

GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

TAENIA SPP.

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Summary

Tapeworms of the genus *Taenia* include over 100 species. Members of the genus are characterised by a ribbon-like appearance comprised of multiple, egg-containing segments called 'proglottids'. *Taenia* spp. lifecycle relies on a vertebrate intermediate host in which the infective larvae (cysticerci) develop and a definitive host which ingests the uncooked flesh of the intermediate host containing the cysticerci which develops in the small-intestine into the adult tapeworm. The tapeworm proglottids are released from the definitive host and intermediate hosts are infected by ingesting the tapeworm eggs from the proglottids. *Taenia* spp. eggs have been demonstrated to survive for periods of months or years on pasture, with low to moderate temperatures and high humidity being associated with long survival times.

Humans are the definitive host for three *Taenia* species (*T. saginata*, *T. solium*, *T. asiatica*). The accidental ingestion of *T. solium* eggs by humans, through poor hand hygiene, contaminated water or faecal contamination of food, can result in the aberrant larval infection human cysticercosis. Neurocysticercosis, infection of the nervous system, is one of the most common causes of preventable epilepsy in endemic countries and has been estimated to be responsible for a loss of 503,000 (379,000-663,000) DALYs yearly.

Human taeniosis infections (with the adult tapeworm) have generally rather mild clinical effects and are susceptible to praziquantel, niclosamide or triple dose albendazole. Neurocysticercosis, however, requires careful surgical or medical management due to the sensitive location of the larval cysts and potential critical complications that can occur.

As well as anthelmintic treatment of the definitive or intermediate hosts, the control of *Taenia* spp. relies on stringent meat inspection, education on safe meat preparation, personal hygiene and, importantly, the careful management of potentially contaminated domestic effluent and sewage sludge.

The zoonotic tapeworms *Taenia saginata* and *Taenia solium*, are known as the beef tapeworm and pork tapeworm, respectively, depending on their intermediate host. A third species, *Taenia asiatica*, the Asian tapeworm, although genetically closest to *T. saginata*, also utilises a porcine intermediate host (Flisser, 2006). In the definitive, human, host infections with the adult stage of *Taenia* spp. (taeniosis) have only mild health implications through gastro-intestinal disturbance. The larval stage of *T. solium*, can however infect humans as aberrant intermediate hosts, an infection known as cysticercosis. This can result in severe disease including epileptic seizures due to cyst development in the brain (neurocysticercosis). Humans acquire cysticercosis through ingestion of *T. solium* eggs for instance in excreta or through contaminated water and food-stuffs. Correct treatment and handling of excreta, raw sewerage and sewerage sludge is therefore of incredible importance for the safety of both sanitation workers and the public.

1.0 Epidemiology of *Taenia Saginata* and *Taenia Solium*

The zoonotic tapeworms *Taenia saginata* and *solium* like their definitive host (humans) have a global distribution and wide ecological niche, *T. saginata* being recorded on every continent with the exception of Antarctica and *T. solium* being recorded in the majority of countries where pork is consumed. Regions reporting *T. solium* infections in the porcine or human hosts include Africa (Braae et al., 2015) and Asia (Rajshekhar et al., 2003), Latin America (Coral-Almeida et al., 2015), North America (Cantey et al., 2014; DeGiorgio et al., 2005) and Europe (Devleesschauwer et al., 2015).

Key risk factors for *Taenia* spp. infection in both intermediate and definitive hosts includes poor sanitary conditions, contaminated water, outdoor and free-range animal husbandry, poor meat inspection and lack of health education. These risk factors are highly prevalent in less developed countries and are reflected in the high prevalence of *Taenia* spp. found in these countries. While it is generally thought that improved pig husbandry and sanitary conditions in Europe have effectively eradicated *T. solium*, a recent review suggests that this may not be entirely true (Devleesschauwer et al., 2015). Valid epidemiological data are still lacking for both parasites on a country-by-country basis, and prevalence levels are difficult to compare due to the differing diagnostic methodologies utilised in many studies.

1.1 Global Burden of Disease

The 2010 Global Burden of Disease (GBD) survey estimated that human cysticercosis (caused by *T. solium*) was responsible for 503,000 (95% C.I. 379,000-663,000) DALYs lost annually (Murray et al., 2012). It is felt by many, however that this under-estimates the true burden. It is also estimated that approximately 30% of epilepsy in endemic areas is attributable to neurocysticercosis (NCC) (Rajshekhar et al., 2006, Ndimubanzi et al., 2010, Bruno et al., 2013) and extrapolating from the DALYs attributable to epilepsy from the 2010 GBD survey it is likely that the DALYs attributable to *T. solium* should be in the region of 2.7million (95% C.I. 2.16-3.61 million) (Torgerson et al., 2015). *T. solium* is also thought to be responsible for approximately 28 thousand deaths worldwide on an annual basis (95% C.I. 21-37,000) (Torgerson et al., 2015).

1.1.1 Symptomatology

Intestinal taeniosis is generally asymptomatic (Garcia et al., 2003), although mild abdominal discomfort has been reported (Tembo and Craig 2015). Rare reported sequelae to intestinal taeniosis include Cholangitis (an infection of the biliary tract) (Uygur-Bayramicli et al., 2012), gall bladder perforation (Hakeem et al., 2012), appendicitis (Kulkarni et al., 2014) and bowel obstruction (Atef and Emna, 2015; Li et al., 2015). Some people suffering from taeniosis will notice the passage of proglottids (parasite segments containing eggs) in their faeces, although this seems most common with the more motile proglottids of *T. saginata*

(Garcia et al., 2003). The major health burden imposed by *T. solium* is human cysticercosis caused by the ingestion of eggs, or via auto-infection within individuals with taeniosis, and the subsequent aberrant encystment of the larval stage within humans. *T. solium* larvae can encyst in various places throughout the human body, although muscular, subcutaneous, ocular and neuro-cysticercosis are the most common presentations (Gonzales et al., 2016). The most common form of human cysticercosis infection, and that causing the majority of associated morbidity, is the presence of a cyst or cysts within the central nervous system (CNS) termed neurocysticercosis (NCC). Larvae invade the CNS and establish a viable cysticerci within the brain parenchyma or in the extra-parenchymal space (Gonzales et al., 2016).

1.1.2 Economic Impact

T. saginata is the most common cause of taeniosis in humans, with an estimated 60 to 70,000 carriers worldwide (Flisser et al., 2011). In comparison to the large public health burden of *T. solium*, however, *T. saginata* is an infection primarily of economic importance through the condemnation, downgrading and processing of carcasses and organs containing cysts (Boone et al., 2007; Torgerson, 2013). Regulations such as EU regulation 854/2004 and the Kenyan Meat Control Act (CAP 356) requires that generalised infections of cattle with cysticercosis requires that the meat is declared unfit for human consumption or, in the case of localised infections the carcass, or uninfected parts of the carcass are declared fit for consumption only after cold treatment (-10°C for 10 days -3 weeks) (Abuseir et al., 2006; Government of Kenya, 2012). The extra handling, condemnation, the weight loss (2 to 5%) and reduced value of meat after freezing, potentially up to 45% of the carcass value are responsible for large economic losses due to bovine cysticercosis. In England alone bovine cysticercosis is estimated to cost \$5.6 million annually although the largest economic burden is imposed upon Africa, where it is estimated that \$1.8 billion is lost annually (Abuseir et al., 2006).

1.2 Taxonomic Classification of the Agent(s)

1.2.1 Taxonomy and physical description of the agents

There are two major subclasses of tapeworms, Cestodaria and Eucestoda. The genus *Taenia* belong to the subclass Eucestoda, order Cyclophyllyidea and family Taenidae. The family Taenidae comprises small to large sized tapeworms. Tapeworms are anchored to the intestine of the host by a holdfast organ named scolex and possess a large tape-like body with segments, called proglottids. There is no alimentary canal and each segment has both female and male sexual organ. The eggs of *Taenia* spp. are small 34 to 36µm and subspherical and cannot easily be identified to the species level morphologically. Almost all the Cyclophyllyidean family includes parasites that have two hosts. Their life cycles always involve vertebrate and invertebrate species for the adult and intermediate forms, respectively (Smith, 1969). Taeniids are unique among the cyclophyllyideans in having mammals as both definitive and

intermediate hosts.

1.3 Transmission

1.3.1 Life cycle, and routes of transmission

The life cycle of *T. solium*, *saginata* and *asiatica* involve humans as the sole definitive hosts, harbouring the adult tapeworm in the intestine. *T. saginata* adult tapeworms produce 1,000 to 2,000 proglottids per worm and may produce up to 100,000 eggs per worm. *T. solium* (pork) produce an average of 1,000 proglottids/worm, and may produce 50,000 eggs per worm and *T. asiatica* tapeworms produce 700 proglottids/worm and may produce 80,000 eggs per proglottid. The metacestode (larval) stage of the parasites is found in pigs, in the case of *T. solium* and *T. asiatica* and in cattle and some other ruminants in the case of *T. saginata* (Flisser et al., 2004; Galan-Puchades and Fuentes, 2013). Transmission between intermediate and definitive host occurs through ingestion of undercooked meat containing metacestodes, with transmission from definitive host and intermediate host occurs through the ingestion of the eggs or egg-filled proglottids excreted by the definitive host.

1.3.1.1 Dispersion of *Taenia* spp. eggs

The way in which the intermediate hosts ingest cestode eggs depends on physiological characteristics of the tapeworms. In *T. saginata*, the beef tapeworm, proglottids are released one by one and they move away from faeces by contracting and expanding longitudinally towards areas where the eggs may be likely to be ingested by cattle (Flisser et al., 2004). Like other cestodes whose adult form infects ruminants, eggs remain within a range of about 180 m of the site of faecal deposition but some eggs rapidly disperse in all directions (Gemmell et al., 1987). On the other hand, dispersion of *T. solium* proglottids and eggs is less likely to occur due to the intermittent release nature of non-motile proglottids (Garcia et al., 2003). Therefore, *T. solium* proglottids are usually ingested directly at the site of deposition by the intermediate hosts (Gonzalez et al., 2005).

1.3.1.2 Invertebrates and *Taenia* spp. transmission

Taenia spp. eggs have been identified in the external body surface and the gut contents of non-biting cyclorrhaphan flies in slum areas of Addis Ababa (Getachew et al., 2007). Experiments have shown that dead blowflies containing eggs of *Taenia hydatigena* can transmit infection if ingested by lambs during grazing (Lawson and Gemmell, 1985). Consumption by pigs or cattle of *Taenia* spp. egg-carrying flies may also be a transmission route for *T. solium* and *T. saginata* resulting in porcine or bovine cysticercosis. Eggs of *Taenia* spp. have been isolated from the gut contents of three *C. megacephala* flies caught on a garbage dump in Nigeria (Adeunusi and Adewoga, 2013). Transmission of eggs by adult flies may occur via A) mechanical dislodgment from the exoskeleton; B) fecal deposition; and C) regurgitation (Graczyk et al., 2001). Oribatid mites may also be involved

in transmission, with the mites potentially consuming tapeworm eggs released in the faeces of definitive hosts and intermediate host potentially acquiring the tapeworm larvae through accidental ingestion of infected mites during grazing (Mullen and O'Connor, 2002).

1.3.1.3 Dung Beetles and Parasite eggs

The ability of dung beetles to act as passive vectors carrying *Taenia* eggs was first suggested by Bily (Bily et al., 1978) and the potential of *Dermestes maculatus* to passage *T. saginata* eggs was suggested by Prokopic (Prokopic and Minar, 1980). These results were later confirmed with an experiment which demonstrated that two genus of paracoprid beetle from Kenya, *Onitis* spp. and *Helicopris* spp., remained infected with *T. saginata* eggs for up to 10 days (Mutinga, 1981). The tenebrid beetle *Ammophorus rubripes*, also coprophagic, can harbour intact *T. solium* eggs for 36 days after ingestion (Gomez-Puerta et al., 2014). Pigs have been demonstrated to eat dung beetles when consuming feces (Gonzales, 2010) and beetles are abundantly attracted to human stools suggesting a possible role of dung beetles within *Taenia* spp. transmission.

1.4 Population and Individual Control Measures

There are various proposed methods for control of *Taenia* spp. at a population level. For discussion of the measures and their efficacy we refer readers to the following reviews: (Carabin and Traoré, 2014; Thomas, 2015; Okello and Thomas, 2017).

1.4.1 Hygiene measures

Poor hand hygiene, such as not using soap to wash hands after defecation, has been associated with greater risk of exposure to cysticercosis (Vora et al., 2008) and therefore education encouraging better personal hygiene practices are important in the control of *T. solium* (Alexander et al., 2011).

1.4.2 Drug and Treatment options

1.4.2.1 Human taeniosis

Infections with the adult stage of *Taenia* spp. are responsive to the common anthelmintic drugs, niclosamide (2g/person as a single dose), praziquantel (5-10mg/kg as a single dose) (Pearson and Guerrant, 1983, Pearson and Hewlett, 1985) and Albendazole (3x400mg/person for three consecutive days) (Steinmann et al., 2011). All treatments have demonstrated between 85 to 100% efficacy (Steinmann et al., 2011) (Pawlowski et al., 2005) with Praziquantel appearing to be the most cost-effective treatment at \$0.05 to 0.1/person (Engels et al., 2003). Reported minor side-effects of praziquantel are abdominal pain, dizziness and diarrhoea (Raso et al., 2004) though there are also concerns that, due to the ability of praziquantel to cross the blood-brain barrier (BBB), there may be neurological consequences due to an inflammatory response to the death of previously undiagnosed NCC (Flisser et al., 2003). Neurological side-effects are a

potential danger in Albendazole treatment which also crosses the BBB (Sotelo and Jung, 1998). Niclosamide, conversely has little systemic absorption and therefore has no effect on NCC (Pawlowski, 2006).

1.4.2.2 Human Cysticercosis

A full discussion of treatment of human cysticercosis is beyond the scope of this chapter. Several recent reviews have provide a good overview of the disease, its symptomology and its treatment. (Garcia et al., 2014; WHO, 2015; Gripper and Welburn, 2017)

1.4.2.3 Porcine and Bovine Cysticercosis

Treatment of the larval stage of *Taenia solium* can be achieved through the use of anthelmintic treatment, with Oxfendazole (30mg/kg) demonstrating the best efficacy (Gonzales et al., 1996; Gonzalez et al., 1997, 1998; Gonzalez et al., 2001; Sikasunge et al., 2008). Oxfendazole has no reported side-effects (Gonzalez et al., 1998), has now been approved in many countries and is now being formulated specifically as Panthic 10% for this indication in pigs (Thomas, 2015). It has been suggested that treatment of pigs with oxfendazole could be utilised as a control strategy and some evidence exists for of its utility when used in combination with human MDA (Garcia et al., 2006) and/or concurrent use of a porcine vaccination against *T. solium* (Assana et al., 2010; Okello et al., 2016). Bovine cysticercosis also responds to anthelmintic treatment with praziquantel (Pawlowski et al., 1978; Thomas and Gönnert, 1978; Harrison et al., 1984) and protection against re-infection appears to last at least 12 weeks (Gallie and Sewell, 1983)

1.4.2.4 Vaccines

Vaccines have been developed against the larval infection in the porcine host and two of these (SP3VAC and TSOL18) have shown high efficacy in protecting pigs from both experimental and natural challenges (Lightowlers, 1999; Plancarte et al., 1999; Huerta et al., 2001; Gonzalez et al., 2005; Scituito et al., 2007a, 2007b; Morales et al., 2008; Lightowlers, 2010; Morales et al., 2011; Jayashi et al., 2012). Vaccination of cattle against *T. saginata* has been attempted with some success, with the TSA9/TSA18 vaccine demonstrating high efficacy in protecting cattle from infection (Rickard et al., 1981; Lightowlers et al., 1996, 2000; Harrison et al., 2005).

2.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Key to the propagation of the *Taenia* spp. lifecycle is the contact between the intermediate hosts (cattle, pigs) and human faecal material containing infective eggs. Sanitation measures such as the use of well-constructed latrines, correct management of excreta and waste water and best practise in animal husbandry all contribute to preventing the intermediate host (or accidental intermediate host in the case of *T. solium*) becoming infected. Best practise in

meat hygiene, including meat inspection and correct cooking techniques can prevent infection in the definitive (human) host. In the case of *T. solium*, human cysticercosis infections can be prevented through stringent hand hygiene to prevent faecal-oral contamination with the infective eggs.

Free-range pigs are often more at risk from infection with *Taenia* spp. than those which are kept under confined conditions (Sarti et al., 1992; Sikasunge et al., 2007; Pondja et al., 2010) and an increase in the popularity of free-range pork in Europe is identified as having the potential to increase the prevalence of porcine cysticercosis found in that region (Zammarchi et al., 2013). Housed cattle and pigs can also be exposed however, through fodder contaminated by slurry containing *Taenia* spp. eggs or due to family or farm workers defecating in the housing unit (Ilsoe et al., 1990; Sarti et al., 1992; Dorny et al., 2002; Shey-Njila et al., 2003).

The absence of a latrine on the homestead has been identified as a risk factor for porcine cysticercosis (Sánchez et al., 1998; Shey-Njila et al., 2003; Ngowi et al., 2004; Mutua et al., 2007; Kagira et al., 2010; Eshitera et al., 2012) as members of the homestead will out of necessity, engage in open defecation, therefore allowing free-ranging pigs easy access to potentially infective human excreta. It has been hypothesised that the practice of open defecation may be associated with outbreaks of bovine cysticercosis in New Zealand (McFadden et al., 2011). Improvements in sanitation have been hypothesised to be responsible for the reduction in NCC cases in Ecuador between 1990 to 2009 (Del Brutto et al., 2012) but there is not yet evidence for successful control of *T. solium* through use of specific latrine provision (Bulaya et al., 2015; Thomas, 2015). Focus group discussions with a community in Zambia where a program of 'community lead total sanitation' was undertaken, identified several barriers to the use of latrines. Barriers to use included taboos surrounding the use of latrines by men if in-laws or grown up children of the opposite sex use the same latrine (Thys et al., 2015). Studies such as this illustrate the importance of taking into account cultural norms when designing and implementing programs aiming to improve the sanitation of communities.

If sanitation infrastructure is available, the correct management of the resultant treated waste-water and sludge is incredibly important for the control of *Taenia* spp. as illustrated by the association of access of cattle to surface water and close proximity of waste water effluent with bovine cysticercosis in Belgium (Boone et al., 2007).

2.1 Detection Methods

Taenia spp. ova have a specific gravity of 1.27 (Maya et al., 2006). Modified flotation and sedimentation techniques have successfully recovered *Taenia* spp. eggs from soil (Huerta et al., 2008; Maikai et al., 2008; Nooraldeen, 2015), water (Scandrett and Gajadhar, 2004; Ayed et al., 2009; Verbyla et al., 2013), and sewage sludge (Cabaret et al., 2002) although the techniques have not been standardised. Egg recoveries appear low, with sensitivities

of 19-47% being reported for the most effective triple-flotation method (Cabaret et al., 2002), potentially due to adherence of particulate matter to the egg (Scandrett and Gajadhar, 2004). Eggs of different *Taenia* spp. cannot be distinguished on appearance (Scandrett and Gajadhar, 2004; Gajadhar et al., 2006; Huerta et al., 2008), molecular methods are available which have been utilised primarily for the diagnosis of Taeniosis, though there is potential that they could be utilised for environmental detection and allow for speciation of the eggs (González et al., 2000). Faust triple flotation technique with ZnSo₄.7H₂O (728g) plus deionised water to a volume of 1,000ml, sp.G of 1.38 (Barbier et al., 1990) had a mean recovery efficacy (E) of 35% (95% C.I. 25.2 to 42.8) (Barbier et al., 1990).

2.2 Data on Occurrence in the Environment

2.2.1 Raw sewage and sludge

Taenia spp. ova can be found in raw sewage and sewage sludge in all communities in which the helminths are endemic as demonstrated in the examples cited in section 3.0. Yet the numbers are not often reported. In Iran 1.25 eggs/l were found in raw wastewater and 0 eggs/l in the effluent from an activated sludge treatment (Mahvi and Kia, 2006). Another study found 15 eggs/g dry matter of sludge (Keller et al., 2004).

2.2.2 Surface water including recreational water

Surface water may easily be contaminated with excreta or raw sewage and in turn with *Taenia* spp.. Access of cattle to surface water has been identified as a risk factor for bovine cysticercosis in Belgium (Boone et al., 2007) and *Taenia* spp.-like ova have been detected in water piped from a local stream to a cattle feedlot in Alberta, USA (Scandrett and Gajadhar, 2004).

2.2.3 Groundwaters

Contamination of groundwater by *Taenia* spp. is unlikely due to the relatively large size of ova (Reynolds and Barrett, 2003).

2.2.4 Drinking waters

Taenia spp. ova have been detected in drinking water in Iran (Yousefi et al., 2009) and from peri-urban settlements around Harare, Zimbabwe (Dalu et al., 2011)

2.2.5 Rainwater harvesting

The authors know of no reports of *Taenia* spp. ova detected in freshly harvested rainwater

2.2.6 Seawater and shellfish

Taenia spp. are not known to infect fish or shellfish.

2.2.7 Soil

Taenia spp. ova have been found in soil samples from households in Mexico (Camacho et al., 1991; Huerta et al., 2008) and from public parks in Iraq (Nooraldeen, 2015).

2.3 Persistence (Survival) of eggs

It has been suggested that Taenia spp. eggs are one of the most resilient parasites found in excreta (Cabaret et al., 2002). The viability of Taenia spp. eggs is generally assessed by hatching studies where they are incubated using a variety of protocols. After prior incubation in acidified pepsin solution ocospheres are incubated at 37-39° in artificial intestinal solutions which contain pancreatin, ox or pig bile and sometimes foetal bovine serum. Observing motility or extrusion of hooks from the onchosphere is taken to be indicative of viability. The reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by mitochondrial enzymes with resulting blue staining of eggs has also been used to assess the viability of Taenia spp. eggs (Maravilla et al., 2011) including in sludge (Cabaret et al., 2002). The tenebrid beetle *Ammophorus rubripes* can harbour intact *T. solium* eggs for 36 days after ingestion (Gomez-Puerta et al., 2014). *T. saginata* eggs on the soil may remain infective for 5.5 to 9.5 months (Storey and Phillips, 1985; Ilsoe et al., 1990). No *T. solium* specific data is available but we would assume that the longevity is similar to that of *T. saginata*. The treatment and disposal of waste water from sewage treatment causes a dilution of the eggs explaining the low intensity of most of the infections in cattle in Western Europe. (Dorny et al., 2000).

3.0 REDUCTIONS BY SANITATION MANAGEMENT

3.1 Excreta and wastewater treatment

3.1.1 Onsite sanitation

3.1.1.1 Dry onsite sanitation systems

3.1.1.1.1 Inactivation by storage

It is assumed that cold storage of liquid sludge (4°C) has little inactivation effect on Taenia spp. eggs, with a 0.32 log₁₀ reduction (48% remaining viable) after 60 days. Increasing temperature has the greatest effect upon egg viability with temperatures of 35°C leading to 1.4 log₁₀ inactivation after 60 days of storage (Storey, 1987). Storage in drying beds appears also to be ineffective at destroying Taenia spp. ova with survival for up to 335 days being reported under cool moist conditions in the UK (Silverman and Griffiths, 1955).

3.1.1.1.2. Pit Latrines, Vault Toilets, Dry Toilets

Ecosan sludge (created after urine separation) mixed with topsoil and stored for 12 months had 0.06 log₁₀ reduction (88% of the helminth ova remained viability including Taenia spp. though specific data on Taenia spp.

was not available) indicating that there is little inactivation purely through storage at a moisture content of 57%. (Jimenez et al., 2006). Urine separation in a composting toilet in Burkino Faso with an average temp 37°C +/-2 and average moisture content 23 +/- 2% was found to be poor conditions for inactivation of helminths and therefore post-composting heat treatment of at least 50°C with moisture contents of <50% is strongly suggested (Darimani et al., 2016). The addition of quicklime (15%) resulted in 0.46 to 1.1 log₁₀ (65 to 92%) inactivation with 2hr contact time (Mendez et al., 2002). The addition of the ovicidal fungi *Pochonia chlamydosporia* has demonstrated efficacy in the inactivation of *T. saginata*. The fungus, which is a natural commensal organism of soil, appears to penetrate and attack the eggs via enzymatic activity. (Silva and Costa-Cruz, 2010).

3.1.1.1.3 Composting and ensilation

Thermal composting with temperatures over 45°C has been recommended as a method to disinfect faecal waste. The reduction in the number of tapeworm eggs is inversely proportional to the time and the final silage temperature (Vinnerås et al., 2003). The effect of ensilation (acid treatment) on Taenia spp. eggs has been modelled using Taenia hydatigena eggs ensiled in minced potatoes at 22°C for 0,7,14,21 and 28 days before being fed to lambs. No cysticerci were detected in the lambs at slaughter and regression analysis suggested that 18.6 days of should achieve a reduction of 3 log₁₀ (99.9%) in viability (Buttar et al., 2013).

3.1.1.2 Water-based onsite sanitation systems

Septic tank sludge appears to be responsible for several large outbreaks of bovine cysticercosis in Denmark (Ilsoe et al., 1990). Data are currently lacking on the viability of Taenia spp. eggs after septic tank storage, but evidence from *Ascaris* spp. indicates that periods of at least 24 months of storage may be needed (Pompeo et al., 2016). Various factors can interfere with the efficiency of a septic tank such as high volumes of rainwater contributing to a sudden increased inflow volume, resulting in dilution of the sludge interfering with the decomposition process (Silverman and Griffiths, 1955).

3.1.2 Waste stabilization ponds and aerated lagoons

Although successful removal of Taenia spp. eggs has been demonstrated through stabilisation in Brazil (Ayres et al., 1993) and Thailand (Wongworapat et al., 2001), ova, including those of *T. solium*, have been detected in the effluent from stabilisation pond systems in Bolivia (Verbyla et al., 2013) and Tunisia (Ayed et al., 2009). This may be due to the increased use of detergents, which interferes with sedimentation processes, allowing eggs to pass relatively freely into the effluent (Kyvsgaard and Murrell, 2005).

Aerated lagoons utilise heat production through exothermic degradation of organic matter by aerobic thermophilic microbes within the slurry mixture and can

achieve temperatures between 55 to 70°C. The efficacy of *Taenia* spp. inactivation depends upon a combination of time and temperature, with eggs being eliminated by exposure to treatments >60°C for 15 minutes and >70°C for 5 minutes. In theory, therefore, *Taenia* spp. should be eliminated by a typical 24hr period in an aerated lagoon (Mohaibes and Heinonen-Tanski, 2004)

3.1.3 Constructed Wetlands

The use of constructed wetlands has been studied outside Marrakech city (Morocco). Reeds were planted at a density of 5 shoots per square metre onto a substratum of organic soil (pH near 7) and superficial soil layer of approximately 1/3 clay, 1/3 silt and 1/3 sand. Four beds were constructed of 20, 30, 40 and 50m, they were irrigated weekly and the retention time varied between 1 and 4 hours depending on the bed length. All samples of untreated waste water contained helminth eggs with 8% of samples containing *Taenia* spp. eggs and the efficacy of helminth egg removal was contingent on bed length. The helminth eggs appear to be removed by sedimentation, with rhizomes and submerged reed stalks enhancing sedimentation by blocking the egg outflow. The 20m bed removed only 71% of helminth eggs with the 50m bed removing 1.3 log₁₀ of helminth eggs and removal >2 log₁₀ has been demonstrated by a combination of 24m wetland with a horizontal flow gravel bed although the *Taenia* spp. specific efficacy was not reported in any study (Mandi et al., 1998; Jimenez, 2007).

3.1.4 Wastewater Treatment and Resource Recovery Facilities

3.1.4.1 Primary /preliminary treatment

Experimental data using an 18-inch column of raw sewage, found that 15 minutes of sedimentation removed 0.3 log₁₀ of *T. saginata* eggs, rising to 1.7 log₁₀ removal after 120 minutes (Newton et al., 1949). Alternative data reported 0.49 log₁₀ reduction due to settlement of *T. saginata* eggs from raw sewage within 2hrs and 0.96 log₁₀ reduction within 3hrs although detergent content, currents and disturbances in the settling basin can affect the efficacy (Hays, 1977).

Chemically enhanced primary treatment (CEPT), utilises chemicals such as lime to cause coagulation-flocculation, which in turn accelerates the sedimentation of helminth ova into the sludge and has demonstrated 1 to 2 log₁₀ egg removal (Jimenez, 2007).

3.1.4.2 Secondary treatment

3.1.4.2.1 Trickling filters

Experimental data from the 1940s suggest that gravel (3.5 ft depth of 1.5" gravel) trickle filters loaded at 0.27 to 0.33lbs biological oxygen demand with a filtration of 3 to 4hrs, were not very effective at removing *Taenia saginata* eggs from waste water removing only 0.15 log₁₀ to 0.21 log₁₀ of eggs (Newton et al., 1949). For helminth eggs in general

efficacies of only 0.3 log₁₀ up to 1.3 log₁₀ reductions have been reported for trickling filtration (including sedimentation, sludge digestion and sludge drying) (Feachem et al., 1983).

3.1.4.2.2 Activated sludge

In Iran 1.25 eggs/l were found in raw wastewater and 0 eggs/l in the effluent from an activated sludge treatment (Mahvi and Kia, 2006) and *Taenia* spp. ova were also successfully removed from treated effluent in Thailand (Wongworapat et al., 2001). In Tunisia, however 0.89 log₁₀ removal (13% (95% C.I. 0-38%) passing through treatment) was observed of *Taenia* spp. ova by examining the effluent (Ayed et al., 2009). Data from the 1980's suggests that for helminth eggs in general removals of 0.3 log₁₀ to 1.0 log₁₀ of eggs has been reported by activated sludge including the sedimentation, digestion and drying processes (Feachem et al., 1983).

3.1.4.3 Anaerobic/ anoxic digestion and biogas

Laboratory scale (1.5 gallon) anaerobic digestion at room temperature was found to be ineffective in destroying *T. saginata* ova for up to 1 months. By 2 months, viability, as assessed by morphological changes, was found to decrease by 0.3 log₁₀ and continued to gradually decrease thereafter with only 30% of eggs recovered having a 'normal' appearance by 203 days in the digester (Newton et al., 1949). Efficacy of anaerobic digestion appears to be dependent on temperature, the situation of the ova (free or bound in proglottids) as well as whether the process is continuous or batch. Digestion at 24-30°C was found to result in 1.5 log₁₀ reduction (3% survival) of *T. saginata* at 6 months (Hays, 1977) compared to a 3 log₁₀ and greater reduction efficacy after 28 days at 35°C (Cabaret et al., 2002). The effect of eggs bound in proglottids was shown in another study with laboratory scale anaerobic digestion at 20°C leading to 2.4 log₁₀ reduction of initial inoculum of free eggs being viable at 52 days whereas some proglottid-bound eggs were found to still be viable at 88 days. The same study also indicated that continuous digestion at 35°C was more effective than batch digestion at the same temperature, with no viable eggs found after 10 days of continuous digestion in comparison to some viable eggs remaining after 30 days of batch digestion (Storey, 1987).

3.1.4.4 Up-flow Anaerobic Sludge Blanket (UASB)

The UASB reactor removes helminth eggs through a combination of sedimentation and egg entrapment within the sludge bed. A laboratory scale UASB with 8hr retention time, demonstrated total removal of helminth eggs (El-Gohary and Nasr, 1999) with another laboratory scale reactor demonstrating 93 +/- 5% removal at low up-flow rates of 0.9 m.h⁻¹, though decreasing in a linear fashion with increasing up-flow rate (Yaya-Beas et al., 2015). This efficacy has not been replicated in the real world, an investigation in Brazil indicated a 0.6 log₁₀ reduction efficacy of the UASB unit with retention time of 5.5hrs in removing helminth eggs (no *Taenia* spp. present in the raw sewage) and in combination with a baffled polishing pond

resulted in complete removal of helminth eggs in 13 of 14 effluent samples with a mean of 0.1 helminth egg/l across all samples (Von Sperling et al., 2002). A study in Bolivia found poor efficacy of a UASB in removing helminth ova with only 0.11 log₁₀ of ova removed with 30% of the ova in the USAB effluent being viable. In this study 78.9% of ova in the raw sewage inflow were identified as *Taenia* spp., and both *Taenia* spp. and *Ascaris* spp. were detected in the system effluent (Verbyla et al., 2013). This indicates that a USAB is not sufficient to remove *Taenia* spp. and must be combined with post-treatment.

3.1.4.5 Filtration

Studies of working rapid sand filtration systems in Coventry (UK) in the 1950's found that the current operational practises were insufficient to remove *Taenia* spp. ova. At a flow of 1400 gallons per hour through sand 2 feet deep *T. saginata* ova were recovered in 13 of 14 samples of the effluent taken between 5 and 130 minutes of addition of the ova to the inflow and over 0.3 log₁₀ removal of the total ova introduced to the filter when examining the final effluent (Silverman and Griffiths, 1955). The speed of filtration, effective particle size and depth of sand however does influence the efficacy of sand filtration systems with slow sand filtration systems around Johannesburg (SA) appearing to remove all *Taenia* spp. ova from the effluent (Newton et al., 1949). Laboratory studies demonstrated removals of 3 to > 3log₁₀ of *T. saginata* ova, with sand of 0.5mm effective size and 2.2 uniformity coefficient and a filtration rate of 1,000,000 gallons per acre per day (Newton et al., 1949).

3.1.4.6 Membranes. Micro, Ultra And Reverse Osmosis

Membrane technologies such as micro filtration, using a very finely woven fabric of stainless steel were introduced to the UK in 1945 and experimental evidence from the 1950's suggests they are capable of removing 90% of *T. saginata* ova (Silverman and Griffiths, 1955). There is some

evidence that membrane bioreactors produce effluent with <1 helminth egg/l (Moeslang and Brockmann). Dual-media filtration is estimated to have a theoretical removal rate of approximately 2 log₁₀ of helminth ova (Jimenez-Cisneros and Maya-Rendon, 2007).

3.1.5 Land treatment

Cysticercosis due to *T. saginata* appears to be one of the major pathological threats when sewage sludge is used to fertilise cattle pastures in temperate areas. Land application of sludge is inefficient in destroying the eggs of *Taenia* spp. with reports of eggs remaining viable on grass for up to 159 days (Straub et al., 1993; Cabaret et al., 2002).

3.2 Disinfection as Tertiary (Post Primary) Treatment

3.2.1 Chemical

Treatment of raw sewage sludge with an initial concentration of 15 egg/g dry matter with quicklime at 30 to 60% was found to eliminate helminth eggs. Concentrations of 10 to 20% quicklime resulted in a few non-viable helminth eggs being detected (Keller et al. 2004). Chlorination of wastewater has been reported to achieve no inactivation up to to 0.1 log₁₀ inactivation of helminth ova under laboratory conditions as reviewed by (Jimenez-Cisneros and Maya-Rendon 2007).

3.2.2 Ultraviolet

There are no data currently available on the efficacy of UV irradiation on *T. solium*.

3.2.3 Pasteurization

Pasteurization at 65 to 70°C for 15 to 30 minutes has been reported to inactivate > 2 log₁₀ of helminth ova from sludge (Keller et al., 2004; Paulsrud et al., 2004)

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