

CYSTICERCOSIS*

SUMMARY

Cysticercosis of farmed and wild animals is caused by the larval stages (metacestodes) of cestodes (tapeworms), the adult stages of which occur in the intestine of humans and dogs or wild Canidae. Bovine cysticercosis (primarily in muscle) and porcine cysticercosis (primarily in muscle, the central nervous system and the liver) are caused by the metacestodes (cysticerci) of the human cestodes Taenia saginata and T. solium, respectively. Cysticerci of T. solium also develop in the central nervous system and musculature of humans. Cysticerci of T. asiatica occur in the liver and viscera of pigs. Cysticercosis and coenurosis of sheep and goats (in the muscles, brain, liver and peritoneal cavity) are caused by T. ovis, T. multiceps and T. hydatigena, adults of which occur in the intestines of dogs and wild canids.

Most adult and larval tapeworm infections cause little or no disease. Exceptions are severe, potentially fatal human neurocysticercosis (NCC) caused by T. solium, and occasionally neuro-coenurosis caused by T. multiceps in humans. These parasites are also occasional causes of muscle or ocular signs in humans. 'Gid' caused by T. multiceps in ruminants can require slaughter of the animal. Acute T. multiceps coenurosis and T. hydatigena cysticercosis in sheep and goats is rare but may be fatal. Cysticercosis causes economic loss through condemnation of infected meat and offal.

Identification of the agent: *Adult Taenia tapeworms are dorsoventrally flattened, segmented and large, reaching from 20 to 50 cm (species in dogs) to several metres (species in humans). Anteriorly, the scolex (head) has four muscular suckers and may have a rostellum, often armed with two rows of hooks, the length and number of these being relatively characteristic of a species. A neck follows the scolex, and this is followed by immature and then by mature reproductive segments, and finally gravid segments filled with eggs. Segment structure, although unreliable, can aid in the identification of the species. Taenia species cannot be differentiated by egg structure. Metacestodes consist of a fluid-filled bladder with one or more invaginated protoscoleces. These 'bladderworms' are each contained within a cyst wall at the parasite–host interface. This structure comprises the cysticercus or coenurus.*

Adult Taenia are recognised at post mortem or by passage of segments or eggs. Metacestodes are grossly visible at post mortem and meat inspection, but light infections are often missed. NCC can be diagnosed by imaging techniques.

Immunological tests: *Adult Taenia infections can be recognised by detection of Taenia coproantigen in faeces using an antigen-capture enzyme-linked immunosorbent assay, but the test does not differentiate species and is not commercially available. Use of species-specific probes remains experimental.*

Serological tests: *Tests for antibodies in serum are not used currently for the diagnosis of cysticercosis in animals except for epidemiological purposes. Diagnosis is by meat inspection. Antigens have been identified for the serological diagnosis of NCC in humans.*

Requirements for vaccines and diagnostic biologicals: *Vaccine antigens have been identified for the metacestodes, but not for the adult stages of T. ovis, T. multiceps, T. saginata and T. solium. A T. ovis vaccine is registered in New Zealand, but is not commercially available. A T. solium vaccine is undergoing the steps for practical production.*

A. INTRODUCTION

The metacestodes (or larval cestodes) of *Taenia* spp. tapeworms are the cause of cysticercosis in various farmed and wild animals and in humans. Adult tapeworms are found in the small intestine of carnivore definitive hosts – humans, dogs, and wild canids. *Taenia saginata* of humans causes bovine cysticercosis, which occurs virtually world-wide, but particularly in Africa, Latin America, Caucasian and South/Central Asian and eastern Mediterranean countries and the infection occurs in many countries in Europe. *Taenia solium* of humans causes porcine cysticercosis and human neurocysticercosis (NCC). It is found principally in Mexico, Central and South America, sub-Saharan Africa, non-Islamic countries of Asia, including India and China where there are free-ranging, scavenging pigs. The cysticerci of *T. asiatica* of humans in South-East Asia occur in the liver of pigs. Dogs and wild canids are the definitive hosts of metacestodes of sheep, goats and other ruminants, which occur throughout most of the world, although *T. multiceps* has disappeared from the United States of America (USA) and New Zealand. *Taenia ovis* occurs in the muscles of sheep, *T. multiceps* in the brain (occasionally in the muscles) of sheep, goats, sometimes other ruminants and rarely humans, and *T. hydatigena* is found in the peritoneal cavity and on the liver of ruminants and pigs. Diagnosis in animals is usually based on the host and the location of the metacestode when identified at meat inspection or necropsy. Adults in definitive hosts are acquired by the ingestion of viable metacestodes in meat and offal that has not been adequately cooked or frozen to kill the parasite.

Gravid segments that are shed by the adult tapeworms migrate spontaneously from the anus to fall to the ground and release eggs on the ground, or the segments and free eggs are passed in the faeces. Eggs may also be disseminated from these sites by physical means or transport hosts, particularly flies. *Taenia solium* segments, however, are often passed in chains. Eggs are immediately infective when passed. Animals acquire infection from ingestion of segments and eggs contaminating herbage or in faeces. It is possible that pigs acquire *T. solium* by coprophagy of the faeces of pigs that have eaten segments. Humans may be infected with *T. solium* by eggs on vegetables, etc., that have been contaminated by faeces or dirty hands, by faeco-oral transmission or through retro-peristalsis and hatching of eggs internally. Routine diagnosis continues to be mainly based on the morphology of the adult tapeworm and the presence of eggs or segments in the faeces of infected definitive hosts.

B. DIAGNOSTIC TECHNIQUES

1. Identification of the agent

Taenia saginata (the beef tapeworm): The adult is large, 4–8 metres long and can survive many years, usually singly, in the small intestine of humans. The scolex (or head) has no rostellum or hooks. Useful diagnostic features are presented in Table 1 (16, 17, 24, 32, 34). Gravid segments usually leave the host singly and often migrate spontaneously from the anus.

The eggs are typical 'taeniid' eggs that cannot be differentiated from other *Taenia* or *Echinococcus* spp. eggs. *Taeniid* eggs measure about 30–45 µm in diameter; contain an oncosphere (or hexacanth embryo) bearing three pairs of hooks; have a thick, brown, radially striated embryophore or 'shell' composed of blocks; and there is an outer, oval, membranous coat, the true egg shell, that is lost from faecal eggs.

Metacestodes (*Cysticercus bovis*) of *T. saginata* usually occur in the striated muscles of cattle (beef measles), but also buffalo, reindeer and deer. They are oval, about 0.5–1 × 0.5 cm long, translucent and contain a single white scolex that is morphologically similar to the scolex of the future adult tapeworm. They are contained in a thin, host-produced fibrous capsule. Cysts occasionally are found in the liver, lung, kidney, fat and elsewhere.

Taenia solium (the pork tapeworm) is smaller than *T. saginata* being up to 3–5 metres. The scolex has an armed rostellum bearing two rows of hooks; the number and size of hooks can aid differentiation of *Taenia* spp. (Table 1). Gravid segments have 7–16 (<17) uterine branches and do not usually leave the host spontaneously, but passively in chains with the faeces.

Metacestodes (*C. cellulosae*) occur in the muscles and central nervous system of pigs (pork measles), bear and dogs and in the muscles, subcutaneous tissues and central nervous system of humans. Cysts are grossly similar to those of *T. saginata*, but may be larger than the *T. saginata* cyst. They have a scolex bearing a rostellum and hooks similar to the adult. Occasionally, in the brain of humans, they develop as racemose cysts up to 2 cm or more across that lack a scolex.

Table 1. Useful features for identification of scoleces and segments of *Taenia* spp.

Parasite species	Number of hooks	Length of hooks (µm)		Number of testes	Layers of testes	Cirrus sac extends to longitudinal vessels	Number of uterine branches	
		Large hooks	Small hooks					
<i>T. hydatigena</i>	28–36 (26–44)	191–218 (170–235)	118–143 (110–168)	600–700	1	Yes	6–10 that re-divide	Lobes of ovary unequal in size. No vaginal sphincter. Testes extend to vitellarium, but not confluent behind.
<i>T. ovis</i>	30–34 (24–38)	170–191 (131–202)	111–127 (89–157)	350–750	1	No	11–20 that re-divide	Lobes of ovary unequal in size. Well developed vaginal sphincter. Testes extend to posterior edge of ovary.
<i>T. multiceps</i>	22–30 (20–34)	157–177 (120–190)	98–136 (73–160)	284–388	2	Yes	14–20 that re-divide	Lobes of ovary equal in size. Pad of muscle on anterior wall of vagina. Testes extend to vitellarium, but not confluent behind.
<i>T. saginata</i>	– without rostellum	–	–	765–1200	1	No	14–32 that re-divide Ratio of uterine twigs to branches 2.3	Lobes of ovary unequal in size with small Well developed vaginal sphincter. Testes extend to vitellarium, but not confluent behind.
<i>T. solium</i>	22–36	139–200	93–159	375–575	1	Yes	7–16 that re-divide	Lobes of ovary unequal in size with small accessory lobe. No vaginal sphincter. Testes confluent behind vitellarium
<i>T. asiatica</i>	– with rostellum on some	–	–	868–904		No	16–32 that re-divide Ratio of uterine twigs to branches 4.4	Ovary, vaginal sphincter and extent of testes as <i>T. saginata</i> . Posterior protuberances on some gravid segments

Taenia asiatica (Asian *Taenia*): Closely related to but genetically distinguishable from *T. saginata* (8), the adult in humans has an ovary, vaginal sphincter muscle and cirrus sac like those of *T. saginata*, but *T. asiatica* has a rostellum and posterior protuberances on segments and 16–32 uterine buds with 57–99 uterine twigs on one side. Segments are passed singly and often spontaneously.

The metacestodes (*C. viscerotropica*) are small, about 2 mm, and have a rostellum and two rows of primitive hooks, those of the outer row being numerous and tiny. They occur mainly in the parenchyma and on the surface of the liver of domesticated and wild pigs; they may be found on the omentum and, rarely, on the lungs and colonic serosa. Occasionally they are found in cattle, goats, and monkeys.

Taenia ovis: Adults in the intestine of dogs and wild canines reach 1–2 metres in length and have an armed rostellum (Table 1). Metacestodes (*C. ovis*) that occur in the musculature (skeletal and cardiac) of sheep and less commonly goats reach 3.5–1.0 x 0.2–0.4 cm. Commonly, the cysticerci are degenerate with a green or cream, caseous or calcified centre. A similar parasite occurs in wild canines and dogs and the muscles of reindeer and deer in northern areas.

Taenia hydatigena: Adults are 1–5 metres long, are found in the intestine of dogs and wild canines, and have an armed rostellum (Table 1). Metacestodes (*C. tenuicollis*) are large, from 1 cm up to 6–7 cm, and the scolex has a long neck. They are found attached to the omentum, mesentery and occasionally on the liver surface, particularly of sheep, but also of other domesticated and wild ruminants and pigs. A wolf and reindeer/deer cycle exists in northern latitudes, in which the metacestodes are found in the liver of the intermediate host; dogs are also infected as definitive hosts.

Taenia multiceps: Adults are 40–100 cm long in the intestine of canines and have an armed rostellum (Table 1). The metacestodes (*Coenurus cerebralis*) are large, white fluid-filled cysts that may have up to several hundred scoleces invaginated on the wall in clusters. Coenuri grow to 5 cm or so in size in the brain of sheep, the brain and intermuscular tissues of goats, and also the brain of cattle, wild ruminants and occasionally humans. In neural tissue the cysts are not encapsulated. The cysts induce neurological signs that in sheep are called 'gid', 'sturdy', etc.

a) Diagnosis of adult parasites in humans or canine carnivores

All parasite or faecal material from humans with possible *T. solium* infections must be handled with suitable safety precautions to prevent accidental infection with the eggs. *Taenia multiceps* and *Echinococcus* spp. also infect humans and, as taeniid eggs in dogs cannot be differentiated to species or genus level, in areas where these are endemic, the same safety precautions apply. In addition to *Taenia* spp., humans and canine carnivores may be infected by *Diphyllobothrium* and *Hymenolepis* spp., while six other cestode genera are recorded occasionally in humans. These are described by Lloyd (22) and all can be differentiated from *Taenia* spp. by egg/proglottid morphology. Recently however, *T. taeniaeformis* with morphologically indistinguishable taeniid eggs was recorded in a child. In canids, *Echinococcus* spp. eggs cannot be distinguished from *Taenia* spp. eggs, but the presence of the former can be determined by tapeworm size and, more recently, *Echinococcus* species-specific antigen-capture enzyme-linked immunosorbent assay (AG-ELISA) (2). Other worms, *Dipylidium*, *Diplopylidium*, *Mesocestoides* and *Diphyllobothrium* spp. have morphologically distinct eggs and proglottids (22, 32).

Adult cestodes can be expelled from humans using an anthelmintic followed by a saline purgative and are identified on the basis of scolex and proglottid morphology. A self-detection tool has been developed and tested in Mexico (10); medical staff in health centres are supplied with preserved tapeworm segments in a bottle and a manual of questions to ask patients to try to identify carriers. In animals, arecoline purgation has been useful; again, the recovered tapeworms are identified morphologically. Arecoline is no longer available as an anthelmintic, but can be obtained from chemical supply companies. As it has side-effects, old, infirm and pregnant animals should be excluded from treatment. A dose of 4 mg/kg should result in purgation in under 30 minutes, provided food has been withheld for several hours (i.e. administer to dogs with empty stomachs). Walking and abdominal massage of recalcitrant cases or enema for constipated dogs may avoid the use of a second dose (2 mg/kg), which should be given only sparingly. Fortunately, arecoline purgation is being replaced rapidly by the copro-antigen ELISA for *Echinococcus* spp. and perhaps in the future this will also be the case for *Taenia* spp. Tapeworms can be recovered after anthelmintic treatment, and require appropriate disposal.

Verster (34) and Loos-Frank (24) have given descriptions of parasitic diagnosis of all the *Taenia* spp. of humans and animals, their hosts and geographical distributions. Keys for identification are given by Khalil *et al.* (16). Mayta *et al.* (25) and Loos-Frank (24) give methods for mounting, embedding, sectioning and staining the proglottids. The following staining technique is that of Loos-Frank (24). Worms, after relaxation in water, can be stained directly, although small worms should be fixed in ethanol for a few minutes. Alternatively, worms can be fixed and stored in 70% ethanol containing 10% lactic acid, the scolex and worm being stored separately. The rostellar hooks of scoleces or protoscoleces should be cut off and mounted *en face* in Berlese's fluid (made by dissolving 15 g gum arabic in 20 ml distilled water and adding 10 ml glucose syrup and 5 ml acetic acid, the whole then being saturated with chloral hydrate, up to 100 g). The stain is lactic acid carmine: 0.3 g carmine is dissolved at boiling point in 42 ml lactic acid and 58 ml distilled water, 5 ml of 5% iron chloride solution ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) is added after cooling and can be used again to refresh older solutions. Specimens are allowed to sink in the stain in a vial and then are left in the stain for some more minutes to allow the stain to penetrate. Specimens are then washed in 1-day-old tap water until blue in colour. They are then fixed in 50–70% ethanol and dehydrated under the slight pressure of plastic foil keeping the segments flat. Salicylic acid methyl ester is used as clearant.

When segments break from the end of the worm, some eggs are expelled in the intestine and can be found in the faeces. Spontaneous migration of *T. saginata* or *T. asiatica* from the anus is likely to be noticed by the patient (>95%). When the segments migrate, the sticky eggs are deposited in the perianal area and might be detected by application and examination of sticky tape. These signs are far less likely for chains of *T. solium* (3). Segments of all three may be found on the faeces, but are passed intermittently. In dogs, approximately 50% of the segments migrate spontaneously from the anus. These segments, when they fall to the ground, will migrate, shedding eggs. The remainder of the segments are passed in the faeces, but commonly, the segments migrate and void the majority of their eggs in trails on the surface of the faeces and

surrounding area. Even if a migrating segment sheds all its eggs, it can be identified as a cestode by the many concentric calcareous corpuscles contained within its tissues. Faeces, after mixing to reduce aggregation, can be examined for eggs. Various techniques are used throughout the world and include ethyl acetate extraction and flotation. For the latter, NaNO₃ or Sheather's sugar solution (500 g sugar, 6.6 ml phenol, 360 ml water), with their higher specific gravities, are superior to saturated NaCl as flotation media for taeniid eggs. Flotation can be carried out in commercially marketed qualitative or quantitative flotation chambers or by centrifugal flotation that includes a modified Wisconsin technique (faeces, diluted in water, are sieved and centrifuged, the pellet is resuspended in sugar or Sheather's solution and centrifuged at 300 **g** for 4 minutes). Eggs adhering to the cover-slip can then be detected. Faecal egg examination will be less sensitive for *T. solium* than the other species. Species cannot be determined by egg morphology, but DNA probes, the polymerase chain reaction (PCR) and PCR restriction fragment length polymorphism (RFLP), have proved useful for differentiation in the laboratory. These have been largely used experimentally to differentiate faecal eggs of *T. solium*, *T. saginata* and *T. asiatica* (11, 12, 13). While equally applicable to differentiation in dogs, the same examinations have not been done.

An AG-ELISA to detect *Taenia* coproantigen in faeces is no longer available commercially, but can be developed if laboratory facilities are available (2, 3). Information on availability for epidemiological studies or collaborative use can be obtained from Professor P.S. Craig, OIE Reference Expert on Echinococcosis (see Table given in Part 3 of this *Terrestrial Manual*). This AG-ELISA was developed experimentally by Allan *et al.* (2) to detect coproantigen in dogs, and so, with appropriate controls, could be used to detect *Taenia* infection in this species. The technique, however, is only *Taenia* species specific. The test is a solid-phase, microwell assay with wells coated with polyclonal, rabbit anti-*Taenia*-specific antibody (TSA). The following is the basic technique:

- i) Faecal supernatants are recovered from fresh, frozen or formalinised (5% formalin at 4°C) faecal samples. The sample is vigorously shaken forming a slurry in an equal weight/volume of 0.15 M phosphate buffered saline (PBS) containing 0.3% Tween 30. The supernatant is recovered by centrifugation at 2000 **g** for 30 minutes.
- ii) A soluble aqueous extract of non-gravid proglottids from *Taenia* are obtained following emulsification in PBS and centrifugation.
- iii) Hyperimmune rabbit antiserum is produced against the soluble proglottid extract and the IgG fraction is isolated by passage into and elution of the bound IgG from Protein A-Sepharose CL 4B (Pharmacia). Some of the IgG fraction is conjugated to peroxidase type VI (Sigma). The sera are stored in small quantities frozen at -20°C. Sera may need to be absorbed by packed normal dog faeces in a ratio 2/1 with mixing for 1 hour and recovered by centrifugation.
- iv) Flat-bottomed microtitre plates (Dynatech) are coated with rabbit anti-*Taenia* IgG (protein content 5–25 µg/ml determined by UV spectrophotometry) using 100 µl/well, the antisera are diluted in 0.05 M NaHCO₃/Na₂CO₃ buffer, pH 9.6. Plates are incubated overnight at 4°C, the wells are washed three times with PBS/0.1% Tween, blocked with PBS/0.3% Tween for 1 hour and washed again. 100 µl of faecal supernatant containing 50% fetal calf serum is added and the plates are incubated for 1 hour and then washed three times. 100 µl of the peroxidase-conjugated anti-*Taenia* IgG (diluted 1/100 or 1/200) is added and the plates are incubated for 1 hour before washing three times. Substrate solution (100 µl of 5-amino-salicylic acid [Sigma] and 0.005% H₂O₂ in 0.1 M phosphate buffer containing 1 mM Na₂EDTA [ethylene diamine tetra-acetic acid] at pH 6.0) is added for 25 minutes and the result is read at 450 nm. Cut-off values are the mean plus 3 standard deviations of values for normal dog faeces.

b) Diagnosis of metacestodes

In live animals, *T. solium* or *T. saginata* metacestodes might be palpable in the tongue but, both in the living animal and on post-mortem examination or meat inspection, tongue palpation is of diagnostic value only in pigs or cattle heavily infected with metacestodes; these will also be difficult to differentiate from large sarcocysts.

- **Meat inspection – the main diagnostic procedure**

Metacestodes are visible first as very small, about 1 mm, cysts, but detection of these requires thin slicing of tissues in the laboratory. Many young cysts are surrounded by a layer or capsule of inflammatory cells (mononuclear cells and eosinophils being prominent histologically). Cysts may later degenerate, but the parasites' abilities to evade the immune response mean that later in infection, as the cyst matures, few inflammatory cells are present in its vicinity and the cysticercus in its intermuscular location is surrounded by a delicate fibrous tissue capsule.

In theory, cysts can be visualised or felt in tissues such as the tongue of heavily infected animals as early as 2 weeks after infection. Cysts are readily visible by 6 weeks and, when mature, are usually oval, about 10 ×

5 mm or larger, with a delicate, fairly translucent, white parasite membrane and host capsule; pale fluid within the cyst and the scolex, visible as a white dot within the cyst, usually invaginates midway along the long axis of the cyst.

At meat inspection many of the cysts detected, often as many as 85–100%, are dead. The rate at which cysts age and die and so degenerate varies with the parasite species and also with the tissue within which the cyst is embedded. Death usually occurs within 9 months of infection of adult cattle with *T. saginata*. However, cysts may remain viable for several years. Cysts of *T. hydatigena* in the peritoneal cavity of sheep and those of *T. solium* also have been described as surviving in sheep and pigs for long periods. *Taenia solium* cysts survive for many years in the brain of humans, and frequently symptoms begin only as the cyst begins to degenerate. In general, cysts tend to die more rapidly in the muscular predilection sites, such as heart. The preferential distribution of parasites in these areas may be because of greater blood circulation to these muscles. Conversely, the higher rate of activity in these muscles may damage the parasites, allowing leakage of fluid and perhaps disrupting the parasite's ability to evade the immune response. Cysts at different stages of viability and degeneration can be found in the same host.

Degenerating cysts vary in appearance. The host's fibrous tissue capsule thickens and becomes opaque, but initially the cyst within remains apparently normal. The fluid gradually becomes colloid and inflammatory cells infiltrate. The cyst cavity becomes filled with greenish (eosinophilic) and then yellow, caseous material and is very unaesthetic, usually being larger in size and certainly more obvious in meat than the original viable cyst. Later the cyst may calcify. While PCR assays have been used largely for the differentiation of adult taeniids in humans, they could be usefully applied to unambiguously identify a metacestode; in a recent study though, PCR identified only 50% of degenerate presumed *T. saginata* cysts (1). Where very young (without a scolex) or degenerate cysts need to be differentiated from other lesions, compression of the cyst, smears of the caseous contents and histological examination of haematoxylin and eosin (H&E) stained sections are used. Microscopic examination may reveal the calcareous corpuscles (concentric concretions of salts that are around 5–10 µm in size). These indicate a cestode origin of the tissue and differentiate, for example, an immature or degenerate cyst from a cyst of another aetiology. The presence of hooks and their length together with knowledge of the host and tissue may aid in identification of cestode species. Experimentally, immunohistochemical staining has differentiated *T. saginata* cysts from non-*Taenia* structures (27). PCR would be useful, but is experimental, in the finding of a new cestode in a host species or geographical area from which, historically, the parasite was absent (1).

After treatment of *T. saginata* and *T. solium* in cattle and pigs with drugs such as albendazole and oxfendazole, the cysts may lose their fluid and collapse. The resultant lesion is much smaller than lesions observed following natural death. However, cysts that have died before treatment of the animal will remain large and visible. Treatment of pigs with oxfendazole (30 mg/kg) 3 months before slaughter has been suggested as a control measure.

Meat inspection procedures vary with the parasite and the host involved, i.e. zoonosis or not, the tissue involved, and regulations within a country. Examinations tend to be more extensive with the zoonotic infections *T. saginata* and *T. solium*.

In general, meat inspection procedures consist of:

- i) Visual inspection of the carcass, its cut surfaces and the organs within it. This may reveal *T. saginata*, *T. solium* and *T. ovis* in the muscles, *T. hydatigena* on the liver or mesenteries and omentum, or *T. multiceps* in the brain.
- ii) The external and internal masseters and the pterygoid muscles each must be examined and one or two incisions made into each, the cuts being parallel to the bone and right through the muscle.
- iii) The freed tongue is examined visually and palpated, particularly for *T. solium*
- iv) The pericardium and heart are examined visually. The heart usually is incised once lengthwise through the left ventricle and interventricular septum so exposing the interior and cut surfaces for examination. Incisions may go from the base to the apex and regulations also may require additional, perhaps four, deep incisions into the left ventricle. Alternately, the heart may be examined externally and then internally after cutting through the interventricular septum and eversion.
- v) The muscles of the diaphragm, after removal of the peritoneum, are examined visually and may be incised.
- vi) The oesophagus is examined visually.
- vii) In African countries in particular, the triceps brachii muscle of cattle is incised deeply some 5 cm above the elbow. Additional cuts into it may be made. The gracilis muscle also may be incised parallel to the pubic symphysis. These cuts are usually also undertaken for *T. solium* in pigs. Such incisions into the legs are made in Africa as it is suspected that more parasites lodge in these muscles in working or

range animals walking long distances due to the exercise and consequent increased blood flow to these muscles. Other countries may also require such incisions into the legs. However, as this devalues the meat, such incisions are made most commonly once one or more cysts have been found at the predilection sites so as to determine the extent of the infection.

Overall, the initial incision into any tissue is the most important, but additional incisions may be required by the regulations or are required if cysts are found on the initial incision(s). Details on meat inspection are supplied by Herenda (15) and by Kyvsgaard & Murrell (17).

Additional or fewer procedures may be required for specific parasites and the judgements on the carcass, viscera, offal and blood will vary dependent on *Taenia* species and regulations within a country. Judgement on infected carcasses will fall into three main categories: i) approve for human consumption; ii) partially condemn and pass the remainder of the carcass, but in the case of the zoonoses, *T. saginata* and *T. solium*, the carcass, meat and viscera must be treated; and iii) totally condemn heavily infected carcasses or emaciated diseased ones.

Taenia saginata: Calves under 6 weeks or <32 kg are not usually examined. Predilection sites are the heart, tongue, masseters and diaphragm, presumably because they receive the greatest circulation. Nonetheless, cysts may be found in any muscle of the body. If one carcass in a lot is found to be infected, all carcasses from the same lot can be held until laboratory confirmation is obtained. If *T. saginata* infection is confirmed, additional incisions are usually made in the carcasses in the lot; all suspicious lesions found in the rest of the lot are considered to be *T. saginata* without laboratory confirmation. Lesions of *T. saginata* may need to be differentiated from *Sarcocystis* sarcocysts and other lesions. In recent PCR studies in Germany, Switzerland and New Zealand up to 20% of viable, presumed *T. saginata* cysts could not be positively identified; this suggests there may be an unidentified cestode infecting cattle (1). Conversely, aberrant *T. saginata* oncospheres can lead to cerebral oncospherical lesions and thus need to be taken into account for differential diagnosis of neurological disorders (9).

Judgement: If a carcass is considered to be heavily infected then the carcass, meat, offal and blood all are condemned. The description of a heavy infection varies, but generally it is the detection of cysts at two of the predilection sites plus two sites in the legs. In the case of a lesser infection, the infected parts and surrounding tissues are removed and condemned. Even a single dead cyst requires that the carcass and edible viscera must then be treated and this is justifiable as about 10% of lightly infected carcasses were found on dissection to have both dead and viable parasites within them. Treatment varies with country and facilities available and includes: i) freezing at lower than -10°C for >10 or 14 days, or lower than -7°C for 21 days; ii) boxes of boned meat are frozen at less than -10°C for >20 days; iii) heated to above 60°C throughout; iv) steamed at moderate pressure (0.49 kg/cm²); v) heated at $95-100^{\circ}\text{C}$ for 30 minutes; or vi) pickled in salt solution for 21 days at $8-12^{\circ}\text{C}$. Blast freezing needs examination; generally a 30 kg box is reported to require 2 × 24-hour cycles at -30.9°C followed by 72 hours cold storage at -23.3°C for death of scoleces. Often no treated meat can be exported although in some countries it can be exported in canned form.

Taenia solium: The predilection sites are as for *T. saginata* although there are reports of higher prevalence in shoulder and thigh. Commonly one or more cuts are required 2.5 cm above the elbow joint. This is said to detect some 13% of infected carcasses that would otherwise have been missed.

Judgement: In some countries, any lightly or heavily infected pigs and their viscera and blood are condemned. In areas where infection is common, lightly infected carcasses can be passed for cooking and pickling and occasionally freezing.

Taenia hydatigena: The parasite migrating in the liver leaves haemorrhagic tracks that then become green/brown with inflammation and later white due to fibrosis. For the records, these must be differentiated from those of liver flukes, if possible, by identification of the cysticerci or adult flukes. White spot from *Ascaris* infection is differentiated as the lesions appear as pale to white, small, isolated foci. Some cysts remain trapped below the liver capsule. These usually are small and degenerate early and then calcify into cauliflower-like lesions. *Taenia hydatigena* cysts usually mature in the omental or mesenteric fat. Those that are retained at the liver surface are usually superficial and subserosal, while *Echinococcus granulosus* hydatid cysts tend to be deeper in the parenchyma. If viable, the former has a long-necked single scolex in a virtually translucent fluid-filled cyst. Fertile hydatid cysts have thicker white outer membranous walls from which brood capsules containing numerous protoscoleces. These appear as a sandy deposit with the cysts. Differentiation can be important in the implementation and monitoring of hydatid disease control measures for which histology may be required. H&E-stained sections will reveal the laminated membrane of very young hydatid cysts as indicated by Lloyd *et al.* (23). Its presence or absence can be confirmed by periodic acid-Schiff staining when the highly glycosylated proteins in the laminated membrane stain red. *Taenia hydatigena* lesions in cattle and pigs can be similar to tuberculosis. However, the portal and mesenteric

lymph nodes are not involved, the contents of parasite cysts are more easily shelled-out and remainders of hooks and calcareous corpuscles may be seen or Ziehl–Neelsen staining may reveal bacteria.

Judgement: Usually only a few cysts or tracks are present and these can be trimmed. Heavily infected livers and omentum are condemned. Rarely, acute infections are seen with large numbers of migrating parasites producing traumatic hepatitis, ascites, oedema, etc., and would result in secondary condemnation of the carcass.

Taenia multiceps: The parasites have a predilection site for the brain and spinal cord.

Early migrating parasites can cause reddish haemorrhagic and later grey purulent tracks in the brain, and in heavy infections, the sheep may have a meningoencephalitis. Clinical signs due to the mature cyst relate to pressure atrophy of adjacent nervous tissue and vary according to location in the brain. There may be impaired vision or locomotion if cysts are in the cerebral hemispheres and the sheep gradually may be unable to feed and will become emaciated. Cerebellar cysts may precipitate more acute and severe signs of ataxia or opisthotonus. In heavy infections, parasites migrate and begin development in other tissues, but they die early. These produce small lesions, 1 mm or so in size, that first contain an encapsulated cyst, then eosinophilic, caseous material that later may calcify.

Judgement: Initially only the head is condemned or occasional cysts in intermuscular or subcutaneous sites are trimmed. With chronic infection, the animal may have been unable to feed, resulting in condemnation due to emaciation, etc.

Taenia ovis: The predilection sites are as for *T. saginata*. Cysts may be confused with large *Sarcocystis gigantea* sarcocysts.

Judgement: Commonly detection of up to 2–5 cysts results in trimming and the carcass is passed. This does not prevent the unaesthetic presence of live or degenerate parasites in other tissues. Ultrasound and X-rays are being tested for detection of these. Some authorities may require that the meat be boned, trimmed and frozen or cooked. In heavy infections the carcass is condemned.

In general, meat inspection procedures detect only about 20–50% of the animals that are actually infected. Light infections are easily missed on palpation and meat inspection – in one study, 78% of carcasses infected with >20 cysts were detected compared with those detected following dissection and slicing, while only 31% of those with fewer cysts were detected (35). Meat inspection efficacy will vary with the number and location of incisions (and the skill and experience of the inspector). For example, in Zimbabwe, 58% of cattle were positive in the head only, 20% in the shoulder only and 8% in the heart only, although overall 81% were found to be infected if all three organs were included. Walther & Koske (35) in Kenya also found that the predilection sites were not necessarily infected in 57% of the cattle found positive on dissection. They also confirmed the importance of the shoulder incisions in detection of infection in Africa as 20% of the cattle found to be infected were positive in the shoulder only. Wanzala *et al.* (36), also in Kenya, described the insensitivity of meat inspection in detecting cysticerci: only 50% of naturally or artificially infested cattle were identified. Their observations indicated that a number of viable cysticerci may be missed.

In humans, the most common presenting sign in *T. solium* NCC is seizures followed by headache, but a range of signs, such as vomiting, psychoses, etc., are seen depending on the number, location and viability or level of degeneration of the cysticerci (viable, transitional dying, calcification) (4, 21, 26). In humans, clinical evaluation and either computerised tomography (CT) scan (best for calcified cysts) or magnetic resonance imaging (MRI) (detects cysts in both parenchymal and extraparenchymal locations and can follow the progression of the lesion) are used to detect the exact locations and viability of *T. solium* and *T. multiceps* metacestodes. These remain the most efficient means of diagnosis, but access to imaging facilities may not be available in endemic areas. Calcified cysts in tissues are detected by radiography. Serology now is available for NCC.

2. Serological tests

The development of an automated sensitive and specific diagnostic test would greatly reduce the costs of damage to the carcass and also the costs of labour. Serological tests for animals have not reached the stage where commercialisation for individual diagnosis or large-scale detection of infected carcasses in slaughter houses is possible. All assays tested – AG-ELISA, antibody ELISA, enzyme-linked immunoelectro transfer blot (EITB) and tongue inspection – show low sensitivity in rural pigs infected naturally with low levels of *T. solium* (7, 29). This contrasts with their high sensitivity and specificity when applied to commercially reared pigs free from infection and such pigs experimentally infected with *T. solium* (29, 30). This finding is also true for *T. saginata* infections in cattle (28, 33). Thus, only a small percentage (13–22%) of cattle carrying fewer than 30–50 viable cysticerci is detected by AG-ELISA. Conversely, antibody has proven most useful for detecting cysts that are no

longer viable. Nonetheless, AG-ELISAs do have a use in field-based epidemiological studies for indicating transmission. For example, the detection of viable infections in cattle or pigs could indicate point sources of infection, season of transmission and age of animals at risk. The development of more sensitive and specific assays with recombinant antigens for diagnosis of NCC should improve immunodiagnosis of *T. solium* in pigs.

A number of EITB and ELISA assays for antibodies to *T. solium* in humans are now widely available commercially (i.e. Immunetics, USA; Cypress Diagnostics, USA; Diagnostic Automation, USA; United Biotech, USA, Arup Laboratories, USA; Biopharm, Germany). An AG-ELISA using polyclonal or monoclonal antibody (used to detect antigen in cerebrospinal fluid) has a specificity and sensitivity of up to 86% in selected patients. The specificity of these tests tends to be very high but sensitivity is lower, this is in part related to cyst number. The hierarchy of clinical symptoms, imaging studies and serological tests has been presented by del Brutto *et al.* (6).

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

Immunochemical identification of protective antigens and their production by recombinant DNA technology has been uniquely successful in the Taeniidae compared with other eukaryotes, and is described by Lightowers & Gauci (18, 19, 20). Vaccination with the resultant products has been highly effective. Overall the success was advanced by the fact that a strong protective immunity occurs after natural infection, high levels of protective immunity are induced by antigens in oncospherical extracts, there is good cross-immunity between *Taenia* species, and immunity is largely antibody mediated as evidenced by passive and maternal transfer of immunity so that antibody could be used to probe for protective antigens. Initially the *T. ovis* vaccine was developed. The *T. solium* 45W antigen was isolated as a recombinant protein from *Escherichia coli*. Potency control is by AG-ELISA and *in vivo* immunogenicity, and the vaccine has provided protection for a period of at least 1 year in field trials. Two other antigens (To16K and To18K) have been isolated and cloned and each individual *T. ovis* protein is protective. Using the benefits of cross-reaction and probing with the *T. ovis* cDNA, homologues of the *T. ovis* antigens, TSA9 and TSA18, equivalent to To45W and To18, were identified in *T. saginata* and cloned from *T. saginata* oncosphere mRNA. In contrast to the *T. ovis* individual antigens, immunisation with both *T. saginata* antigens was required to produce high level protection. Homologues of the *T. ovis* antigens also were cloned from *T. solium* mRNA and TSO45 and TSOL18 antigens of *T. solium*. Both the *T. ovis* and *T. saginata* vaccines have given >94% and 98% protection in cattle and sheep, respectively. Comparable antigens now have been identified for *T. multiceps*. The *T. ovis* vaccine was registered in 1994 by the New Zealand Animal Remedies Board. However, due to market changes in New Zealand, the vaccine is not available commercially. Costs of large-scale production of antigens, processing conditions and potential variants in expressed antigens have been outlined by Lightowers & Gauci (20). The *T. solium* antigens, in particular TSOL18, have given high levels of protection experimentally (99%) (18); the antigen is undergoing the steps similar to the *T. ovis* antigens to develop a practical vaccine. Other avenues for vaccine antigens are being explored. Synthetic peptides from the sequences of the recombinant Taeniidae antigens induced antibody but not protection, indicating that the protective epitopes seemed conformational. Reasonable levels of protection were induced experimentally in piglets exposed to natural *T. solium* challenge using synthetic peptides based on protein sequences of the murine parasite *T. crassiceps* (14). The S3Pvac *T. solium* subunit vaccine has had some field efficacy in protecting against natural infection but requires further examination (5, 31). It is possible that cost-benefit analyses concerning the use of the *T. saginata* vaccine could obviate its use in many countries, as cost of the vaccine is very important to the livestock industries. The importance of *T. solium* in humans increases the costs of the disease, but it remains to be seen whether the significance of the disease in endemic countries will be sufficient to push commercial production of the vaccine for use in pigs. Sensitive immunodiagnosis for metacestodes still requires development to access vaccine efficacy.

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