglottid is gravid

(full of eggs) and constitutes about half of the total length of the tapeworm (Constantine et al. 1993; Muller et al. 2002; Zhang et al. 2021; Teroj 2022).

The scolex (head) of *E. granulosus* is characteristic of taeniids. It has a rostellum (a protrusion) with 28-40 small hooks (22-34 µm) and large hooks (30-40 µm) in length (Fig. 3). It also has four muscular suckers that help to attach to the intestinal mucosa of the host. These features are essential for the survival and reproduction of the tapeworm in its adult stage. The scolex can also be used for identification purposes (Shield 1969; Reissenweber et al. 1975; Constantine et al. 1993; Teroj 2022).



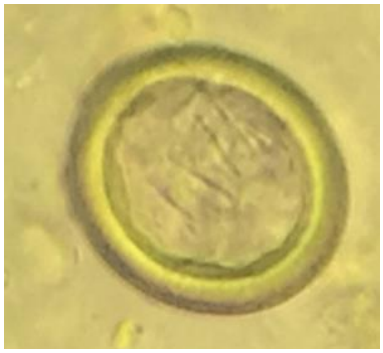



**Fig. 1:** Morphological structure of *E. granulosus* egg (Zhang et al. 2021 and Teroj 2022)

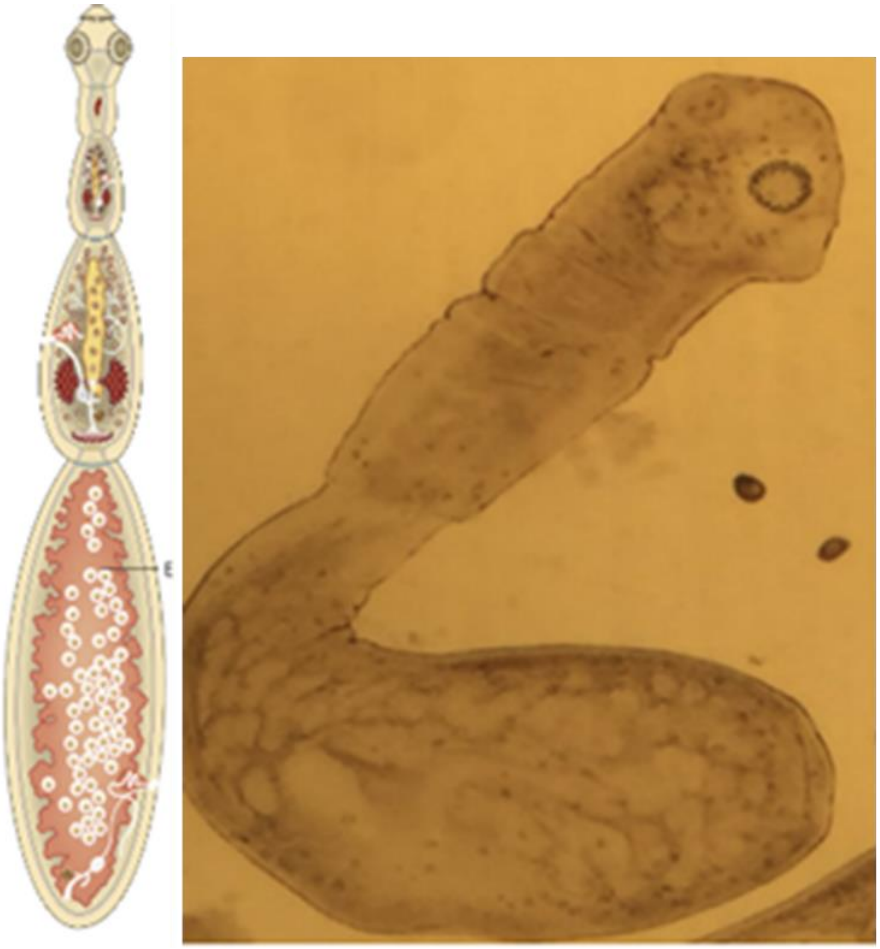


**Fig. 1:** Morphological structure of adult *E. granulosus* (Zhang et al. 2021 and Teroj 2022)

### 1.2.3 HYDATID CYST

The hydatid cyst is the larval stage of *E. granulosus* and can develop in various organs of the intermediate host, especially the liver and lungs. It has a complex structure composed of several distinct components. The outermost component is the laminated membrane, which forms a non-nucleated hyaline cuticle of about 1 mm thickness. It is produced by the germinal layer, which lines the inside of the cyst and overlays the laminated layer. The germinal layer is attached to the laminated layer by finger-like processes that extend into it (Fig. 4). This layer is permeable to water (PUMP 1963; Richards et al. 1983; Ingold et al. 1998; Brunetti et al. 2010).

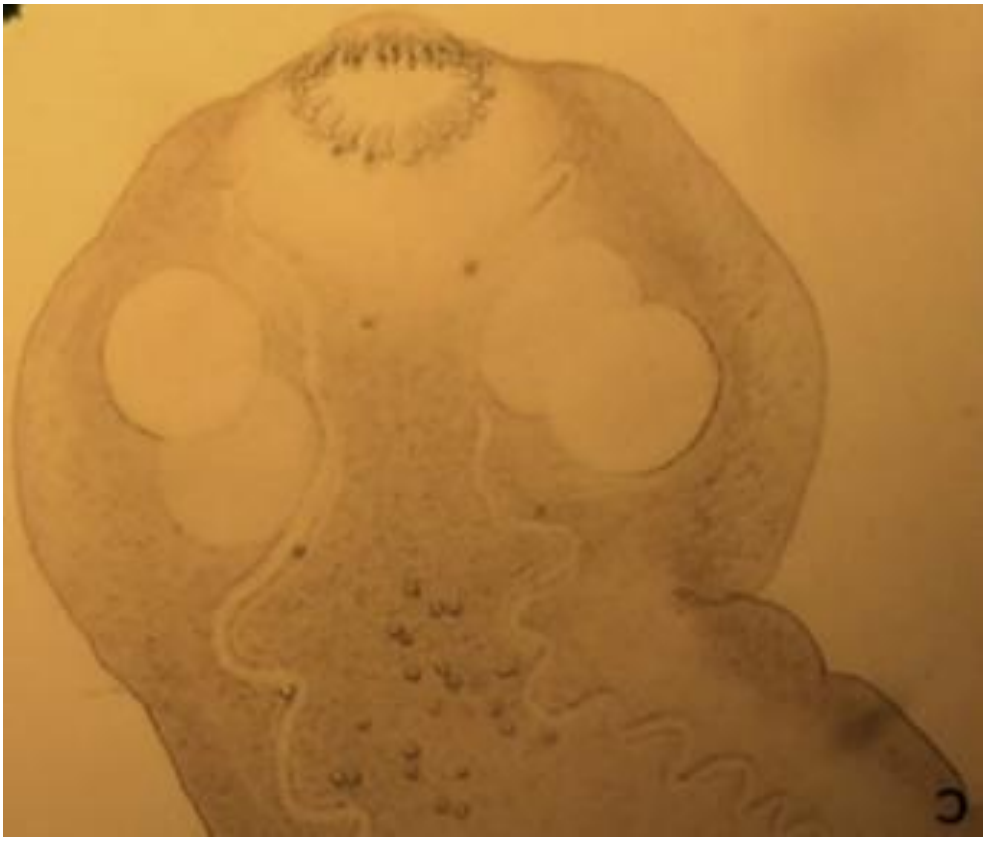


**Fig. 2:** Morphological structure of *E. granulosus* scolex (Teroj 2022)

The cyst contains a fluid called hydatid fluid, which is typically colorless or slightly yellowish. It has a specific gravity of 1.012 and a pH ranging from 6.7 to 7.2. The hydatid fluid comprises various components such as albumin, creatinine, lecithin, urea, a small amount of glucose, sodium chloride, sodium, calcium, and enzymes (Kassis and Tanner 1976).

Another essential component of the cyst is the brood capsule, which has a highly vacuolated wall with nuclei present at irregular intervals. Within the brood capsule, debris from degenerated protoscolices can be observed. Surrounding the entire cyst is a fibrous tissue capsule (Gottstein 1992). In some cases, the cysts may be sterile and fail to produce brood capsules (acephalocyst), while in others, the brood capsules may not generate scolices (Paul and Stefaniak 1997).

The protoscolices are another critical element of the cyst. They consist of scolices with a rostellum and suckers that can be either invaginated or evaginated. Microscopically, they appear grain-like, and hydatid sand refers to the granular material containing free protoscolices, daughter cysts, hooks, and calcareous bodies (Mehlhorn 2008).

The hydatid cyst is a significant stage in the life cycle of *E. granulosus* and can cause cystic echinococcosis in humans and animals. The complex components of the hydatid cyst highlight the remarkable adaptability of this parasite within its hosts, making it a challenging and significant public health concern (Zeibig 1997; Budke et al. 2013).

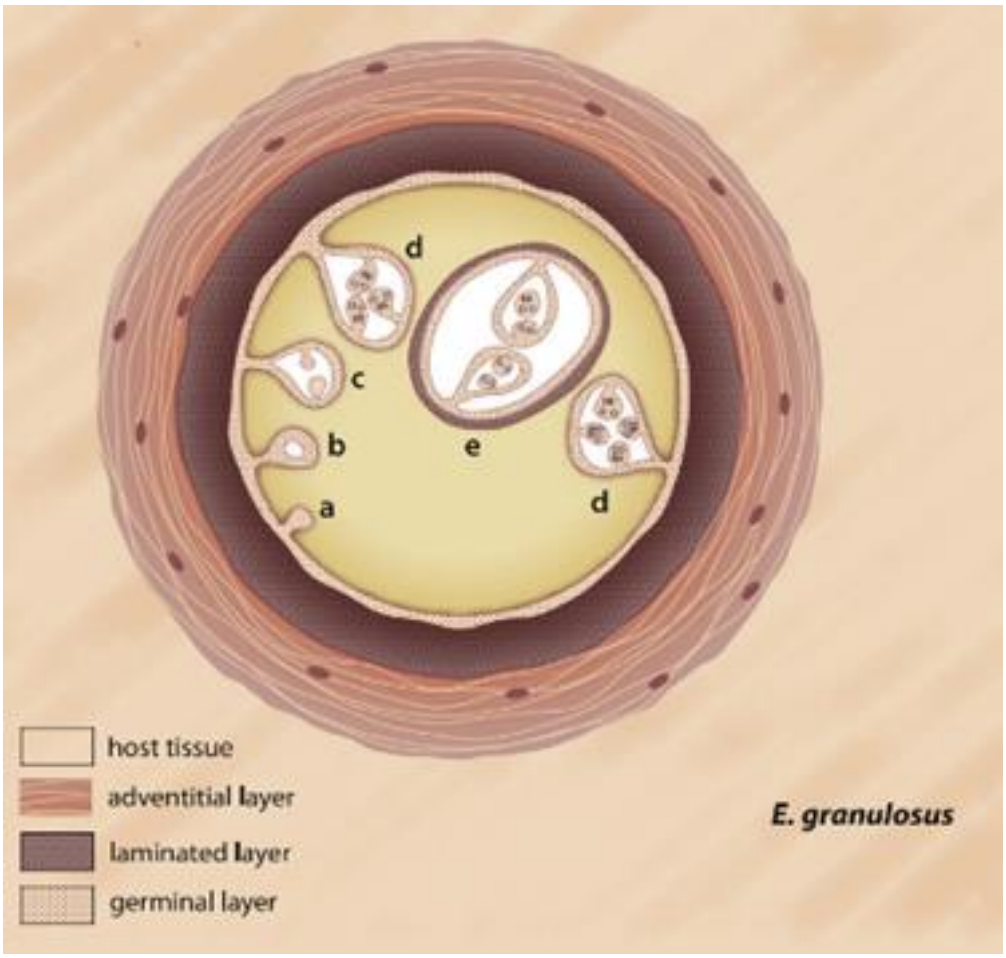



**Fig. 3:** Morphological structure of *E. granulosus* hydatid cyst (Brunetti et al. 2010)

### 1.3 LIFE CYCLE OF *ECHINOCOCCUS GRANULOSUS*

The life cycle of the genus Echinococcus can be divided into two main kinds based on the hosts involved: the domestic or pastoral life cycle and the natural or sylvatic life cycle. Each kind involves different definitive and intermediate hosts, highlighting the adaptability and complexity of the parasite's life cycle (Sing 2015; Bowman 2020).

#### 1.3.1 DOMESTIC OR PASTORAL LIFE CYCLE

In the domestic or pastoral life cycle, dogs serve as the definitive host, where the adult tapeworms reside in their small intestine. The infection in dogs occurs when they ingest uncooked offal, such as the liver, lungs, or other organs from intermediate hosts, which containing fertile metacestodes. Domestic farm animals, including sheep, goats, and cattle act as the intermediate hosts in this cycle. These intermediate

hosts become infected by ingesting the eggs of Echinococcus through contaminated food, water, or vegetation (Morar and Feldman 2003).

Once inside the dog's small intestine, the eggs hatch, releasing the oncosphere. The oncosphere penetrates the intestinal wall and is passively carried by the bloodstream to various organs, where it develops into the metacestode stage, forming hydatid cysts. The hydatid cysts primarily develop in the liver and lungs of intermediate hosts but can also affect other organs (Morar and Feldman 2003) (Fig. 5).

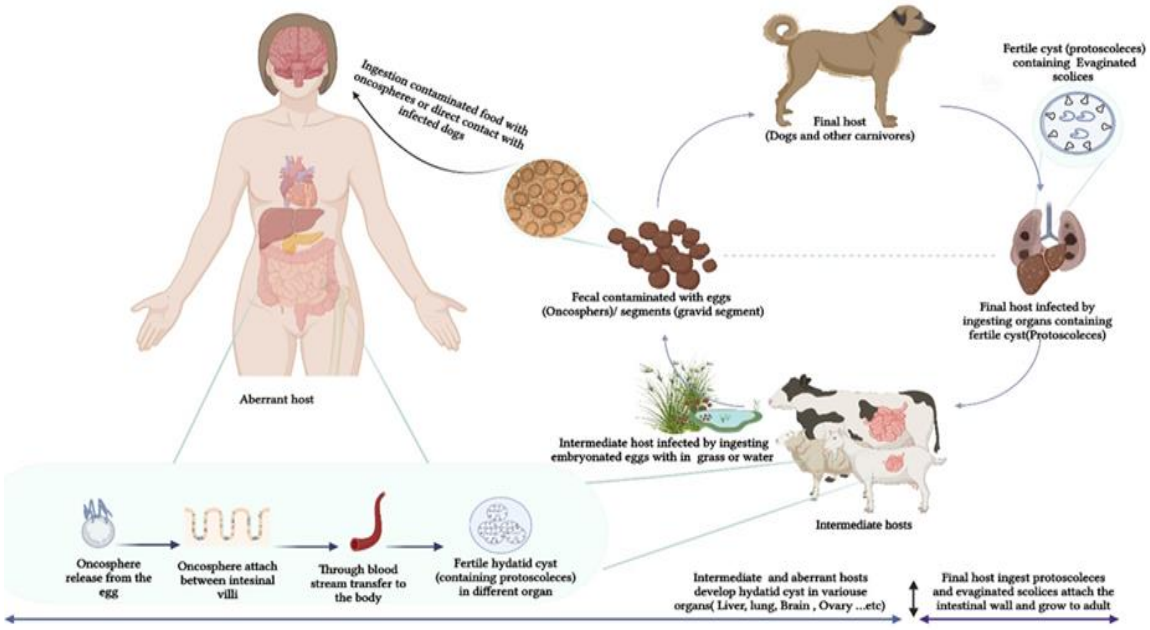



**Fig. 4:** *E. granulosus* life cycle.

In this cycle, the dog serves as the definitive host, shedding infectious eggs through proglottids in its feces. If dogs roam freely in areas where livestock graze, they can contaminate the environment with these eggs, leading to the infection of intermediate hosts and perpetuating the life cycle (Craig et al. 1995).

### 1.3.2 NATURAL OR SYLVATIC LIFE CYCLE

In the natural or sylvatic life cycle, the life cycle primarily involves wildlife. Wolves and certain species of cervids (e.g., moose, caribou, and reindeer) act as the definitive hosts in this cycle. The definitive hosts become infected by consuming the organs or tissues of intermediate hosts harboring metacestodes. Similar to the domestic cycle, the intermediate hosts become infected by ingesting the eggs of Echinococcus from contaminated food, water, or vegetation (Almulhim and John 2023).

The life cycle in wildlife settings is more complex and involves interactions among different animal species. Wild carnivores, such as wolves, play a crucial role as definitive hosts, shedding infectious eggs into the environment through their faeces. The contamination of the environment with these eggs can lead to the infection of intermediate hosts, which, in turn, can be consumed by the definitive hosts, completing the life cycle (Craig et al. 1995). Additionally, hunters who handle and consume the meat of infected wildlife may accidentally become hosts of the parasite if proper food hygiene practices are not followed (Morar and Feldman 2003).

The natural or sylvatic life cycle is often self-sustaining within wildlife populations, and human involvement as accidental hosts is relatively less common compared to the domestic cycle (Morar and Feldman 2003). Both life cycles are summarized in the Table 3.

## 2. *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS

Canine echinococcosis, caused by the parasitic tapeworm *E. granulosus*, poses a significant zoonotic threat. Domestic dogs (Canis familiaris) serve as the primary host for this parasite.

**Table 3:** *E. granulosus* life cycles

| Life Cycle | Definitive Host | Intermediate Host | Transmission | Prevention |
|---|---|---|---|---|
| Domestic or Pastoral | Dog | Sheep, goats, cattle | Ingesting uncooked offal from intermediate hosts, releasing oncospheres that develop into hydatid cysts in the liver and lungs of intermediate hosts. | Deworming dogs, improving food hygiene practices, and vaccination of intermediate hosts. |
| Natural or Sylvatic | Wolves, cervids | Small mammals, livestock | Ingesting the organs or tissues of intermediate hosts harboring metacestodes, which develop into hydatid cysts in the liver and lungs of intermediate hosts. | Education, improved food hygiene practices, and vaccination of intermediate hosts. |

Dogs are particularly susceptible to the intestinal form of echinococcosis, making them crucial in the transmission cycle of this parasitic infection. Remarkably, *E. granulosus* infections in dogs do not manifest as disease, even in cases of heavy infestations (Grosso et al. 2012). However, in young dogs heavily burdened with the parasite, a potbellied appearance may be observed, and there is a risk of small intestine obstruction (Soulsby 1982).

The intricate interaction between Echinococcus parasites and their canine hosts occurs within the small intestine. The tapeworms adeptly penetrate between the intestinal villi, make their way into the crypts of Lieberkühn. There they firmly attach themselves using suckers and rostellar hooks to the epithelial lining. Despite this intimate relationship between the parasites and their host's gut, the infection usually does not cause significant harm. Nevertheless, some minor changes may occur, including slight cellular infiltration of the intestinal mucosa and localized flattening of epithelial cells. Additionally, the presence of the parasites stimulates an increase in mucus production in the small intestine (OIE 2022).

## 3. DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS

As Echinococcus tapeworms reside in the intestines of dogs, where they release excretory/secretory products from their scolex region into the surrounding environment. This process can trigger the production of circulating antibodies in the dog's immune system. These antibodies are believed to play a role in the host's defense against the parasites and may have implications in the diagnostic process (OIE 2022).

Diagnosing *E. granulosus* infection in definitive hosts, such as dogs, can be challenging due to the morphological similarities among the eggs of various Echinococcus species and Taenia parasites. Additionally, the characteristic small segments of Echinococcus tapeworms may be absent from the dog's feces or easily overlooked during routine examinations. To overcome these difficulties, veterinarians and parasitologists employ various diagnostic methods for both living and deceased dogs (OIE 2022).

### 3.1 DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN LIVING DOGS

In living dogs, fecal examinations using flotation or sedimentation techniques are commonly employed to detect parasite eggs (Jenkins et al. 2023). However, due to intermittent egg shedding, these tests may yield false-negative results. As an alternative, coproantigen tests offer higher sensitivity and specificity by detecting specific antigens produced by adult Echinococcus worms. Additionally, serological tests, such as ELISA and Western blot, play a crucial role in detecting specific antibodies produced by the dog's immune system in response to the parasitic infection. These serological assays are particularly useful for identifying chronic infections or when eggs are not present in the faeces (Chamekh et al. 1992; Paduraru et al. 2023). These coproantigen tests significantly improve the accuracy of diagnosis (Abbasi et al. 2003).

### 3.2 DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN DEAD DOGS

For the systematic analysis of canine intestinal content, commence by making a sterile incision in the dog's abdomen to carefully access the intestine (El-Shehabi et al. 2000). With precision, tie off both the pyloric and anal ends of the intestine. Immediately collect the intestinal contents in sterile containers, ensuring no contamination. Given the sensitivity of the samples, they should be stored in an icebox to maintain integrity and be transported to the lab within a maximum of three hours. Following the collection, for ethical and environmental safety, incinerate the canine remains on-site. Upon arrival in the lab, segment the intestine into four equal portions. Subsequently, longitudinally slice open each segment and immerse it in a 0.15 M phosphate buffer saline solution with a pH of 7.2 for exactly five minutes. Using a sterilized spatula, scrape the mucosal lining gently, depositing the content into clean glass dishes. Let the samples settle in 1,000 ml conical Nalgene graduates. After multiple rinses with the phosphate buffer solution, inspect the aliquots meticulously under a dissecting microscope for detailed analysis (El-Shehabi et al. 1999).

## 4. TRADITIONAL METHODS FOR DIAGNOSING *ECHINOCOCCUS GRANULOSUS* INFECTION

The diagnosis of *E. granulosus* in the definitive host, such as domestic dogs, can be challenging due to the morphological similarities between *E. granulosus* eggs to those of *E. multilocularis*, and various Taenia species. Moreover, egg excretion is irregular, making it difficult to reliably identify them in fecal samples microscopically. However, there are specific techniques available to aid in the detection process (El-Shehabi et al. 1999).

### 4.1 DETECTION OF EGGS AND PROGLOTTIDS

Faecal samples can be examined using flotation techniques, wherein the eggs are concentrated by suspending the feces in a liquid with a higher specific gravity. Additionally, the perineal skin of dogs can be examined using clear adhesive tape, which is pressed against the skin, transferred to a microscopic slide, and examined. Despite these methods being helpful, distinguishing between the eggs of different parasites can still be a challenge (Varcasia et al. 2004; Benito et al. 2006; OIE 2022).

Apart from eggs, proglottids of *E. granulosus* can also be detected in faecal samples. Proglottids are tapeworm segments that are spontaneously discharged by dogs and can often be found on the surface of samples. Examining these segments can aid in correctly diagnosing the presence of *E. granulosus* in the definitive host (Ajlouni et al. 1984; Craig et al. 1995).

### 4.2 ARECOLINE HYDROBROMIDE PURGATION

The method of arecoline purging is recognized as a reliable technique for the detection of *E. granulosus* infection in canine populations, as documented by Benito et al. (2006). The central component, arecoline hydrobromide, is a parasympathomimetic drug that predominantly affects the small intestine's smooth muscle, resulting in tapeworm paralysis. For effective outcomes, it is critical to administer the drug, available in both tablet and liquid forms, either orally or, in some instances, per rectum. Based on extensive research by Craig et al. (1995), Dakkak et al. (2017), and OIE (2022), the suggested dose spans from 1.75 mg/kg to 3.5 mg/kg of body weight, demonstrating effectiveness in a majority of dogs. Post-administration, the drug induces purgation, facilitating the tapeworms' expulsion via faeces. It's imperative to promptly collect this purged material in leak-proof bags and to preserve it by saturating in either a 10% formalin or 85% ethanol solution, as recommended by Benito et al. (2006). Following preservation, the samples are ready for a comprehensive examination to determine the degree of *E. granulosus* infection.

### 4.3 IMMUNODIAGNOSTIC METHODS

Immunodiagnostic methods for *Echinococcus spp.* in the final host has significantly advanced over the years. Early attempts at immune-diagnosis were made in the early 20$^{th}$ century, and several immunological and molecular methods have since been applied to the diagnosis of intestinal stages of both *Echinococcus spp.* and *Taenia spp.* (Gasser et al. 1988; JC et al. 1992; Deplazes et al. 1992; Benito et al. 2006).

Among the major methods developed and assessed are serological tests and molecular techniques. Serological tests, such as enzyme-linked immunosorbent assay (ELISA) and Western blot (Gasser et al. 1988; Craig et al. 1995; Kouguchi et al. 2010), rely on detecting specific antibodies produced by the host's immune system in response to the presence of *Echinococcus* antigens (Al-Khalidi and Barriga 1986). The choice of diagnostic method depends on a number of factors, including the stage of infection, the availability of resources, and the clinical presentation of the patient. In general, serological tests are a good first-line option, as they are relatively inexpensive and easy to perform (Benito et al. 2006).

## 5. COPROANTIGEN TESTS

The development of immunodiagnostic methods has significantly advanced the detection of canine echinococcosis. Among these methods, the Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test stands out as a powerful tool for diagnosing this parasitic infection (Allan and Craig 1989; JC et al. 1992; Deplazes et al. 1992; Deplazes et al. 1994).

To perform the Coproantigen ELISA test, researchers have generated polyclonal antibodies against somatic or excretory/secretory antigens of adult *E. granulosus* (Deplazes et al.,1992; Deplazes et al. 1994; Allan and Craig 2006). This test can detect coproantigens highly specific to the genus *Echinococcus* in dogs as early as 5-10 days after infection, even in cases where eggs are not yet present in the faeces (Deplazes et al. 1992).

One significant advantage of detecting specific antigen(s) in faecal samples from definitive hosts is its high probability of correlation with current infection, as it directly indicates the presence of the parasite in the intestine. This method has been successfully utilized in various countries to diagnose canine echinococcosis (Varcasia et al. 2004; OIE 2022).

The Coproantigen ELISA test represents a valuable tool in canine echinococcosis surveillance and control programs, enabling early and accurate diagnosis of infected dogs. By promptly identifying infected animals, health authorities can implement appropriate measures to prevent the spread of the parasite to humans and other susceptible hosts, ultimately contributing to public health and the reduction of zoonotic

risks associated with this disease. As research in immunodiagnosis continues to progress, it is likely that even more sensitive and specific methods will be developed to enhance our ability to combat this significant zoonotic threat effectively (WHO 2012).

## 5.1 VALIDATION AND SENSITIVITY OF COPROANTIGEN TESTS

### 5.1. SPECIFICITY

The detection of *E. granulosus* coproantigens using the Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test has been shown to be highly specific (Deplazes et al. 1992; Allan and Craig 2006). The test, which employs antibodies raised against *Echinococcus spp.* antigens, has proven its capability to specifically detect infected faeces from dogs harboring *E. granulosus* parasites while yielding negative results in hosts infected with other types of parasites, including *Taenia* species. The specificity of this test has been reported to reach an impressive 98% and higher (Allan and Craig 2006).

### 5.2. SENSITIVITY

The sensitivity of the coproantigen test for *E. granulosus* detection is influenced by the worm burden within the definitive host's intestine. In cases where faecal samples are obtained from animals harboring only a few worms, the sensitivity of the coproantigen test may be lower. However, as the worm burden increases, so does the sensitivity of the test. For instance, the sensitivity of coproantigen testing reaches 100% when the worm burden exceeds 1000 worms, and it remains high at 93.3% when the worm burden ranges between 200 and 1000 worms. The test's sensitivity is still satisfactory, ranging between 85% and 93.1%, even when the worm burden is in the range of 50 to 100 worms (Allan and Craig 2006).

### 5.3. ADVANTAGES

The Coproantigen ELISA test offers several advantages as enlisted in Table 4 that make it a valuable tool in the diagnosis of canine echinococcosis (Craig et al. 1995; Allan and Craig 2006).

## 5.4 APPLICATION OF COPROANTIGEN TESTS FOR MONITORING INFECTION IN DOGS

The Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test is an essential tool for monitoring and controlling Echinococcosis in the final host (dogs). This highly specific and sensitive diagnostic method allows for the early and accurate detection of *E. granulosus* infections in the definitive hosts, primarily domestic dogs. By employing this diagnostic tool in a systematic manner, public health authorities and veterinarians can implement effective measures to reduce the prevalence of the parasite and its transmission to humans and other susceptible hosts (Allan and Craig 2006).
The Coproantigen ELISA test can be used for a variety of purposes, including:

### 5.4.1. ACTIVE SURVEILLANCE OF *E. GRANULOSUS* INFECTIONS IN DOG POPULATIONS

Veterinarians and public health officials can conduct regular testing campaigns, especially in areas known to have high prevalence rates of the parasite. By testing faecal samples obtained either directly from the

rectum or from the ground, authorities can identify infected dogs early in the course of the disease, even before the shedding of eggs. This early detection allows for prompt treatment and isolation of infected animals, minimizing the risk of transmission to humans and other animals (Allan and Craig 2006; WHO 2002).

### 5.5.2. MONITORING THE EFFECTIVENESS OF CONTROL MEASURES AND TREATMENT INTERVENTIONS

Following the administration of anti-parasitic drugs to infected dogs, veterinarians can use the test to assess treatment efficacy. The coproantigen level in faecal samples typically decreases within a few days after successful treatment. By monitoring this decline, veterinarians can ensure that infected dogs are effectively treated and pose a reduced risk of transmission (Allan and Craig 2006).

**Table 4:** Copro-antigen advantages

| Advantage | Notes |
|---|---|
| Sample Collection Flexibility | Faecal samples can be obtained either directly from the rectum or from the ground where dogs defecate. Some studies have even demonstrated that dry faeces can be used for testing. |
| Ease of Sample Storage | Faecal samples can be tested on the same day of collection or stored in a refrigerator or deep-frozen at -20°C until use. |
| High Genus-Specificity | The coproantigen test exhibits a high level of genus-specificity and accurately identify the presence of *Echinococcus spp.* without cross-reacting with antigens from other parasites. This specificity has been reported to exceed 95%. |
| Early Detection | The coproantigen can be detected in faeces even before the shedding of eggs by the adult worms. Additionally, the level of antigen does not depend on the number of eggs present, making it a reliable indicator of infection. |
| Environmental Stability | The coproantigen remains stable in environmental conditions for several months, and it retains its detectability even across a range of temperatures from -80°C to 35°C. This stability allows for the safe storage and transportation of faecal samples for testing purposes. |
| Monitoring Treatment Efficacy | The coproantigen level in faecal samples decreases within 1 to 5 days after treatment, providing a means to monitor the efficacy of therapeutic interventions. |

### 5.5.3. SURVEILLANCE OF DOGS IN REGIONS WHERE *E. GRANULOSUS* IS ENDEMIC

By conducting regular testing of canine populations in these areas, health authorities can identify high-risk zones and target control efforts more effectively. This information aids in developing localized prevention and control programs, including public awareness campaigns and interventions targeting specific risk factors (Craig et al. 1995; Varcasia et al. 2004).

### 5.5.4. MASS SCREENING OF DOGS IN COMMUNITIES WHERE THERE IS A HIGH LIKELIHOOD OF HUMAN EXPOSURE TO THE PARASITE

In such settings, identifying and treating infected dogs can play a crucial role in breaking the transmission cycle and reducing the incidence of human echinococcosis (Ahmad and Nizami 1998; De et al. 2010).

## 5.6 LIMITATIONS AND CHALLENGES

The Coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) test, despite its numerous advantages and valuable applications for monitoring and controlling *E. granulosus* in dogs, faces certain limitations and challenges that need to be addressed for its optimal use in combating this zoonotic disease. One of

the main challenges is the variation in sensitivity depending on the worm burden within the definitive host's intestine. The test's sensitivity is generally higher when the worm burden is high, but it decreases when the number of worms is low, potentially leading to false-negative results in dogs with low parasite loads. Cross-reactivity with closely related parasites, such as other *Taenia* species, also poses a risk of false-positive results in regions where multiple taeniid parasites coexist (Siavashi and Motamedi 2006). Additionally, the timing of detection can be critical as the presence of antigen in faecal samples may decline within a few days after effective treatment, leading to false-negative results shortly after treatment. Proper sample collection, transportation, and storage are crucial to obtaining accurate results, which can be logistically challenging, especially in free-roaming or uncooperative animals. Furthermore, the test's implementation requires specialized laboratory equipment and trained personnel, which can increase the overall cost of testing and may limit its accessibility in regions with limited resources. To overcome these limitations, complementary diagnostic methods, such as serological tests, imaging techniques, and coproscopy (egg detection in faecal samples), should be used in combination with the Coproantigen ELISA test to improve overall accuracy. By addressing these challenges and employing a comprehensive approach to diagnostics, veterinary and public health efforts can better combat *E. granulosus*, protect human health, and reduce transmission in dog populations. Efforts should be made to improve the accessibility and affordability of the test in regions with high disease burden, ultimately contributing to the successful control of this zoonotic disease (Craig et al. 1995; Guarnera et al. 2000; WHO 2002; Allan and Craig 2006).

## 6. CONCLUSION

Coproantigen ELISA test offers a valuable tool for monitoring and controlling Echinococcosis in dogs, the definitive host. Its high specificity and sensitivity allow for early detection of infections, enabling effective control measures to be implemented. However, limitations such as reduced sensitivity with low worm burden and cross-reactivity with other parasites necessitate a comprehensive approach. Combining the ELISA test with other diagnostic methods, improving accessibility in resource-limited settings, and addressing logistical challenges are key to maximizing its effectiveness. By overcoming these hurdles and employing a multifaceted approach, veterinarians and public health officials can effectively combat Echinococcosis, protect human health, and safeguard dog populations.

## REFERENCES

Abbasi I et al., 2003. Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. The American Journal of Tropical Medicine and Hygiene 69(3): 324–330.

Ahmad G and Nizami WA, 1998. Coproantigens: early detection and suitability of an immunodiagnostic method for echinococcosis in dogs. Veterinary Parasitology 77(4): 237–244.

Ajlouni AQ et al., 1984. Intestinal cestodes of stray dogs in Jordan. Zeitschrift fur Parasitenkunde (Berlin, Germany) 70(2): 203–210.

Al-Khalidi NW and Barriga OO, 1986. Cell-mediated immunity in the prepatent primary infection of dogs with Echinococcus granulosus. Veterinary Immunology and Immunopathology 11(1): 73–82.

Allan JC and Craig PS, 1989. Coproantigens in gut tapeworm infections: Hymenolepis diminuta in rats. Parasitology Research 76(1): 68–73.

Allan JC and Craig PS, 2006. Coproantigens in taeniasis and echinococcosis. Parasitology International 55: S75–S80.

Almulhim AM and John S. 2019. Echinococcus granulosus.

Ananda KJ et al., 2015. Sero-diagnosis of Echinococcus granulosus infection in dogs using faecal supernatant antigen. Journal of Parasitic Diseases 39(3): 461.

WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern [Accessed 1 August 2023]. Available from: https://apps.who.int/iris/handle/10665/42427?show=full.
Benito A et al., 2006. Dog echinococcosis in northern Spain: comparison of coproantigen and serum antibody assays with coprological exam. Veterinary Parasitology 142(1–2): 102–111.
Borji H et al., 2013. Copro-DNA test for diagnosis of canine echinococcosis. Comparative Clinical Pathology 22(5): 909–912.
Bowman DD, 2020. Georgis' Parasitology for Veterinarians. Georgis' Parasitology for Veterinarians 2020: 1–519.
Brunetti E et al., 2010. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Tropica 114(1): 1–16.
Budke CM et al., 2013. A systematic review of the literature on cystic echinococcosis frequency worldwide and its associated clinical manifestations. American Journal of Tropical Medicine and Hygiene 88(6): 1011–1027.
Chamekh M et al., 1992. Diagnostic value of a synthetic peptide derived from Echinococcus granulosus recombinant protein. The Journal of Clinical Investigation 89(2): 458–464.
Constantine CC et al., 1993. Morphological characterization of adult Echinococcus granulosus as a means of determining transmission patterns. Journal of Parasitology 79(1): 57–61.
Craig PS et al., 1995. Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. Veterinary Parasitology 56(4): 293–301.
Dakkak A et al., 2017. Echinococcus granulosus infection in dogs in Sidi Kacem Province (North-West Morocco). Acta tropica 165: 26–32.
De S et al., 2010. Detection of canine echinococcosis by coproantigen ELISA. Asian Pacific Journal of Tropical Medicine 3(7): 519–522.
Deplazes P et al., 1992. Detection of Echinococcus coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes. Parasitology Research 78(4): 303–308.
Deplazes P et al., 1994. Detection of Echinococcus coproantigens in stray dogs of northern Spain. Applied Parasitology 35(4): 297–301.
El-Shehabi FS et al., 1999. Prevalence of intestinal helminths of dogs and foxes from Jordan. Parasitology Research 85(11): 928–934.
El-Shehabi FS et al., 2000. Diagnosis of canine echinococcosis: comparison of coproantigen detection with necropsy in stray dogs and red foxes from northern Jordan. Parasite (Paris, France) 7(2): 83–90.
Gasser RB et al., 1988. Evaluation of a serological test system for the diagnosis of natural Echinococcus granulosus infection in dogs using E. granulosus protoscolex and oncosphere antigens. Australian Veterinary Journal 65(12): 369–373.
Guarnera E et al., 2000. Canine echinococcosis: an alternative for surveillance epidemiology. Veterinary parasitology 88:131–134
Gottstein B, 1992. Molecular and immunological diagnosis of echinococcosis. Clinical Microbiology Reviews 5(3): 248–261.
Grosso G et al., 2012. Worldwide epidemiology of liver hydatidosis including the Mediterranean area. World Journal of Gastroenterology 18(13): 1425.
Howorth MB, 1945. Echinococcosis of bone. JBJS 27(3): 401-411.
Hüttner M et al., 2008. Genetic characterization and phylogenetic position of Echinococcus felidis (Cestoda: Taeniidae) from the African lion. International Journal for Parasitology 38(7): 861–868.
Ingold K et al., 1998. Identification of a laminated layer-associated protein in Echinococcus multilocularis metacestodes. Parasitology 116(4): 363–372.
JC A et al., 1992. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology 104(2): 347–355.
Jenkins EJ et al., 2023. Intestinal infection with Echinococcus multilocularis in a dog. Journal of the American Veterinary Medical Association 2023: 1–3.
Kassis AI and Tanner CE, 1976. The role of complement in hydatid disease: In vitro studies. International Journal for Parasitology 6(1): 25–35.
Kouguchi H et al., 2010. Echinococcus multilocularis: two-dimensional Western blotting method for the identification and expression analysis of immunogenic proteins in infected dogs. Experimental Parasitology 124(2): 238–243.

Kumaratilake LM and Thompson RCA, 1982. A review of the taxonomy and speciation of the genus Echinococcus Rudolphi 1801. Zeitschrift für Parasitenkunde Parasitology Research 68(2): 121–146.

Lahmar et al., 2007. Screening for Echinococcus granulosus in dogs: Comparison between arecoline purgation, coproELISA and coproPCR with necropsy in pre-patent infections. Veterinary Parasitology 144(3–4): 287–292.

Mehlhorn H, 2008. Encyclopedia of parasitology, Volume 1.

Morar R and Feldman C, 2003. Pulmonary echinococcosis. European Respiratory Journal 21(6):1069-1077.

Muller R et al., 2002. Worms and human disease, CABI.

Nakao M et al., 2007. A molecular phylogeny of the genus Echinococcus inferred from complete mitochondrial genomes. Parasitology 134(5): 713–722.

OIE 2022. Echinococcosis (Infection with *Echinococcus Granulosus* and with *E. Multilocularis*). OİE Terrestrial Manual: 1–17.

Paduraru AA et al., 2023. Seroprevalence of Anti-Echinococcus granulosus Antibodies and Risk Factors for Infection in Blood Donors from Western Romania Life 13(4): 871.

Paul M and Stefaniak J, 1997. Detection of specific Echinococcus granulosus antigen 5 in liver cyst bioptate from human patients. Acta Tropica 64(1–2): 65–77.

Pump KK, 1963. Echinococcosis (Hydatid Disease): Review and Report of a Case of Secondary Echinococcosis. Canadian Medical Association Journal 89(2): 73.

Reissenweber NJ et al., 1975. Histochemistry and histoenzymology of the hydatid cyst (Echinococcus granulosus Batsch, 1786) - II. Scolices and brood capsules. Zeitschrift für Parasitenkunde 48(1): 25–33.

Richards KS et al., 1983. Echinococcus granulosus equinus: an ultrastructural study of the laminated layer, including changes on incubating cysts in various media. Parasitology 86(3): 399–405.

Shield JM, 1969. Dipylidium caninum, Echinococcus granulosus and Hydatigera taeniaeformis: Histochemical identification of cholinesterases. Experimental Parasitology 25(C): 217–231.

Sing A, 2015. Zoonoses—infections affecting humans and animals: Focus on public health aspects. Zoonoses-Infections Affecting Humans and Animals: Focus on Public Health Aspect 1–1143.

Siavashi MR and Motamedi GR. 2006. Evaluation of a coproantigen enzyme-linked immunosorbent assay for the diagnosis of canine echinococcosis in Iran. Helminthologia, 43:17-19.

Soulsby EJL, 1982. Helminths, arthropods, and protozoa of domesticated animals.

Tappe D et al., 2008. Emergence of Polycystic Neotropical Echinococcosis. Emerging Infectious Diseases 14(2): 292.

Teroj M, 2022. Movement of Echinococcus granulosus under a light microscope. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases 28(9):1236–1237.

Thompson RCA, 2017. Biology and Systematics of Echinococcus. Advances in Parasitology 95: 65–109.

Torgerson PR et al., 2006. Present situation of cystic echinococcosis in Central Asia. Parasitology International 55: S207–S212.

Varcasia A et al., 2004. The diagnosis of Echinococcus granulosus in dogs. Parassitologia 46(4): 409–412.

Zeibig EA, 1997. Clinical parasitology: a practical approach.

Zhang W et al., 2021. Molecular Epidemiology of *Echinococcus* spp. Molecular Food Microbiology 2021: 431–440