Echinococcus species, neglected food borne parasites: taxonomy, life cycle and diagnosis

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ABSTRACT

Echinococcosis is a parasitic disease caused by a cestode belonging to the genus Echinococcus. These are small tapeworms belonging to the phylum Plathelmintes, family Taeniidae, class Cestoda with a worldwide distribution from the North Hemisphere to the tropics. The biology of the parasite and the transmission patterns offer enough reasons to consider the species of this genus (Echinococcus) as food borne parasites. The adult stage of the worm lives in the digestive tract of the definitive host (canids, felids, hyenids), these are able to excrete infected eggs in the environment. The intermediate host (usually herbivores, ungulates and accidental human) become infected through ingestion of the cestodes eggs. The metacestodes develop in the body of the intermediate host, in different organs (liver and lung most frequent) where are described cystic, polycystic and alveolar echinococcosis. The diagnosis of the disease is based on the screening and confirmatory methods. The imagery is an important tool that has to be combined with serology for increasing the accuracy of the diagnostic. The level of the hygiene, the proximity with domestic animals and occupation are important opportunities for the transmission of the parasite. Human echinococcosis is a neglected parasitic disease which asks more attention and improvements to the diagnostic tools.

Keywords: Echinococcus spp; hydatid cyst; metacestode; alveolar echinococcosis; serology; molecular diagnosis.

1. INTRODUCTION

Echinococcosis is a parasitic disease with an impact on public health has implications in both human and veterinary medicine. The damage produced is economic and social, by the invalidity produced on the patients, the large number of days of hospitalization from a minimum of 4 days to 30 – 40 days in severe cases associated with high costs, and the damage to the populations of domestic animals that play the role of intermediate hosts in the biological cycle of the parasite (sheep, cattle). The species in the Echinococcus complex are geographically spread on all inhabited continents, preferring these hosts that are close to human populations. This explains the direct relationship between the parasite and the human being, connection which has mandatory importance for the transmission of the parasitic organism.

The study of the species of Echinococcus has been preoccupied the researchers since antiquity. Greek doctors of the ancient world, Hippocrates, Aretaeus and Galen mentioned these parasites in their writings [1]. The etiology of this disease remained somewhat unsolved until the clarification of the origin of the parasitic organisms.

Since the introduction of the binary nomenclature, in 1758, and by the end of the nineteenth century, the species of Echinococcus have received not less than 85 bi or trinomial names [2]. The classification and ordering of Echinococcus species constituted a constant challenge for the scientific world.

Batsch (1786), morphologically described hydatid cysts in sheep and provided the name Hydatigena granulosa [2]. Von Siebold in 1852 conducted experimental studies feeding dogs with protoscolices obtained from hydatid cysts from sheep, thereby achieving adult parasites. In this way von Siebold and those who followed his example (Haubner, Leuckart, Kuchenmeister and Nettleship) have brought important clarification regarding the life cycle of the parasite [3,4], connecting the two stands of the parasite (adult-in the body of canids the definitive hosts and larval stage - in the body of herbivores, intermediate hosts). In 1801 Rudolphi, defined the genus Echinococcus, starting from the small, round protoscolices and hooks found in cysts, suggesting the name of Echinococcus granulosus, the name that is used and currently.

In 1855, two types of hydatids were described, which triggered a controversy in the scientific world regarding the existence of two species producing these types of lesions. The existence of the two species, Echinococcus granulosus for cystic and Echinococcus multilocularis for the alveolar (Leuckart, 1863) was scientifically demonstrated only in 1957 when Vogel succeeded in reproducing the life cycle of Echinococcus multilocularis in the laboratory. Until this time Echinococcus multilocularis was considered a variant of Echinococcus granulosus. Between 1910 - 1972, were described 14 species belonging to the genus Echinococcus [5], of which, a part, were disagreed on morphological criteria: Rausch (1953), Vogel (1957), Rausch and Nelson (1963) [6]. Only Echinococcus oligarthrus (Diesing, 1863) and Echinococcus vogeli [7] have maintained the status recognized by the species.

2. TAXONOMY

In the early 1980’s, the scientific researches proved the existence of four species defined by Echinococcus: granulosus, multilocularis, oligarthrus and vogeli [5]. It was also acknowledged the existence of numerous intraspecific variations
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based on morphological differences, host specificity, biochemical parameters and geographical distribution.

The late 1980 and early 1990 were marked by molecular studies initiated by a group of Australian researchers. The advanced techniques facilitated the identification of species in the Echinococcus genus considering that morphological differences were difficult to distinguish. Molecular taxonomic analysis was done using short sequences of mitochondrial DNA: cytochrome c oxidase subunit 1 (cox1) and NADH dehydrogenase subunit 1 (nad1). The studies led to the conclusion: the presence of the 10 specific genotypes (G1 – G10) grouped in the Echinococcus granulosus sensu lato complex. This complex comprises species that show preferences for the intermediate host as follows: in sheep - two species (G1, G2), in cattle - two species (G3, G5), equines - one species (G4), in camelides - one species (G6), two species - in pigs (G7, G9) and two species - in cervids in the North American/Scandinavian region (G8, G10) [8-10]. Genotypes G1 - G3 are closely grouped into a specific cluster forming the taxon Echinococcus granulosus sensu stricto, G1 being responsible for most human cases [11]. Despite the distant position of the G6-G10 taxon, this also includes species that cause infections in humans, but in a much lower percentage than G1 - G3 [11,12]. In 2005, it was described also a new species Echinococcus shiquiquis located in the Tibet Plateau [13].

3. MORPHOLOGY

The morphological description of the species of Echinococcus complex is based on the differences between species, concerning the number of segments, the morphology of the hooklet, the number and distribution of uterine diverticula, the position of genital pores and other morphological criteria. Echinococcus granulosus is a small cestode (2-9mm long). The adult is attached to the intestinal mucosa of the definitive host (domestic and/or wild canids) and has the characteristic structure of the class (Cestode). The parasite is built of as follow: scolex (head), neck and body(strobila). The scolex has a globular shape, with a diameter of about 0, 3mm, contains 4 suction cups and a double crown of hooks (large and small). The number of hooks is variable between 25-50, there is a connection between the number of large and small hooks. The scolex is followed by the neck, with one/two immature segments. The last segment, the mature one contains a fully developed male and female genital apparatus, the uterus containing about 500 eggs that are released into the external environment together with the gravid segment. The position of the genital pores differs according to the species of Echinococcus [14].

![Figure 1. Echinococcus granulosus – cyst.](image1)

Echinococcus multilocularis is also a small tapeworm 1.5 – 3.0 mm length, with a typical cestode organization: head (scolex), neck and body (strobila). The scolex is a globular equipped with a double crown of hooks (28-30) and four suckers located on the dorsolateral side. The head and the body are connected through a long and thin neck [15].

![Figure 2. Echinococcus granulosus (a) membranes: germinal&laminar; (b) daughter vesicules; (c) hydatic fluid.](image2)

The Echinococcus spp. eggs are identical to those of other Taenia species with approximately 30-40 µm in diameter and contain the hexacant embryo. The eggs are covered with two layers, extremely durable, heavily keratinized that offer the pigmented look. The membrane is double with radiating striations [16].

The metacestode represents the larval stage of the platyhelminth, which develops in the body of the intermediate host and whose evolution differs according to the species of Echinococcus.
The location could be single and/or multiple at the level of different internal organs. The intermediate host (herbivores, rodents, accidentally humans) enters in the life cycle of this cestode ingesting the infected eggs. In the small intestine, oncospheres hatches crosses the intestinal wall and via the blood stream are transported to various internal organs. The liver is the preferred target organ, followed by the lung and other locations (kidneys, spleen, heart, bone, central nervous system) where metacestode occurs less frequently [17]. When the oncospheres arrive at the destination (the target organ), there will start his process of cellular differentiation. The evolution at this level is different related to the species. The metacestode of *Echinococcus granulosus*, called the hydatid cyst, is a globular cystic formation (Figure 1) whose evolution occurs over a long period of time (months even years). Initially, the presence of the parasite is asymptomatic because the metacestode achieves only mechanical compression in the affected organ, leading to damage to adjacent tissues, compression of blood vessels or even organs. The whole process is dependent on the affected organ and the position of the cyst.

The cyst is surrounded (figure 2) with a wall called the germinal membrane. This membrane, towards the internal cavity of the cyst, produces protoscolices and daughter vesicles that are capable of developing in the body. On the other hand, there are patients who succeed by their own internal means inactivating the metacestode [17].

The hydatid fluid has a clear, perfectly transparent appearance, rich in specific antigens. The laminar membrane has the role to protect the physical integrity of the cyst and allow the germinal membrane to carry out its activity. Laminar membrane has been specially designed in the evolutionary process to ensure the protection of the cyst from the action of the host's immune system. The presence of an active cyst in the host's body causes the formation of a protective layer produced by the host, the adventitial membrane (pericyst), a coating that aims to supervise the cyst evolution (Figure 3).

**Figure 3. Echinococcus granulosus pericyst.**

*Echinococcus multilocularis* metacestode has a different evolution from that of *Echinococcus granulosus*. In the body of the intermediate host, the oncosphere is released, and via blood circulatory system with various target organs as destinations. The liver is among the preferred organs, where metacestode shows as a dispersion of fibrous tissue with groups of small cavities whose diameter varies from a few mm to cm in diameter.

In chronic cases, the lesion could develop a central cavity containing a viscous fluid. As a characteristic of the disease is the appearance of calcified areas, especially in the tissue belonging to the parasite. The central lesion can be surrounded by small cavities, resulting in the appearance of bunches comprising liver tissue. The host's immune system acts against parasitic invasion, which is an explanation for the fact that not all people who come into contact with the cestode eggs will develop the larval stage in the body. On the other hand, there are patients who succeed by their own internal means inactivating the metacestode [17].

### 4. LIFE CYCLE

The species were spread across the globe at first by chance, being associated with the movements of dogs and sheep accompanying human populations in migration, and then with the expansion of shepherding in Eurasia and the massive expansion and colonization of European states. The geographic distribution of *Echinococcus granulosus* species began early in the Neolithic Era (around 10,000 BC) and was accelerated in the time of scientific revolution (15th and 17th centuries).

*Echinococcus granulosus* and *Echinococcus multilocularis* have the greatest social and economic impact, affecting the largest number of human individuals worldwide. According to statistics, 188,000 new cases with 184,000 DALYs (Disability-Adjusted Life Year) of cystic echinococcosis are recorded annually and 18,500 new cases with 688,000 Alveolar Equinococcosis DALYs. The DALY system is currently the most widespread way of measuring and comparing the incidence of the disease, negative effects and risk factors both at the level of a country and internationally [18,19]. World Health Organisation considered the echinococcosis to be an emerging zoonose (WHO/FAO/OIE in the meeting with the subject 'Emerging Zoonoses' Geneva, 3-5 May 2004). The definition of emerging zoonosis is: 'newly recognized zoonoses, which has a new evolution, or occurring in the past but registering an increase in incidence, an increased geographical distribution as regards the spread area, vectors and hosts' ([http://www.who.int/zoonoses/en/](http://www.who.int/zoonoses/en/)).

The biological cycle of cestode species belonging to this genus implies the existence of two hosts: definitive (a species of domestic/wild canids) and intermediates (domestic/wild ruminants, omnivores). The adult stage develops and reaches sexual maturity, produces eggs, which are excreted by the host, thus contaminating the environment. The intermediate host (domestic and/or wild herbivore species, accidentally human being), is the second host involved in the biological cycle. The intermediate host ingest the infected products (eggs) with the water and food contaminated and develop—the metacestode. The locations are in various internal organs, the more common being in the liver and lungs. The life cycle is complete when the definitive host consumes the metacestode, thus allowing the development of the adult organism in the digestive tract of the definitive host (canids).

*Echinococcus granulosus sensu stricto*. Definitive host (species of canids) releases pregnant, egg-carrying segments into
the environment. The eggs contain the hexacanth embryo and are infected immediately after disposal. The intermediate host (sheep, goats, accidentally man, and/or cats) [20] ingest these eggs with contaminated foods, and in its small intestine the embryo hatching. They penetrate the intestinal wall and enter circulation. They travel with the blood stream reach several important points in the host's body (liver, lung, pancreas, spleen and other organs). 12 hours after ingestion, it reaches the liver, where, if not destroyed by lymphocytes, it will turn into the larval stage of the cestode (metacestode - the future cyst). Cysts are mainly localized in the liver but can also be established in other organs: lungs, spleen, pancreas, CNS, thyroid [21], the evolution being very slow. In the first 10-14 days, cell proliferation phenomena occur, with the formation of a central cavity and laminal and germinal membranes. If metacestode is ingested by the definitive host (a canid), the protoscolices released in the small intestine evaginated and they will attach to the vilosities of enterocytes on the intestinal mucosa, a process that precedes the transformation the parasitic stage in adult worm (approximative 40-50 days). The adult cestode survives in the body of the definitive host between 5-29 months. The excretion of eggs is rhythmic, and it is done every time the gravid segment is released into the external environment (at about 2 weeks).

*Echinococcus ortleppi* is a species that was established by Lopez-Neyra and Soler Planas (1943), based on the reassessment of the observations made by Ortlepp (1934). In 1965, Verster situated the species in a new taxonomic position, namely the subspecies *Echinococcus granulosus ortleppi* following morphological descriptions of a number of specimens collected from South Africa, including the original ones of Ortleppi, and added more individuals resulting from the experimental infection of dogs with protoscolices from cattle. In 2002 Thompson and McManus [21], published morphological and genetic evidence, studying the sequences of nucleotides and molecular aspects. The results of molecular studies came in support of morphological conclusions of a phylogenetic point of view, and further research. Molecular and morphological studies have gained high attention and further research. **Echinococcus ortleppi** is a species that was established by Lopez-Neyra and Soler Planas (1943), based on the reassessment of the observations made by Ortlepp (1934). In 1965, Verster situated the species in a new taxonomic position, namely the subspecies *Echinococcus granulosus ortleppi* following morphological descriptions of a number of specimens collected from South Africa, including the original ones of Ortleppi, and added more individuals resulting from the experimental infection of dogs with protoscolices from cattle. In 2002 Thompson and McManus [21], published morphological and genetic evidence, studying the sequences of nucleotides and molecular aspects. The results of molecular studies came in support of morphological arguments on the role of cattle as intermediate hosts in the biological cycle of the parasite *Echinococcus ortleppi* and geographical spread in Europe, Africa, southern Asia and the Americas [8], [22-25].

Molecular and morphological studies have gained high weight when, from an epidemiological point of view, this species has proven its pathogenicity to humans [26]. Following molecular analyses on mitochondrial genes [8, 27], concluded that there is a close connection between the *Echinococcus ortleppi* and *Echinococcus canadensis* species, and they were positioned side by side in the phylogenetic tree as sister species. Although the species *Echinococcus ortleppi* is recognised as having affinity for cattle, cases of fertile hydatids belonging to *Echinococcus ortleppi* have been reported in pigs, cervides (*Rusa alfredi*) [28, 29], on the other hand, the G6 genotype, which is part of group E. *canadensis*, was also included in cattle [30-32]. In the last year were also reported human cases with *Echinococcus ortleppi*, even with lung location [33-35].

*Echinococcus canadensis* include a group of G6-G10 genotypes, each with a special composition and a specific geographical distribution. In 1960 Cameron, following morphological and serological studies conducted on individuals belonging to the genus *Echinococcus*, from deer in Canada, proposed the introduction of *Echinococcus granulosus var. canadensis*. Subsequent research has led to some aspects of structure being clarified and conclusions such as that *Echinococcus canadensis* is genetically closer to the bovine strain (*Echinococcus ortleppi*) than to that of sheep (*Echinococcus granulosus sensu stricto*). The group comprises several genotypes (G6-G10), and geographically they are distributed both in the extreme north, namely Canada and the Scandinavian peninsula and in Africa.

The species from the camel, the G6 genotype, has been identified in Africa, specifically in Kenya. Using the DNA hybridization technique, G6 comprises individuals other than the other species of *Echinococcus* identified in other geographical areas of the globe [23] (McManus and Rishi, 1989). The species found in the camel is also particular from a biochemical point of view, and morphological examination of adult parasites from experimental infections clearly shows that individuals enrolled in G6 differ from those identified in sheep (*Echinococcus granulosus*), horses (*Echinococcus equinus*) or cattle (*Echinococcus ortleppi*) [37].

The G8 genotype was genetically characterized in moose (*Alces alces*) in Minnesota, USA [38] and G10 in deer and moose in Finland and designated as the fennoscandian strains of the group due to their geographical distribution [10].

*Echinococcus canadensis* species were considered as having reduced involvement in human pathology [39, 40]. Subsequently, a higher number of positive cases were identified with G6 in Mongolia [41] and the G7 in Austria and Poland [42], and an increased incidence of cases caused by G6 in Argentina [43]. In 2002, an extremely severe case was reported on the Scandinavian peninsula [44]. All these results have led to the conclusion that *Echinococcus canadensis* require increased attention and further research.

*Echinococcus felidis* is a species whose status was until recently uncertain [45]. Although the morphology of rostellum with hooks is characteristic and has a preference for a particular definitive host (*Panthera leo*), this species remained indefinite until 2008. Adults of *Echinococcus felidis* have been collected from the African lion but it is not very clear which of the species of ungulate serves as an intermediate host. Using mitochondrial and nuclear DNA as criteria of genetic analysis, from a phylogenetic point of view, the lion's cestode was found to be sister species (directly related) to *E. granulosus sensu stricto* [46].

The two species *Echinococcus felidis* and *Echinococcus granulosus sensu stricto* have a common Asian ancestor [45, 47]. *Panthera leo* (African lion) evolved from an Asian ancestor to the late Pliocene and invaded Africa in early Pleistocene (1.5- 2 million years) [48]. Assuming that the bifurcation between the two species was carried out in Asia, the hypothesis claims that *Echinococcus felidis* would have entered Africa with the lions, in the same period [49].

*Echinococcus felidis* is a cestode that has as its definitive host the African lion (*Panthera leo*), and as an intermediate host is found in many species (zebra, giraffe, buffalo, wild pig and others). Cestode does not have a preference for the intermediate host and no data are known as regards its pathogenicity in humans and domestic animals. On the other hand, the close relation with
Echinococcus granulosus sensu stricto does not exclude its zoonotic potential.

Echinococcus multilocularis, the tapeworm of the fox is spread in the northern hemisphere, in the Holarctic region [50] and is responsible for the disease called alveolar echinococcosis. In this sylvatic life cycle, the definitive host of the cestode is the fox (red Vulpes vulpes or arctic Vulpes lagopus). Involving the domestic dog (Canis lupus familiaris) in the life cycle, the connection to the human being is marked, marking the time of human intervention in biology of the parasite. The existence of these species was difficult to prove until the 1950s, followed by the discovery of alveolar lesions in patients on the island of St Lawrence in Alaska. The researchers suggested that it could be the same species that causes alveolar echinococcosis in Europe and Russia [44]. In 1957 Vogel using morphological analyses of an adult parasite collected from a lesion of a patient with alveolar echinococcosis in Germany demonstrated the independence of the Echinococcus multilocularis taxon. Vogel also performed the artificial infection of two dogs by feeding them with an infected organ from a patient with alveolar echinococcosis, obtaining the adult stage of the parasite. He went further using the artificial infection of some rodents, Microtus arvalis, with infected eggs collected from the dogs who were carrying the adult. Thus, Vogel, obtained the larval stage of the cestode. By morphological analysis of adults obtained and comparing them with adults of Echinococcus granulosus, Vogel defined the species as a standalone one.

Genetic variation within the species Echinococcus multilocularis was first studied using mitochondrial DNA sequences [22,51]. Only a few substitutions were identified that led to two distinct genotypes: M1 (Europe) and M2 (China, Alaska and North America).

Other researches have presented data such as isolated from Echinococcus multilocularis in the Svalbard archipelago in the Arctic Ocean, which are genetically similar to those in St. Lawrence Island, Alaska [52]. All these studies bring data proving that Echinococcus multilocularis persists in the Arctic (boreal forests), although these were considered ecological barriers to the spread of this cestode, taking into account the low diversity of intermediate host species [53].

5. DIAGNOSIS

The diagnosis of echinococcosis is carried out by several methods, both at the level of the final host (the canid) and at the level of the intermediate host (herbivores and/or human being). The domestic dog (Canis lupus familiaris), wild canids (Canis lupus, Vulpes vulpes) and wild felides (Panthera leo) are the main source of infection throughout the globe, both in humans and animals. The dog is susceptible to infections with all species of the genus Echinococcus, developing different stages from egg ingestion to proglottids elimination for each genotype[32], [56].

Diagnosis of echinococcosis in the body of the definitive host Necropsy.

As regards the identification of parasitic elements in the body of the definitive host (canids - domestic dog, wolf, fox), this can be achieved by very careful examination of necropsy of small intestine fragments and identification of small parasites (2-9 mm for Echinococcus granulosus and 1.5 – 3.0 mm respectively for Echinococcus oligarthra). The process includes processes of washing the intestines, incubation and cooling of the intestinal mucosa for microscopic examination of preparations; the intensity of the infection is appreciated according to the number of parasites identified, a severe infection is that in which >1000 cestodes are highlighted along the intestine, and a low intensity infection <20 cestodes observed.

Parasitological examination.

Routine parasitological examination is carried out by collecting feces through a procedure with arecoline hydrobromide. The method is specific (100%), but sensitivity is low (20% of infested dogs fail to eliminate anything), is laborious and generates substances whose elimination involves special treatment regarding toxic residues. Despite all the disadvantages the method has long provided important data in the circle regarding parasitic loading [57].
**Immunological/serological diagnosis.**

Immunodiagnosis should prove the existence of the specific antibodies and/or the detection of specific coproantigens [58]. These methods offer an alternative to the diagnosis with arecoline hydrobromide. Immunological methods by detecting specific coproantigens of *Echinococcus* have a specificity (97%) and higher sensitivity (98%) compared to previous methods (PAHO 1994) and should be applied to a parasitic load of more than 50 parasites and the use of feces fixed with 5% formaldehyde[57], [59-60]. Reactions are based on the presence of polyclonal antibodies/secretors antibodies (ES) against the adults of *Echinococcus granulosus*. Despite the fact that the value of the optical density of Ag-Ac immune complexes increases with the degree of infection, ELISA reactions for the detection of parasitic load cannot be used as a quantitative method of determining the number of parasites in the body of the definitive host.

Detection of the level of antibodies uses diagnostic antigens extracted from different stages of the parasite (fluid, protoscolices, surface of adult worm) natives or recombinants. The disadvantage of this method is that you cannot distinguish between present and past infections.

Methods of detection of antigens and antibodies are gen - specific. Differentiation of *Echinococcus granulosus* from other species requires molecular methods of hybridization and amplification of genetic material from the feces of the canine host. Taking into account that the parasite eggs are no constant released in the host feces, there are required new methods for obtaining genetic material from other products of the parasite (outside the eggs)[59], [61-62].

**Diagnosis in the body of the intermediate host.**

Intermediate hosts (herbivores and occasionally humans) become infected if they ingest foods contaminated with *Echinococcus* eggs, in their body developing the metacestode (larval stage). The location and the number of larvae formations in the host body differ depending on the species of *Echinococcus* and its ecological particularities.

Since the evolution of metacestode is slow (1-50 mm/year) (Brunetti and Col., 2010) and persists in the absence of specific symptomatology, early diagnosis is almost impossible to achieve. In some situations, there may be ruptures of hydatid or simply its spontaneous disappearance without surgery or medication [63-66]. The diagnosis of echinococcosis is the result of corrobororation of serological tests, imaging and complete anamnesis of the patient [67].

**Clinical diagnosis.**

Medical history and physical examination of the patient are indications for the orientation of the diagnosis to hydatid disease. It is necessary to corroborate information, which provides indications about the contact and risk of egg infestation or the parasite itself, not only recent but in the last 10 years: on/off to/from endemic areas; contact with dogs, foxes, domestic animals (cattle, sheep, goats); work in a pastoral area, involving either contact with sheep and herd guard dogs or contact with wild animals; work in a slaughterhouse.

**Cystic echinococcosis.**

More than 90% of cysts are localized in the liver/lung or both organs and remain without symptomatology for a long time. The main signs depend on the affected organ, the size and positioning of the cyst(s), adjacent tissues and complications from rupture of the cyst/cysts and/or secondary echinococcosis. In the first stage it is an asymptomatic disease. In this phase the oncospheres are released from the ingested eggs; they cross the intestinal wall and penetrate the circulatory system (port vein), thus having access to important organs such as liver, lungs and other locations [68]. The presence of the parasite produces discomfort in the upper abdominal area, reduced appetite, at abdominal palpation can be discovered a mass on the surface of the abdominal organs (the liver being the most affected - in 2/3 patients). Hepatomegaly and/or abdominal distension is recorded. Chest pain, hemoptysis could indicate the existence of a lung cyst. Rupture of the cyst in the bronchi can be completed with the expel of fluid and/or hydatid membranes. In the liver, cysts compress or erode the bile ducts, causing pain, jaundice, cholangitis or sometimes become infected due to a biliary fistula. Secondary echinococcosis could develop from disseminating the contents of a cyst as a result of surgery or rupture of the cyst wall for various reasons. Damaged cysts cause immunological reactions of the body due to an IgE immune response, which most commonly leads to allergic reactions completed with urticaria, membrane elimination, and other systemic anaphylactic shock reactions [69].

**Alveolar echinococcosis** has a longer latent phase, reaching up to 15 years before being diagnosed as chronic disease. The predominant location is in the right liver lobe, the lesions being 15-20 mm diameter in areas of inflammatory infiltration. There is no knowledge about primary extrahepatic localizations. Metastasis leads to the formation of secondary alveolar infiltrations into the lungs, spleen and central nervous system[70-73], diffuse abdominal pain (in the upper right quadrant – 30% of cases) the liver is the starting point, jaundice (25% of cases), fatigue, weight loss, fever, chills. When palpation can be observed hepatomegaly, splenomegaly occurs in complicated cases with portal hypertension. Other clinical manifestations can be associated with metastatic lesions (if the lungs are involved) [74].

**Imaging diagnosis.**

The slow evolution of metacestode in the body of the intermediate host, the existence of a very long (asymptomatic) latent period (sometimes reaching 15 years) and the absence of specific signs, all of these lead to a major difficulty in diagnosis of early echinococcosis and chronic disease. For these reasons, a simple physical examination is not enough, imagery could bring an additional intake of information [75]. The imaging techniques used are: ultrasound, X-ray, computed tomography and nuclear magnetic resonance.

Ultrasound is used in the diagnosis of cystic echinococcosis, both individually and populationally [76,77]. With the help of abdominal ultrasound can be viewed cysts in various abdominal organs and sometimes even in the lung, when located on the periphery [78,79].

Since 1970s the scientists used this method to identify various pathological lesions in various parasitic diseases, including echinococcosis[80,81]. Lately has been used to monitor the evolution of the disease, bringing important information regarding the appearance of the cyst, its size and changes after
drug therapy [63,64], [82]. According to the WHO classification the cysts were grouped into four categories: CL – liquid cysts - non-differentiated; Active – CE1 si CE2; Transitional - CE3 (CE3a with detached endocyst; CE3b predominantly solid, but with daughters vesicles); Inactive – CE4 and EC5

This classification can also be interpreted as follows: CE1–CE2 early stages, CE3a-CE3b transitional stages and CE4-CE5 late stages.

In the diagnosis of hydatid disease, x-ray is recommended in case of suspicion of hydatid cyst with pulmonary and bone localization [78].

It is one of the newer technologies used in medical imaging. Although ultrasound is a commonly used method for screening, there are situations where it turns out not to be the most appropriate method. As a result, in patients suffering from obesity, accumulation of gas in the intestinal region or have had surgery, ultrasound is not effective. In these cases, it is recommended to recommend computed tomography.

MRI is a noninvasive technique that allows scanning the human body by inserting it inside a magnet. Comparing this method with computed tomography, MRI is not mandatory, but can provide additional information required. The cyst shows a hyperintense image, surrounded by a low-signal area, which represents the outer shell rich in collagen (pericyst). Daughters vesicles attached to the germinal membrane, generate a lower intensity signal comparing with the rest of the cyst. The detached membrane appears as a curved, irregular line inside the cyst [83]. Calcifications in the membrane can be detected using both computed tomography and nuclear magnetic resonance. Although irregularities in the cyst membrane are easier to detect by CT, the MRI technique is more faithful in identifying the imperfections of the cystic coating [84].

The second method has the advantage of providing information in the early stages of diagnosis [68]. The MRI technique is used if there are the following suspicions: subdiaphragmatic location, dissemination of hydatid fluid into cavities, extra abdominal location, cysts complications (abscesses, gallstones) and in case of prior evaluation of surgery [78].

Identification of parasitic elements directly in biological materials.

The presence of protoscolices is proven by the microscopic examination of biological materials/histopathological preparations resulting from surgical treatment (where indicated). The surgical approach of hydatid cysts is done by several methods, depending on the specifics of each patient, the location of the cyst, the number of cysts as well as the type of cyst (from CL to CE5) [85]. Considering the criteria listed above, there are low invasive, conservative and radical techniques. The biological material extracted is different depending on the procedure used (membranes, fluid, daughters vesicles). The element identified in the samples could be protoscolices viables and/or damaged, hooks, daughter vesicles, crystals (figure 4- a,b,c).

Immunological diagnosis.

The presence of Echinococcus in the human body induce production of the specific antibodies. For the detection of these were elaborated a large number of serological tests Immunological methods for the diagnosis of echinococcosis are based on the determination in circulating blood of immune antigen–antibody complexes (Ag-Ac). Different techniques are used to do this: screening - ELISA enzyme linked immunosorbent assay, IHA - indirect hemagglutination, IFA - indirect fluorescent antibodies; confirmatory: immunoblotting [86]. The main disadvantage of the serological test is low sensitivity for the cysts with lung, extrapleural, central nervous system, eye location, for very young (small) and calcified cysts [87]. Tests recorded also different performances related to the cyst stage according the WHO classification[86].

Serological diagnosis is very difficult to realize because there are necessary more than one molecule available. These molecules are different in various stages of the cyst [88], so there are necessary more than one antigen for immunodiagnosis of the cyst in different stages of evolution. The antigens used in the serological diagnosis of the echinococcosis are obtained from different sources: hydatid fluid, protoscolices or adult worm excretor/secretor antigen and extract of adult parasite or larval stage[89]. Echinococcus infection in the human body induces the synthesis of antibodies of class IgG (increased levels of IgG1 and IgG4), but increased levels of IgM, IgA and IgE can also be detected. In 30-40% of patients, no specific antibodies are detected, although circulating antigen [90] can be detected. This
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suggests that the infection is associated with inhibition of the host's immune mechanisms. In time, the metacestode leads to active inhibition of the host's immune defense mechanisms [90]. Factors leading to the different reactivity of intermediate hosts to the presence of metacestode are largely unknown. Thus, it was demonstrated on different lines of laboratory mice treated with oncospheres/eggs of Echinococcus spp reacts differently to the presence of cestode, producing a different immune response [90].

In the early stages of infection, oncospheres are transported into the target organs (most commonly liver, lung) where they will develop, evolving into a hydatic cyst. The newly formed cyst will have to evade the action of the host's immune system. Compared to the immune response of experimentally infected hosts, in the case of the human host, the cellular and humoral immune response varies greatly, with patients reacting differently to the different types of parasitic antigens. 8-10 weeks after infection the growth of the cyst is maintained and specific complex antigens are released, and the number of lymphocytes belonging to the Th2 subpopulation (which stimulates lymphocytes B differentiation and the humoral mediated immune response in specific antibodies) is balanced with that of Th1 (the subpopulation that stimulates the differentiation of T effector lymphocytes – Tc/cytotoxic).

Currently the parasite produces significant amounts of specific antigens, which participate in modulating the host's immune response. IgG levels (IgG1, IgG4), IgM and IgE are high. When the cyst becomes inactive (dead), or surgically removed, Th2 levels decrease rapidly, while the Th1 level slowly decreases. IgG level is maintained in the host body for a long time (sometimes years after surgical removal of the cyst, due to activation of immunological memory and lymphocytes B memory differentiation). In the case of reactivations/relapses, Th2 levels increase rapidly while other markers have a slow evolution [90].

Immunoblot method records on the nitrocellulose membrane, the existence of specific band patterns, patterns caused by the production of immune complexes between antibodies in the patient serum and the specific antigen impregnated (antigen derived from hydatic fluid – Ag 5, AgB). The antigens used have different molecular weights, characteristic for each species of Echinococcus granulosus and/or multilocularis. Parasitic proteins of importance in the immunological diagnosis of cystic disease are antigen 5 (Ag5) and antigen B (AgB). Antigen 5 has a subunit with a molecular weight of 38kDa and comprises a component, phosphorylcholine, responsible for most cross-reactions [91,92]. Studies have shown that antigen 5 is not a safe means of diagnosis and its use is limited applicable [93]. Antigen B consists of components with a molecular weight between 8 – 25 kDa. The most important areas are those located at the molecular weights: 8, 16 and 24 kDa. When applying antigens to nitrocellulose membranes used in the immunoblotting method, specific recognition and differentiation of ag- antibodies specific complexes for alveolar and cystic echinococcosis is more difficult to be done and cross-reactions have been encountered with cysticercosis and fasciolosis. In patients with alveolar echinococcosis were recorded patterns indicated activity in low molecular weight bands (approximately 14-20 kDa) and it was used the specific antigen Em18. The specificity and sensitivity of the method ranges between 51-100% and 70-100%, respectively. The serum of patients diagnosed with hydatid cyst reacts securely and consistently to existing antigens in the bands of 7/20kDa [94]. The highest sensitivity is immunoblotting method (80%), followed by ELISA (72%) immunoelectrophoresis (31%) [95].

Molecular diagnosis.

The methods of molecular biology, through numerous techniques and their variants, have provided new means of diagnosis of echinococcosis [96]. Using advanced methods of isolation and purification, it was achieved by completely new and highly characterized antigenic molecules. By classical, immunochemical methods, the amount of product obtained is always insufficient, so the introduction of advanced techniques was a necessity. Cloning and expression of Echinococcus genes using specific vectors solved these problems. The progress made led to the formation of a cloned DNA bank, using messenger RNA molecules (RNA) from different stages of the parasitic organism (egg, larvar stage, adult). The use of different types of antigens obtained by recombinant DNA technology is applicable in the immunological diagnosis of echinococcosis. Molecular cloning of Echinococcus genes that encode epitopes with potential in immunological diagnosis is of overwhelming importance for obtaining new standardized diagnostic kits [97]. The identification of specific DNA sequences from the parasitic species allowed them to be used in the process of hybridization of genetic material for diagnostic purposes. The use of these products is limited and is used more for epidemiological than clinical purposes. One of the great disadvantages of the hybridization technique is reduced sensitivity. The final target of hybridization techniques is to obtain techniques/methods capable of differentiating the eggs of a single taeniid species [98]. Molecular diagnosis is used for epidemiological and research purposes and is applicable to patients undergoing surgery.

At this moment the serological diagnosis for echinococcosis has a lot of problems. Considering that there are necessary many molecules of antigens for each stage of the cyst, and the sensitivity is also different related to the location and stage of the cyst, serology alone is not a reliable method for echinococcosis diagnosis. This is the reason that immunological diagnosis should be combined with imagery techniques and clinical findings.

6. CONCLUSION

Echinococcus is a complex which includes various species involved in human and veterinary pathology. The genetic variability of these species is influencing the response of their intermediate hosts to the presence of the cestode and other aspects of the parasite biology. Molecular analyses allowed us to separate the complex in more groups. All the species belonging to this complex have a common pattern of transmission. The intermediate host becomes infected through alimentary way consuming food and water contaminated with parasites eggs. These cestodes have a great ability to adapt to various species as intermediate hosts. Most of them are domestic animals who are living nearby the man’s house (sheep, goats, cattle). The human being enters accidentally in the life cycle of the parasite and it is necessary a log time until the disease became visible. In the beginning there are no specific
symptoms, the parasite being able to avoid the immune system of the host. The livestock is affected by the presence of the cestode, the economic damage is significant.

The diagnosis of this parasitic disease is relatively limited and should be combined with different methods to increase the accuracy of it. The serology techniques evolved a lot and with important input from molecular techniques, new molecules were discovered, allowing to increase quality of the diagnosis kits (sensitivity/specificity). Even so, we have many more details to establish because the parasite has a huge genetic variability inside the group and inside the same species. These facts led to the conclusion that serology should be used besides other techniques.

7. REFERENCES


Imagery is an important tool used in the diagnosis of this parasitic disease, from screening -ultrasound, to advanced-CT, MRI.

All these aspects describe a complex picture, very dynamic where the characters involved (the parasite and their hosts-definitive and intermediate) are morphological and physiological changing all the time. The molecular techniques are the future and the hope! They will bring more and more knowledge in these complex host-parasite mechanisms allowing us to understand and explain better the behavior of the species involved and at least not at last to reduce the important economic and social damages produced by this interaction.


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