

## CHAPTER 3.10.2.

# CYSTICERCOSIS (INCLUDING INFECTION WITH *TAENIA SOLIUM*)<sup>1</sup>

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### SUMMARY

**Description of the disease:** Cysticercosis of farmed and wild animals is caused by the larval stages (metacestodes) of cestodes of the family Taeniidae (tapeworms), the adult stages of which occur in the intestine of humans, dogs, cats or wild Canidae and Mustellidae. Bovine cysticercosis (primarily in muscle) and porcine cysticercosis (primarily in muscle and the central nervous system [CNS]) are caused by the metacestodes (cysticerci) of the human cestodes *Taenia saginata* and *T. solium*, respectively. Cysticerci of *T. solium* also develop in the CNS, musculature and subcutaneous tissue of humans. *Taenia asiatica* is a less widespread cause of cysticercosis in pigs, with the cysts locating in the liver and viscera and the adult tapeworm occurring in humans. Cysticercosis and coenurosis of sheep and goats, and occasionally cattle, with the cysts occurring in the muscles, brain, liver or peritoneal cavity, are caused by *T. ovis*, *T. multiceps* and *T. hydatigena*, with the adult tapeworms occurring 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. Rare but severe cysticercoses in humans can be caused by the fox tapeworm *Taenia crassiceps* and the mustelid tapeworms *Taenia martis* and *Versteria* spp. 'Gid' caused by *T. multiceps* in ruminants can require surgery or slaughter of the animal. Acute *T. multiceps* coenurosis and *T. hydatigena* cysticercosis in sheep and goats are rare but may be fatal. Cysticercosis causes economic loss through condemnation of infected meat and offal.

**Detection 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. Adult *Taenia* are recognised at post-mortem or by passage of segments or eggs in faeces. *Taenia* species cannot be differentiated by egg structure. Metacestodes consist of a fluid-filled bladder with one or more invaginated scoleces. These 'bladderworms' are each contained within a cyst wall at the parasite–host interface. This structure comprises the cysticercus or coenurus. Metacestodes are grossly visible at post mortem and meat inspection, but light infections are often missed. NCC can be diagnosed by imaging techniques.

**Immunological and molecular tests:** Adult human *Taenia* infections can be diagnosed by detection of *Taenia* coproantigen in faeces using an antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA). Several species-specific DNA-based techniques have been developed that can be applied on parasite material or on faecal extracts, but remain to be fully validated.

**Serological tests:** Commercial antibody-detecting tests (enzyme linked immunoelectrotransfer blot) are available for the diagnosis of *T. solium* cysticercosis in people and in pigs, though for the latter the diagnostic performance is limited due to a high false positive rate. A commercial test (ELISA format)

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<sup>1</sup> Although certain diseases caused by Taeniidae are included in some individual species sections of the WOAHL, this chapter covers several species and thus gives a broader description.

is available for detection of circulating parasite-derived antigen in the serum of pigs or humans with *T. solium* cysticercosis, but the use in pigs is limited due to a high false positive rate and low positive predictive value.

**Requirements for vaccines:** Excellent vaccines based on recombinant antigens derived from oncosphere proteins have been developed for immunising animals against cysticercosis caused by *T. ovis* in sheep, *T. saginata* in cattle and *T. solium* in pigs. Currently, no vaccines are available for the adult stages of *Taenia* spp. A *T. solium* vaccine for pigs is commercially available; it is approved in some countries and undergoing regulatory approval in other countries. A combination of vaccination and oxfendazole treatment was highly effective in experimental control of natural transmission to pigs. Immunisation of pigs for *T. solium* cysticercosis requires at least two vaccinations.

## 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, cats and wild canids and mustelids. *Taenia saginata* of humans causes bovine cysticercosis, which occurs virtually world-wide, but particularly in Africa, Latin America, Caucasian and South/Central Asia and eastern Mediterranean countries. The infection occurs in many countries in Europe and sporadically in the United States of America (USA), Canada, Australia and New Zealand. *Taenia solium* of humans causes porcine cysticercosis and human (neuro)cysticercosis ((N)CC), the most important cause of acquired epilepsy in endemic areas. It is found principally in Mexico, Central and South America, sub-Saharan Africa, non-Islamic countries of Asia, including India and China (People's Rep. of) in regions with poor sanitation and free-ranging, scavenging pigs. In 2016, central nervous system syndromes, including seizures and depressed spirit, have been observed in pigs naturally infected with *T. solium* (Trevisan *et al.*, 2016). The cysticerci of *T. asiatica* in South-East Asia occur in the liver of pigs. Dogs and wild canids are the definitive hosts of *Taenia* spp. that have sheep, goats and other ruminants and pigs as intermediate hosts, which occur throughout most of the world, although *T. multiceps* has disappeared from the USA, Australia and New Zealand. *Taenia ovis* cysticerci occur 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. *Taenia crassiceps*, a species mainly occurring in foxes, causes, though rarely, ocular and neural cysticercoses in immunocompetent human patients and severe subcutaneous disseminated and proliferative cysticercoses in immunodeficient patients; *T. martis*, a tapeworm of martens in Europe causes rare cysticercosis in immunocompetent humans. A *Versteria* sp. distinct from *V. mustelae* caused severe and lethal cysticercoses in heavily immunosuppressed patients in North America (Deplazes *et al.*, 2019). Diagnosis in animals usually is based on identification of the metacestode 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 are shed by the adult tapeworms. Tapeworm eggs, within proglottids or separate, are released into the environment with the faeces. For certain species (*T. saginata*, canid taeniids), active migration of the proglottids out of the anus occurs. Eggs may be disseminated from faeces by physical means or transport hosts. Flies particularly ingest eggs and transport these eggs, so eggs are deposited at high intensity within 150 m of the faeces and at low intensity for 10 km (Lawson & Gemmell, 1990). Eggs are immediately infective when passed. Animals acquire infection from ingestion of food or water contaminated with sticky eggs, ingestion of segments or faeces containing eggs. or by eating arthropods such as dung beetles that carry eggs. Humans may be infected with *T. solium* by eggs on vegetables, in water, etc., that have been contaminated by faeces, or food contaminated by dirty hands, by faeco-oral transmission or possibly through retro-peristalsis and hatching of eggs internally (auto-infection). Disease clusters where a human carrier exists. Routine diagnosis of taeniosis 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.

*Taenia* spp. should be handled with appropriate biosafety and biocontainment measures 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*).

## B. DIAGNOSTIC TECHNIQUES

### 1. Identification of the agent

#### 1.1. *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 morphological features are presented in Table 1 (Khalil *et al.*, 1994; Loos-Frank, 2000; Soulsby, 1982; Verster, 1969). Gravid segments have >14 uterine branches. They usually leave the host singly and many migrate spontaneously from the anus. Tapeworm eggs are also released separately, outside the proglottid, in the faeces.

The eggs are typical 'taeniid' eggs that cannot be differentiated morphologically from other *Taenia* or *Echinococcus* spp. eggs. *Taeniid* eggs measure about 25–45 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 of *T. saginata* usually occur in the striated muscles of cattle (beef measles), but also buffalo, and various *Cervidae*. Viable cysts are oval, fluid-filled, about 0.5–1 × 0.5 cm, 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.

#### 1.2. *Taenia solium* (the pork tapeworm)

*Taenia solium* is typically smaller than *T. saginata* being 1–5 metres and is generally assumed to survive for 2–3 years. The scolex has an armed rostellum bearing two rows of hooks. Gravid segments have <14 uterine branches and do not usually leave the host spontaneously, but passively (in small chains) with the faeces. Tapeworm eggs are also released separately, outside the proglottid, in the faeces.

*Taenia solium* cysticerci occur primarily in the muscles and central nervous system (CNS) of pigs (pork measles), and in the muscles, subcutaneous tissues, CNS and, rarely the eye, of humans. Cysts are grossly similar to those of *T. saginata*. They have a scolex bearing a rostellum and hooks similar to the adult. Occasionally, in the brain cisterns of humans, cysts can develop in the space available as racemose cysts up to 10 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)		No. testes	Layers of testes	Cirrus sac extends to longitudinal vessels	No. 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.

Parasite species	Number of hooks	Length of hooks (µm)		No. testes	Layers of testes	Cirrus sac extends to longitudinal vessels	No. uterine branches	
		Large hooks	Small hooks					
<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	7–14 that re-divide	Lobes of ovary unequal in size with small accessory lobe. No vaginal sphincter. Testes confluent behind vitellarium
<i>T. asiatica</i>	Vestigial hooks some with small rostellum	–	–	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

### 1.3. *Taenia asiatica* (Asian *Taenia*)

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

The metacestodes 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 mesenteries and, rarely, are described in cattle, goats, and monkeys.

### 1.4. *Taenia ovis*

Adults in the intestine of dogs and wild canines reach 1–2 metres in length and have an armed rostellum; the number and size of hooks can aid differentiation of *Taenia* spp. (Table 1). Metacestodes that occur in the musculature (skeletal and cardiac) of sheep and less commonly goats reach 0.5–1.0 × 0.5 cm. A similar parasite (*T. ovis krabbei*) occurs in wild canines and dogs and the muscles of reindeer and deer in northern areas.

**1.5. *Taenia hydatigena***

Adults are up to 1 metre or more long, are found in the intestine of dogs and wild canids, and have an armed rostellum (Table 1). Metacestodes can be 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 protruding from 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; canids are definitive hosts.

**1.6. *Taenia multiceps***

Adults, up to a metre long in the intestine of canids, 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 more 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. The cysts induce neurological signs that in sheep are called 'gid', 'sturdy', etc.

**1.7. 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 (2011) and all can be differentiated from *Taenia* spp. by egg/proglottid morphology. 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 *Echinococcus* -specific antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA) (Allan *et al.*, 1992). Other worms in canids, *Dipylidium*, *Diplopylidium*, *Mesocestoides* and *Diphyllobothrium* spp., have morphologically distinct eggs and proglottids (Lloyd, 2011; Soulsby, 1982).

Adult cestodes can be expelled from humans using an anthelmintic (praziquantel, niclosamide, albendazole) followed by a saline purgative and are identified on the basis of scolex and proglottid morphology, though scolices are often not recovered and comparison of proglottid morphology is not always a reliable method. A self-detection tool was used in Mexico (Flisser *et al.*, 2011); 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 (no species identification). In animals, an anthelmintic, such as praziquantel, can be used as well; again, the recovered tapeworms are identified morphologically. Arecoline can no longer be recommended because of its side-effects. Tapeworms can be recovered after anthelmintic treatment, and require appropriate disposal.

Verster (1969) and Loos-Frank (2000) 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.* (1994). Loos-Frank (2000) gives methods for mounting, embedding, sectioning and staining the proglottids. 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 rostellum, hooks and suckers 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 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, eggs are expelled in the intestine and can be found in the faeces. Segments of *T. saginata*, *T. asiatica* and the dog *Taenia* spp. may migrate spontaneously from the anus and this is likely to be noticed (>95% in the case of *T. saginata*). 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 *T. solium*. Segments of all three may be found on the faeces, but are passed intermittently. Even if a segment has shed 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, NaNO<sub>3</sub> 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 (Mc Master technique) 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. Cheesbrough (2005; 2006) reports that *T. saginata* eggs can be differentiated from *T. solium* on staining with Ziehl–Neelsen as used for acid-fast bacilli: the striated embryophore of *T. saginata* is acid fast (stains red), that of *T. solium* is not acid fast. DNA probes, the polymerase chain reaction (PCR), PCR restriction fragment length polymorphism (RFLP) or multiplex PCR, have proved useful for differentiation though largely used experimentally to differentiate faecal eggs of *T. solium*, *T. saginata* and *T. asiatica* (Gasser & Chilton, 1995; Geysen *et al.*, 2007; Gonzalez *et al.*, 2004; Yamasaki *et al.*, 2004). While equally applicable to differentiation in dogs, the same examinations have not been done for *Taenia* spp.

Ag-ELISAs to detect *Taenia* coproantigen have been developed and can be established in-house if laboratory facilities are available (Allan *et al.*, 1992; Deplazes *et al.*, 1991), with prior evaluation of appropriate controls. One Ag-ELISA was developed experimentally by Allan *et al.* (1992) 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*-genus specific. A modification of the test, including polyclonal antibodies directed against excretory secretory antigens has been developed, rendering the test species specific (Guezala *et al.*, 2009). The test is an in-house, solid-phase, microwell assay with wells coated with polyclonal, rabbit anti-*Taenia*-specific antibody (TSA).

## 1.8. Diagnosis of metacestodes

*Taenia solium* 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 heavily infected with metacestodes; these will also be difficult to differentiate from large sarcocysts, or other (mechanical) lesions. Nevertheless, tongue palpation is a common diagnostic method used by pig traders in endemic, resource poor areas. A sensitivity of 21% was determined by Dorny *et al.* (2004).

### 1.8.1. 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). 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 and viable, are usually oval, about 10 × 5 mm or larger (depending on the species), 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 *T. saginata* 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 the tissue within which the cyst is embedded, the host's immunological status and possibly with host age at infection. In general, cysts tend to die more rapidly in the muscular predilection sites, such as the 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 parasites' abilities to evade the immune response. Cysts at different stages of viability and degeneration can be found

in the same host. Death in skeletal muscles may occur within 2 months of infection of adult cattle with *T. saginata*, but cysts may remain viable for several years. Cysts of *T. hydatigena* in the peritoneal cavity of sheep and those of *T. solium* in pigs also have been described as surviving for long periods.

Degenerating cysts vary in appearance. Marked infiltration of eosinophils, macrophages, lymphocytes and collagen deposition thickens the capsule, which 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, usually being larger in size and certainly more obvious in meat than the original viable cyst. Later the cyst may calcify. 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, 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. 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. Molecular tools can be used as well, especially for species identification (see above), also in the finding of a new cestode in a host species or geographical area from which, historically, the parasite was absent. In one study PCR identified only 50% of degenerate presumed *T. saginata* cysts (Abuseir *et al.*, 2006), whereas Eichenberger *et al.* (2011) using different primers identified 80% of calcified lesions.

After treatment of *T. solium* in pigs with drugs such as oxfendazole (single dose, 30 mg/kg), the cysts may lose their fluid and collapse. The resultant lesion is smaller than lesions observed following natural death but can take 3–6 months to resolve.

Meat inspection procedures are defined, amongst others, by the regulations within a country or region (e.g. EU) and the animal species. Additional inspections can be performed on doubt or identification of a lesion, or specific origin of an animal. Examinations tend to be more extensive with the zoonotic infections *T. saginata* and *T. solium*.

In general, meat inspection procedures related to *Taenia* spp. 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 (and *T. asiatica*), mesenteries or omentum, or *T. multiceps* in the brain in the specific animal species.
- ii) The external and internal masseters and the pterygoid muscles are each 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 some countries, 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 undertaken for *T. solium* in pigs. Such incisions into the legs are made, particularly in African countries as it is suspected that more parasites lodge in these muscles in working or range animals walking long distances because of 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.

Additional incisions may be required either by the regulations or if cysts are found on the initial incision(s). Eichenberger *et al.* (2013) reported an increase in sensitivity by multiple incisions. Details on meat inspection are supplied by Herenda *et al.* (2000).

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* spp. and regulations within a country.

### 1.8.2. Meat inspection – species differentiation and decision making

#### i) *Taenia saginata*: predilection site

Calves under 6 weeks are not examined in certain countries (e.g. EU regulation). Predilection sites are the heart, tongue, masseters and diaphragm, presumably because they receive the greatest blood circulation. Nonetheless, cysts may be found in any muscle (or less frequently organs) of the body. Lesions of *T. saginata* may need to be differentiated from *Sarcocystis* sarcocysts and other lesions. In PCR studies in Germany, Switzerland and New Zealand up to 20% of viable, presumed *T. saginata* cysts could not be positively identified (Abuseir *et al.*, 2006). Meat inspection has a very low sensitivity (<16%) for the detection of *T. saginata*, especially with low infection levels (Dorny *et al.*, 2000; Eichenberger *et al.*, 2013; Kyvsgaard *et al.*, 1990). In general, meat inspection procedures detect only about 15–50% of the animals that are actually infected. Light infections are easily missed on palpation and meat inspection – in one study involving *T. saginata*, 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 (Walther & Koske, 1980). 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. In Kenya Walther & Koske (1980) 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 African countries as 20% of the cattle found to be infected were positive in the shoulder only. Animals infected as very young calves may have few or no cysts in tongue and masseters. Wanzala *et al.* (2003), also in Kenya, described the insensitivity of meat inspection in detecting cysticerci: only 50% of naturally or artificially infected cattle were identified. Their observations indicated that a number of viable cysticerci may be missed. A recent large-scale study on Belgian cattle, characterised by mostly light infections, even estimated the sensitivity as low as 0.54% (Jansen *et al.*, 2018a). While Eichenberger *et al.* (2011) detected substantially more cases when implementing additional incisions in the heart muscle (estimated sensitivity of 24.2%), this could not be confirmed by Jansen *et al.* (2018b) who defined a sensitivity of 2.87%.

#### ii) *Taenia solium*: predilection site

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. Incisions to be made are country regulation dependent. As for *T. saginata*, meat inspection has a low sensitivity (22.1%), especially for light infections (Dorny *et al.*, 2004). A recent study in South Africa found that the level of agreement (Kappa statistic) between carcass dissection (gold standard) and meat inspection was negative, which is an indication of disagreement between the two methods and confirms that the current meat inspection procedures alone are not sufficiently sensitive to detect all cases of porcine cysticercosis (Sithole *et al.*, 2019).

#### iii) *Taenia asiatica*: predilection site

The small size means detection of cysts in the liver is difficult except in heavy infection.



iv) *Taenia hydatigena*: predilection site

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. Those that are retained at the liver surface are usually superficial and subserosal, while most of an *Echinococcus granulosus* hydatid cyst is deeper in the parenchyma. *Taenia hydatigena* cysts usually mature in the omental or mesenteric fat. If viable, the *T. hydatigena* cyst has a long-necked single scolex in virtually translucent cyst fluid. Fertile *Echinococcus* hydatid cysts have thicker walls and may contain many brood capsules containing protoscolexes; these appear as a sandy, whitish deposit within the cysts. Differentiation can be important in the implementation and monitoring of hydatid disease control measures so histology may be required. H&E-stained sections will reveal the laminated membrane of very young hydatid cysts as indicated by Lloyd *et al.* (1991). 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.

v) *Taenia multiceps*: predilection site

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 caused by 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. The site of *T. multiceps* cysts in sheep brain might be identified by clinical signs presented and, possibly, softening of the skull overlying the coenurus. Sometimes, the coenurus can be observed at the superficial surfaces of affected muscles, especially in the neck region.

vi) *Taenia ovis*: predilection site

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

## 1.9. Detection of circulating antigens

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 at meat inspection. Sensitivity of serological tests for animals has 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* (Dorny *et al.*, 2005; Scitutto *et al.*, 1998). This finding is also true for *T. saginata* infections in cattle (Jansen *et al.*, 2018b; Van Kerckhoven *et al.*, 1998). For example, only a small percentage (13–22%) of cattle carrying fewer than 30–50 viable cysticerci is detected by Ag-ELISA. Recent results estimate a sensitivity and specificity of 26.9 (increasing to 40% if only viable cysts are considered) and 99.4%, respectively for Ag ELISA in Belgian cattle (Jansen *et al.*, 2018b). Results of test performances can vary substantially between studies, representing different populations and study designs/analyses. In a Swiss study, a sensitivity and specificity of 14.3% (increasing to 40% if only viable cysts are considered) and 93.7%, were determined, respectively for the same Ag ELISA (Eichenberger *et al.*, 2013).

For the diagnosis of *T. solium* in pigs based on the B158/B60 Ag ELISA, for which a commercial kit is now available, latest results indicate a rather poor sensitivity and specificity (Chembensofu *et al.*, 2017; Chilundo *et al.*, 2018; Kabululu *et al.*, 2020; Sithole *et al.*, 2019). The Ag ELISA was recently shown to have a low positive predictive value of 35.2 % (Kabululu *et al.*, 2020).

Nonetheless, Ag-ELISAs do have a use in field-based epidemiological studies for indicating transmission. 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.

## 2. Serological tests for antibodies

Tests for circulating antibodies have little role in animals, except for epidemiological studies. A number of EITB and ELISAs for antibodies to *T. solium* in humans are now widely available. These were reviewed by Rodriguez *et al.* (2012) with comparisons of sensitivity and specificity. Recent results indicate a sensitivity and specificity of 13.8% and 92.9%, respectively for antibody detection in Belgian cattle (Jansen *et al.*, 2018b), while the same test used in Swiss cattle in a different study, performed better with sensitivity and specificity of 81.6% and 96.3%, respectively (Eichenberger *et al.*, 2013).

In pigs, specific antibody detection, based on EITB and assuming the presence of one reactive band as a positive test result leads to a fairly good sensitivity of 89%, but a poor specificity of 48%. The latter can be improved by setting the cut-off at three bands needed for test positivity, leading to an increase in specificity reaching 76%, but with sensitivity decreasing to 78% (Jayashi *et al.*, 2013). Recent results suggest a cross-reactivity to GP50 in pigs infected with *Taenia hydatigena* (Muro *et al.*, 2017).

## C. REQUIREMENTS FOR VACCINES

Effective vaccines have been developed against infection with the larval stages of several *Taenia* spp., but internationally accepted standards for the production of vaccines are not available. Considerable information is available in the scientific literature on immunogenic molecules, their recombinant technology and extraction, and their efficacy experimentally and in a number of field trials.

A recombinant vaccine based on the 45W antigen was developed in Australia and New Zealand against infection with *Taenia ovis* (Rickard *et al.*, 1995). The vaccine was approved for use in sheep in New Zealand but is not available commercially.

An effective recombinant vaccine has been developed against bovine cysticercosis based on the TSA9/18 antigens of *T. saginata* (Lightowlers *et al.*, 1996), but it has never been developed as a commercial product.

For *Taenia solium*, a vaccine based on the recombinant antigen TSOL18 (Flisser *et al.*, 2004) has been evaluated in independent experimental studies conducted in Mexico, Peru, Honduras and Cameroon in which the vaccine achieved 99–100% protection (Lightowlers, 2013). Subsequently, the vaccine has been used in the field in Nepal, Tanzania, Zambia, Uganda, as well as in Peru where it was used in a project involving >55,000 pigs (Gabriel *et al.*, 2020; Garcia *et al.*, 2016; Kabululu *et al.*, 2020; Poudel *et al.*, 2019). The TSOL18 vaccine has been found to be both safe and efficacious. There is only one commercially available approved TSOL18 vaccine for pigs, produced in India. The TSOL18 vaccine targets the parasite during its early development in the pig, preventing infection. It does not affect cysticerci if they were already established in the tissues prior to vaccination. For that reason, use of the vaccine is recommended in combination with treatment using oxfendazole (single dose, 30 mg/kg), where the drug clears any pre-existing infection, while the vaccine prevents infection from any subsequent exposure to the parasite.

The commercial TSOL18 vaccine incorporates 150 µg of the TSOL18 antigen expressed in yeast, together with an oil adjuvant. Pigs of 2 months of age or older are immunised by intramuscular injection with 1 ml of the vaccine. A booster vaccination is required after 3–4 weeks. Booster vaccination may be applied after 6 months, however recent evidence suggests that pigs become naturally resistant to new *T. solium* infections as they age (Poudel *et al.*, 2019), suggesting that booster vaccination of animals fully vaccinated as young animals may not be required. Generally, no significant side effects are noticed, however in some animals, temporary pyrexia, lethargy for 1–2 days and local injection site reactions for up to 7 days may be observed after vaccination. Following administration of a 5-fold overdose of vaccine, no adverse reactions were observed under farm conditions other than those described above.

The vaccine should be stored and transported between 2°C and 8°C; it should not be frozen. Although the manufacturer recommends the vaccine be maintained refrigerated, being a defined antigen, non-living vaccine, the vaccine is known to be relatively insensitive to exposure to room temperatures.

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**NB:** At the time of publication (2021) there were no WOAHO Reference Laboratories for cysticercosis (including infection with *Taenia solium*) (please consult the WOAHO Web site: <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

**NB:** FIRST ADOPTED IN 1991. MOST RECENT UPDATES ADOPTED IN 2021.