CHAPTER 2.1.6.

ECHINOCOCCOSIS
(INFECTION WITH ECHINOCOCCUS GRANULOSUS AND WITH E. MULTILOCULARIS)

SUMMARY

Human cystic echinococcosis, caused by Echinococcus granulosus, and alveolar echinococcosis, caused by E. multilocularis, are important public health threats in many parts of the world. Diagnosis of echinococcosis in dogs or other susceptible carnivores relies on the detection of adult cestodes of the Echinococcus genus or their eggs in their faeces or small intestine. Coproantigen and copro-DNA assays have proven useful for safe, fast and accurate diagnosis. In intermediate hosts, diagnosis is dependent on post-mortem detection of the larval cyst form that can infect almost any organ, particularly the liver and lungs.

Identification of the agent: It was previously accepted that there were five valid species of the genus Echinococcus: The current view however, informed by biology, epidemiology and particularly molecular genotyping, recommends the inclusion of at least nine species in the genus. All those species of Echinococcus known to cause cystic echinococcosis in the intermediate host may be referred to as E. granulosus sensu lato (s.l.), whereas strains G1–G3 (which are closely related) are now referred to as E. granulosus sensu strictu (s.s.). Larval forms of Echinococcus can usually be visually detected in organs. Special care has to be taken for a specific diagnosis of E. granulosus in instances where Taenia hydatigena in sheep is also a problem. Histological examination may confirm the diagnosis after formalin-fixed material is processed by conventional staining methods. The presence of a periodic-acid-Schiff positive, acellular laminated layer with or without an internal cellular, nucleated germinal membrane can be regarded as a specific characteristic of metacestodes of Echinococcus. Genotyping via polymerase chain reaction (PCR) is the only method available to confirm the exact species of Echinococcus infecting animals. The identification of larval E. multilocularis in rodents and other hosts is possible by macroscopic or microscopic examination and confirmed by DNA detection using the PCR.

The small intestine is required at necropsy for the detection of adult Echinococcus spp. in wild or in domestic carnivores. Handling infected material needs detailed safety precautions to avoid risk to the operator of contracting a potentially fatal disease.

Coproantigen or CoproDNA tests: Significant progress is being made in the development of immunological tests for the diagnosis of intestinal Echinococcus infections by use of coproantigen detection. The technique has been used successfully in surveys of E. granulosus in dogs and is currently used in surveys for E. multilocularis in populations of dogs and foxes. Coproantigen detection is possible in faecal samples collected from dead or living animals or from the environment. PCR DNA methods for the detection of E. multilocularis and more recently E. granulosus in definitive hosts have now been established as diagnostic techniques.

Serological tests: Antibodies directed against oncosphere, cyst fluid and protoscolex antigens can be detected in the serum of infected dogs and sheep, but this approach is presently of limited practical use as it does not distinguish between current and previous infections. The sensitivity in cases of low worm burden is poor. Cross-reactivity between Echinococcus and Taenia species also may occur.

Requirements for vaccines: Progress has been made in the development of an effective vaccine (Eg95) against infection with the larval stage of E. granulosus in sheep and cattle.
A. INTRODUCTION

The species within the genus Echinococcus are small tapeworms of carnivores with larval (metacestode) stages known as hydatids that proliferate asexually in various mammals including humans. Until recently it was accepted that there were five morphologically distinct species in this genus: *E. granulosus*, *E. multilocularis*, *E. oligarthrus*, *E. vogeli* and *E. shiquicus*. *Echinococcus granulosus*, formerly regarded as a single species with a high genotypic and phenotypic diversity, is now recognised as an assemblage of cryptic species, which differ considerably in morphology, development, host specificity (including infectivity or pathogenicity for humans). This diversity is reflected in the mitochondrial and nuclear genomes. Based on phenotypic characters and gene sequences, *E. granulosus* (sensu lato [s.l.]) has now been subdivided into *E. granulosus* (sensu stricto [s.s.]) (including the formerly identified genotypic variants G1–3), *E. felidis* (the former 'lion strain'), *Echinococcus equinus* (the 'horse strain', genotype G4), *E. ortleppi* (the 'cattle strain', genotype G5) and *E. canadensis*. The latter species, as recognised here, shows the highest diversity and is composed of the 'camel strain', genotype G6, the 'pig strain', genotype G7,and two 'cervid strains', genotypes G8 and G10 (Nakao et al., 2013; Romig et al., 2015). *Echinococcus granulosus* (s.l.) has a global distribution; *E. multilocularis* occurs in wide areas of the Northern Hemisphere, *E. shiquicus* is found in China (People’s Rep. of) and *E. oligarthrus* and *E. vogeli* are confined to Central and South America. All five of the originally described species are infective to humans causing various forms of echinococcosis although in the most recent taxonomic classification there is no evidence of *E. equinus* infections in humans. Human cystic echinococcosis, caused by *E. granulosus*, and alveolar echinococcosis, caused by *E. multilocularis*, are important public health threats in many parts of the world (WHO/OIE, 2001).

<table>
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<tr>
<th>Table 1. Useful characteristics for identification of Echinococcus species in definitive hosts (source: Xiao et al., 2006)</th>
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<td><strong>Species</strong></td>
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<td><em>E. granulosus</em></td>
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<td><em>E. shiquicus</em></td>
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1. *Echinococcus granulosus* (sensu lato)

The parasite is transmitted between the domestic dog and a number of domestic ungulate species. The dog or sheep cycle of *E. granulosus* (s.s.) is most important. Sylvatic definitive and intermediate hosts also occur, e.g.
wolf or cervid (E. canadensis). The adult varies between 2 and 11 mm in length and usually possesses from two to seven segments, averaging from three to four segments. The penultimate segment is mature, and the genital pore normally opens posterior to the middle in both mature and gravid segments. The last (gravid) segment is usually more than half the length of the entire worm. There are rostellar hooks of various sizes on the protoscolex in two rows. The size of the hooks varies between 25 to 49 µm in the first row, and between 17 and 31 µm in the second row. The gravid uterus has well-developed sacculations.

The larval stage is a fluid-filled bladder or hydatid cyst that is unilocular, although communicating chambers also occur. Growth is expansive, and endogenous daughter cysts may be produced. Individual bladders may reach up to 30 cm in diameter and occur most frequently in liver and lungs, but may develop in other internal organs. The infection with this stage is referred to as cystic echinococcosis.

The cryptic species of E. granulosus (s.l.) in domestic cycles include, dog or sheep in the Mediterranean region, South America (Argentina, Brazil, Chile, Peru and Uruguay), Africa (Ethiopia, Kenya and Sudan), the Middle East and Levant regions, Russia, Central Asia (Kazakhstan, Kyrgyzstan and Uzbekistan), Mongolia, China (People’s Rep. of), Oceania and the United Kingdom; dog or horse in Belgium, Ireland and the United Kingdom; dog or cattle in Belgium, Germany, South Africa and Switzerland; dog or swine in Poland; dog or wolf/reindeer in sub-Arctic regions of Norway, Finland and Alaska; dog or camel in the Middle East, Africa, Central Asia and China (People’s Rep. of).

2. Echinococcus multilocularis

The parasite is transmitted primarily between wild definitive hosts (e.g. Vulpes vulpes, V. corsac, Alopex lagopus) and small arvicolid rodents (voles and lemmings). The adult varies between 1.2 and 4.5 mm in length and usually possesses from two to six segments, with an average of four to five. The penultimate segment is characteristically mature, and the genital pore is anterior to the midline in both mature and gravid segments. The gravid uterus is sac-like. On the rostellum, the larger hooks of the first row vary in size between 24.9 and 34.0 µm and the smaller hooks of the inner row between 20.4 and 31.0 µm.

The metacestode is a multivesicular structure consisting of conglomerates of small vesicles, usually not exceeding a few millimetres in diameter. Unlike E. granulosus, the larval mass often contains a semisolid rather than a fluid matrix. It proliferates by exogenous budding, which results in infiltration of tissues. Infection with this stage is commonly referred to as alveolar echinococcosis. There is no clear evidence for distinct strains or genotypes of E. multilocularis, though regional variations at the continental scale have been described (WHO/OIE, 2001).

This zoonotic parasite is found mainly in the Northern Hemisphere, and its life cycle is mainly maintained in wildlife (Kamiya et al., 2007). The sylvatic cycle involves foxes and many species of wild rodents. Coyotes, raccoon dogs, wolves, wild cats, domestic dogs and cats however, may serve as definitive hosts while pigs, horses, primates and humans can be infected as intermediate hosts (Kamiya et al., 2007). Dogs may also rarely be infected with larval stages producing lesions in internal organs, even simultaneously with adult stages present in the gastro-intestinal tract.

3. Echinococcus oligarthus

The parasite typically uses neotropical wild felids as definitive hosts (e.g. Felis concolor, F. jaguarundi) and large rodents (e.g. Dasyprocta sp., Cuniculus paca) as intermediate hosts. The adult varies between 2.2 and 2.9 mm in length, and normally possesses three segments, the penultimate of which is mature. The genital pore is anterior to the middle in mature segments and approximately at the middle in gravid segments. The gravid uterus is sac-like.

The metacestode is polycystic and fluid-filled with a tendency to become septate and multichambered. The rostellar hooks of the protoscolex vary in length between 25.9 and 37.9 µm. The hooks are described in more detail in the next section where they are also compared with those of E. vogeli. The single cyst may reach a diameter of approximately 5 cm. Predilection sites are internal organs and muscles. To date, there have only been a few reports of human disease. The parasite appears not to mature in dogs.

4. Echinococcus vogeli

The parasite typically uses the South American bush dog (Speothus venaticus) as a wild definitive host, but the domestic dog is susceptible, as are large rodents (e.g. Cuniculus paca) as intermediate hosts. The adult varies between 3.9 and 5.5 mm in length, and usually has three segments, the penultimate of which is mature. The genital pore is situated posterior to the middle in both the mature and gravid segments. The gravid uterus has no
lateral sacculations and is characterised by being relatively long and tubular in form, compared with the other segments, which are sac-like.

The metacestode is similar to that of *E. oligarthrus*. It has been reported that the two species can be distinguished by comparing differences in the dimensions and proportions of the rostellar hooks on the protoscolex. The hooks of *E. oligarthrus* vary in length between 25.9 and 37.9 µm (average 33.4 µm) and between 22.6 and 29.5 µm (average 25.45 µm) for large and small hooks, respectively. Those of *E. vogeli* vary between 19.1 and 43.9 µm (average 41.64 µm) and between 30.4 and 36.5 µm (average 33.6 µm) for the large and small hooks, respectively. Also the hook-guard for *E. oligarthrus* divides the hook 50:50, compared with 30:70 for *E. vogeli*.

*Echinococcus vogeli* is a zoonotic agent with approximately 200 human cases in total reported in South America. The infection caused by the larval stage of this species is commonly referred to as polycystic echinococcosis.

5. *Echinococcus shiquicus*

The parasite was found in the Tibetan fox (*Vulpes ferrilata*) its definitive host and the plateau pika (*Ochotona curzoniae*), the intermediate host. In most species of *Echinococcus*, the gravid segment is connected to a mature segment; however, a strobila consisting of only two segments (a gravid segment directly attaching to a premature segment) is unique to this species (WHO/OIE, 2001). The adult stage is morphologically similar to *E. multilocularis* but differs by its smaller hooks, fewer segments, upper position of genital pore in the premature segment and fewer eggs in the gravid segment. It is easily distinguishable from *E. granulosus* by its shorter length, branchless gravid uterus and the anterior position of the genital pore in the gravid segment. The adult measures 1.3 to 1.7 mm.

The metacestode is found in the liver and is essentially a unilocular minicyst containing fully developed brood capsules; however, oligovesicular forms have also been observed. It is differentiated from *E. granulosus* by the absence of daughter cysts within the fertile cyst (WHO/OIE, 2001).

A detailed description of echinococcosis in humans and animals can be found in the WHO/OIE Manual on echinococcosis (WHO/OIE, 2001).

### B. DIAGNOSTIC TECHNIQUES

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<tr>
<th>Method</th>
<th>Population freedom from infection</th>
<th>Individual animal freedom from infection prior to movement</th>
<th>Contribute to eradication policies</th>
<th>Confirmation of clinical cases</th>
<th>Prevalence of infection – surveillance</th>
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*Table 2. Test methods available for the diagnosis of echinococcosis and their purpose*
Table 2. (cont.) Test methods available for the diagnosis of echinococcosis and their purpose

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<th>Method</th>
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<th>Individual animal freedom from infection prior to movement</th>
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Detection of immune response

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<th>Method</th>
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Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.

PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

1. Identification of the agent

In the intermediate host, diagnosis depends on the meat inspection or post-mortem detection of the larval cyst form, which can occur in almost any organ, particularly in the liver and lungs. The diagnosis of echinococcosis in dogs or other carnivores requires the demonstration of the adult cestodes of *Echinococcus* spp. in their faeces or the small intestine or the detection of specific coproantigens or coproDNA.

Investigators carrying out these procedures are exposed to the risk of infection and severe disease, which must be minimised by appropriate biosafety and containment procedures as determined by biorisk analysis (see Chapter 1.1.4 Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities). Infective (egg/adult) material can be decontaminated by freezing at -80°C (core temperature) for 5 days, or by heating to 70°C for 12 hours. Face masks, disposable gloves and an apron must be worn. Chemical disinfection is not reliable, although sodium hypochlorite (10% bleach) can be used to destroy eggs (Craig, 1997). Contaminated material must be destroyed by incineration or autoclaving.

1.1. Diagnosis of *Echinococcus* eggs in environmental samples

1.1.1. Faecal samples (Ito, 1980; Thienpond et al., 1979)

Eggs of *Echinococcus* are identical to those of other taeniid cestodes. It is therefore not possible to make a species-specific diagnosis using microscopy alone. However isolation of eggs can allow molecular techniques to be used for more accurate identification. One method is a concentration method in which a saturated solution is used to separate *Echinococcus* eggs from faeces. A faecal sample of 0.5–2 g is mixed with water or 0.3% Tween 20 in 1% formalin (Nonaka et al., 1998) in a 10–15-ml test tube and centrifuged (1000 g for 10 minutes) once or twice until the supernatant is clear. Sediment is mixed with either zinc sulphate 33% (1.18 sp. Gr.) or sucrose solution (1.27 sp. Gr.) and centrifuged at 1000 g for 5–10 minutes. The test tube is filled to the top and a cover-glass is placed on the tube. The cover-glass is examined microscopically 2–16 hours later.

1.1.2. Soil samples (Matsuo & Kamiya, 2005)

A 20-g soil sample is placed in a 50-ml conical tube to which is added 40 ml of 0.05% Tween 80. The mixture is stirred vigorously and sieved through a 100-µm mesh. The suspension is centrifuged at 1000 g for 5 minutes and the supernatant is discarded. The remaining procedure follows the concentration method used for faecal sample examination.
1.2. Diagnosis of larval echinococcosis

1.2.1. Necropsy

Whereas surveillance for *E. granulosus* in domestic animals may take place in licensed slaughter houses, that for *Echinococcus* sp. in wildlife must be done by field surveys. When undertaking surveillance work with *E. granulosus* in intermediate hosts, it is vitally important that data are stratified and reported according to the age of animals slaughtered. Prevalence rates are strongly age dependent and reports from abattoirs that may slaughter only young animals will substantially under-represent the true situation. This is because older animals may be heavily infected even when animals have very few larvae.

1.2.1.1 Investigative procedure

Hydatid cysts can be observed in many organs, but in large animals, such as sheep and cattle, palpation or incision should be done. Pigs, cattle, sheep and goats may also be infected with larval *Taenia hydatigena*, and it is sometimes difficult to differentiate between these two parasites when they occur in the liver. In wild animals, such as ruminants and rodents, several other larval cestodes should be considered for differential diagnosis. Please refer to Chapter 2.9.5 *Cysticercosis* for information on other cestodes found at meat inspection.

i) Suspect parasite material should be removed from the organ by cutting with a scalpel to include the immediate host tissue, and kept in a cool location. (NB hydatid cyst tissue in intact cysts will remain viable for more than 24 hours after death even at ambient temperatures. However viability will be prolonged by storage at 4°C for up to 72 hours. If material cannot be examined within this time, it should be stored in either 10% formal saline for subsequent microscopic examination or in 90% ethanol for subsequent DNA analysis. Ideally a sample of parasite material should be preserved in both media. Parasite tissues that are frozen will not be viable but can be examined morphologically on thawing and subjected to DNA analyses.)

ii) For morphological analysis of cyst contents, fluid should be removed and retained using a syringe. The material inside the cyst should then be washed with saline and the contents examined under the microscope (×4 objective) for the presence of protoscoleces. Note that some hydatid cysts may be sterile and not contain protoscoleces. If no protoscoleces are present, the laminated layer on the inside of the cyst cavity may be observed as a gelatinous structure that can easily be pulled away. Formalin-fixed material can be stained by conventional histological techniques. The presence of a periodic-acid-Schiff (PAS) positive acellular laminated layer, underlying a connective tissue layer, and with or without an internal cellular, nucleated germinal membrane can be regarded as a specific characteristic of the metacestodes of *Echinococcus* spp.

iii) In all cases exact species identification can only be made through extraction of DNA from ethanol-fixed material (Dinkel et al., 1998) and subsequent genotyping by polymerase chain reaction (PCR). Specific primers for all *Echinococcus* species and related taeniids are summarised by Roelfsema et al. (2016).

1.3. Diagnosis of adult parasites in carnivores

1.3.1. Necropsy

Necropsy is invariably employed in studies of echinococcosis in wildlife and is useful if domestic dogs are humanely culled. It should be emphasised that it is necessary to isolate and identify the adult *Echinococcus*, because under normal conditions of faecal examination, the eggs of *Echinococcus* cannot be differentiated from those of *Taenia* spp. The eggs of *E. granulosus* and *E. multilocularis* can now be identified and differentiated from other taeniid eggs by PCR.

The small intestine is removed as soon as possible after death, and tied at both ends. If the material is not frozen or formalin fixed (4–10%), it should be examined quickly, as the parasite can be digested within 24 hours. Formalin does not kill eggs. The fresh intestine is divided into several sections and immersed in 0.9% saline at 37°C for examination. Worms adhering to the intestinal wall may be observed and counted by means of a hand lens (for *E. granulosus* and *E. vogeli*). For accurate counts, the unfixed intestine is best divided into four or six sections, opened up and immersed in 0.9% saline at 37°C for 30 minutes to release the parasites. The contents are washed into another container for detailed examination, and the intestinal wall is scraped with a spatula. All material is boiled and washed by sieving to eliminate most of the particulate material and to make it noninfectious. The washed intestinal contents and scrapings...
are placed on a black tray, and the worms are counted with the aid of a hand lens or stereoscopic microscope. *Echinococcus granulosus* is usually found in the first third of the small intestine of dogs and *E. multilocularis* in the mid/posterior sections. This approach has a greater than 95% sensitivity, except under low worm burdens where false negative results may occur.

Necropsy is considered to be the most reliable form of diagnosis for *E. multilocularis* in definitive hosts. It is an inexpensive method for determining the prevalence in a population and the best way to determine worm burden. Carcasses or intestines of definitive hosts for examination should be deep frozen at between −70°C and −80°C for 3–7 days before necropsy to kill any eggs. Eggs of *E. multilocularis* are resistant to freezing to −50°C.

1.3.2. Sedimentation and counting technique (SCT) (Eckert, 2003)

This technique has been regarded as the ‘gold standard’ for assessing the sensitivity and specificity of other techniques, however the copro-DNA (PCR) test has a greater sensitivity than SCT.

i) The small intestine is incised longitudinally and cut into 20 cm long segments or into 5 pieces of approximately the same length. These pieces are transferred to a glass bottle containing 1 litre physiological saline (0.9% NaCl) solution.

ii) The glass bottle is shaken vigorously for a few seconds and the pieces of intestine are removed. The superficial mucosal layer is stripped by exerting pressure between thumb and forefinger to dislodge attached helminths.

iii) The glass bottle is left for 15 minutes for sedimentation to occur; the supernatant is then decanted. The glass bottle is refilled with physiological saline solution. This procedure is repeated 2–6 times until the supernatant is cleared of coloured particles.

iv) The sediment fraction is examined in small portions of about 5–10 ml in rectangular plastic or Petri dishes with a counting grid (9 × 9 cm) in transmission light under a stereomicroscope at a magnification of ×120.

v) If up to 100 worms are found, the entire sediment fraction is checked; if higher numbers are present, the total worm burden is calculated from the count of one subsample.

1.3.3. Intestinal scraping technique (IST) (Deplazes & Eckert, 1996)

i) Deep mucosal scrapings are taken at nearly equal distances from the small intestine using microscope slides (75 × 25 × 1 mm). Five mucosal scrapings from proximal, middle and posterior thirds of the small intestine (total 15) are recommended. Adherent materials are transferred to a square plastic Petri dish.

ii) Scrapings are squashed between slides and examined under a stereoscopic light microscope (×120). Three slides are placed in one plastic dish and examined. *Echinococcus multilocularis* is usually found in the second half of the small intestine.

1.3.4. ‘Shaking in a vessel’ technique (SVT) (Duscher et al., 2005)

i) A plastic vessel (1 litre) that has a plastic screw-on lid with a central hole 6–7 cm in diameter is used. The hole is covered with a high-grade steel mesh (mesh size 500 μm) fixed into the remaining plastic ring with a hot soldering iron. Silicone is applied to seal the edges of the steel mesh.

ii) The longitudinally opened small intestine is transferred to the vessel with all its contents; the vessel is closed with the lid and filled with water.

iii) The vessel is inverted and shaken; the water is decanted. The vessel is refilled with water, and the process is repeated until the decanted water is clear.

iv) The half-filled vessel is opened and the intestines are removed. The intestines are stripped between the thumb and forefinger to dislodge parasites stuck to the mucosa into the vessel.

v) The vessel is closed again, refilled and shaken one last time draining as much water from it as possible.

vi) The remaining sediment is filled into a 1 litre plastic jug and stored at 4°C. For prolonged storage, a 0.9% NaCl solution is added to the sediment to prevent the parasites from shrinking.

vii) For analysis, the materials are placed into small glass Petri dishes and scanned along engraved lines using the stereomicroscope as above.
1.3.5. Preserving specimens

Intact worms are fragile and for morphological studies are best handled in normal saline with a Pasteur pipette. They are washed free of other material and left for approximately 30 minutes for all movement to cease. After removal of the fluid, cold 5–10% formalin (5°C) or FAA fixative (95% ethanol [80 ml], 37–40% formaldehyde [10 ml], and glacial acetic acid [5 ml]) is added and the worms are left for a further 12 hours. For staining, the worms are washed in water for 15 minutes and transferred to Mayer’s paracarmine (carminic acid [1.0 g], aluminium chloride [0.5 g], calcium chloride [4.0 g], and 70% ethanol [100 ml]) for 12–24 hours. Excess stain is removed by immersion in 0.5–1.0% hydrochloric acid solution for a few seconds. Dehydration is accomplished by serial passage in ascending concentrations of alcohol (41, 50, 70, 85, 95, and 100%) for at least 15 minutes in each, with two changes in 100%. The alcohol is removed by xylol (10 minutes) and cleared with methyl salicylate or creosote. Prior to mounting in any suitable medium such as balsam, picolyte, etc., the specimens should be returned to the xylol for a few minutes. Persons involved in such examinations should receive serological screening for anti-Echinococcus serum-antibodies at least once a year (WHO/OIE, 2001).

Over the past 15 years, methods have been developed with the aim of simplifying and improving epidemiological investigations in final host populations and of allowing diagnosis in living animals. These methods include the detection of coproantigens and PCR DNA detection (see below).

1.4. Arecoline surveys and surveillance

Arecoline has been used to perform surveys of tapeworm infections in dog populations. Its use as a control agent has been superseded by praziquantel.

2. Coprological tests

Adult Echinococcus worms inhabiting the intestine will release both surface or secretory molecules (antigens) and DNA (usually contained within eggs). Both types of molecules can be detected by assaying faecal samples. The sensitivity of the tests is strongly influenced by the worm burden and stage of maturity.

2.1. Coproantigen tests

A specific and sensitive laboratory test for antigen detection in canid faecal samples (coproantigen) has the potential to replace arecoline purgation and is preferable to serology for detection of current infection (Allan et al., 1992; Deplazes et al., 1992). Coproantigen ELISA (enzyme-linked immunosorbent assay) or coproELISA provides an alternative method for diagnosing canine echinococcosis, and both polyclonal and monoclonal antibodies have been used: directed against either somatic or excretory/secretory (ES) antigens. To create polyclonal antibodies against Echinococcus spp., rabbits were hyperimmunised with Echinococcus antigens, such as adult or protoscoleces ES extracts, or somatic extracts of adult tapeworms. Alternatively, monoclonal antibodies have been produced using donor mice hyperimmunised with E. granulosus somatic or ES antigens (Craig et al., 2015) CoproELISAs are usually genus-specific for Echinococcus spp. (Allan & Craig, 2006), although depending on the endemic region and study aims, coproELISAs have been developed and validated to test for infection with E. multilocularis in foxes and dogs or primarily for E. granulosus. For canine echinococcosis caused by E. granulosus, most authors report reasonable sensitivity (78–100%) and good genus specificity from 85% to greater than 95% as well as a degree of pre-patent detection (Craig et al., 2015). Where cross-reactions occur these generally appear to be caused by infection with Taenia hydatigena, the most common taenid of dogs, and attempts to improve specificity by using monoclonal antibodies in coproELISAs have not been able to eliminate this problem. CoproELISA sensitivity broadly correlates with worm burden of E. granulosus, however some low intensity infections (worm burdens <50–100) may give false negatives in coproELISA (Allan & Craig, 2006). Coproantigens can be detected prior to release of eggs by Echinococcus worms, and therefore are not related to egg antigen(s). This has the advantage of detection of prepatent infections. Furthermore, coproantigen levels return to the preinfection baseline within 5 days of anthelmintic treatment of infected dogs (Deplazes et al., 1992). More importantly, it reduces the biohazardous risk of exposure of personnel to potentially infective eggs during purgation or necropsy.

For detection of E. multilocularis infection of foxes, necropsy is time-consuming. Coproantigen testing by ELISA offers a specific practical alternative. Fox faecal samples should be taken at post-mortem from the rectum rather than from the small intestine. Echinococcus coproantigens are also stable in fox or dog faeces left at 20°C for 1 week and in frozen dog faeces. Coproantigen testing has also been successfully used to evaluate the efficacy of deworming wild foxes infected with E. multilocularis using praziquantel-laced bait, which proved to be a successful combination of eliminating the source of infection.
2.1.1. Coproantigen test procedure (*Echinococcus granulosus*) (Allan et al., 1992; Craig et al., 1995)

i) The faecal sample (collected per rectum or from the ground) is mixed with an equal volume of phosphate buffered saline (PBS), pH 7.2, containing 0.3% Tween 20 (PBST), in a capped 5 ml disposable tube. This is shaken vigorously and centrifuged at 2000 g for 20 minutes at room temperature. Faecal supernatants can be tested immediately or stored at −20°C or lower. Supernatants that appear very dark or viscous are still acceptable for use.

ii) A 96-well ELISA microtitre plate is coated with optimal concentration (typically 5 µg per ml) of a protein A purified IgG fraction of rabbit anti-*E. granulosus* proglottid extract (Allan et al., 1992) in 0.05 M bicarbonate/carbonate buffer, pH 9.6 (100 µl per well). The plate is covered and incubated overnight at 4°C.

iii) The wells are rinsed three times in PBST with 1 minute between washes; 100 µl of the same buffer is added to each well, and the plate is incubated for 1 hour at room temperature.

iv) The PBST is discarded and 50 µl of neat fetal calf serum is added to all wells. This is followed by the addition of 50 µl per well of faecal sample supernatants is added (in duplicate wells). The plate is incubated at room temperature for 1 hour with plastic film to seal the plate.

v) The wells are rinsed as in step iii, but the contents are discarded into a 10% bleach (hypochlorite) solution.

vi) An optimal dilution concentration of around 1 µg/ml of an IgG rabbit anti-*E. granulosus* proglottid extract peroxidase conjugate (Allan et al., 1992) in PBST is prepared and 100 µl per well is added to all wells. The plate is incubated for 1 hour at room temperature (22–24°C).

vii) The wells are rinsed as in step iii.

viii) Next, 100 µl per well of tetramethyl benzidine (TMB) substrate is added and the plate is left in the dark for 20 minutes at room temperature (22–24°C).

ix) Absorbance of wells is read at 650 nm. The enzyme-substrate reaction can be stopped by adding 100 µl of 1 M phosphoric acid (H₃PO₄) to each well. The colour turns from blue to yellow if positive and is read at 450 nm.

x) Laboratories should establish their own end-point criteria using standard positive and negative samples. Standards can also be obtained from the OIE Reference Laboratories (see Table given in Part 4 of this Terrestrial Manual). Usually, the positive to negative threshold is taken as 3 standard deviations above the mean absorbance value of control negatives, or against a reference standard control positive using absorbance units equivalence.

2.1.2. Coproantigen test procedure (*Echinococcus multilocularis*) (Morishima et al., 1999; Nonaka et al., 1998)

Sandwich ELISA using a monoclonal antibody EmA9 raised against adult *E. multilocularis* somatic antigen.

i) 0.5 g of each faecal sample is placed in a centrifuge tube and a 1% formalin solution containing 0.3% Tween 20 is added to a total volume of 15 ml.

ii) After adequate mixing, the faecal solution is centrifuged at 1200 g for 10 minutes at room temperature. A supernatant fraction is used for the coproantigen detection assay.

iii) Flat-bottomed microtitre plates are coated with 50 µl/well of 1 µg/ml rabbit IgG directed against adult *E. multilocularis* excretory/secretory (ES) products in 0.05 M NaHCO₃/Na₂CO₃ buffer (pH 9.6) and are left overnight at 4°C.

iv) The plates are washed three times with 250 µl/well PBS (pH 7.4) containing 0.05% Tween 20 (PBST), and blocked using 100 µl/well 1% bovine serum albumin (BSA) in PBS for 1 hour at room temperature (22–24°C).

v) The plates are washed three times (with the wash disinfected with 10% bleach) and 50 µl of faecal supernatant is added to each well and the plates are incubated for 2 hours.
vi) The plates are again washed four times and 0.5 µg/ml of the biotinylated monoclonal antibody in 0.5% BSA/0.5% casein in PBST is added to each well and the plates are incubated for 1 hour.

vii) The plates are washed four times and streptavidin-biotinylated horseradish peroxidase complex, diluted 1/1000 in 0.5% BSA/0.5% casein in PBST is added to each well and the plates are incubated for 1 hour.

viii) The plates are washed five times and 100 µl/well of substrate solution (1 mg of tetramethyl benzidine in 10 ml of 0.05 M phosphate citrate buffer, pH5.0 with 2 µl of H_2O_2) is added.

ix) The plates are shaken immediately and placed in a 37°C incubator for 30 minutes. The reaction is stopped by adding 50 µl/well of 4 N H_2SO_4. The optical densities (OD) of the plates are read at 450 nm.

x) The cut-off value is calculated as the mean OD value plus 3 standard deviations of samples from uninfected animals.

2.2. Copro-DNA methods

2.2.1. Definitive hosts

Copro-DNA has proven to be of value for the diagnosis of Echinococcosis in animal definitive hosts and in the identification of individual parasite species. DNA isolation from the faeces, however, is laborious.

PCR is a technically demanding and expensive technique. It is currently used mainly for confirmatory testing of coproantigen-positive samples or for identification of taenid eggs recovered from faeces. Table 3 presents the different PCR primers used for identification of copro-DNA from faeces in definitive hosts of genus *Echinococcus.*

**Table 3.** PCR primers used for copro-DNA detection (modified from Mathis & Deplazes, 2006)

<table>
<thead>
<tr>
<th>Primer designation: primer sequences (5’–3’)</th>
<th>Ref.</th>
<th>Target, comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. multilocularis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTG-AGG-CGA-TGT-GTG-ATG-GAG-AGG</td>
<td>Bretagne et al., 1993</td>
<td>U1 sRNA gene: may yield non-specific products when used with metacestode material containing host DNA (unpublished observation)</td>
</tr>
<tr>
<td>CAA-GTG-GTC-AGG-GGC-AGT-AG</td>
<td></td>
<td>Mitochondrial 12S RNA gene; used in two-tube nested PCR</td>
</tr>
<tr>
<td>Outer primers:</td>
<td>Dinkel et al., 1998</td>
<td>Mitochondrial 12S RNA gene; modified from Dinkel et al., 1998 for use in one-tube nested PCR</td>
</tr>
<tr>
<td>(P60 forward)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTA-AGA-TAT-AGG-TAC-AGG-ATT-AGA-TAC-CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P375 reverse)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC-CGA-GGG-GTG-GCC-GCG-GTG-TGT-ACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner primers:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Pnest forward)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACA-ATA-CCA-TAT-TAC-AAC-ATT-CCT-ATC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Pnest reverse)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATA-TTT-TGT-AAG-GTT-GT-TCT-CTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer primers:</td>
<td>Van der Giessen et al., 1999</td>
<td>Mitochondrial 12S RNA gene; modified from Dinkel et al., 1998 for use in single PCR</td>
</tr>
<tr>
<td>(Em-1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAA-GAT-ATA-TGT-GGT-ACA-GGA-TTA-GAT-ACC-C</td>
<td></td>
<td></td>
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<tr>
<td>(Em-2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT-GAC-GGG-GTG-TGT-TGT-A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner primers:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Em-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATA-TTA-CAA-CAA-TAT-TCC-TAT-C</td>
<td></td>
<td></td>
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<tr>
<td>(Em-4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATA-TTT-TGT-AAG-GTT-GTT-CTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primer designation: primer sequences (5’–3’)</td>
<td>Ref.</td>
<td>Target, comments</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>------</td>
<td>-----------------</td>
</tr>
<tr>
<td>(EM-H15) CCA-TAT-TAC-AAC-AAT-ATT-CCT-ATC (EM-H17) GTG-AGT-GAT-TCT-TGT-TAG-GGG-AAG</td>
<td>Stieger et al., 2002</td>
<td>NADH dehydrogenase subunit 1 (ND1) of mtDNA; cleavage with enzyme Cfo1 distinguish <em>E. multilocularis</em> from <em>E. granulosus</em></td>
</tr>
<tr>
<td>*(E. multilocularis and <em>E. granulosus)</em> ND1 (NDfor2-) AGT-TTC-GTA-AGG-GTC-CTA-ATA (NDrev2-) CCC-ACT-AAC-TAA-CTC-CCT-TTC</td>
<td>Moks et al., 2005</td>
<td>Repeated sequences from <em>E. granulosus</em>, ’sheep strain’; yields banding pattern upon electrophoresis Mitochondrial 12SRNA gene; specific for <em>E. granulosus</em> ’sheep strain’ Amplyfy a fragment of the <em>cox1</em> genespecifc to <em>E. granulosus</em></td>
</tr>
<tr>
<td><em>(E. granulosus)</em> (Eg1121a) GAA-TGC-AAG-CAG-ATG (Eg1122a) GAG-AGT-GAG-AAG-GAG-TG</td>
<td>Abbasi et al., 2003</td>
<td></td>
</tr>
<tr>
<td><em>(Eg1f)</em> CATTAATGTATTTTGAAGTTG <em>(Eg1r)</em> CAC-ATC-ATC-TTA-CAA-TAA-CAC-C</td>
<td>Stefanic et al., 2004</td>
<td></td>
</tr>
<tr>
<td><em>(EgO/DNA-IM1)</em> forward TGA-TAT-TTG-TTT-GAG-KAT-YAG-TKC reverse GTA-AAT-AAM-ACT-ATA-AAA-GAA-AYM-AC</td>
<td>Naidich et al., 2006</td>
<td></td>
</tr>
<tr>
<td><em>(Eg1f)</em> CAT-TAA-TGT-ATT-TTG-TAA-AGT-TG (Eg1r) CAC-ATC-ATC-TTA-CAA-TAA-CAC-C</td>
<td>Trachsel et al., 2007</td>
<td><em>Echinococcus granulosus</em> (sheep strain)</td>
</tr>
<tr>
<td><em>(Cest1)</em> TGC-TGA-TTT-GTT-AAA-GTT-AGT-GAT-C (Cest2) CAT-AAA-TCA-ATG-GAA-ACA-ACA-ACA-AG</td>
<td>Trachsel et al., 2007</td>
<td><em>E. multilocularis</em></td>
</tr>
<tr>
<td><em>(Cest4)</em> GTT-TTT-GTG-TGT-TAC-ATG-AAG-GGT-G</td>
<td>Trachsel et al., 2007</td>
<td><em>E. granulosus</em></td>
</tr>
</tbody>
</table>
Differential diagnosis of *E. granulosus* and *E. multilocularis* infections in definitive hosts may be achieved by specific detection of PCR-amplified DNA from eggs present in faeces. In practice, it is recommended to screen definitive hosts (e.g. foxes) using the coproantigen test and confirm with the PCR DNA test. In Europe, transmission of *E. multilocularis* generally occurs in regions where *E. granulosus* is not endemic or appears very infrequently. In other regions, including parts of the Near East (Turkey and Iran), North Africa (Tunisia and Morocco), Central Asia, Russia and China (People’s Rep. of); these two species may occur together (Craig et al., 1996). Further evaluation of *E. multilocularis* infection is required to investigate intermittent shedding and duration of shedding of parasite DNA.

As PCR is generally used as a confirmatory test, it is suggested to concentrate the taeniid eggs by sequential sieving and an in-between concentration method step. DNA isolation from these eggs can be achieved using a simplified protocol of the alkaline lysis method combined with a commercial kit with no need for organosolvent extractions (Lightowlers & Gottstein, 1995). The sensitivity of egg isolation followed by PCR is 78% for *E. granulosus* and 50% for *E. multilocularis* infection in dogs (Ziadino et al., 2008).

### 2.2.2. Intermediate hosts

Molecular methods are important in identification of *Echinococcus* spp. in samples collected from livestock at abattoirs for epidemiological purposes (Roelfsema et al., 2016). For the identification of small, degenerated or calcified lesions of *E. multilocularis* in intermediate or aberrant hosts, PCR is of great value (Conraths & Deplazes, 2015).

### 3. Serological tests

#### 3.1. Intermediate hosts

Serological diagnosis of ovine echinococcosis has long been considered a potentially important tool for epidemiological studies in endemic areas, as well as for surveillance of hydatid control programmes. It has been known for many years that sheep infected experimentally with *E. granulosus* can mount detectable specific IgG responses within weeks. However serum antibody levels varied greatly in natural infections resulting in reduced sensitivity and cross-reactions with *Taenia hydatigena* or *T. ovis* infected animals. At present this approach cannot replace necropsy (Craig et al., 2015; McManus, 2014).

#### 3.2. Definitive hosts

Serodiagnostic tests for canine echinococcosis were considered to have good potential for practical testing of dogs for *E. granulosus* infection and, initially, as a potential substitute for arecoline purgation. Diagnostic specificity was good (>90%) but sensitivity was generally poor (35–40%) with natural infections, and was much lower when compared directly with coproantigen detection. Further research to assess existing or develop better recombinant antigens may improve the sensitivity of serological tests for canine echinococcosis.

### C. REQUIREMENTS FOR VACCINES

#### 1. Intermediate hosts

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**Primer designation: primer sequences**

<table>
<thead>
<tr>
<th>Primer designation</th>
<th>Ref.</th>
<th>Target, comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cest5) GCG-GTG-TGT-ACM-TGA-GCT-AAA-C</td>
<td>Trachsel et al., 2007</td>
<td><em>Taenia</em> spp.</td>
</tr>
<tr>
<td>(Cest5) YGA-YTC-TTT-TTA-GGG-GAA-GGT-GTG</td>
<td>Trachsel et al., 2007</td>
<td><em>Taenia</em> spp. (sequencing primer for the 267 bp amplicon of the multiplex PCR)</td>
</tr>
<tr>
<td>(Cest5) GCG-GTG-TGT-ACM-TGA-GCT-AAA-C</td>
<td>Trachsel et al., 2007</td>
<td><em>Taenia</em> spp.</td>
</tr>
<tr>
<td>(Cest5seq) GAT-TCT-TTT-TAG-GGG-AAG-G</td>
<td>Trachsel et al., 2007</td>
<td><em>Taenia</em> spp. (sequencing primer for the 267 bp amplicon of the multiplex PCR)</td>
</tr>
</tbody>
</table>
Application of an effective vaccine to reduce hydatid infection in livestock is likely to have a substantial impact on the rate of transmission of the disease to humans (Lightowlers, 2006). As E. granulosus belongs to the Taenid family, many aspects of its immunological relationship with its intermediate host are similar to that occurring in Taenia species. Moreover, it was considered that the vaccine development approach used in Taenia species, such as the native host-protective antigens of T. ovis, would also be successful for E. granulosus. A recombinant antigen vaccine, EG95, was therefore developed in 1996 using an E. granulosus oncosphere protein expressed in Escherichia coli. The vaccine has been shown to produce high levels of protection (96–100%) against an experimental challenge infection with E. granulosus in sheep. Thereafter, the vaccine has been successfully applied in experimental trials in different countries in sheep and in other intermediate hosts. The EG95 vaccine has been licensed in some countries (Lightowlers, 2006).

2. Definitive hosts

Development of E. granulosus vaccines for dogs would ideally reduce worm fecundity and populations, and would be a crucial step towards the reduction (prevention) of the infection pressure on intermediate hosts, and thus reduce (prevent) infection in dogs. However no clear candidate molecules have yet been identified.

REFERENCES


McMANUS D.P. (2014). Immunodiagnosis of sheep infections with Echinococcus granulosus: in 35 years where have we come? Parasite Immunology, 36, 125–130.


* * *

**NB:** There are OIE Reference Laboratories for Echinococcosis (see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/). Please contact the OIE Reference Laboratories for any further information on diagnostic tests, reagents and vaccines for Echinococcosis/Hydatidosis

**NB:** First adopted in 1989 as *Echinococcosis/Hydatidosis*. Most recent updates adopted in 2017