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TOPIC HIGHLIGHT

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Hepatic echinococcosis: Clinical and therapeutic aspects

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Abstract

Echinococcosis or hydatid disease (HD) is a zoonosis caused by the larval stages of taeniid cestodes belonging to the genus *Echinococcus*. Hepatic echinococcosis is a life-threatening disease, mainly differentiated into alveolar and cystic forms, associated with *Echinococcus multilocularis* (*E. multilocularis*) and *Echinococcus granulosus* (*E. granulosus*) infection, respectively. Cystic echinococcosis (CE) has a worldwide distribution, while hepatic alveolar echinococcosis (AE) is endemic in the Northern hemisphere, including North America and several Asian and European countries, like France, Germany and Austria. *E. granulosus* young cysts are spherical, unilocular vesicles, consisting of an internal germinal layer and an outer acellular layer. Cyst expansion is associated with a host immune reaction and the subsequent development of a fibrous layer, called the pericyst; old cysts typically present internal septations and daughter cysts. *E. multilocularis* has a tumor-like, infiltrative behavior, which is responsible for tissue destruction and finally for liver failure. The liver is the main site of HD involvement, for both alveolar and cystic hydatidosis. HD is usually asymptomatic for a long period of time, because cyst growth is commonly slow; the most frequent symptoms are fatigue and abdominal pain. Patients may also present jaundice, hepatomegaly or anaphylaxis, due to cyst leakage or rupture. HD diagnosis is usually accomplished with the combined use of ultrasonography and immunodiagnosis; furthermore, the improvement of surgical techniques, the introduction of minimally invasive treatments [such as puncture, aspiration, injection, re-aspiration (PAIR)] and more effective drugs (such as benzoimidazoles) have deeply changed life expectancy and quality of life of patients with HD. The aim of this article is to provide an up-to-date review of biological, diagnostic, clinical and therapeutic aspects of hepatic echinococcosis.

Key words: Hydatidosis; Cystic echinococcosis; Alveolar echinococcosis; Liver; PAIR; Albendazole; Treatment; Diagnosis

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INTRODUCTION

Echinococcosis or hydatid disease (HD) is a zoonosis caused by the larval stages of taeniid cestodes belonging to the genus *Echinococcus*. Six species of *Echinococcus* are known, but only four of them are responsible for human disease: *Echinococcus granulosus* (*E. granulosus*) (which causes cystic echinococcosis), *Echinoccus multilocularis* (*E. multilocularis*) (which causes alveolar echinococcosis), *E. vogeli* and *E. oligarthrus* (which cause polycystic echinococcosis). Recent studies have identified two new species, *E. felidis* and *E. shiquicus*, even if no data are available about their pathogenicity to humans.

Hepatic alveolar and cystic echinococcosis are both life-threatening diseases because of their medical and economical impact and their wide geographical distribution. Polycystic echinococcosis is, on the contrary, confined to Central and South America and only few cases of this condition have been reported in man[1,2].

The liver is the major site of HD involvement (about 75% of cases) both in the alveolar and in the cystic form[3]. This review is focused on the biological, epidemiological, clinical and therapeutic aspects of hepatic echinococcosis, with particular reference to *E. granulosus* cystic and *E. multilocularis* alveolar hydatidosis (Table 1).

HEPATIC ECHINOCOCCOSIS CAUSED BY *E. GRANULOSUS*

Cystic echinococcosis (CE) occurs as the result of infection by the larval stages of *E. granulosus*. CE is the most common form of HD, with a worldwide distribution, and it can be regarded as an emerging or re-emerging disease in several countries of the world.

E. granulosus: The parasite biology and life cycle

E. granulosus is a small tapeworm (length of 2-7 mm), whose body is made up by a mean number of three proglottids. There are ten distinct genetic types (G1-10) within *E. granulosus*, with a different geographical distribution, and these have been identified by molecular studies based on mitochondrial DNA sequences[4,5]: 2 sheep strains (G1-G2), 2 bovid strains (G3 and G5), a horse strain (G4), a camelid strain (G6), a pig strain (G7), a cervid strain (G8), a swine strain (G9) and a reindeer strain (G10).

The parasite life cycle involves dogs and other canids (coyotes, dingoes, red foxes) as definitive hosts and ungulates (sheeps, pigs, goats, horses) as intermediate hosts. Definitive hosts are infected by ingestion of offal containing hydatid cysts; the adult worms reside in the canine small bowel and their eggs or gravid proglottids are shed in the feces. After oral uptake of eggs by intermediate hosts, an oncosphere larva is released from the egg and penetrates the intestinal lamina propria, reaching the blood and lymph vessels which transport it to liver, lungs and other organs, where oncosphere larvae can develop into metacestodes (also known as hydatid cysts). Humans can accidentally become "aberrant" intermediate hosts, after ingestion of *Echinococcus* eggs excreted by infected carnivores.

Hydatid cysts are spherical, fluid-filled, unilocular vesicles, consisting of an internal cellular layer (germinal layer) and an outer acellular, laminated layer. The parasite cysts gradually expand and cause a granulomatous host reaction, followed by the development of a fibrous tissue layer (pericyst). Brood capsules and protoscolices bud from the germinal membrane; with time, internal septations and daughter cysts usually develop, modifying the unilocular morphology that is typical of young hydatid cysts. When definitive hosts ingest the cyst-containing organs of intermediate herbivore hosts, the *Echinococcus* life cycle can restart, as the protoscolices evaginate, attach to the intestinal mucosa and develop to adult stage in 30-80 d[6,7].

Molecular crosstalk between human host and parasite

Several studies have focused on the mechanisms of host-parasite interplay in CE.

The immune response to *E. granulosus* infection has been investigated through both clinical studies on patients with hydatidosis and sheep and mouse experimental models[8]. In the early stage of hydatid cyst development, a cell-mediated response involving macrophages, neutrophils and eosinophils is established[9-11]; antibody response is usually undetectable during the first weeks after infection, but IgE, IgG2 and IgG4 levels subsequently significantly increase[8]. Elevated levels of IgE for echinoccocal antigens are responsible for allergic reactions, such as itching, urticaria and anaphylactic shock[12].

E. granulosus induces both TH1 and TH2 response: elevated levels of TH1 cytokines, especially interferon-γ (IFN-γ)[13], but also TH2 cytokines, such as IL-4, IL-5 and IL-6, have been recorded in patients with HD[8,11]. The reason for this duplex cytokine secretion pattern is not known: TH1 and TH2 responses usually down-regulate each other, with a cross-inhibitory mechanism; it is assumed that the complex antigenic organization of *Echinococcus* may stimulate both T-cell subsets[14]. After chemotherapy treatment, surgical removal or natural death of a cyst, TH2 response quickly drops and TH1 response becomes predominant[15].

The metacestode attempt to escape from the host protective response involves complex and intriguing strategies aimed at modulating host response and protecting itself from elimination. *Echinococcus* tries, in fact, to minimize host reaction by exposing several immunomodulatory molecules to its host[16], interfering with complement

Table 1 Hydatid disease epidemiology and characteristics[6,7]

	Cystic echinococcosis	Alveolar echinococcosis
Causative agent	*E. granulosus*	*E. multilocularis*
Definitive hosts	Dogs and other canids (coyotes, dingoes, red foxes)	Red foxes, arctic foxes, coyotes, dogs and cats
Intermediate hosts	Ungulates	Rodents
Geographic distribution	Worldwide	North America, northern and central Eurasia
Worldwide incidence	1-200/100 000	0.03-1.2/100 000
Organ localization	Mainly liver and lungs	Mainly liver
Characteristics of hydatid lesions	Young cysts: spherical, fluid-filled, unilocular vesicles (diameter: 1-15 cm) Old cysts: internal septations, daughter cysts Three-layered structure: germinal layer, laminated layer, pericyst	Alveolar-like pattern, with numerous vesicles (< 1 mm up to 15 cm in diameter) and surrounding dense connective tissue, no cyst fluid, sometimes central necrosis
Type of growth in human organs	Concentric expansion	Tumor-like, infiltrative behaviour
Therapeutic options	Surgery, PT (especially PAIR), chemotherapy	Surgery, chemotherapy, EPIs

PTs: Percutaneous treatments; PAIR: Puncture, aspiration, injection, re-aspiration; EPIs : Endoscopic percutaneous interventions.

activity[17], altering leukocyte function[18] or using molecular mimicry[19].

Epidemiology and infection risk

E. granulosus has a worldwide distribution; the highest prevalence is recorded in the Mediterranean countries, Russia and China (in Sichuan Province human CE had a prevalence of 2.1% in 1997-1998[20]). Other hyperendemic areas are North and East Africa (prevalence > 3%), South America and Australia[21]. CE infection has re-emerged in certain parts of the world where it was once believed to be controlled, including Israel, Central Asia and Eastern Europe[21,22]. In Bulgaria the annual incidence of CE in children has increased from 0.7 per 100 000 in 1971-1982 to 5.4 in 1995[23]; in Kazakhstan the annual surgical incidence of CE over the whole country was below 1.4 per 100 000 inhabitants from 1988 until 1995 but has increased to 5.9 in 2000[24,25].

CE is typically a rural and occupational disease, since certain human activities, such as feeding dogs with the viscera of slaughtered livestock, increase the risk of infection. Humans acquire the parasite through fecal-oral contact, generally by handling infected domestic dogs or egg-containing feces. *Echinococcus* eggs adhere to the coat of animals, especially to hairs around the anus and on the muzzle and paws[26]. Eggs can also be ingested with contaminated water or vegetables; it is also possible that the contamination of surfaces and foodstuffs with *Echinococcus* eggs occurs *via* wind, flies, birds or beetles.

Some studies have evaluated several risk factors for infection: Campos-Bueno *et al*[27] studied a Spanish cohort of 127 CE infected patients, matched with 127 healthy controls, associating an increased risk for CE with having a higher number of dogs in the family and with dogs' ease of access to raw viscera of slaughtered animals. In Tibet a rise of infective risk was associated with nomadic life, age, playing with dogs, not protecting food from flies and raising yaks or sheep. Water wells were suspected to be a source of infection in African arid lands, where animals and humans often share the same water points[28].

Clinical aspects

After infection, humans are usually asymptomatic for a long period of time, since cyst growth is usually slow; in the liver the growth rate is variable, ranging from 1 mm to 5 mm in diameter per year. Most primary infections consist of a single cyst, but up to 20%-40% of infected people have multiple cysts. Presenting symptoms depend not only on the size and number of cysts, but also on the mass effect within the organ and upon surrounding structures. The signs and symptoms of liver hydatidosis include hepatomegaly, right/epigastric pain, nausea and vomiting. Cyst leakage or rupture may be responsible for systemic immunological responses, causing anaphylaxis; in one series, anaphylaxis complicated 10% of all intraperitoneal ruptures.

Cyst rupture in the peritoneal cavity may cause secondary CE, with the release of protoscolices and/or small cysts, which can grow to larger cysts.

Portal vein or bile duct obstruction, caused by the expanding cysts, may be responsible for segmental or lobar liver atrophy in the cyst-bearing lobes[29].

Other complications are rupture in the biliary tree with secondary cholangitis[30], biliary obstruction by daughter cysts, portal hypertension, ascites, intracystic or subphrenic abscess formation, development of a bronchobiliary fistula[31,32]. Hydatid cyst suppuration has been reported as occurring in 5% to 40% of patients[33]. Perforation in the biliary tree has been described in up to 90% of HD[34].

Diagnosis

Considering that the early stages of infection are usually asymptomatic, the diagnosis of liver CE may often be incidental, associated with an abdominal ultrasonography performed for other clinical reasons. In endemic areas, the presence of symptoms suggestive of CE in a person with a history of exposure to sheepdogs supports the suspicion of hydatidosis.

A non-invasive diagnosis of hepatic CE is usually accomplished with the combined use of radiologic imaging

and immunodiagnostic techniques. Abdominal ultrasonography is considered the gold standard for defining the number, site, dimensions and vitality of cysts[32,35,36] and it is also important to evaluate treatment options. A standardized ultrasonographic classification system for hepatic cysts has been developed by the World Health Organization (WHO)[37], in order to update the older Gharbi classification[38].

Ultrasonography is not always able to differentiate hydatid cysts from other space-occupying lesions, like tumors or liver abscesses, so that additional imaging techniques, such as magnetic resonance imaging (MRI) and CT scans, may be required. MRI should be preferred to CT, due to better visualization of liquid areas within the matrix[39]. MRI is also important for pre-surgical evaluation of CE.

Immunodiagnosis is useful to confirm a radiologic diagnosis and can also be an important tool for the follow-up after surgical or pharmacological treatment, even if not all patients with CE have a detectable immune response[40-42]. Serological test sensitivity is indeed inversely related to the degree of sequestration of the echinococcal antigens inside cysts; for instance, healthy, intact cysts can elicit a minimally detectable response, whereas previously ruptured or leaking cysts are associated with stronger immune responses.

Almost all traditional immunodiagnostic methods (e.g., Casoni intradermal test, complement fixation test, indirect hemagglutination test, indirect immunofluorescence antibody test, immunoelectrophoresis and latex agglutination test) have now been replaced by the enzyme-linked immunosorbent assay (ELISA) and/or immunoblotting[43]. In order to detect antibody response to parasite, several hydatid antigens have been extracted and used for serological diagnosis. Hydatid cyst fluid antigen B (AgB) and antigen 5 (Ag5) from *E. granulosus* are considered the most specific native antigens for the immunodiagnosis of CE[40,41], even though lack of sensitivity and specificity, technique standardization and cross-reactivity with antigens of other parasites[44-46] are major problems associated with immunodiagnosis of CE.

In doubtful cases, for example undetectable anti-*Echinococcus* antibodies in patients with small lesions resembling hydatid cysts or in patients whose hepatic cysts cannot be differentiated from liver abscess or neoplasms, ultrasonography-guided fine needle puncture may represent an additional diagnostic option. The demonstration of protoscolices or hydatid membranes or echinococcal antigens/DNA in the aspirated cyst fluid can confirm, in fact, the diagnosis of CE. Anthelmintic coverage is important to minimize the risk of secondary CE: albendazole should be recommended for 4 d before the procedure and should be continued for at least 1 mo after having punctured a lesion recognized as an *E. granulosus* cyst[32,47]. Detection of parasite-specific IgE has no significant diagnostic advantages, even if eosinophilia is often present after rupture/leakage of the cyst[48].

Treatment

The goals of hepatic hydatid cyst treatment are a complete elimination of the parasite and prevention of recurrence, minimizing mortality and morbidity risk. In order to achieve these aims, it is essential to choose the most appropriate treatment with regard to disease-specific characteristics (cyst number, size, site, presence of cystobiliary communication), to patient clinical conditions, availability of an experienced surgeon or an interventional radiologist.

Three therapeutic modalities are available to treat hepatic CE: chemotherapy, surgery (with open or laparoscopic approach) and percutaneous treatments (PTs). A stage-specific approach is recommended[49].

Surgery: Until the 1980s, surgery was the only therapeutic option for patients with CE. Surgery is still the first choice for large CE2-CE3b cysts with multiple daughter cysts or for single superficial cysts, considering the likelihood of spontaneous or traumatic rupture, when PT is not available. Presence of complicated cysts, e.g., infected cysts or cysts communicating with the biliary tree, and cysts exerting pressure on other vital organs, are other indications for surgical approach. Surgery is contraindicated in patients whose preexisting medical conditions put them at risk or in patients having inactive asymptomatic cysts or multiple cysts which are difficult to access. If feasible, surgical removal of hydatid cysts has the best chance to completely remove cysts and to immediately cure CE.

Surgical options can be divided into radical (pericystectomy) and conservative approaches (for instance unroofing or capitonnage)[50-53]. Radical procedures are associated with a lower risk of recurrence, but also with a higher operative risk; conservative procedures, on the contrary, are easier to perform but have a higher likelihood of recurrence. Recurrence is usually due to either inadequate cyst removal or to previously undetected cysts; reported recurrence rates range from 2% to 25%[54].

Whichever technique is used, a benzimidazole (BMZ) agent is usually used to reduce the risk of anaphylaxis and secondary CE[55]. BMZ is administered from 1 d before surgery to 1 mo after surgery but, again, no conclusive data about the best timing are available. Major complications of surgery are postoperative hemorrhage, cholangitis, sepsis and fistulae formation. Operative mortality varies from 0.5% to 4%[55].

Percutaneous treatments: PTs of hepatic CE can aim at the destruction of the germinal layer [puncture, aspiration, injection, re-aspiration (PAIR)] or the evacuation of the entire endocyst ("modified catheterization technique").

PAIR is an acronym that stands for "puncture, aspiration, injection, re-aspiration". PAIR consists of four steps: (1) percutaneous puncture of the cyst using ultrasound guidance; (2) aspiration of the cyst fluid; (3) injection of a protoscolicidal agent (e.g., 95% ethanol or 20% NaCl) for at least 15 min; and (4) re-aspiration of the fluid[37,56].

PAIR is indicated for CE1 and CE3a cysts > 5 cm[49,56]; CE2 and CE3b cysts treated by PAIR tend to relapse. PAIR has also been used for patients who refused surgery

or relapsed after surgical treatment. It is contraindicated for inaccessible or superficially located liver cysts and for inactive or calcified cystic lesions. The possibility of secondary echinococcosis can be minimized by concurrent treatment with benzimidazoles; indeed, combined treatment (PAIR plus albendazole) may yield better results than those of either chemotherapy or PAIR alone[57,58]. The length of administration of chemotherapy with albendazole usually ranges between 4 h before and 1 mo after PAIR, in order to reduce the risk of disease recurrence and intraperitoneal seeding of infection. PAIR must be avoided in patients with cystobiliary communications, to prevent the risk of sclerosing cholangitis.

Chemotherapy: Mebendazole (MBZ) and albendazole (ABZ) are the BMZ agents used for the treatment of hepatic CE. They interfere with the absorption of glucose through the wall of the parasite, causing glycogen depletion and degenerative changes in echinococcal mitochondria and endoplasmic reticulum. BMZ may be favorably used alone for the treatment of small (< 5 cm) CE1-CE3a liver cysts[59] or for inoperable patients; BMZs are also usually associated with PAIR or surgery to prevent secondary CE[55]. BMZs are not indicated for the treatment of inactive or calcified asymptomatic cysts, unless they are complicated lesions[49].

Both ABZ and MBZ are effective, but ABZ is considered the drug of choice, because it is more active *in vitro* and it has a better gastrointestinal absorption and bioavailability[60,61]. The usual dose of orally-administered ABZ is 10-15 mg/kg per day in two divided doses; if MBZ, the daily dose is 40-50 mg/kg in three divided doses. Treatment with BMZ should be administered continuously, for 3-6 mo[49].

Clinical and radiographic improvement (in most studies defined as > 25% reduction in cyst size, membrane separation, or cyst calcification[62]) is quite frequent and is favorably influenced by the duration of treatment. Unfortunately, complete cure (i.e., cyst disappearance) only occurs in approximately a third of patients treated with BMZ alone and, interestingly, the number of patients with cure does not significantly increase by extending the duration of treatment[60]. A recent systematic review[63] has confirmed that the size and stage of cysts are the key factors to evaluate the likelihood of response to chemotherapy.

Usual adverse effects include nausea, hepatotoxicity, neutropenia and occasionally alopecia. Thus, all patients should have regular monitoring of leukocyte counts and liver function tests. Contraindications to chemotherapy include pregnancy, chronic hepatic diseases and bone marrow depression.

Praziquantel has been used (40 mg/kg once a week) with ABZ for combined treatment of CE; this therapeutic association seems to be more effective than ABZ alone[64].

For uncomplicated CE4 and CE5 cysts a "watch and wait" strategy is currently advised[49].

HEPATIC ALVEOLAR ECHINOCOCCOSIS CAUSED BY *E. MULTILOCULARIS*

Hepatic alveolar echinococcosis (AE) results from infection by the larval forms of *E. multilocularis*. The echinococcal metacestode develops in the liver and is characterized by an alveolar structure, made up by several vesicles surrounded by large granulomas. Human AE is a severe and emerging disease, whose prognosis is bleak in absence of treatment or if it is not diagnosed at an early stage of disease.

E. multilocularis: The parasite life cycle

E. multilocularis is a small cestode (1.2-4.5 mm), whose definitive hosts are wild carnivores such as red fox and arctic fox (sylvatic cycle) or domestic dogs and cats (synanthropic cycle). The adult tapeworms, whose bodies are characterized by a mean number of five proglottids, reside in the small bowel of their definitive hosts, where gravid proglottids release eggs which are passed in the feces. Intermediate hosts, usually small rodents, or aberrant hosts such as humans, become infected by ingestion of embryonated eggs. Human infection can happen through direct contact with the definitive host or it can be indirect, through contamination of food or water with parasite eggs[7,65]. The echinococcal metacestode develops in the liver and is characterized by an alveolar structure, made up by several vesicles whose diameter varies from < 1 mm up to 15-20 cm[47,65]. Each vesicle has a wall structure similar to that of the *E. granulosus* cyst, consisting of a germinal and a laminated layer[66]. Brood capsules or protoscolices are only occasionally seen and lesions may be complicated by central necrosis, producing a cavity or pseudocyst after liquidization. Small cysts are surrounded by a dense connective tissue and they usually do not contain fluid but instead a semisolid matrix[6].

Host-parasite interaction

E. multilocularis is able to elicit a strong cellular immune response: in the liver, parasitic lesions appear to be surrounded by large granulomas made up by macrophages, T-lymphocytes and myofibroblasts[67-69]. Observations in humans and experiments with rodents have shown that cellular immunity, related to TH1 cytokine profile, has a crucial role in host defense against the parasite[70]. IL-12, a key factor in the induction of TH1 profile, has been shown to inhibit, in mice, the development of alveolar lesions, leading to the formation of abortive parasitic vesicles surrounded by fully efficient periparasitic immune cell infiltration and fibrosis[71]. In mice treated with IFN-γ, a typical TH1 cytokine, a partial reduction in larval growth has been observed[72]. In contrast, a TH2 cytokine profile has been associated with disease progression: high levels of IL-5 and IL-10 have been detected in serum of patients with progressive disease, compared with individuals with abortive forms[73-76]. As in the case of *E. granulosus*, several mechanisms have been proposed

to explain *E. multilocularis* avoidance from host-protective responses, including antigenic disguise[77], immunomodulation[78-80], molecular mimicry[81], antigen and DNA polymorphism[82,83].

Epidemiology and infection risk

Data on human AE are difficult to be evaluated due to its low prevalence[21], which does not allow a reliable recognition of temporal developments or differences in spatial distribution. The long asymptomatic period also makes it difficult to determine time and place of infection[84]. *E. multilocularis* is endemic in the Northern hemisphere, including North America (Alaska, Canada), Asia (some of the newly independent states of the former Soviet Union[85], China[86] and Japan) and some European countries[87] (mainly France, Switzerland, Austria, Germany)[21,22]. In endemic areas, annual incidence of AE ranges from 0.03 to 1.2/100 000 inhabitants[88,89]. Increasing fox population, increased fox encroachment into urban areas and *E. multilocularis* spillover from wild carnivores to domestic hosts, are all factors that may explain *E. multilocularis* spreading from endemic areas to previously non-endemic European countries[21,90].

Considering the parasite life cycle, exposure of humans to echinococcal eggs may be influenced by occupational and behavioral factors. Hunters, trappers and persons who work with fox fur should be more frequently exposed to *E. multilocularis* eggs, but there is no evidence that these groups are at increased risk[91,92].

Clinical aspects

Slow larval growth results in an asymptomatic phase of several years (5-15 years). Initially, the liver, usually the right lobe, is the organ where the metacestodes establish themselves; then, later in the infection, it is possible to find blood metastasis to lung, brain, bones and local extension of the lesion (abdomen, retroperitoneum, diaphragm)[66]. First symptoms are usually vague: patients may complain of fatigue, weight loss or may have hepatomegaly. One third of them have cholestatic jaundice; one third present with abdominal pain[54,66,93]. In advanced stages, liver failure usually occurs and it is frequently associated with portal hypertension, ascites and splenomegaly. The prognosis in untreated or inadequately treated patients with AE is poor. Treatment has radically changed average life expectancy at diagnosis from 3 years in the 1970s to 20 years in 2005[94].

Diagnosis

As for CE, AE diagnosis is based on clinical and epidemiologic findings, imaging techniques, nucleic acid detection and serology.

Among the imaging techniques, ultrasonography is the method of choice to identify hydatid lesions: ultrasound (US) typical aspect shows a pseudotumoral mass, with irregular limits and scattered calcification, where hypoechogenic and hyperechogenic areas are juxtaposed; central necrosis may give to the mass the appearance of a cystic-like structure, surrounded by a hyperechogenic ring[95,96]. Color doppler may be useful to evaluate biliary and vascular infiltration. Abdominal CT gives further anatomical details and information about the lesion pattern of calcification[65]. MR imaging is the best standard to study the invasion of adjacent structures and may help in unclear cases[97]. Pre-surgical percutaneous cholangiography is important to assess the presence of communication between the biliary tree and the alveolar lesions[96]; it is also fundamental to exclude extra-hepatic involvement, through pulmonary and cerebral radiological examination.$^{(18F)}$Fluorodeoxyglucose positron emission tomography (FDG-PET) scanning gives indirect information on the parasite metabolic activity, especially if combined with MRI or CT scan; if negative, this finding does not mean that the parasite is not viable but that there is a suppressed periparasitic inflammatory activity[98].

WHO classification of AE is based on imaging findings and it is useful to have an internationally recognized, uniform standard for disease diagnosis and treatment strategies. The WHO-IWGE PNM classification system[65,99] is similar to tumor TNM classification: "P" refers to the extent of parasite localization inside the liver, "N" establishes the involvement of neighboring organs, "M" evaluates the absence (M0) or presence (M1) of distant metastasis, after having performed a chest X-ray and a cerebral CT.

As in CE, immunodiagnosis has a complementary role to other procedures, not only in primary diagnosis but also for follow-up of patients after surgical treatment or chemotherapy[100,101] and for the specific differential diagnosis between AE and CE in those regions where the diseases are co-endemic[102,103]. Immunodiagnosis (with indirect hemoagglutination test or ELISA) is more reliable for the diagnosis of AE than for CE, because more specific antigens are available. For example, the Em2plus-ELISA, which is a mixture of affinity purified *E. multilocularis* metacestode antigens (Em2-antigen) and a recombinant antigen (Em Ⅱ/3-10), has shown a great sensitivity and specificity[104], but it is not able to discriminate between active and inactive lesions; in fact, Em2-ELISA may be positive for years after spontaneous or pharmacological-induced dying out of the metacestode in patients with calcified lesions, because the Em2 antigen main source is the laminated layer of the parasite which obviously persists in these inactive lesions. Surgical removal of the dried-out lesion results in an immediate seroconversion to negative anti-Em2 antibodies[105,106]. Considering that the protoscolex is the most active component of echinococcal tissues, protoscolex antigens Em16 and Em18 have been isolated and used for immunoblot tests, in order to discriminate between active and inactive lesions[107]; recombinant (r) Em18 appears to be a promising immunodiagnostic tool for serological differentiation between AE and CE[107,108]. Combining US and serological data, it is possible to classify seropositive patients into three groups: patients with active hepatic lesions, patients with calcified lesions and patients with no

evidence of hepatic lesions[49]. The latter cases are a consequence of immune system pressure, which can cause larval degeneration and death, so that the only radiological sign of the host-parasite interaction may be the US finding of calcifications[109].

Some studies have shown that patients with AE have high levels of IgG1 and IgG4 antibodies and that after treatment they usually become seronegative for IgG4 antibodies[110-113]; IgG4 antibody reappearance can be considered a warning sign of disease reactivation.

Liver needle biopsy can be performed in uncertain cases and it can confirm AE diagnosis if histopathological examination identifies the presence of alveolar vesicles. RT-PCR on liver specimens, obtained by biopsy or surgery, has been used to assess parasite viability, while PCR can detect *E. multilocularis* DNA. These tests have a good positive predictive value, but a negative result does not exclude parasite activity and parasite presence in the liver, respectively[114].

Treatment

The key concept of AE treatment is to adopt a multidisciplinary approach to disease. Surgery and chemotherapy are the cornerstones of AE treatment and, as for CE, a stage-specific approach is recommended[49].

Surgery: Surgery is the first-choice option in all operable patients. Radical resection of the entire hepatic parasitic lesions is the only curative procedure, even though it is often difficult to achieve because of echinococcal dissemination into host tissues. Palliative liver surgery is almost always contraindicated, because it does not offer advantages when compared with conservative treatment[115,116]. Preoperative evaluation is important to establish lesions full resectability; WHO-IWGE PNM classification estimates quite well the likelihood to achieve radical resection[99].

Liver transplant (LT) has been employed in otherwise terminal cases[117]. Indications for LT are the presence of severe liver failure or recurrent life-threatening cholangitis and the inability to perform a radical liver resection. The absence of extra-hepatic AE localizations is mandatory for LT[49].

BMZ chemotherapy should be carried out for at least 2 years after surgery and patients should be monitored for at least 10 years, because of the risk of recurrence: in fact, unrecognized or invisible parasites can re-grow, even after some years, especially in post-LT immunosuppressed patients[118].

Chemotherapy: Inoperable AE patients should receive continuous BMZ treatment for life; moreover, long-term BMZ administration (at least 2 years) is mandatory after surgical treatment. Pre-surgical BMZ therapy is advised only in the case of LT. ABZ is given orally at a dosage of 10-15 mg/kg per day, in two divided doses; if it is not tolerated, MBZ may be given at daily doses of 40-50 mg/kg per day, split into three divided doses with fat-rich meals[49]. Conventional and liposomal amphotericin B has been used in patients who did not tolerate BMZ[119]. In a recent study nitazoxanide has not shown any efficacy for AE treatment[120].

Therapy with BMZ has resulted in an increased 10-year survival rate of approximately 80% (6%-25% in untreated historical controls)[121]. BMZs are parasitostatic, not parasiticidal: after several years of BMZ treatment, in the absence of progression of AE lesions, it is possible to discuss whether treatment should be continued or not. Decision-making should be supported by the evaluation of parasite viability, usually by PET-CT[98], and serum specific antibodies[101,102]. These tools may also be useful for the follow-up after BMZ withdrawal.

All AE patients should be monitored by US at frequent intervals and CT and/or MRI at intervals of 2-3 years, to evaluate disease recurrence or progression[49].

Endoscopic percutaneous interventions: Interventional procedures may be considered in inoperable patients in the presence of complications such as liver abscesses, jaundice due to biliary duct obstruction, portal vein thrombosis or bleeding esophageal varices associated with portal hypertension[96]. EPIs with BMZ avoid palliative surgery and may improve the patient life expectancy and quality of life.

CONCLUSION

Liver echinococcosis is a severe, neglected, often misdiagnosed disease; both AE and CE may be considered emerging public health problems, since CE is endemic in several countries in the world and AE is one of the most lethal helminthic diseases.

The last years have been characterized by significant advances in the knowledge of *Echinococcus* biology and interaction with the immune system; the development of more specific and sensitive immunological tests and the introduction of PCR for detection of parasite nucleic acid have increased the range of diagnostic tools. Furthermore, the improvement in surgical techniques, the introduction of effective drugs (e.g., BMZ) and minimally invasive treatments (e.g., PAIR) have deeply changed the life expectancy and quality of life of patients with HD.

Despite diagnostic and therapeutic progress, many unresolved problems are still waiting for a solution; for instance, there is a need for prevention programs able to monitor and control parasite spreading. Additionally, randomized, controlled trials comparing different therapeutic options, especially for CE, are urgently required, in order to provide new evidence to guide treatment decision-making.

REFERENCES

1 **Rausch R,** D'Alesandro A. The epidemiology of echinococcosis caused by Echinococcus oligarthrus and E vogeli in the neotropics. In: Craig P, Pawlowksi Z. Cestode zoonoses: echinococcosis and cysticercosis. Amsterdam: IOS Press, 2002: 107-130

2 **D'Alesandro A.** Polycystic echinococcosis in tropical America: Echinococcus vogeli and E oligarthrus. *Acta Trop* 1997; **67**: 43-65
3 **Polat P**, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid disease from head to toe. *Radiographics* 2003; **23**: 475-494; quiz 536-537
4 **McManus DP**, Thompson RC. Molecular epidemiology of cystic echinococcosis. *Parasitology* 2003; **127** Suppl: S37-S51
5 **Thompson RC**, McManus DP. Towards a taxonomic revision of the genus Echinococcus. *Trends Parasitol* 2002; **18**: 452-457
6 **Thompson RC,** McManus DP. Aetiology: parasites and lifecycles. In: Eckert J, Gemmell M, Meslin FX, Pawlowski Z. WHOI/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 1-19
7 **Thompson RC.** Biology and systematics of Echinococcus. In: Thompson RCA, Lymbery AJ. The biology of Echinococcus and hydatid disease. Wallingford: CAB International, 1995: 1-50
8 **Zhang W**, Li J, McManus DP. Concepts in immunology and diagnosis of hydatid disease. *Clin Microbiol Rev* 2003; **16**: 18-36
9 **Archer GT**, Robson JE, Thompson AR. Eosinophilia and mast cell hyperplasia induced by parasite phospholipid. *Pathology* 1977; **9**: 137-153
10 **Riganò R**, Profumo E, Siracusano A. New perspectives in the immunology of Echinococcus granulosus infection. *Parassitologia* 1997; **39**: 275-277
11 **Riganò R**, Profumo E, Teggi A, Siracusano A. Production of IL-5 and IL-6 by peripheral blood mononuclear cells (PBMC) from patients with Echinococcus granulosus infection. *Clin Exp Immunol* 1996; **105**: 456-459
12 **Vuitton DA**. Echinococcosis and allergy. *Clin Rev Allergy Immunol* 2004; **26**: 93-104
13 **Touil-Boukoffa C**, Bauvois B, Sancéau J, Hamrioui B, Wietzerbin J. Production of nitric oxide (NO) in human hydatidosis: relationship between nitrite production and interferon-gamma levels. *Biochimie* 1998; **80**: 739-744
14 **McManus DP,** Bryant C. Biochemistry, physiology and molecular biology of Echinococcus. In: RCA Thompson and AJ Lymbery, editors. The biology of Echinococcus and hydatid disease. Wallingford: CAB International, 1995: 355-410
15 **Riganò R**, Profumo E, Ioppolo S, Notargiacomo S, Ortona E, Teggi A, Siracusano A. Immunological markers indicating the effectiveness of pharmacological treatment in human hydatid disease. *Clin Exp Immunol* 1995; **102**: 281-285
16 **Vuitton DA**. The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Trop* 2003; **85**: 119-132
17 **Ferreira AM**, Würzner R, Hobart MJ, Lachmann PJ. Study of the in vitro activation of the complement alternative pathway by Echinococcus granulosus hydatid cyst fluid. *Parasite Immunol* 1995; **17**: 245-251
18 **Annen JM**, Köhler P, Eckert J. Cytotoxicity of Echinococcus granulosus cyst fluid in vitro. *Z Parasitenkd* 1981; **65**: 79-88
19 **Dixon JB,** Jenkins P. Immunology of mammalian metacestode infections. I. Antigens, protective immunity and immunopathology. *Helm Abstr* 1995; **64**: 532-542
20 **Wang Q**, Qiu JM, Schantz P, He JG, Ito A, Liu FJ. Investigation of risk factors for development of human hydatidosis among households raising livestock in Tibetan areas of western Sichuan province. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 2001; **19**: 93-96
21 **Eckert J,** Schantz P, Gasser R. Geographic distribution and prevalence. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, editors. WHOI/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 100-141
22 **Ito A**, Urbani C, Jiamin Q, Vuitton DA, Dongchuan Q, Heath DD, Craig PS, Zheng F, Schantz PM. Control of echinococcosis and cysticercosis: a public health challenge to international cooperation in China. *Acta Trop* 2003; **86**: 3-17
23 **Todorov T**, Boeva V. Human echinococcosis in Bulgaria: a comparative epidemiological analysis. *Bull World Health Organ* 1999; **77**: 110-118
24 **Shaikenov BS,** Torgerson PR. Distribution of Echinococcus multilocularis in Kazakhstan. In: Craig P, Pawlowski Z. Cestode zoononoses: echinococcosis and cysticercosis, an emergent and global problem. Amsterdam: IOS Press, 2002: 299-307
25 **Shaikenov BS**, Vaganov TF, Torgerson PR. Cystic echinococcosis in Kazakhstan: an emerging disease since independence from the Soviet Union. *Parasitol Today* 1999; **15**: 172-174
26 **Moro PL**, Cavero CA, Tambini M, Briceño Y, Jiménez R, Cabrera L. Identification of risk factors for cystic echinococcosis in a peri-urban population of Peru. *Trans R Soc Trop Med Hyg* 2008; **102**: 75-78
27 **Campos-Bueno A**, López-Abente G, Andrés-Cercadillo AM. Risk factors for Echinococcus granulosus infection: a case-control study. *Am J Trop Med Hyg* 2000; **62**: 329-334
28 **Macpherson CNL.** Epidemiology of Echinococcus granulosus in transhumant situations. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, editors. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 156-163
29 **Prousalidis J**, Tzardinoglou E, Kosmidis C, Katsohis K, Aletras O. Surgical management of calcified hydatid cysts of the liver. *HPB Surg* 1999; **11**: 253-259
30 **Atli M**, Kama NA, Yuksek YN, Doganay M, Gozalan U, Kologlu M, Daglar G. Intrabiliary rupture of a hepatic hydatid cyst: associated clinical factors and proper management. *Arch Surg* 2001; **136**: 1249-1255
31 **Kammerer WS**, Schantz PM. Echinococcal disease. *Infect Dis Clin North Am* 1993; **7**: 605-618
32 **Pawlowski Z,** Eckert J, Vuitton DA, Ammann RW, Kern P, Craig PS. Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski Z, editors. WHO/OIE Manual on Echinococcosis in humans and animals. Paris: Office International des Epizooties, 2001: 20-71
33 **Ergüney S**, Tortum O, Taspinar AH, Ertem M, Gazioğlu E. [Complicated hydatid cysts of the liver]. *Ann Chir* 1991; **45**: 584-589
34 **Pedrosa I**, Saíz A, Arrazola J, Ferreirós J, Pedrosa CS. Hydatid disease: radiologic and pathologic features and complications. *Radiographics* 2000; **20**: 795-817
35 **Cohen H**, Paolillo E, Bonifacino R, Botta B, Parada L, Cabrera P, Snowden K, Gasser R, Tessier R, Dibarboure L, Wen H, Allan JC, Soto de Alfaro H, Rogan MT, Craig PS. Human cystic echinococcosis in a Uruguayan community: a sonographic, serologic, and epidemiologic study. *Am J Trop Med Hyg* 1998; **59**: 620-627
36 **Shambesh MA**, Craig PS, Macpherson CN, Rogan MT, Gusbi AM, Echtuish EF. An extensive ultrasound and serologic study to investigate the prevalence of human cystic echinococcosis in northern Libya. *Am J Trop Med Hyg* 1999; **60**: 462-468
37 **WHO Informal Working Group.** International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003; **85**: 253-261
38 **Gharbi HA**, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatic liver. *Radiology* 1981; **139**: 459-463
39 **Hosch W**, Junghanss T, Stojkovic M, Brunetti E, Heye T, Kauffmann GW, Hull WE. Metabolic viability assessment of cystic echinococcosis using high-field 1H MRS of cyst con-

tents. *NMR Biomed* 2008; **21**: 734-754
40 **Zhang W**, McManus DP. Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol Med Microbiol* 2006; **47**: 24-41
41 **Ito A**. Serologic and molecular diagnosis of zoonotic larval cestode infections. *Parasitol Int* 2002; **51**: 221-235
42 **Gottstein B**. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev* 1992; **5**: 248-261
43 **Craig PS**, Rogan MT, Campos-Ponce M. Echinococcosis: disease, detection and transmission. *Parasitology* 2003; **127** Suppl: S5-20
44 **Liu D**, Rickard MD, Lightowlers MW. Assessment of monoclonal antibodies to Echinococcus granulosus antigen 5 and antigen B for detection of human hydatid circulating antigens. *Parasitology* 1993; **106** (Pt 1): 75-81
45 **Ortona E**, Riganò R, Margutti P, Notargiacomo S, Ioppolo S, Vaccari S, Barca S, Buttari B, Profumo E, Teggi A, Siracusano A. Native and recombinant antigens in the immunodiagnosis of human cystic echinococcosis. *Parasite Immunol* 2000; **22**: 553-559
46 **Poretti D**, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. *Am J Trop Med Hyg* 1999; **60**: 193-198
47 **Hira PR**, Shweiki H, Lindberg LG, Shaheen Y, Francis I, Leven H, Behbehani K. Diagnosis of cystic hydatid disease: role of aspiration cytology. *Lancet* 1988; **2**: 655-657
48 **Khabiri AR**, Bagheri F, Assmar M, Siavashi MR. Analysis of specific IgE and IgG subclass antibodies for diagnosis of Echinococcus granulosus. *Parasite Immunol* 2006; **28**: 357-362
49 **Brunetti E**, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010; **114**: 1-16
50 **Erdener A**, Ozok G, Demircan M. Surgical treatment of hepatic hydatid disease in children. *Eur J Pediatr Surg* 1992; **2**: 87-89
51 **Perdomo R**, Alvarez C, Monti J, Ferreira C, Chiesa A, Carbó A, Alvez R, Grauert R, Stern D, Carmona C, Yarzabal L. Principles of the surgical approach in human liver cystic echinococcosis. *Acta Trop* 1997; **64**: 109-122
52 **Cirenei A**, Bertoldi I. Evolution of surgery for liver hydatidosis from 1950 to today: analysis of a personal experience. *World J Surg* 2001; **25**: 87-92
53 **Dziri C**, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004; **28**: 731-736
54 **Ammann RW**, Eckert J. Cestodes. Echinococcus. *Gastroenterol Clin North Am* 1996; **25**: 655-689
55 **Arif SH**, Shams-Ul-Bari NA, Zargar SA, Wani MA, Tabassum R, Hussain Z, Baba AA, Lone RA. Albendazole as an adjuvant to the standard surgical management of hydatid cyst liver. *Int J Surg* 2008; **6**: 448-451
56 **Informal Working Group on Echinococcosis.** PAIR: Puncture, Aspiration, Injection, Re-Aspiration. An Option for the Treatment of Cystic Echinococcosis. Geneva: WHO, 2003
57 **Khuroo MS**, Dar MY, Yattoo GN, Zargar SA, Javaid G, Khan BA, Boda MI. Percutaneous drainage versus albendazole therapy in hepatic hydatidosis: a prospective, randomized study. *Gastroenterology* 1993; **104**: 1452-1459
58 **Smego RA**, Bhatti S, Khaliq AA, Beg MA. Percutaneous aspiration-injection-reaspiration drainage plus albendazole or mebendazole for hepatic cystic echinococcosis: a meta-analysis. *Clin Infect Dis* 2003; **37**: 1073-1083
59 **Vutova K**, Mechkov G, Vachkov P, Petkov R, Georgiev P, Handjiev S, Ivanov A, Todorov T. Effect of mebendazole on human cystic echinococcosis: the role of dosage and treatment duration. *Ann Trop Med Parasitol* 1999; **93**: 357-365
60 **Senyüz OF**, Yeşildag E, Celayir S. Albendazole therapy in the treatment of hydatid liver disease. *Surg Today* 2001; **31**: 487-491
61 **Nahmias J**, Goldsmith R, Soibelman M, el-On J. Three- to 7-year follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). *Ann Trop Med Parasitol* 1994; **88**: 295-304
62 **Kumar A**, Lal BK, Chattopadhyay TK. Hydatid disease of the liver--non surgical options. *J Assoc Physicians India* 1993; **41**: 437-438
63 **Stojkovic M**, Zwahlen M, Teggi A, Vutova K, Cretu CM, Virdone R, Nicolaidou P, Cobanoglu N, Junghanss T. Treatment response of cystic echinococcosis to benzimidazoles: a systematic review. *PLoS Negl Trop Dis* 2009; **3**: e524
64 **Cobo F**, Yarnoz C, Sesma B, Fraile P, Aizcorbe M, Trujillo R, Diaz-de-Liaño A, Ciga MA. Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatisosis caused by Echinococcus granulosus. *Trop Med Int Health* 1998; **3**: 462-466
65 **Eckert J,** Gemmell MA, Meslin FX, Pawłowski ZS. WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organisation for Animal Health (Office International des Epizooties) and World Health Organization. Paris: WHO, 2001
66 **Eckert J.** Alveolar echinococcosis (Echinococcus multilocularis) and other forms of echinococcosis (Echinococcus oligarthrus and Echinococcus vogeli). In: Palmer SR, Soulsby EJL, Simpson DIH, editors. Oxford: Oxford University Press, 1998: 689-716
67 **Ricard-Blum S**, Bresson-Hadni S, Guerret S, Grenard P, Volle PJ, Risteli L, Grimaud JA, Vuitton DA. Mechanism of collagen network stabilization in human irreversible granulomatous liver fibrosis. *Gastroenterology* 1996; **111**: 172-182
68 **Grenard P**, Bresson-Hadni S, El Alaoui S, Chevallier M, Vuitton DA, Ricard-Blum S. Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. *J Hepatol* 2001; **35**: 367-375
69 **Vuitton DA**, Guerret-Stocker S, Carbillet JP, Mantion G, Miguet JP, Grimaud JA. Collagen immunotyping of the hepatic fibrosis in human alveolar echinococcosis. *Z Parasitenkd* 1986; **72**: 97-104
70 **Gottstein B**, Hemphill A. Immunopathology of echinococcosis. *Chem Immunol* 1997; **66**: 177-208
71 **Emery I**, Leclerc C, Sengphommachanh K, Vuitton DA, Liance M. In vivo treatment with recombinant IL-12 protects C57BL/6J mice against secondary alveolar echinococcosis. *Parasite Immunol* 1998; **20**: 81-91
72 **Liance M**, Ricard-Blum S, Emery I, Houin R, Vuitton DA. Echinococcus multilocularis infection in mice: in vivo treatment with a low dose of IFN-gamma decreases metacestode growth and liver fibrogenesis. *Parasite* 1998; **5**: 231-237
73 **Sturm D**, Menzel J, Gottstein B, Kern P. Interleukin-5 is the predominant cytokine produced by peripheral blood mononuclear cells in alveolar echinococcosis. *Infect Immun* 1995; **63**: 1688-1697
74 **Godot V**, Harraga S, Deschaseaux M, Bresson-Hadni S, Gottstein B, Emilie D, Vuitton DA. Increased basal production of interleukin-10 by peripheral blood mononuclear cells in human alveolar echinococcosis. *Eur Cytokine Netw* 1997; **8**: 401-408
75 **Godot V**, Harraga S, Beurton I, Deschaseaux M, Sarciron E, Gottstein B, Vuitton DA. Resistance/susceptibility to Echinococcus multilocularis infection and cytokine profile in humans. I. Comparison of patients with progressive and abortive lesions. *Clin Exp Immunol* 2000; **121**: 484-490
76 **Godot V**, Harraga S, Beurton I, Tiberghien P, Sarciron E, Gottstein B, Vuitton DA. Resistance/susceptibility to Echinococcus multilocularis infection and cytokine profile in humans. II. Influence of the HLA B8, DR3, DQ2 haplotype. *Clin Exp Immunol* 2000; **121**: 491-498
77 **Bresson-Hadni S**, Liance M, Meyer JP, Houin R, Bresson JL, Vuitton DA. Cellular immunity in experimental Echinococcus multilocularis infection. II. Sequential and comparative

phenotypic study of the periparasitic mononuclear cells in resistant and sensitive mice. *Clin Exp Immunol* 1990; **82**: 378-383
78 **Rakha NK**, Dixon JB, Carter SD, Craig PS, Jenkins P, Folkard S. Echinococcus multilocularis antigens modify accessory cell function of macrophages. *Immunology* 1991; **74**: 652-656
79 **Kizaki T**, Ishige M, Bingyan W, Day NK, Good RA, Onoé K. Generation of CD8+ suppressor T cells by protoscoleces of Echinococcus multilocularis in vitro. *Immunology* 1993; **79**: 412-417
80 **Vuitton DA**, Bresson-Hadni S, Laroche L, Kaiserlian D, Guerret-Stocker S, Bresson JL, Gillet M. Cellular immune response in Echinococcus multilocularis infection in humans. II. Natural killer cell activity and cell subpopulations in the blood and in the periparasitic granuloma of patients with alveolar echinococcosis. *Clin Exp Immunol* 1989; **78**: 67-74
81 **Frosch PM**, Frosch M, Pfister T, Schaad V, Bitter-Suermann D. Cloning and characterisation of an immunodominant major surface antigen of Echinococcus multilocularis. *Mol Biochem Parasitol* 1991; **48**: 121-130
82 **Gottstein B**. Echinococcus multilocularis: antigenic variance between different parasite isolates. *Parasitol Res* 1991; **77**: 359-361
83 **Bretagne S**, Assouline B, Vidaud D, Houin R, Vidaud M. Echinococcus multilocularis: microsatellite polymorphism in U1 snRNA genes. *Exp Parasitol* 1996; **82**: 324-328
84 **Romig T**, Dinkel A, Mackenstedt U. The present situation of echinococcosis in Europe. *Parasitol Int* 2006; **55** Suppl: S187-S191
85 **Shaikenov BS**. Distribution and ecology of Echinococcus multilocularis in Central Asia. *Parasitol Int* 2006; **55** Suppl: S213-S219
86 **Craig PS**, Deshan L, MacPherson CN, Dazhong S, Reynolds D, Barnish G, Gottstein B, Zhirong W. A large focus of alveolar echinococcosis in central China. *Lancet* 1992; **340**: 826-831
87 **Kern P**, Bardonnet K, Renner E, Auer H, Pawlowski Z, Ammann RW, Vuitton DA, Kern P. European echinococcosis registry: human alveolar echinococcosis, Europe, 1982-2000. *Emerg Infect Dis* 2003; **9**: 343-349
88 **Eckert J**, Deplazes P. Alveolar echinococcosis in humans: the current situation in Central Europe and the need for countermeasures. *Parasitol Today* 1999; **15**: 315-319
89 **Schweiger A**, Ammann RW, Candinas D, Clavien PA, Eckert J, Gottstein B, Halkic N, Muellhaupt B, Prinz BM, Reichen J, Tarr PE, Torgerson PR, Deplazes P. Human alveolar echinococcosis after fox population increase, Switzerland. *Emerg Infect Dis* 2007; **13**: 878-882
90 **Eckert J**, Conraths FJ, Tackmann K. Echinococcosis: an emerging or re-emerging zoonosis? *Int J Parasitol* 2000; **30**: 1283-1294
91 **Hildreth MB**, Sriram S, Gottstein B, Wilson M, Schantz PM. Failure to identify alveolar echinococcosis in trappers from South Dakota in spite of high prevalence of Echinococcus multilocularis in wild canids. *J Parasitol* 2000; **86**: 75-77
92 **Doi R,** Seo H, Fukuyama Y, Nakao M, Inaoka T, Kutsumi S. Epidemiology of multilocular echinococcosis in Hokkaido. A sero-epidemiological study of hunters. *Jap J Pub Health* 1987; **34**: 357-365
93 **Sato N,** Aoki S, Matsushita M, Uchino J. Clinical features. In: Uchino J, Sato N, editors. Alveolar echinococcosis of the liver. Sapporo: Hokkaido University School of Medicine, 1993: 63-68
94 **Torgerson PR**, Schweiger A, Deplazes P, Pohar M, Reichen J, Ammann RW, Tarr PE, Halkik N, Müllhaupt B. Alveolar echinococcosis: from a deadly disease to a well-controlled infection. Relative survival and economic analysis in Switzerland over the last 35 years. *J Hepatol* 2008; **49**: 72-77
95 **Bartholomot G**, Vuitton DA, Harraga S, Shi DZ, Giraudoux P, Barnish G, Wang YH, MacPherson CN, Craig PS. Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. *Am J Trop Med Hyg* 2002; **66**: 23-29
96 **Bresson-Hadni S**, Delabrousse E, Blagosklonov O, Bartholomot B, Koch S, Miguet JP, André Mantion G, Angèle Vuitton D. Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. *Parasitol Int* 2006; **55** Suppl: S267-S272
97 **Reuter S**, Nüssle K, Kolokythas O, Haug U, Rieber A, Kern P, Kratzer W. Alveolar liver echinococcosis: a comparative study of three imaging techniques. *Infection* 2001; **29**: 119-125
98 **Stumpe KD**, Renner-Schneiter EC, Kuenzle AK, Grimm F, Kadry Z, Clavien PA, Deplazes P, von Schulthess GK, Muellhaupt B, Ammann RW, Renner EL. F-18-fluorodeoxyglucose (FDG) positron-emission tomography of Echinococcus multilocularis liver lesions: prospective evaluation of its value for diagnosis and follow-up during benzimidazole therapy. *Infection* 2007; **35**: 11-18
99 **Kern P**, Wen H, Sato N, Vuitton DA, Gruener B, Shao Y, Delabrousse E, Kratzer W, Bresson-Hadni S. WHO classification of alveolar echinococcosis: principles and application. *Parasitol Int* 2006; **55** Suppl: S283-S287
100 **Scheuring UJ**, Seitz HM, Wellmann A, Hartlapp JH, Tappe D, Brehm K, Spengler U, Sauerbruch T, Rockstroh JK. Long-term benzimidazole treatment of alveolar echinococcosis with hematogenic subcutaneous and bone dissemination. *Med Microbiol Immunol* 2003; **192**: 193-195
101 **Ma L**, Ito A, Liu YH, Wang XG, Yao YQ, Yu DG, Chen YT. Alveolar echinococcosis: Em2plus-ELISA and Em18-western blots for follow-up after treatment with albendazole. *Trans R Soc Trop Med Hyg* 1997; **91**: 476-478
102 **Xiao N**, Mamuti W, Yamasaki H, Sako Y, Nakao M, Nakaya K, Gottstein B, Schantz PM, Lightowlers MW, Craig PS, Ito A. Evaluation of use of recombinant Em18 and affinity-purified Em18 for serological differentiation of alveolar echinococcosis from cystic echinococcosis and other parasitic infections. *J Clin Microbiol* 2003; **41**: 3351-3353
103 **Ito A,** Ma L, Paul M, Stefaniak J, Pawlowski ZS. Evaluation of Em18-, Em16-, antigen B-western blots, Em2plus-ELISA and four other tests for differential serodiagnosis of alveolar and cystic echinococcosis patients in Poland. *Parasitol Int* 1998; **47**: 95-99
104 **Gottstein B**, Jacquier P, Bresson-Hadni S, Eckert J. Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2plus antigen. *J Clin Microbiol* 1993; **31**: 373-376
105 **Lanier AP**, Trujillo DE, Schantz PM, Wilson JF, Gottstein B, McMahon BJ. Comparison of serologic tests for the diagnosis and follow-up of alveolar hydatid disease. *Am J Trop Med Hyg* 1987; **37**: 609-615
106 **Rausch RL**, Wilson JF, Schantz PM, McMahon BJ. Spontaneous death of Echinococcus multilocularis: cases diagnosed serologically (by Em2 ELISA) and clinical significance. *Am J Trop Med Hyg* 1987; **36**: 576-585
107 **Ito A**, Schantz PM, Wilson JF. Em18, a new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. *Am J Trop Med Hyg* 1995; **52**: 41-44
108 **Ito A**, Xiao N, Liance M, Sato MO, Sako Y, Mamuti W, Ishikawa Y, Nakao M, Yamasaki H, Nakaya K, Bardonnet K, Bresson-Hadni S, Vuitton DA. Evaluation of an enzyme-linked immunosorbent assay (ELISA) with affinity-purified Em18 and an ELISA with recombinant Em18 for differential diagnosis of alveolar echinococcosis: results of a blind test. *J Clin Microbiol* 2002; **40**: 4161-4165
109 **Vuitton DA**, Zhang SL, Yang Y, Godot V, Beurton I, Mantion G, Bresson-Hadni S. Survival strategy of Echinococcus multilocularis in the human host. *Parasitol Int* 2006; **55** Suppl: S51-S55
110 **Wen H**, Craig PS, Ito A, Vuitton DA, Bresson-Hadni S, Allan JC, Rogan MT, Paollilo E, Shambesh M. Immunoblot evaluation of IgG and IgG-subclass antibody responses for im-

munodiagnosis of human alveolar echinococcosis. *Ann Trop Med Parasitol* 1995; **89**: 485-495
111 **Wen H**, Craig PS. Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. *Am J Trop Med Hyg* 1994; **51**: 741-748
112 **Dreweck CM**, Lüder CG, Soboslay PT, Kern P. Subclass-specific serological reactivity and IgG4-specific antigen recognition in human echinococcosis. *Trop Med Int Health* 1997; **2**: 779-787
113 **Wen H**, Bresson-Hadni S, Vuitton DA, Lenys D, Yang BM, Ding ZX, Craig PS. Analysis of immunoglobulin G subclass in the serum antibody responses of alveolar echinococcosis patients after surgical treatment and chemotherapy as an aid to assessing the outcome. *Trans R Soc Trop Med Hyg* 1995; **89**: 692-697
114 **Yamasaki H,** Nakaya K, Nakao M, Sako YAI. Significance of molecular diagnosis using histopathological specimens in cestode zoonoses. *Trop Med Health* 2007; **35**: 307-321
115 **Kadry Z**, Renner EC, Bachmann LM, Attigah N, Renner EL, Ammann RW, Clavien PA. Evaluation of treatment and long-term follow-up in patients with hepatic alveolar echinococcosis. *Br J Surg* 2005; **92**: 1110-1116
116 **Buttenschoen K**, Carli Buttenschoen D, Gruener B, Kern P, Beger HG, Henne-Bruns D, Reuter S. Long-term experience on surgical treatment of alveolar echinococcosis. *Langenbecks Arch Surg* 2009; **394**: 689-698
117 **Koch S**, Bresson-Hadni S, Miguet JP, Crumbach JP, Gillet M, Mantion GA, Heyd B, Vuitton DA, Minello A, Kurtz S. Experience of liver transplantation for incurable alveolar echinococcosis: a 45-case European collaborative report. *Transplantation* 2003; **75**: 856-863
118 **Reuter S**, Jensen B, Buttenschoen K, Kratzer W, Kern P. Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. *J Antimicrob Chemother* 2000; **46**: 451-456
119 **Reuter S**, Buck A, Grebe O, Nüssle-Kügele K, Kern P, Manfras BJ. Salvage treatment with amphotericin B in progressive human alveolar echinococcosis. *Antimicrob Agents Chemother* 2003; **47**: 3586-3591
120 **Kern P,** Abboud P, Kern W, Stich A, Bresson-Hadni S, Guerin B, Buttenschoen K, Gruener B, Reuter S, Hemphill A. Critical appraisal of nitazoxanide for the treatment of alveolar echinococcosis. *Am J Trop Med Hyg* 2008; **79**: 119
121 **Eckert J**, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004; **17**: 107-135

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