Network for wildlife health surveillance in Europe

Diagnosis Card

Alveolar Echinococcosis

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Etiology

Echinococcosis is caused by parasitic cestodes of the genus *Echinococcus* which belongs to the family *Taeniidae*. Currently, a number of nine species are attributed to the genus *Echinococcus*: *Echinococcus granulosus sensu stricto, E. multilocularis, E. oligarthrus, E. vogeli, E. felidis, E. equinus, E. ortleppi, E. canadensis and E. shiquicus.* There is an ongoing discussion on the taxonomy regarding the paraphyletic *Echinococcus granulosus* complex. Therefore phylogeny and taxonomy are in constant flux due to changing data and new knowledge.

Affected species (wildlife, domestic animals, humans)

Definitive hosts of *E. multilocularis* are typically canid species especially the red fox (*Vulpes vulpes*), the raccoon dog (*Nyctereutes procyonoides*) and the wolf (*Canis lupus*), but domestic animals (dogs and to a lesser extent cats) can also be infected. Several genera of rodents serve as regular sylvatic intermediate hosts. Humans, dogs and many other mammals can also be infected as aberrant intermediate host. For *E. granulosus*, jackals and wolves represent potential definitive hosts.

Epidemiological characteristics and disease course

Transmission among animals: Infected definitive hosts harbor the mature tapeworms and excrete tapeworm eggs with their feces. Intermediate hosts get infected by oral ingestion of tape worm eggs leading to the development of larval stages (“metacestodes”) which are mostly found in the liver. The life cycle of the parasite is completed when definitive hosts eat infected intermediate hosts.

Clinical signs

In definitive hosts there are usually no clinical signs.

E. multilocularis causes alveolar echinococcosis (AE) in intermediate and aberrant hosts. In humans, AE represents a severe disease which is often lethal if left untreated.

Intermediate hosts: Signs include enlargement of the abdomen, increased total body weight (due to metacestode proliferation), but loss of total body mass, weakness, apathy, anorexia, cholestatic jaundice and/or epigastric pain, fatigue, ascites, hepatomegaly, masses due to spread of the metacestode to other other abdominal organs and sometimes elsewhere in the body, and finally death.

Aberrant hosts: Asymptomatic liver infections can occur in domestic pigs and wild boar.

Gross lesions

E. multilocularis does normally not induce lesions in definitive hosts. In normal and aberrant intermediate hosts, gross pathological lesions in the liver include multilocular fluid-filled cysts (more common in normal intermediate hosts), solid and caseous white-yellow nodules (more common in aberrant intermediate hosts), and cholestasis. Other lesions depend on location of metastasis. Abdominal effusion is commonly present in advanced cases.

Histological lesions

Lesions of the liver in intermediate and aberrant hosts: numerous echinococcal ‘cysts’; PAS-positive laminated layer, with an adjoining germinal layer from which multiple protoscolices may protrude. Around the cystic vesicles, layers of mature connective tissue with multifocal mineralisation and a moderate cellular infiltrate of lymphocytes, macrophages and neutrophils. Liver parenchyma adjacent may show moderate proliferation of bile ducts and moderate cellular infiltration by lymphocytes, histiocytes and neutrophils. The morphology and size of protoscolex hooks should be taken into

account if possible (Eckert et al., 2002). PCR (or immunohistochemistry) should be used for confirmation. In living animals, serological techniques (Em2-ELISA or Westernblot) in conjunction with medical imaging techniques can be used. Alveolar hydatid larvae do not pose a biohazard to people.

Differential diagnosis

Infections with *Taenia* species in definitive hosts: Taeniid eggs can normally not be differentiated on the basis of their morphology. Species identification of taeniid eggs and also of adult worms and larval stages can be achieved using molecular techniques (Trachsel et al., 2007). Morphology of intact adult *Echinococcus* worms is similar to E. *granulosus*, but differentiation is possible on the basis of morphological criteria (number of proglottids, position of the genital pores in anterior half of the proglottid, no lateral extensions of the uterus). Differential diagnosis of AE in intermediate hosts: malignant liver tumours, cysticerci and coenurus in early stages, granulomatous hepatic inflammation.

Criteria for diagnosis

In definitive hosts, adult cestodes can be recovered using the intestinal scraping technique (Eckert et al., 2002; Tackmann et al. 2006). Trained laboratory personnel can differentiate adult *E. granulosus* and *E. multilocularis* The adult worm of *E. multilocularis* is 1.2 mm to 4.5 mm long (smaller than *E. granulosus*) and possesses four to five proglottids; however age of infection and sample preservation (i.e. freezing) can render morphological identification difficult.

Taeniid eggs can be detected and recovered using a sedimentation and flotation technique of faecal samples, but cannot be morphologically differentiated from *Taenia* and other *Echinococcus* spp.. However, the differentiation of taeniid eggs by PCR is possible (e.g. Trachsel et al., 2007). Direct PCR for the detection of *E. multilocularis* from DNA isolated from fecal samples may lack diagnostic sensitivity (versus DNA extracted from eggs concentrated from feces). A new real time PCR (Knapp et al. 2014, Vet. Parasitology) and magnetic capture techniques are under development (Øines et al. 2014; Isaksson et al., 2015).

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Before any investigation, the material (intestines, carcasses or faeces) should be deep-frozen at -70°C to -80°C for at least 3-7 days to minimize the infections risk for personnel handling potentially infectious material (Eckert et al. 2002).

Intestinal scraping technique (IST) can used to detect E. multilocularis in the intestine of definitive hosts as previously described in WHO/OIE manual on Echinococcosis (Eckert et al. 2002; Tackmann et al., 2006). Alternatively, the sedimentation and counting technique (SCT) can be used which is more laborious, but may also be more sensitive (Eckert et al., 2003). A modified Segmental Sedimentation and Counting Technique (SSCT) has been validated for large epidemiological studies on fox intestines segments, to save time, without loss of specificity and sensibility in regards of the gold standard SCT (Umhang et al. 2013),

Combined sedimentation flotation process to identify and count eggs in faecal samples:

- Add to approx. 10 g faeces H2O and suspend
- Pass through sieve with 250-300 µm mesh
- Flush with water in funnel (total volume 250 ml)
- 30 min sedimentation (discard remaining coarse constituents in sieve)
- decant supernatant
- re-suspend sediment in remaining water
- transfer 3 ml of suspension in 15 ml centrifuge tubes
- add concentrated sugar solution (1000g sugar + 800 ml of tap water + 18.18 ml of 37% formaldehyde solution) up to 15 ml (formaldehyde may be omitted if molecular identification of eggs is planned)
- Centrifugation at 2100 g for 10 min
- take samples with an inoculation loop from 5-7 different places on the surface of the flotation suspension and transfer to a microscope slide
- add 18x18 coverslip
- Count taeniid eggs by microscopic examination (magnification 200x - 400x).

Another method is to concentrate the eggs in faecal samples by the flotation and sieving technique (Deplazes et al. 2003).

For the differentiation and identification of eggs and worm fragments, use a multiplex PCR that distinguishes among Taenia spp., E. multilocularis and E. granulosus (Trachsel et al. 2007)), or a simplex PCR for mitochondrial genes such as NAD1 or COX1.

APHAEA protocol (for harmonization at large scale)

Sample treatment as mentioned above for safety reasons. Use the sedimentation and counting technique (SCT) as described in Eckert et al., 2003 or SSCT, that is less time consuming without specificity and sensibility decrease (Umhang, 2011). A more time saving but at least in some variants less sensitive method is the intestinal scraping technique (IST). The protocol described by Tackmann et al., 2006 may have a similar sensitivity as the SCT. SCT, SSCT and IST are inexpensive, easy to learn and to perform. For faecal samples, the flotation and sieving technique (Deplazes et al. 2003), which may be more expensive, or the combined sedimentation flotation technique are suitable, followed by PCR (e.g.Trachsel et al. 2007).

Laboratories that can be contacted for diagnostic support

FLI, Institute for Epidemiology, National Reference Laboratory for Echinococcosis, (https://www.fli.de/de/institute/institut-fuer-epidemiologie-ife/referenzlabore/nrl-fuer-echinokokkose/)

Institut für Parasitologie, Universität Zürich, Switzerland (www.paras.uzh.ch)

Universität Bern, Institut für Parasitologie der Vetsuisse Fakultät und der Medizinischen Fakultät, Switzerland (www.itpa.vetsuisse.unibe.ch)

EU RL for parasites: Istituto Superiore di Sanità (ISS) Italy (http://www.iss.it/crlp/)

Anses, EcoEpidemiological Wildlife Unit, National reference Laboratory for echinococcosis, France, (https://www.anses.fr/en/content/nancy-laboratory-rabies-and-wildlife)

Recommended literature

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