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Role of Serological Techniques in Detection of Echinococcosis

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Abstract

Human echinococcosis, a dangerous zoonotic parasitic disease that is still a major public health concern in many developing countries, is caused by the larval stages of Echinococcus species. The disease frequently affects the liver and lungs, which can lead to the development of hydatid cysts. These cysts may not exhibit any symptoms for years, which increases the risk of complications and delays in diagnosis. In order to manage the illness and avoid major consequences, early and accurate detection is crucial.

Serological methods have been shown to be useful supplemental tools in the diagnosis of echinococcosis when paired with radiological investigations. Enzyme-Linked Immunosorbent Assay (ELISA) is one of the most widely used immunodiagnostic methods due to its low cost, ease of use, and relatively high sensitivity. Other serological methods are immunoblotting, indirect hemagglutination assay, latex agglutination, and recombinant antigen-based assays, which have also enabled improved diagnostic methods.

The pathophysiology, epidemiology, and serological diagnosis of human echinococcosis are summarized in this review, with a focus on ELISA-based detection techniques. The benefits, drawbacks, sensitivity, specificity, and most recent developments in immunodiagnostic methods are also covered in this review. It also emphasizes how crucial it is to integrate imaging modalities with serological results in order to make an accurate diagnosis.

All things considered, especially in endemic areas, serological techniques are still crucial for the early identification and clinical treatment of echinococcosis. Future disease surveillance, treatment results, and preventative measures may all be enhanced by ongoing research and development of more sensitive and accurate diagnostic instruments.

Keywords: ELISA, Echinococcus, Serology, Hydatid Disease, Immunodiagnostics.

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1.0 Introduction

Globally, parasitic diseases are still a major public health concern, especially in developing and low-resource nations where veterinary control programs, sanitation, and healthcare services are frequently insufficient. Poor hygiene, raising livestock, and frequent human-domestic animal contact are all strongly linked to a number of zoonotic parasite infections. Echinococcosis is one of these infections that is regarded as one of the most neglected tropical diseases due to its slow progression, delayed diagnosis, and severe health consequences if treatment is not received (WHO, 2023).

The larval stage of tapeworms in the genus Echinococcus is the cause of echinococcosis, also referred to as hydatid disease. *Echinococcus multilocularis* causes alveolar echinococcosis, whereas *Echinococcus granulosus* causes cystic echinococcosis. The more prevalent type of echinococcosis in humans, cystic echinococcosis, is commonly reported in regions where raising sheep and cattle is a common occupations (Johannes & Deplazes, 2004).

The transmission cycle of Echinococcus involves dogs and other canines as definitive hosts, whereas animals such as sheep, goats, cattle, pigs, and camels act as intermediate hosts. Humans become accidental hosts after ingesting parasite eggs through contaminated food, water, soil, or direct contact with infected dogs. Once inside the body, the eggs hatch in the intestine and release embryos that enter the bloodstream and travel to different organs. The liver is the most commonly affected organ because it acts as the first filter for circulating embryos, followed by the lungs. In some patients, cysts may also develop in the spleen, kidneys, bones, brain, or heart (McManus et al., 2003).

One of the major concerns associated with echinococcosis is that the disease often develops silently over many years. Hydatid cysts grow slowly and may not produce symptoms during the early stages of infection. As a result, many patients remain undiagnosed until the cysts become large enough to cause pressure effects or complications. Clinical manifestations depend mainly on the location and size of the cysts. Liver involvement may cause abdominal pain, nausea, hepatomegaly, or jaundice, whereas lung cysts may lead to cough, chest discomfort, breathlessness, or fever. In severe cases, rupture of the cyst may result in secondary spread of infection or serious allergic reactions such asanaphylaxis (Brunetti et al., 2010).

Echinococcosis continues to be endemic in several regions of the world, including Central Asia, China, South America, the Middle East, Mediterranean countries, and parts of Africa. In India, cases have been reported from different states such as Rajasthan, Punjab, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, and Jammu & Kashmir. However, the actual burden of the disease may be much higher than reported figures because many infections remain asymptomatic or are not diagnosed properly, especially in rural populations with limited access to healthcare and laboratory facilities (Bhatia, 2010).

Other than its impact on human well-being, echinococcosis is also an economically burdensome condition. It involves extended periods of medication, diagnostics, operations, and hospital stay, among other factors. Hydatid cyst infection in animals causes condemned organs upon meat inspection, hence economic losses to the farmers and animal husbandry sector. Thus, the infection has economic impacts in addition to those associated with human health in endemic areas (Torgerson, 2003).

Early diagnosis of echinococcosis is critical for the prevention of possible complications and effective management. The diagnostic tools commonly utilized include ultrasound (USG), computed tomography (CT), and magnetic resonance imaging (MRI). Ultrasound is viewed as the best tool for diagnosing liver hydatid cysts due to its simplicity, lack of invasiveness, affordability, and wide availability (Alvi et al., 2023). CT and MRI offer more detailed pictures and are highly informative when dealing with complicated cases (Pedrosa et al., 2000).

While there is a role played by various imaging procedures in the detection of hydatid cysts, the results of radiological examination alone may not always offer enough evidence in terms of a definitive diagnosis especially in the case of infections that are in their early stages or when there are atypical infections. This is why serological testing has often proven effective as a supplementary method in the identification of antibodies that react to Echinococcus antigens. Different approaches based on immunology such as Enzyme-Linked Immunosorbent Assay (ELISA), indirect hemagglutination assay (IHA), latex agglutination, immunoblotting, and tests using recombinant antigens have all been used. However, of all the different types, ELISA has emerged as one of the more popular diagnostic options available today (Knapp et al., 2023).

ELISA works on the principle of antigen-antibody interaction, where the reaction produces a measurable color change that can be analyzed spectrophotometrically. In most cases, samples showing optical density values higher than the established cut-off value are considered positive for infection:

$OD_{sample} > OD_{cutoff}$ =Positive

Even though ELISA test is a widely used test that plays a significant role in disease diagnosis, there are certain limitations associated with this test. The test may produce false-negative results due to the presence of inactive, calcified, or pulmonary cysts. In such cases, the amount of antibody present in the body will be minimal. Additionally, false-positive tests can result from cross-reaction among antibodies generated from other parasitic diseases (Virginio et al., 2012).

Some of the recent developments in immunodiagnosis are improvements in the sensitivity and specificity of serological tests owing to the purification of antigens and recombinant antigens. Additionally, techniques such as PCR are increasingly being used in the diagnosis of diseases for confirmation purposes and determining species. However, despite all this, ELISA continues to remain one of the most practical tests used clinically (Wen et al., 2019).

The last decade has been also seen the rise of the concept of "One Health," which focuses on joint efforts by human medical practitioners, veterinarians, ecologists, and communities in combating and preventing zoonotic diseases such as echinococcosis. It is critical that actions aimed at prevention be taken into account, such as regular deworming of infected dogs, proper disposal of organs from sick animals, clean operations at the abattoirs, personal hygiene, and education of the community (Craig et al., 2017).

In this study, an attempt has been made to present a comprehensive overview of human echinococcosis with special emphasis on serology. This includes the epidemiology of the disease, symptoms of the disease, different means of diagnosis, advantages and disadvantages of serology, advances in immunodiagnosis and its future prospects.

2.0 Current Perspective on Serological Diagnosis of Echinococcosis.

Echinococcosis remains one of the important zoonotic parasitic infections with serious health consequences in human, as well as negative economic effects in many endemic countries worldwide. In most cases, the parasite occurs in those settings where farming activities and human interaction with dogs and domestic animals take place. Several scientific studies have indicated that echinococcosis is still a public health challenge due to the problem of late diagnosis, long-term illness and lack of awareness by patients suffering from this condition (Hogea et al., 2024).

Echinococcosis cases have been recorded in different endemic areas in the world such as central Asia, south America, Middle East, China, Mediterranean region, and even some parts of Africa. As reported by the World Health Organization, there are millions of people who are at risk of developing this disease, especially those who are residing in rural areas and depending on animal rearing for their means of income. Echinococcosis is one of the neglected tropical diseases recognized by the WHO (WHO, 2023).

Many studies conducted internationally reveal that the liver and lungs are the most commonly involved body organs. The studies show that most of the cases are usually caused by hepatic involvement since the liver plays a crucial role in filtering the parasite after its entry into the circulatory system. The second most common involvement involves the lungs, while involvement of the spleen, kidneys, brain, and bones occurs to a lesser extent. One of the problems with echinococcosis is its delayed presentation, as the cysts grow very slowly and become symptomatic after many years (Mihmanli et al., 2016).

Despite several measures taken against the disease, echinococcosis continues to be endemic in various Indian states such as Rajasthan, Punjab, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, and Jammu & Kashmir. Some Indian scientists opine that poor sanitation, unhygienic animal slaughter practices, improper disposal of infected animals' organs, and close contact with infected dogs are some major causes responsible for the proliferation of this disease. Besides, ignorance about the lifecycle of this parasite among the rural population and lack of veterinary control have been identified by scientists as additional factors (Bhatia, 2010).

Prevalence studies conducted in various tertiary care hospitals of India have proved that hydatid disease is an important entity which affects those people who are clinically identified as patients of hydatid disease. It is found through most studies that hydatid disease affects

mainly adults because adults come under productive age group owing to the nature of their profession which is usually agriculture and animal rearing (Ahmad et al.).

Due to the advances in imaging methods,tremendous progress has been made with regard to the diagnostic approaches to echinococcosis in th past few decades.Some of the methods Some of the methods that can be mentioned are ultrasonography, CT scan, and MRI scan, which help identify hydatid cysts, locate sites infected with hydatid disease, and determine potential complications caused by such diseases. It would also be worth mentioning that ultrasound technique is very useful in diagnosing hepatic hydatid cysts because of its relatively low cost and ease of use

In fact, comparative diagnostic tests have indicated differences in the sensitivity and specificity of different serological approaches. It is suggested by some authors that immunoblot and Western blot testing offer higher levels of specificity in case of uncertainty, whereas the use of ELISA proves to be more efficient for routine diagnosis due to its speediness and economic advantages. Moreover, ELISA with recombinant antigens is considered to perform better than crude antigen-based approaches (Virginio et al., 2012).

Though serological tests have proven to be quite effective, there are also some limitations in diagnosis. False negative ELISA tests are possible in the case of individuals with dormant, calcified, or pulmonary cysts as the production of antibodies may be minimal here. On the other hand, false positives may also happen due to cross-reactions with other helminthic infections. This is why it is usually advised to take into account both clinical as well as radiological tests with serological tests (Wen et al., 2019).

These new improvements in immunodiagnosis are associated with the development of more sensitive and specific tests relying on the application of pure and recombinant antigens. Tests based on the use of recombinant antigen B and antigen 5 have demonstrated high potential for improving the efficiency of serology. At the same time, the use of molecular techniques, such as PCR, became an area of interest regarding the confirmation of infections, detection of a particular strain, and epidemiological studies. However, despite the developments in the sphere of immunodiagnostics, ELISA remains one of the most practical serological approaches used by laboratories (Brunetti et al., 2010).

There has been a recent rise in the application of the concept of "One Health" in the prevention and control of zoonotic diseases like echinococcosis. This kind of cross-disciplinary approach requires the coordination of efforts from different specialists including medical professionals, veterinarians, environmentalists, and representatives of local communities. It has been stated that regular deworming of dogs, adequate hygiene in the slaughterhouses, proper handling of animal organs affected by the disease, educating people, and better sanitary conditions should be ensured to prevent further infection spreading (Craig et al., 2017).

Overall, available literature shows that echinococcosis continues to impose a substantial health burden globally as well as in India. Although radiological investigations remain central to diagnosis, serological methods—particularly ELISA—play a crucial supportive role in early detection and disease management. Continuous improvements in immunodiagnostic techniques, combined with effective public health interventions and community awareness programs, may contribute significantly toward reducing the burden of this neglected parasitic disease in endemic populations.

3.0 Etiology and Life Cycle of Echinococcus.

Echinococcosis is an important disease, which is caused by the larvae stages of tapeworms of the Echinococcus genus. Hydatid disease is the common name used for this disease, and at present, the disease continues to pose a threat to both animals and humans living in several parts of the world. Several types of Echinococcus have been described, but among all these parasites, the Echinococcus granulosus is the one considered as the most important parasite responsible for causing cystic echinococcosis, and the Echinococcus multilocularis is associated with causing the aggressive form of the disease, alveolar echinococcosis. (Moro & Schantz, 2009).

Two types of hosts exist in the life cycle of Echinococcus: the intermediate and definitive hosts. The definitive hosts are dogs because it is where the adult worm resides in its small intestines to develop fully. There may also be other carnivorous hosts such as foxes, wolves, and jackals depending on the location. The adult worms produce eggs, which are then transmitted through the environment via feces of the affected host organism (Vuitton, 2009).

Intermediate hosts mainly include herbivorous animals such as sheep, goats, cattle, pigs, camels, and other livestock animals. These animals become infected after consuming food or water contaminated with parasite eggs. Inside the intestine of the intermediate host, the eggs hatch and release embryos known as oncospheres. These embryos penetrate the intestinal wall and enter the bloodstream, through which they migrate to various organs, particularly the liver and lungs, where they gradually develop into hydatid cyst (McManus et al., 2003).

Humans are accidental intermediate hosts, and infections are acquired through the consumption of Echinococcus eggs found in contaminated food, drinkable water, or direct exposure to dogs with the parasite. Poor hygiene practices, frequent contact with livestock, and improper slaughtering techniques may greatly contribute to the transmission of the disease. However, unlike livestock, human hosts are not involved in the reproduction and survival of the parasites and are thus referred to as dead-end hosts (Efa et al., 2026).

Post consumption, the larvae emerge from the eggs in the small intestine of humans and burrow into the wall of the intestine. The larvae gain entry into the portal system and travel via the bloodstream to various organs. Liver acts as the primary filtration point; hence, it is the most frequently affected organ. There are some larvae that manage to bypass filtration at the level of the liver and enter the lungs or any other organ via systemic circulation. Gradually, these larvae evolve into hydatid cysts containing liquid (Sarkari et al., 2007).

The hydatid cysts have a highly organized structure that comprises two layers – the outer layer called laminated and the inner layer known as the germinal layer. The germinal layer develops the brood capsules and the protoscolices, which serve as infective stages for further growth into adult tapeworms once consumed by the final host. The cyst fluid is very rich in antigens and can cause severe allergic response after cyst rupture (Tamarozzi & Brunetti, 2018).

The lifecycle of the parasite is completed when dogs or other definitive hosts consume the raw infected organs of intermediate hosts containing viable hydatid cysts. Inside the intestine of the definitive host, the protoscolices develop into mature adult worms, results in continuing the transmission cycle. In endemic areas, feeding infected animal like dogs and poor slaughterhouse hygiene are considered major factors responsible for maintaining the lifecycle of the parasite (Thys et al., 2019).

The lifecycle (figure 1) of Echinococcus clearly demonstrates the close relation between animals, humans, and environmental contamination in the spread of the disease.

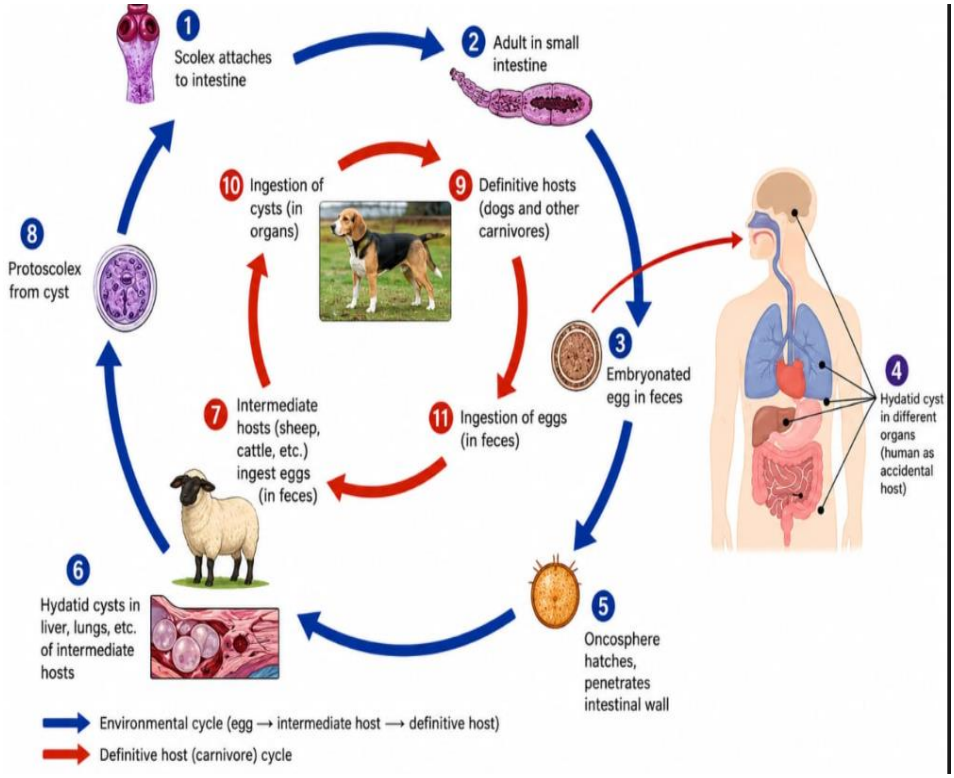

Figure1. *Lifecycle of Echinococcus granulosus showing definitive hosts, intermediate hosts, and accidental transmission to humans.*

4.0 Pathogenesis and Clinical Manifestation.

Echinococcosis develops when humans accidentally ingest Echinococcus eggs through contaminated food, water, or by direct contact with infected dogs. After entering the small intestine, the eggs hatch and release larvae called oncospheres. These larvae penetrate the intestinal wall and enter the bloodstream, through which they travel to different organs of the body. Since the liver acts as the first filtration site, it becomes the organ most commonly affected. Some larvae may escape from the liver and later reach the lungs or other organs through systemic circulation (Moro & Schantz, 2009).

After reaching the organs, the larvae slowly develop into fluid-filled hydatid cysts. The growth of these cysts is usually very slow, due to which the disease may remain unnoticed for many years. Hydatid cysts contain an outer laminated layer and an inner germinal layer that produces brood capsules and protoscolices (figure 2). The cyst fluid contains highly antigenic substances, and leakage or rupture of the cyst may lead to allergic reactions in the body (Vuitton, 2009).

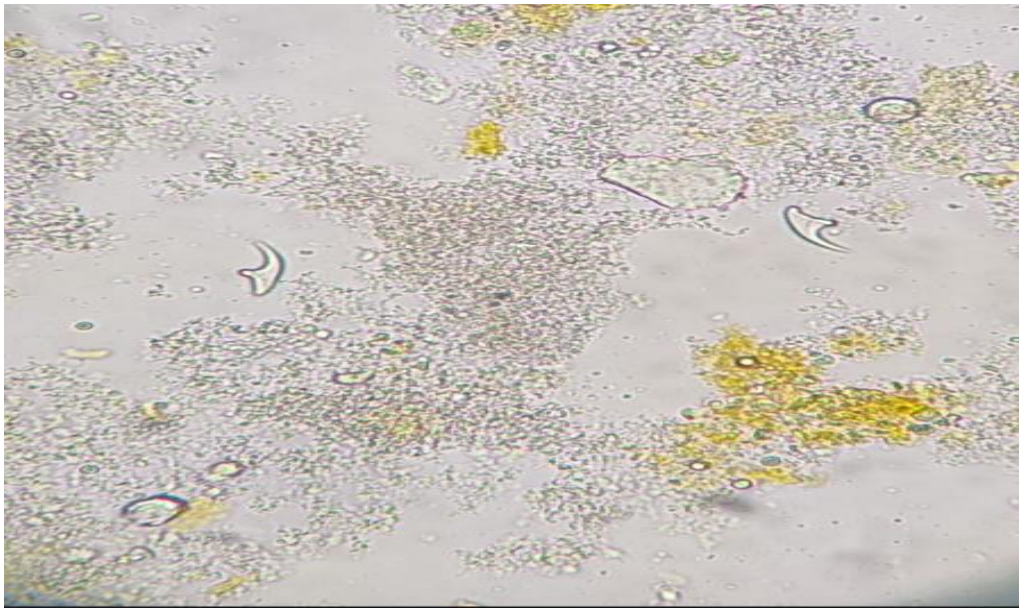

Figure 2. *Microscopic appearance of protoscolices and hooklets of Echinococcus obtained from Hydatid cyst fluid*

The liver and lungs are the most commonly involved organs in human echinococcosis. Liver involvement is seen in the majority of patients because most larvae become trapped in the portal circulation after crossing the intestinal wall. Lung involvement is considered the second most common manifestation and is more frequently observed in younger individuals. In rare conditions, cysts may also develop in organs such as the spleen, kidneys, brain, bones, heart, and muscles (Aydin et al., 2022).

Clinical manifestations mainly depend on the size, number, location, and stage of the cysts. Small cysts may remain asymptomatic for long periods, whereas larger cysts can produce symptoms because of pressure on surrounding tissues and organs. Patients with hepatic hydatid disease commonly complain of abdominal pain, nausea, vomiting, abdominal discomfort, hepatomegaly, and jaundice. In pulmonary echinococcosis, symptoms such as chronic cough, chest pain, fever, breathlessness, and sometimes hemoptysis may occur (Sarkari et al., 2007).

Complications usually arise when cysts enlarge significantly or rupture either spontaneously or after trauma. Rupture of hydatid cysts may result in the spread of infection into nearby tissues or body cavities. Leakage of cyst fluid can also trigger hypersensitivity reactions ranging from mild allergy to severe anaphylactic shock. Other complications may include secondary bacterial infection, biliary obstruction, pulmonary collapse, and compression of nearby vital structures (Akbulut et al., 2019).

The pathological effects of echinococcosis are mainly related to the pressure caused by enlarging cysts and the immune response generated by the host. Over time, some cysts may undergo calcification or degeneration, which may further contribute to tissue damage and organ dysfunction. Therefore, early diagnosis and proper treatment are very important for preventing serious complications and improving patient outcomes.

5.0 Diagnostic Approaches in Echinococcosis.

Early and proper diagnosis of echinococcosis is vital for the management of the disease, complication avoidance, and effective treatment results. Diagnosis of the condition depends mainly on the clinical presentation, epidemiology, imaging studies, and serological testing of the patient. Given the asymptomatic nature of hydatid cysts for many years, some instances of detection occur by accident during radiographic exams carried out for other diseases (Moro & Schantz, 2009).

5.1 Imaging Techniques

Imaging methods are considered to be the crucial point in diagnosing cases of cystic echinococcosis because they will help establish such parameters as the size, location, shape, and stage of hydatid cysts.

5.2 Ultrasonography (USG)

Ultrasonography is one of the most widely used diagnostic tools for hepatic hydatid disease. It is a simple, non-invasive, affordable, and easily available imaging technique that helps visualize cyst morphology, daughter cysts, septations, and calcification. Ultrasonography is particularly valuable in endemic regions because it is suitable for screening large populations and for regular follow-up of patients undergoing treatment. The WHO Informal Working Group on Echinococcosis has also introduced ultrasound-based classification systems for staging hydatid cysts (WHO-IWGE Guidelines).

5.3 Computed Tomography (CT)

Computed tomography provides more detailed anatomical information compared to ultrasonography and is especially useful for detecting cysts in deep tissues or unusual anatomical locations. CT scans are highly effective in identifying calcifications, internal septations, cyst wall characteristics, and complications such as rupture or secondary infection. This technique is also useful in evaluating multiple organ involvement and complicated hydatid disease (Pedrosa et al., 2000).

5.4 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) provides better soft tissue visualization and helps in the detailed examination of cystic lesions, especially in organs such as the brain, spinal cord, and soft tissues. MRI is particularly useful in complicated hepatic echinococcosis and neurological involvement because it clearly shows the relationship between hydatid cysts and surrounding structures. It also helps in identifying internal cyst details and associated complications more accurately. However, due to its high cost and limited availability, MRI is generally preferred only in selected or complicated cases (Wen et al., 2019).

5.5 Serological Techniques

Serological investigations play an important supportive role in the diagnosis of echinococcosis, especially in cases where imaging findings alone are not enough for confirmation. These techniques mainly detect antibodies produced against Echinococcus antigens in patient serum samples. Over the years, several immunological methods have been developed to improve the sensitivity and specificity of diagnosis.

Among the available serological methods, Enzyme-Linked Immunosorbent Assay (ELISA) is considered one of the most commonly used techniques because it is simple, economical, sensitive, and suitable for testing a large number of samples within a short period of time. The test is based on antigen-antibody interaction, which produces a measurable color reaction indicating the presence of infection (Santucciu et al., 2020).

Apart from ELISA, other serological techniques such as indirect hemagglutination assay (IHA), latex agglutination, immunoblotting, and Western blot analysis are also used in the diagnosis of echinococcosis. Immunoblotting methods are generally regarded as more specific and are mainly used for confirming doubtful or borderline ELISA results. In recent years, recombinant antigen-based assays have further improved the accuracy and reliability of serological diagnosis (Virginio et al., 2012).

Despite their usefulness, serological investigations also have certain limitations. False-negative results may occur in patients with inactive, calcified, or pulmonary cysts because antibody production may be comparatively low in such conditions. Similarly, false-positive reactions can occur because of cross-reactivity with antibodies produced against other parasitic infections. Therefore, serological results should always be interpreted together with imaging findings and clinical evaluation for better diagnostic accuracy (Brunetti et al., 2010).

Recent advancements in immunodiagnostic research mainly focus on the development of purified and recombinant antigens to improve the sensitivity and specificity of serological tests. Molecular methods such as polymerase chain reaction (PCR) are also increasingly being explored for species identification, confirmation of diagnosis, and epidemiological investigations. However, despite these advancements, ELISA still remains one of the most practical and widely used serological techniques in routine clinical laboratories, particularly in endemic and resource-limited settings.

6.0 Role of ELISA in the Diagnosis of Echinococcus.

The serological tests are very important in the diagnosis of human echinococcosis especially when there are no signs and symptoms present for confirmation through physical examination and imaging tests. There are different serological methods that are commonly used but one method which is being widely used is the ELISA (Enzyme-Linked Immunosorbent Assay). The ELISA test has been popular among other methods because it is

simple to use, inexpensive, sensitive, and is suitable for high volume testing.

Detection of IgG antibodies is the most commonly employed approach in serological diagnosis of echinococcosis. IgG-based ELISA assays are widely preferred because IgG antibodies remain detectable for longer durations and provide useful information regarding exposure to the parasite. Several commercial diagnostic kits are specifically designed for anti-Echinococcus IgG detection and are commonly used in diagnostic laboratories (Sarkari et al., 2007).

The sensitivity and specificity of ELISA may vary depending on several factors including the type of antigen used, location of the cyst, stage of infection, and host immune response. Studies have shown that hepatic hydatid cysts generally produce higher antibody responses compared to pulmonary cysts, resulting in better diagnostic sensitivity in liver involvement. Use of purified or recombinant antigens has significantly improved the specificity and overall diagnostic performance of ELISA in recent years.

One of the major advantages of ELISA is that it is relatively easy to perform and can process a large number of samples within a short period of time. The method is economical, less time-consuming, and suitable for routine laboratory screening as well as epidemiological surveys. ELISA also provides quantitative or semi-quantitative results that help in monitoring antibody levels during treatment and follow-up of patients. Because of these benefits, ELISA remains one of the most practical serological techniques in endemic and resource-limited settings.

Despite its important role in the serological diagnosis of echinococcosis, ELISA also has certain limitations. False-positive reactions may sometimes occur due to cross-reactivity with antibodies produced during other parasitic infections such as cysticercosis, schistosomiasis, and fascioliasis. In some cases, autoimmune disorders and non-specific immune responses may also interfere with the accuracy of the test. Therefore, ELISA findings should always be interpreted along with clinical history, radiological imaging, and other laboratory investigations for proper confirmation of the disease.

False-negative results may also be observed, especially in patients having inactive, calcified, intact, or pulmonary cysts where antibody production remains comparatively low. Similarly, during the early stages of infection, the host immune response may not be sufficiently developed to produce detectable antibody levels. Hence, a negative serological result does not completely rule out echinococcosis, particularly when imaging studies strongly indicate hydatid disease (Zhang et al., 2012).

Nowadays, commercial kit-based ELISA systems are widely used in diagnostic laboratories because they provide standardized reagents, better reproducibility, and easier interpretation of results. These kits generally contain ready-to-use controls, calibrators, conjugates, substrate solutions, and washing buffers for detecting specific anti-Echinococcus antibodies. Standardization of these assays has significantly improved diagnostic consistency and reduced inter-laboratory variation (Tamarozzi et al., 2021).

Several comparative studies have evaluated ELISA with other serological techniques such as indirect hemagglutination assay (IHA), latex agglutination, immunoblotting, and Western blot analysis. ELISA is considered more practical for large-scale screening due to its high sensitivity, cost-effectiveness, and simple procedure. However, immunoblotting methods usually provide greater specificity and are therefore commonly used for confirmation of doubtful or borderline ELISA results. In recent years, recombinant antigen-based ELISA assays have shown improved sensitivity and specificity in the serodiagnosis of cystic echinococcosis. Recent studies have also highlighted the usefulness of recombinant antigen B and glutaredoxin-based ELISA systems in improving diagnostic performance and reducing cross-reactivity (Sharifi et al., 2023).

Current research in immunodiagnostics is mainly focused on developing highly sensitive recombinant antigen-based ELISA techniques along with molecular methods such as polymerase chain reaction (PCR) for confirmation and species identification. Although newer molecular approaches are emerging rapidly, ELISA still remains one of the most widely accepted and practically useful serological methods for routine diagnosis of echinococcosis because of its affordability, accessibility, and reliable diagnostic efficiency.

7.0 Other Serological Techniques used in the Diagnosis of Echinococcosis.

Although ELISA is considered one of the most widely used serological techniques for the diagnosis of

echinococcosis, several other immunological methods are also available for detection and confirmation of infection. Different serological tests vary in their sensitivity, specificity, technical procedure, cost, and overall diagnostic usefulness. In routine laboratory practice, a combination of more than one serological method is often preferred to improve diagnostic accuracy and reduce the possibility of false-positive or false-negative results (Peruzzu et al., 2022).

7.1 Western Blot Technique

Western blot analysis is considered as one of the most specific serological techniques used in the diagnosis of echinococcosis. This method identifies antibodies directed against particular Echinococcus antigenic proteins that are separated according to their molecular weight. Because of its higher specificity, Western blot is frequently used for confirming doubtful or borderline ELISA findings. Studies have shown that this method is more effective in distinguishing true infections from cross-reactive parasitic diseases (Brunetti et al., 2010).

When compared with ELISA, Western blot generally provides better specificity; however, the procedure is more expensive, technically demanding, and time-consuming. It also requires specialized laboratory equipment and trained personnel. For this reason, Western blot is usually preferred as a confirmatory technique rather than a routine screening method in large populations (Tamarozzi & Brunetti, 2018).

7.2 *Indirect Hemagglutination Assay (IHA)*

Indirect hemagglutination assay (IHA) is another important serological method used for the detection of antibodies against Echinococcus antigens. In this technique, red blood cells coated with parasite antigens show agglutination when specific antibodies are present in the patient's serum sample. The procedure is relatively simple, cost-effective, and easy to perform, which makes it useful for routine laboratory investigations (Moro & Schantz, 2009).

Despite its usefulness, several studies have shown that IHA usually possesses lower sensitivity and specificity compared to ELISA, especially in patients with inactive, calcified, or pulmonary cysts. In addition, cross-reactivity with antibodies produced during other parasitic infections may sometimes interfere with the test results and reduce diagnostic accuracy. Because of these limitations, IHA is generally used as a supportive serological technique rather than a confirmatory diagnostic method for echinococcosis (Sarkari et al., 2007).

7.3 Latex Agglutination Test

The latex agglutination test is based on the interaction between antibodies present in patient serum and latex particles coated with specific parasite antigens. If antibodies are present, visible agglutination occurs. This method is rapid, simple, and does not require sophisticated laboratory infrastructure, which makes it useful in smaller healthcare settings and screening programs (Vuitton, 2009).

Despite these advantages, the diagnostic performance of latex agglutination is generally lower when compared with ELISA and immunoblotting techniques. Test sensitivity may vary depending on the type of antigen preparation used and the stage of infection. In addition, false-positive reactions due to cross-reactivity with other helminthic infections can reduce its specificity. Consequently, latex agglutination is usually employed as an adjunctive rather than an independent diagnostic method (Zhang et al., 2012).

7.4 *Immunochromatographic Tests*

Immunochromatographic assays, commonly known as rapid diagnostic tests, have become increasingly important in recent years because they provide quick and easy results. These tests work on antigen-antibody reactions occurring on membrane strips and usually give results within a few minutes. Due to their simple procedure and easy interpretation, they are especially useful in field areas and peripheral healthcare centers where advanced laboratory facilities may not be available (WHO-IWGE Guidelines, 2003).

Compared to conventional ELISA, immunochromatographic tests are faster and require less technical expertise. However, their sensitivity and specificity may vary depending on the quality and type of antigen used in the assay (Tamarozzi et al., 2021).

Overall, every serological method has its own advantages and limitations. ELISA still remains the preferred method for routine screening because of its affordability, simplicity, and comparatively high sensitivity. On the other hand, techniques such as Western blot and recombinant antigen-based assays provide better specificity and are more useful for confirmatory diagnosis. Therefore, combining serological methods with imaging investigations often

provides more accurate diagnosis and better clinical management of echinococcosis.

8.0 Recent Advances in Immunodiagnosis of Echinococcosis.

In recent years, significant progress has been made in the field of immunodiagnosis of echinococcosis. Although conventional serological methods such as ELISA and indirect hemagglutination assay are still widely used, certain limitations including cross-reactivity, variable sensitivity, and occasional false results have encouraged researchers to develop more reliable and accurate diagnostic techniques. Modern immunodiagnostic approaches mainly focus on improving sensitivity, specificity, rapid detection, and overall diagnostic accuracy (Wen et al., 2019).

8.1 *Recombinant Antigen-Based Diagnostics*

One of the major recent developments in serodiagnosis is the use of recombinant antigen-based assays. Conventional serological tests often use crude hydatid cyst fluid antigens, which may sometimes react with antibodies produced during other parasitic infections and reduce test specificity (Güreser et al., 2023). To overcome this limitation, recombinant antigens such as antigen B and antigen 5 have been developed for better and more specific detection of Echinococcus infection (Sarkari & Rezaei, 2015).

Several studies have reported that recombinant antigen-based ELISA systems provide improved sensitivity and specificity compared to traditional serological methods. These assays also help in reducing non-specific reactions and produce more consistent and reproducible results. In addition, recombinant antigen technology improves standardization among laboratories, which is beneficial for routine diagnosis as well as epidemiological studies. Because of these advantages, recombinant antigen-based assays are increasingly considered promising alternatives to conventional serological techniques (Yang et al., 2024).

8.2 *Molecular Methods in Diagnosis*

Molecular diagnostic methods have also emerged as valuable tools for the diagnosis and characterization of echinococcosis. Unlike conventional serological tests that mainly detect host antibodies, molecular techniques directly identify the genetic material of the parasite, thereby improving diagnostic precision. These methods are especially useful in complicated or doubtful cases where routine serological findings may not provide definite confirmation (Wen et al., 2019).

Molecular techniques also play an important role in differentiating various Echinococcus species and their genotypes. This information is useful for understanding transmission dynamics, geographical distribution, and epidemiological patterns of the disease. In recent years, molecular epidemiology has significantly contributed to expanding knowledge regarding the genetic diversity and global spread of echinococcosis (Torgersopn & Deplazes, 2010).

9.0 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is one of the most important molecular techniques used for the diagnosis of echinococcosis. This method works by detecting and amplifying specific DNA sequences of the parasite from clinical samples such as cyst fluid, tissue samples, or blood. Because of its high sensitivity and specificity, PCR can identify even very small amounts of parasitic DNA (Kırış et al., 2022).

Compared to routine serological methods, PCR provides more accurate identification of parasite species and genotypes. It is especially useful in doubtful cases, confirmation of infection, monitoring recurrence after treatment, and epidemiological studies. Advanced methods such as real-time PCR and multiplex PCR have further improved diagnostic accuracy by allowing rapid and simultaneous detection of multiple targets (Knapp et al., 2023).

Despite these advantages, PCR-based techniques require advanced laboratory facilities, skilled personnel, and higher costs compared to conventional serological tests. These factors may limit their routine use, particularly in low-resource healthcare settings (Knapp et al., 2023).

10.0 Nanotechnology-Based Assays

In recent years, nanotechnology has gained increasing attention in the diagnosis of infectious diseases, including echinococcosis. Nanoparticle-based immunoassays use materials such as gold nanoparticles, magnetic nanoparticles, and quantum dots to improve antigen-antibody detection. These advanced techniques provide better sensitivity, faster reaction time, and improved signal detection compared to conventional serological methods (Tiwari et al., 2023).

Several studies suggest that nanotechnology-based assays may help in the early detection of echinococcosis

with improved diagnostic accuracy. These methods usually require smaller sample volumes and shorter processing time, making them useful for rapid laboratory diagnosis. Although most of these technologies are still under development, they show promising potential for future clinical and diagnostic applications (Hajjafari et al., 2024).

11.0 Point-of-Care Diagnostic Tests

Point-of-care diagnostic methods are gaining increasing importance for the rapid and convenient diagnosis of echinococcosis, especially in endemic and low-resource regions. These tests are designed to provide quick results without requiring highly advanced laboratory equipment or extensive technical expertise. Immunochromatographic strip tests and portable biosensor-based systems are among the most commonly studied point-of-care diagnostic approaches (WHO-IWGE Guidelines, 2003).

Compared with conventional laboratory methods, point-of-care tests are simpler, faster, and more suitable for field screening programs and peripheral healthcare centers. These techniques can help reduce diagnostic delays and improve accessibility in remote areas where proper laboratory facilities may not be available. Although further validation and standardization are still needed, these rapid diagnostic systems show promising potential for future surveillance and control of echinococcosis (Tamarozzi et al., 2021).

Overall, recent advances in immunodiagnostic research have greatly improved the diagnosis and understanding of echinococcosis. Recombinant antigen-based assays, molecular diagnostic methods, PCR techniques, nanotechnology-based systems, and point-of-care tests are all contributing towards the development of more sensitive, specific, and rapid diagnostic approaches. However, ELISA still remains one of the most widely used and practically important serological techniques because of its affordability, accessibility, and usefulness in routine clinical diagnosis.

12.0 Challenges and Limitation in the Diagnosis of Echinococcosis.

Despite major advances in serological and molecular diagnostic methods, the diagnosis of echinococcosis still faces several important challenges. Variation in clinical presentation, differences in immune response among patients, and limited healthcare facilities often make early and accurate diagnosis difficult. In many endemic and resource-limited regions, delayed detection of the disease continues to increase the risk of complications, morbidity, and economic burden on healthcare systems (Agudelo Higuita et al., 2016).

12.1 Cross-Reactivity in Serological Tests

One of the major problems associated with serological diagnosis is cross-reactivity with other parasitic infections. Antibodies produced against parasites such as *Taenia solium, Schistosoma, and Fasciola s*pecies may sometimes react with Echinococcus antigens used in diagnostic tests, leading to false-positive results. This problem is more commonly seen when crude hydatid cyst antigens are used in serological assays (Güreser et al., 2023).

Cross-reactivity decreases the specificity of serological tests and may create difficulty during interpretation of laboratory results, particularly in regions where multiple parasitic infections are common. Although recombinant antigen-based techniques have improved specificity and reduced non-specific reactions to some extent, complete elimination of cross-reactivity still remains a major challenge in immunodiagnostic research (Virginio et al., 2012).

12.2 Reduced Sensitivity in Pulmonary and Inactive Cysts

Another important limitation of serological diagnosis is the comparatively lower sensitivity observed in patients with pulmonary, inactive, or calcified hydatid cysts. In such conditions, the host immune response may become weaker because the parasite remains less exposed to the immune system. As a result, antibody levels may remain low and serological tests such as ELISA may sometimes produce false-negative results even in infected individuals (Brunetti et al., 2010).

Studies have shown that hepatic cysts usually produce a stronger antibody response than lung cysts, resulting in better serological positivity in liver involvement. Therefore, diagnosis of pulmonary echinococcosis often depends more on imaging techniques such as CT scan and MRI along with supportive serological findings (Tamarozzi & Brunetti, 2018).

12.3 High Cost of Advanced Diagnostic Techniques

Modern diagnostic techniques such as PCR, real-time PCR, recombinant antigen-based assays, and nanotechnology-based diagnostic systems have

significantly improved the accuracy of echinococcosis diagnosis. However, the routine use of these advanced methods is often limited because of their high cost. These techniques require sophisticated laboratory equipment, costly reagents, and trained technical personnel for proper performance (Kırış et al., 2022).

In many developing and endemic countries, financial limitations restrict the regular use of advanced molecular diagnostic methods. Because of this, conventional serological tests such as ELISA are still preferred in most laboratories as they are comparatively affordable, simple, and easier to perform.

12.4 Limited Diagnostic Facilities in Rural and Endemic Areas

Lack of proper healthcare infrastructure is another major challenge in the diagnosis of echinococcosis, especially in rural and endemic regions. Many healthcare centers do not have advanced imaging facilities, molecular laboratories, or trained professionals required for specialized diagnostic procedures. Consequently, several infections remain undetected until the disease reaches advanced stages (Craig et al., 2017).

People living in remote areas often face difficulty in accessing specialized healthcare services for confirmatory diagnosis and treatment. Delayed diagnosis not only increases the risk of severe complications but also contributes to continued transmission of infection within the community. Therefore, strengthening rural healthcare systems and improving access to diagnostic services are essential for effective disease control and early detection (WHO-IWGE Guidelines, 2003).

12.5 Lack of Public Awareness and Health Education

Poor public awareness regarding echinococcosis also plays a major role in delayed diagnosis and continued transmission of infection. In many endemic communities, people are still unaware about the modes of transmission, preventive strategies, and importance of seeking timely medical attention. Practices such as close interaction with infected dogs, unsafe slaughtering of livestock, poor sanitation, and inadequate personal hygiene continue to support the lifecycle and spread of the parasite (Thys et al., 2019).

In addition, limited awareness among healthcare workers in peripheral healthcare settings may further delay suspicion and diagnosis of the disease. Public health education programs, routine deworming of dogs, improved slaughterhouse hygiene, and awareness campaigns are therefore important measures for reducing disease burden and promoting early diagnosis (Agudelo Higuita et al., 2016).

Overall, although substantial advancements have been achieved in the field of immunodiagnosis, several diagnostic and public health challenges still remain unresolved. Issues such as cross-reactivity, false-negative reactions, expensive diagnostic technologies, inadequate rural healthcare facilities, and insufficient public awareness continue to affect the effective diagnosis and control of echinococcosis (Liu et al., 2025). Addressing these limitations through improve through improved diagnostic strategy, healthcare acceptability and awareness programs will be essential for better disease management and prevention in the future.

13.0 Prevention and Control.

The prevention and control of echinococcosis mainly focus on breaking the chain of transmission between dogs, livestock , humans ,and the surrounding environment. Since the disease is strongly linked with poor sanitation, unhygienic slaughtering practices, and close interaction with infected animals, effective control requires a combined effort from both veterinary and public health sectors. Long-term reduction of infection is only possible through proper awareness, environmental hygiene, and active community participation in endemic regions (Siles- Lucas et al., 2023).

One of the most effective preventive measures is the regular deworming of dogs, as dogs act as the primary definitive hosts of Echinococcus species. Routine administration of anti-parasitic drugs helps reduce the release of parasite eggs into the environment and lowers the risk of transmission to humans and livestock (Efa et al., 2026).

Maintaining proper slaughterhouse hygiene is another essential step in disease prevention. In many rural areas, infected animal organs are sometimes discarded carelessly or fed to dogs, allowing the parasite life cycle to continue. Therefore, proper meat inspection, hygienic slaughtering practices, and safe disposal of infected organs are necessary to prevent further transmission of infection (Badwaik et al., 2024).

Good personal hygiene and sanitation also help in protecting humans from accidental infection. Simple

practices such as washing hands after handling dogs, drinking clean water, eating properly washed vegetables, and maintaining hygienic surroundings can significantly reduce the risk of ingesting parasite eggs. These precautions are especially important for farmers, butchers, livestock handlers, and people living in close contact with domestic animals (Badwaik et al., 2024).

knowledge regarding the transmission, prevention, and health risks associated with echinococcosis. Educational campaigns through schools, healthcare centers, and community programs can improve awareness about safe animal handling, sanitation, and the importance Public awareness and health education programs also play an important role in disease prevention and control. In many endemic communities, people still have limited of early diagnosis and treatment (Hajjafari et al., 2024).

In recent years, the "One Health" approach has received increasing attention for the prevention and control of zoonotic diseases such as echinococcosis. This approach promotes coordination between doctors, veterinarians, environmental authorities, and local communities. Combined efforts involving dog deworming, animal surveillance, environmental sanitation, and public education are considered essential for long-term disease control and reduction of transmission in endemic areas (Erganis et al., 2024).

14.0 Conclusion

Echinococcosis is still an important zoonotic parasitic disease, especially in rural and endemic areas where healthcare facilities are limited. The disease usually grows slowly and may remain unnoticed for many years. Because of this, diagnosis is often delayed until serious complications develop. Therefore, early and accurate diagnosis is very important for proper treatment and better patient outcomes (Moro & Schantz, 2009).

Serological methods play an important supportive role in the diagnosis of echinococcosis, particularly when imaging findings alone are not enough for confirmation. Among all serological techniques, ELISA remains one of the most commonly used methods because it is simple, affordable, sensitive, and suitable for routine screening. It is also useful in epidemiological studies, treatment monitoring, and follow-up of infected patients (Vuitton, 2009).

Although ELISA has some limitations such as false-positive and false-negative reactions, it still remains one of the most practical and easily available diagnostic techniques in clinical laboratories. Recent developments such as recombinant antigen-based assays, PCR techniques, molecular diagnostics, circulating free DNA analysis, and nanotechnology-based methods have further improved the sensitivity and specificity of diagnosis and may enhance early detection of echinococcosis in the future (Luo et al., 2024).

Early detection of echinococcosis is necessary to prevent serious complications like cyst rupture, secondary spread of infection, anaphylactic reactions, and permanent organ damage. Combining serological tests with imaging techniques and clinical evaluation usually provides more reliable diagnosis and better disease management. Public awareness, proper sanitation, regular deworming of dogs, and improved healthcare accessibility are also important for effective prevention and control of the disease (Tamarozzi & Brunetti, 2018).

Future research should mainly focus on developing rapid, accurate, affordable, and field-friendly diagnostic methods that can be easily used in endemic and resource-limited regions. Advances in molecular techniques, recombinant antigen technology, PCR-based diagnosis, and nanotechnology may further improve early detection and overall management of echinococcosis in the future (Sharifi et al., 2023).

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- Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies

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