

2. LITERATURE REVIEW

2.1 *Taenia solium* complex

2.1.1 General overview

Porcine cysticercosis is a parasitic zoonotic disease in pigs caused by the larval form of the tapeworm, *Taenia solium* that is transmitted between pigs and humans as well as among human being (Ngowi *et al.*, 2004).

Man is the only natural definitive host while pigs are the natural intermediate hosts. When humans eat the larval stage in raw or inadequately cooked infected pork, an adult tapeworm develops in the small intestine resulting into a condition known as taeniosis. Gravid proglottids or worm eggs are released and come out with feces from tapeworm carriers and may contaminate environments in case of uncontrolled fecal disposal. When the pig ingests infective eggs, the oncospheres hatch and develop into the larval stage (*Cysticercus cellulosae*) in muscles and organs, completing the life cycle of the infection stages for humans, resulting in a condition known as human cysticercosis (Soulsby, 1982).

The tapeworm eggs are also infective if ingested by humans as in pigs; they develop into cysts, which are found in the muscles and in nervous tissue. The cysticerci may lodge in the brain causing cerebral cysticercosis (neurocysticercosis), a very serious zoonosis-causing headache, epileptic seizures, epilepsy, mental disturbance and death (Garcia *et al.*, 1997; White, 2000). Neurocysticercosis is recognized as a serious zoonosis of public health concern because it causes disability of the infected persons and may be fatal if left untreated (WHO, 1979). Studies from countries where neurocysticercosis is endemic have shown that up to 50% of all cases of adult-onset epilepsy cases globally are due to neurocysticercosis and that the prevalence is rising (Tsang and Wilson, 1995). In addition, porcine cysticercosis represents one of the most important constraints to increased pig production in the

developing world, especially affecting the economic and nutritional well being of the rural poor and veterinary public health importance. *T. solium* causes great economic losses in the pig industry due to condemnation of infected carcasses. A very conservative and rough economic estimate indicates that the annual losses due to porcine cysticercosis in developing countries are millions of Euros per year (Zoli *et al.*, 2003).

The transmission of *T. solium* is associated mostly with environments with low socio-economic or low sanitary hygienic concerns, deficient sanitary facilities, poor meat inspection and control or poor pig husbandry practices where pigs can gain access to human feces (Phiri *et al.*, 2002).

Infection with *Taenia solium* is widely prevalent in humans and swine hosts and most common in many developing countries of Latin America, Africa, and Asia (Plancarte *et al.*, 1999). However, with tourism and increasing migration of people harboring tapeworms, human cysticercosis is now transmitted worldwide and it is considered as an emerging disease in the United States of America (Flisser *et al.*, 1998; Schantz *et al.*, 1998). Human cysticercosis has been reported in non-pork consuming communities including an Islamic community and an Orthodox Jewish community (Schantz *et al.*, 1992). In India, more than 95% of patients with neurocysticercosis are vegetarians or do not consume pork (Rajshekhar *et al.*, 2003).

Small-scale producers in rural areas raise most pigs on free ranges. Organizations, private companies and schools within an intensive system, however, raise a small fraction. Land scarcities, rapid turnover of the pigs, and increased pork consumption in some urban areas of the country have contributed to the increase in the rural pig production. Most of the pigs in small-scale farming are allowed to scavenge, looking for their foods especially when there are no crops in the field. The majority of these pigs only spend the night in poorly constructed houses. This practice predisposes pigs to malnutrition, intake of human feces, and various infections.

2.1.2 Morphology

An adult cestode always lives as a single in the middle of the small intestine. The adult tapeworm grows up to 5 meters in length. The head or scolex is armed with four suckers and rostellar hooks, an elongated neck and strobila. The strobila is segmented in proglottids containing male and female reproductive organs. The immature, mature and gravid proglottids differ from each other in size, shape and stage of development. The mature proglottids are almost rectangular up to 12 mm in length, located towards the very distal end of the strobila and each is packed with an uterus full of eggs (Singh *et al.*, 2002). The gravid segments have 7-13 lateral uterus branches and they do not usually leave the host spontaneously, but leave passively in chains with the feces, *i.e.*, gravid proglottids detach from the strobila by apolysis. The ovary has three lobes, there is no vaginal sphincter muscle and the cirrus sac extends to the excretory vessels (OIE, 2004). *Taeniid* eggs are round or sub-spherical in form, measure approximately 30-45 μm in diameter and are characterized by a thick brownish shell containing an oncosphere 30x20 μm in size, bearing 3 pairs of hooklets. The adult tapeworm can shed up to 3,000,000 eggs daily. Each gravid proglottid of the tapeworm has approximately 40,000 eggs (De-Bittencourt *et al.*, 1996; Hoberg, 2002)

2.1.3 Life Cycle

The life cycle of *Taenia solium* includes different developmental stages:

- (a) Pre-adult: a stage after ingesting the cysticercus, and growing up to mature (pre-patent period)
- (b) Adult: the reproductive stage with mature proglottids (patent period).
- (c) Egg: a small embryo covered by an embryophore and a thick shell, a stage responsible for dissemination to the external environment surviving up to one year.
- (d) Oncosphere: a hexacanth embryo, which migrates from the intestine to internal tissues or organs within the intermediate host.

- (e) Post-oncosperal form: the migrating intermediate stage between an oncosphere in the tissues and fully developed cysticercus.
- (f) Cysticercus: a bladder metacestode form with one invaginated scolex that parasitizes tissues of the intermediate host, mainly pigs and humans (Singh *et al.*, 2002).

T. solium requires two hosts to complete its life cycle.

Development in pig: A human infected with an adult tapeworm excretes eggs or gravid proglottids into the environment through feces, and the eggs can survive outside for several months up to one year. Pigs become infected by ingesting the eggs or proglottids via contaminated food or water. The oncospheres hatch in the small intestine under influence of gastric and intestinal juices, penetrate the intestinal wall into the blood, venous vessels or lymph system, and then are distributed by circulation to the muscle via liver, lung, heart and CNS where they grow up to cysticerci. Cysts have different sizes depending on the period of infection, for example 20 days-pinhead, 60 days-pea with the head visible and up to 2-3 months the cysts containing a translucent bladder with the invaginated scolex, surrounded by a capsule of host connective tissue (Joseph *et al.*, 1999).

Development in humans: Humans can become infected with the adult tapeworm by eating raw or uncooked pork containing cysticerci or infected with the larval tapeworm by accidentally ingesting *T. solium* eggs from the environment or contaminated food and water, which develop into a single adult worm inhabiting in the small intestine. After ingestion of cysticercus, the scolex evaginates and attaches to the mucosa of the small intestine. Proglottids develop from the base of the neck. The mature proglottids are 1 cm wide, 1.2 cm long, and 2–3 mm thick. Eggs and/ or proglottids are shed intermittently in the stool. Tapeworm carriers usually note few symptoms other than observing proglottids passed with stool. Excretion is intermittent, thus stool examinations for ova can be negative. Human cysticercosis, however, results from ingestion of ova shed by a human tapeworm carrier. Close personal contact with or perhaps food preparation by a tapeworm carrier is noted in

most cases. Autoinfection may also occur. In the intestines the larvae hatch from *Taenia* eggs, penetrate the intestinal mucosa, enter the blood stream, migrate to the tissues, and develop into cysticerci in muscles and also in brain (White, 2000).

Cysticercus can develop in muscles and in the brain. In the latter organ, different types of cysticerci exist in CNS called neurocysticercosis: sterile (unfertilized), fertile, or cluster-shaped cysts, which are called *Cysticercus racemosus*. Sometimes, they reach infectivity also in other organs such as the eyes and subcutaneous tissues (Botero *et al.*, 1998).

2.1.4 Pathogenicity and clinic

(a) Symptoms in pigs with cysticercosis: Infected pigs are usually asymptomatic except in heavy infection pigs may have muscular stiffness and possible loss of condition.

(b) Symptoms in humans with cysticercosis: Symptoms may appear months to years after infection, usually when the cysts are in the process of dying and calcifying. Symptoms will depend on the location and number of cysticercus at various sites of body.

Cysticerci in muscles: Humans can be affected directly by the eggs of *T. solium* from environment or autoinfection by tapeworm carriers growing to pea like cysts in different muscles or subcutaneous tissues, called muscle cysticercosis. Humans with cysticercus in muscles do not have symptoms of infection.

Cysticerci in the eyes: Eye infection can cause blurry or distributed vision in the cyst stage. Infection may also cause swelling or detachment of the retina (Cardenas *et al.*, 1992).

Cysticerci in the brain and spinal cord: Neurocysticercosis is a severe disease. Symptoms depend upon where and how many cysticerci are found in the brain.

Seizures and headaches are the most common symptoms. When these happen, the brain can swell. The pressure caused by swelling is what causes most of the symptoms of neurocysticercosis. However, confusion, lack of attention to people and surroundings, difficulty with balance, swelling of the brain (called hydrocephalus) may also occur. Death can occur suddenly in the case of epilepsy-like symptoms in heavy infections (White, 2000). Inactive: is located in parenchymal calcification enhancement and chronic hydrocephalus. A typical symptom is seizures and symptoms of increased intracranial pressure (White, 2000). Active: is located in parenchymal, ventricular, subarachnoid (cisternal), ocular, spinal subarachnoid radiculopathy or myelopathy and spinal intramedullary. Typical symptoms are seizures, hydrocephalus, stroke, visual changes and myelopathy (White, 2000).

(c) Adult tapeworm infection in man: The first symptom is itching around the anus due to the migrating proglottids. Other clinical signs include abdominal pain, digestive disturbances, diarrhoea, constipation, nervousness, nausea and vomiting as well as loss of weight (Garcia *et al.*, 1999).

2.1.5 Epidemiology

Porcine cysticercosis is worldwide in distribution. *Taenia* eggs are very highly resistant and can long live in the environment (Schantz, 2002). The main source of infection of pigs is pollution of the environment by sewage and uncontrolled distribution of human feces. Free-range pigs with the disposal of infected human feces particularly in rural and suburban areas, the poor hygiene, low education and the lack of or improperly conducted meat inspection in urban areas lead to a high prevalence (Martin *et al.*, 1987; Singh *et al.*, 2002).

Autoinfection in humans: Cysticercosis in man may also be acquired by direct transfer of *T. solium* eggs from the feces of individuals harboring an adult worm through internal and external autoinfection. External autoinfection implies fecal-oral infection with *T. solium* eggs in an individual with intestinal taeniosis. The reason is neglect of hygienic standards such as washing hands after defecation and before

consuming meals. Internal autoinfection implies infection with eggs through reverse peristalsis and appears improbable since eggs are required to pass through a brief period of peptic digestion that is necessary for disintegration of the embryophores before being invasive to human tissues. Autoinfection is also caused vomiting and swallowing proglottids of *Taenia solium* carriers (Garcia-Noval *et al.*, 1996).

Taenia solium cysticercosis has long been recognized as highly endemic in Latin America. However, in the past few years more data have become available from Asia and Africa, which reveal that the prevalence of *T. solium* in these continents is as high as or higher than those in Latin America (Geerts *et al.*, 2002; Ito *et al.*, 2002; Singh *et al.*, 2002)

Transmission may occur in the highly endemic rural areas in Asia, where pigs usually get infected by food or roaming in areas contaminated by human feces (which can come from sewage water or direct pollution). It has been stressed that *T. solium* cysticercosis in Asia manifests as both neurocysticercosis and subcutaneous cysticercosis. In Japan and South Korea as well as Central Europe, where hygiene is good, *T. solium* has been eradicated (Pawlowski, 2002; Gilman *et al.*, 1999).

Occurrence of neurocysticercosis: Neurocysticercosis is highly prevalent in developing countries, where it constitutes a serious public health problem. Millions of people are affected by *Taenia solium*/cysticercosis in Latin America, Asia and Africa, where the disease is a factor in the relatively high prevalence rate of epilepsy (Preux *et al.*, 1996). Neurocysticercosis is commonly found in India, China, Central and South America and Mexico as well as Southeast Asia countries. Local people live with pigs, which scavenge and are therefore infected with cysticerci of *T. solium* (Gemmell *et al.*, 1983; De-Aluja *et al.*, 1998). Eating dog meat is still not rare in Asia, especially in Korea, China and Vietnam, some parts of Indonesia, including North Sumatra, Java, Sulawesi, and Papua. Therefore the life cycle of *T. solium* could be maintained through the dog-human cycle as well as the pig-human cycle because exceptionally, *cysticercus cellulosae* can develop in dogs and cats (Gonzalez *et al.*, 1994). The situation with *T. solium* taeniosis/cysticercosis in other islands in

neighbouring countries, including East Timor and Papua New Guinea, needs to be studied (Geerts *et al.*, 2002).

2.1.6 Diagnosis

Diagnostic methods of confirmation focus on:

Human taeniosis: The specimens usually required for diagnosis are feces or bloods.

Adult tapeworm infection: Adult cestodes can be expelled from infected humans using an anthelmintic followed by a purgative and are identified on the basis of a single proglottid or chains of segments migrating through the anus. In addition, DNA probes and polymerase chain reactions (PCR) by using *Taenia* material in feces can differentiate human *Taenia* spp. Today, several methods have been used to identify *Taenia* eggs for the diagnosis of *Taenia solium*:

(a) Detection of eggs: Eggs or embryophores with a thick, striated, brownish shell can be detected using different methods. The simplest procedure is the flotation method with a saturated salt solution to detect the eggs in stool specimens or excreted proglottids (Allan *et al.*, 1996; Rodriguez-Canul *et al.*, 1999). *Taenia solium* eggs, however, can not be distinguished from the eggs of other *Taenia* species, *i.e.*, *T. saginata* and *T. asiatica*.

(b) Identification of proglottids: Worms can be obtained from egg-positive humans by giving anthelmintic drugs followed by a purgative. Alternatively, proglottids sometimes appear in feces. Species identification is done by injecting India ink into the uterus of gravid proglottids and counting the number of lateral uterine branches. For mature proglottids, carmine stain reveals the number of ovarian lobes (Morakote *et al.*, 2000).

(c) Copro-antigen detection: Adult *Taenia* infections in humans can be recognized by detection of *Taenia* antigen in human feces using antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA), but the test does not differentiate species (OIE, 2004). This method greatly improved convenience and sensitivity in comparison with the detection of eggs in stool specimens. On the other hand, this method is very costly and is not commercially available.

(d) Antibody detection: Immunoblot assay for the detection of antibodies against *Taenia solium* has been developed (Wilkins *et al.*, 1999).

Currently, both the copro-antigen test and immunoblot assay are very useful for the detection of taeniosis patients, with confirmation of expelled worms by morphology as well as DNA analysis recommended.

Human cysticercosis: Imaging techniques and biopsy of the tissues containing parasites provide direct diagnosis of cysticercosis. Alternatively, Ag-ELISA, antibody AB-ELISA, and immunoblot provide indirect diagnosis.

(a) Tissue biopsy: specimens of subcutaneous or brain tissues are sometimes removed surgically. Tissue section and stain in pathological laboratory reveals scolex with hooks typical of *T. solium* cysticercus.

(b) Imaging techniques: usually, the diagnosis of neurocysticercosis can be determined on the presence of cysts or typical calcifications by magnetic resonance imaging (MRI) or computerized tomography (CT) scan. In the clinical practices, computerized tomography (CT) scan and magnetic resonance imaging (MRI) are used to detect the exact locations and viability of *T. solium* metacestodes. Calcified cysts are also detected by radiography (White, 2000).

(c) Serological detection: several serological techniques are used for detection of anti-cysticercus antibodies such as ELISA and immunoblot. However, not all cysticercosis patients are positive. For neurocysticercosis, the enzyme-linked immunoelectrotransfer blot (EITB) or immunoblot assay is highly specific and commercially available (OIE, 2004).

(d) Molecular technique: while PCR tests have been used largely for the differentiation of adult taeniids in humans, they could be usefully applied to identify species of metacestode infection too.

Porcine cysticercosis: Diagnosis of cysticercosis in pigs can be difficult due to short life and absence of symptoms and may require several testing methods as follows:

(a) Ante mortem procedure: Palpation of tongues in living animals, particularly for the presence of cysts *T. solium*, still remains widely used in surveys, but the most common diagnosis of cysticercosis in pigs has been meat inspection.

(b) Post mortem procedure: in slaughter animals visual meat inspection searching for cysts in whole carcasses including organs is employed. Cysts are essentially found in the following muscles: hearts, tongue, esophagi, masseters and diaphragm, shoulder muscles, intercostals muscles and livers (Evans *et al.*, 1997).

(c) Serological Test: the serum antibody detection such as ELISA is not used for the diagnosis of cysticercosis due to lack of commercial availability on the market. On the other hand serology is too expensive for routine, particularly because of several false positives (Sciutto *et al.*, 1998).

(d) Molecular detection: commonly, PCR can be used to differentiate cysticercus species, but the kits are not available commercially (Sciutto *et al.*, 1998).

2.1.7 Therapy and prevention:

In humans: Therapy exists for humans when diagnosis is made in time.

(a) Adult tapeworm infection: For treatment of individuals with *T. solium*, intestinal taeniosis, the drug of choice is praziquantel at dose 5–10 mg/kg body weight (Sarti *et al.*, 2000). A single dose of praziquantel or niclosamide is sufficient to expel adult worms of *T. asiatica*, or even *T. solium* (Allan *et al.*, 1997). Moreover, adult cestodes can be expelled from humans using an anthelmintic followed by a purgative, in the case of niclosamide praziquantel worms are expelled immediately.

(b) Larval tapeworm infection: In the case of neurocysticercosis, albendazole is the treatment of choice, although praziquantel may also be useful (Garcia, 2003). They are treated with albendazole at a dosage of 15 mg/kg/day for 2 weeks and praziquantel at a dosage of 100 mg/kg/day for 2 weeks additionally. To decrease inflammation in active disease, immunosuppressive agents may be used such as corticosteroids (except pregnant women), but prolonged treatment with steroids is not recommended (Evans *et al.*, 1997). In this case efficacies are up to 80% complete disappearance of the cyst, 10% decrease in the size of the cyst and 10% failure. In heavy infection, surgery is sometimes necessary for treatment, such as ocular cysticercosis (Gonzalez *et al.*, 1999).

According to OIE guidelines for meat inspection: The infected carcasses or meats containing living cysts are inactivated by cooking or heating at 60°C for 15 to 20 minutes and prevention is done through hygiene and proper meat inspection at the slaughterhouse. Deep-freezing at -20°C for 4 days can destroy cysts. In cases of moderate infestation, the meat has been processed using one of the methods provided in the "Recommended International Code of Practice for ante and post mortem judgment of slaughter animals and meat", namely: freezing or heat treatment at 60°C (140°F) (FAO/WHO - CAC/RCP 34-1985; OIE, 2004; Hillwig, 1987). No vaccines have been developed so far. Many investigations have been done to vaccinate against cysticercosis in pig, but unfortunately no commercial vaccines is on the market.

The prevention and control of *T. solium* cysticercosis can be applied through health education and better sanitation. In the high risk areas there are two strategies for health education of people free of taeniosis. First, people in endemic areas do not eat uncooked or undercooked meat containing viscera (cysts). The second is to keep pigs indoors without contact with human feces (Lightowers, 1999). Cutting off the life cycle of *T. solium* depends on sustainable public-health education, such as washing hands with soap and water after using the toilet and before handling food; not touching contaminated raw meats; cleaning and peeling all raw vegetables and fruits before eating; drinking only bottled or boiled water or carbonated (bubbly) drinks in

cans or bottles, and not drinking fountain water. Another way to make water safe is by filtering it through a filter and dissolving iodine into it (Sarti *et al.*, 1997).

Porcine cysticercosis: the therapy of cysticercus stage in pigs is not available (economical or unfit) and vaccination is not completely safe as well, as it is not yet commercially available.

2.2 *Taenia asiatica* complex

A new taeniid species named *Taenia asiatica* is closely related to but genetically distinguishable from *T. saginata* (Eom and Rim *et al.*, 1993). The adult worm in humans has an ovary, vaginal sphincter muscle and cirrus sac like those of *T. saginata*, but Asian *Taenia* does have a rudimentary rostellum with rudimentary hooklets, posterior protuberances on segments, and total length and number of proglottids less than *T. saginata*. *T. asiatica* is 5-8 meters in length, proglottids and side branches of the uterus seem similar to *T. solium*. The metacestodes are small and sometime have two rows of primitive hooks. Otherwise, *T. asiatica* metacestode (*Cysticercus viscerotropica*) was different morphologically from *T. saginata* metacestode (*Cysticercus bovis*) in having wart-like formations on the external surface of the bladder wall (Eom *et al.*, 1998). They occur in viscera, mainly in the liver of domesticated and wild pigs, occasionally in cattle, goats, and monkeys. Based on the morphologic characteristics of adult and metacestodes of Asian *Taenia saginata*, the third kind of human taeniid tapeworm is known to spread in Asian countries, particularly Southeast Asian countries. The life cycle of *T. asiatica* appears to be completed in rather remote areas, where pigs roam with free access to human feces in many Southeast Asian countries, similar to the situation with *T. solium* in Asia (Fan *et al.*, 1995). *T. asiatica* has been also found in Taiwan, Korea, China, Vietnam, and Indonesia; at least where people eat uncooked pork or pig viscera after killing pigs at home. But it has been difficult to differentiate between *T. saginata* from beef and Asian *Taenia* from pork (Simanjuntak *et al.*, 1997; Eom *et al.*, 2002; Erhart *et al.*, 2002). The life cycle of this cestode was also different from classical *T.*

saginata in its intermediate host animals as well as infected organs such as the liver, omentum serosa and lung of pigs in its larval stage, but CNS penetration is never recorded (Eom, 1993).

2.3 Differentiation of cysticercus species

The tapeworms of the genus *Taenia* that infect human beings are *T. solium*, *T. saginata* and *T. saginata asiatica*. *Taenia solium* and *T. saginata* exhibit unequivocal features that characterize them. In contrast, only recent DNA studies, morphological characteristics, and epidemiological and sanitary aspects indicate that *T. saginata asiatica* is a subspecies of *T. saginata*. These 3 tapeworms occur in humans in their adult stage, and the intermediate hosts are pigs for *T. solium* and *T. asiatica* and cows for *T. saginata*. Their identification is crucial considering the migratory increase from Asia to the Western Hemisphere and the fact that these tapeworms coexist in the same environment in Asia; furthermore, it is estimated that movement in both directions across the United States–Mexico border exceeds 200 million persons per year, and thus, opportunities for acquiring and transporting *T. solium* infections are multiplied. It is not easy to distinguish among these tapeworms; therefore, a comparative diagram of the 3 parasites is shown in this article, which will facilitate their identification. All morphological features, some of which allow for identification, are clear and can be easily distinguished among the 3 tapeworms (Flisser *et al.*, 2004).

Today, difference among these parasites can be definitely demonstrated by using molecular study such as PCR. Species-specific identification of human tapeworm infections is important for public health purposes, because prompt identification of *Taenia solium* carriers may prevent further human cysticercosis infections (a major cause of acquired epilepsy). Two practical methods for the differentiation of cestode proglottids:

- (a) Routine embedding, sectioning, and hematoxylin-eosin (HE) staining,
- (b) PCR with restriction enzyme analysis (PCR-REA) (Mayta *et al.*, 2000).

The morphological differentiation of three tapeworms is indicated in Table 1.

Table 1: Morphological differentiation between *T. solium*, *T. saginata* and *T. asiatica*

Characteristics	Morphological differentiation		
	<i>T. solium</i>	<i>T. saginata</i>	<i>T. asiatica</i>
Metacestodes			
Intermediate host	Pigs, humans, dogs and wild boars	Cattle, buffalo and reindeer	Pigs, cattle, goats and wild boars
Site of location	Brain, skin, eye, tongue and muscle	Muscles, viscera & brain	Viscera, mainly livers
Size (mm)	5.6-8.5 x 3.1-6.5	7-10 x 4-6	2 x 2
Scolex	Rostellum with hooklets	No rostellum, no hooks	Rostel. with no or rudiment. hooks
Bladder surface	Wart-like formation	Rugae	Wart-like format.
Adult tapeworms			
Scolex:	Rostel. with hooks	No rostell. hooks	R. with no hooks
Diameter (mm)	0.6-1.0	1.5-2	0.8
No. of suckers	4	4	4
Diameter of sucker	0.4-0.5	0.7-0.8	0.24-0.29
Rostellum	Present	Absent	Present
No. of hooks	22-32	Absent	Absent
Proglottides: N ^o	700-1000	2000	200-1016
Length (mm)	10-16	4-13	5-9.5
Maximal breadth	7-10	8-11	3-5
Mature of progl.: No.	375-575 (testes)	800-1200	868-904
Ovary	Three lobes	Two lobes	Two lobes
Vaginal sphincter	Absent	Present	Present
Gravid proglottides:			
No. of uterine branch	7-12	15-32	16-21
Branching pattern	Dendritic	Dichotonous	Dichotonous
Expulsion from host	Mainly in group passively	Single, actively and spontaneously	Single, actively and spontaneously

R., Rostel.= Rostellum; Rudiment.=Rudimentary; Format.= Formation

2.4 *Trichinella* complex

2.4.1 General overview

Trichinellosis is mainly important, parasitically and zoonotically, because of its public health significance for more than 150 years (Ljungstrom *et al.*, 1998). The *Trichinella* species was first reported in man in 1835 and was first recorded in the United States in 1846. It is a globally distributed zoonotic disease caused by the ingestion of raw or undercooked meat harboring larval muscle parasites of the genus *Trichinella* (Gamble, 1997). The adults of *Trichinella* are at least 3-5 mm in length and may be found in the small intestine of humans, pigs, rats, bears and many other flesh-eating mammals, but may also occur in horses that have eaten fodder containing dead infected rodents (Dupouy-Camt, 1997). *Trichinella* larvae have low host specificity and are capable of infecting a broad range of carnivores and omnivores i.e. at least all vertebrates including birds and reptiles (Kapel *et al.*, 1998).

The discovery of *Trichinella zimbabwensis* in farm crocodiles of Zimbabwe has opened up a new frontier in the epidemiology of the *Trichinella* genus (Murrell *et al.*, 2000). There have been ten *Trichinella* genotypes described so far, eight of which are considered to warrant valid species status:

(a) *Trichinella spiralis* (T-1) belongs to the domesticated cycle and is found in temperate regions worldwide and is commonly associated with domestic pigs and rats living in food competition. It is one of the main zoonotic helminthosis and is highly infective for pigs, mice and rats, as well as man.

(b) *Trichinella nativa* (T-2) is a cold-climate-adapted species and belongs to the sylvatic cycle. It has limited infectivity for pigs, but is commonly found in wild canids, bear, walrus and wild pig-like carriers, only in remote areas far from the influence of human civilization. It is further distinguished its resistance to freezing (Kapel *et al.*, 1997).

(c) *Trichinella britovi* (T-3) belongs to the sylvatic cycle and is found predominantly in wild animals, although it may occasionally be found in pigs or horses. It occurs in the temperate regions of Europe and Asia. *Trichinella britovi* has some of the intermediate characteristics of other species; including some resistance to freezing, moderate infectivity for swine and slow capsule formation (larvae have been confused with non-encapsulating species in some cases).

(d) *Trichinella* T-8 is an isolate from Africa that is similar to *T. britovi* and *Trichinella* T-9 from Japan, but is found to differ by molecular analysis.

(e) *Trichinella pseudospiralis* (T-4) does not form a capsule in muscle, is cosmopolitan in distribution, and has been recovered from raptorial birds, wild carnivores, rats and marsupials in Asia, North America and the Australian subcontinent (Raue *et al.*, 2000).

(f) *Trichinella murrelli* (T-5) is a North American species found in wildlife and occasionally horses and humans. It has low infectivity for domestic pigs, but poses a risk to humans who eat game meat.

(g) *Trichinella papuae* (T-6 or T10) is a very small 2-4 mm long hair-like worm, dwelling deep in the mucus membranes of the small intestine and does not form a capsule in muscle. To date, it has only been reported from Papua New Guinea. *Trichinella papuae* is found in North America. It is resistant to freezing, has low infectivity for pigs, is found in a variety of wild mammals and has been implicated in human disease. But all species may infect man (Pozio *et al.*, 1999).

(h) *Trichinella nelsoni* (T-7) has been isolated sporadically from wildlife in Africa. It is characterized by greater resistance to elevated temperatures as compared with other species of *Trichinella*.

(i) *Trichinella zimbabwensis* adult worms were collected from the intestine and larvae from the muscles of reptile species (OIE, 2004; Dick *et al.*, 2001).

2.4.2 Morphology

Trichinella species belong to the family *Trichuridae* and mainly measure approximately 1-7 mm in length, are unsegmented, cylindrical and tapered at both ends; the esophagus is extremely elongated surrounded by stichosoma, living deeply pierced in the small intestinal wall. Male worms are about 1.4 - 1.6 mm long with an almost terminal anus, they have no spicules, but they have twin terminal appendages and papillae. Moreover, they have a stichosome with a short muscular esophagus and die soon after copulation. Females are about twice the length of males with a similarly located anus. The vulva is located about half way along the pharynx. The single uterus is filled with developing eggs in its posterior region. The females are viviparous, laying first larvae and then dying shortly after the completion of oviposition (Corwin *et al.*, 1999).

2.4.3 Life Cycle

Trichinella species are not very host specific. They infect a very broad range of host species including vertebrates. The definitive host becomes infected when raw or poorly cooked meat containing the infectious stage of the muscle larvae (first-stage larva) is eaten. Larvae are encysted in the muscle fibers. On passage through the stomach of the host, the larvae are released from the cyst. In the small intestine the larvae rapidly penetrate the mucus membrane where they undergo successive moultings to become young adults, within 30 hrs post-infection. In 5-6 days they moult 4 times to become adult worms and shed larvae. The adults mate deep within the mucus membrane where they are regarded as being intracellular parasites, lying within a serial row of host cells. The female worms produce about 1,500 larvae over 4-6 weeks. Males die soon after copulation and the females die shortly after the completion of larvae-position. Newborn larvae enter the blood or lymph vessels, flood into all organs by way of the circulatory system, and after 3 weeks spread throughout the body. Finally the larvae reach striated muscle where they penetrate the individual muscle fiber. They have predilection sites for highly active muscles such as the tongue, masticatory muscles, intercostal muscles, diaphragm, eye muscles and the

muscles of the arms and legs. The larvae absorb nutrients from the muscle cells and increase their length to about 1 mm. They finally coil and remain dormant until eaten and enter the digestive system of the next host. The larvae for nourishment require the muscle cell, and the worm induces changes in muscle cell structures so the larvae stay alive as long as the muscle fiber does not degenerate. The changing muscle fibers are called “nurse cells”, enclosed by the hyaline capsule. In the late phase the cyst wall becomes gradually thicker and eventually calcified (Kapel, 2000; Jospeh *et al.*, 1999).

2.4.4 Pathogenicity and clinic

Trichinellosis is rarely detected clinically in animals.

In humans the pathogenicity of all the different species of *Trichinella* has not yet been totally explored. For the most common species, *T. spiralis*, clinical signs are well documented.

Intestinal phase: the intestinal phase is characterized by a self-limiting bout of abdominal pain and diarrhea with expulsion of mature worms, anorexia, fever, weakness and myositis causing unwillingness to move.

Parasitaemical phase: The newborn larvae enter circulation systems, spread to different organs and invade host cells causing lesions. The symptoms observed include heart attacks, inflammation of the brain, and heart failure. Life-threatening complications include myocarditis, central nervous system involvement, and pneumonitis. Deaths are common (up to 40%) either due to anaphylactic shock or due to the consequences of the myocarditis. Symptoms such as edema around the eyes, muscle pain, fever, itchiness in the skin, and lesions of the skin have been described. More serious cases in humans have caused breathing difficulties as a result of an infected diaphragm.

Muscle phase: Larvae are able to penetrate any cell in various tissues in the body resulting in cell death and associated inflammation and subsequent granuloma formation (Despommier, 1999). Later on they penetrate the cells of the skeleton

muscles and have the ability to become encapsulated. The muscle phase is very complex due to the penetration of *Trichinella* larvae into striated skeletal muscle cells and their permanent residence there (Zarlenga, *et al.*, 2001).

2.4.5 Epidemiology

Trichinellas are some of the most widespread parasites infecting people and other mammals all over the world, regardless of climate. The global prevalence of the disease is difficult to evaluate, but worldwide trichinellosis is estimated to affect at least 11 millions people with different epidemiological patterns (Dupuoy-Camet, 2000).

The International Commission reported more than 10,000 cases of human trichinellosis on trichinellosis from 1995 to June 1997 and about 10,000 porcine infections were reported by the Office International des Epizooties in 1998. The present global status of trichinellosis is determined as a worldwide zoonosis. In contrast to animals where *Trichinella* infection proceeds without clinical symptoms, food borne infection in man usually entails typical trichinellosis with the threat to human health (Marinculic *et al.*, 2001).

A variety of patterns exist which determine the way of *Trichinella* along the food chain to human beings. Two main cycles, *i.e.*, the sylvatic and the domestic cycles have been recognized in the epidemiology of trichinellosis (Campbell, 1988). The main agent is transmitted among domestic pigs by infected pock scraps or infected rats. The natural cycle occurs in sylvatic carnivores and omnivorous animals, mainly in those with cannibalistic and scavenger behaviors (Pozio, 1998). There are natural and artificial factors, which contribute to the maintenance of *Trichinella* within the domestic and sylvatic cycles.

Natural factors which influence the *Trichinella* cycle: the sylvatic cycle is regularly completed by cannibalism and the scavenger behavior of wild animals (Pozio, 2001). Domestic animals, which are infected with these species, represent a

dead end for the sylvatic *Trichinella* species. Another aspect is that they may be transmitted to the sylvatic, where wild animals have other food sources such as garbage dumps or carcasses of domestic animals. Further on, the population density plays an important role for the spread of *Trichinella* in the sylvatic cycle.

Otherwise, domestic pigs and synanthropic rats play an essential role for *Trichinella* infection in the domestic habitat. Infections in domestic habitats occur where domestic animals have access to the environment, and thus may be in contact with synanthropic or others (Pozio, 1998). Rats might play an important role in the flow of *Trichinella* infection between domestic and sylvatic cycles (Pozio, 2000).

Artificial factors which influence the *Trichinella* cycle: there is no doubt that human behavior strongly influences the transmission routes within domestic and sylvatic cycles (Pozio, 2000). The prevalence of trichinellosis is strongly related to hunting practices due to the improper disposal of fox carcasses, which may be a new infection source of other wild carnivores (Kapel *et al.*, 1997). On the other side, domestic animals like pigs and dogs are fed with the remains of sylvatic animals, or domestic pigs are kept under outdoor conditions where close contact to the sylvatic *Trichinella* cycle may exist.

Infection sources in human trichinellosis: in comparison to domestic and sylvatic animals, which may show a variation in susceptibility for different species and genotypes, all *Trichinella* species are pathogenic for human (Ramisz *et al.*, 2001; Kapel *et al.*, 1995; Dupouy-Camet *et al.*, 2002). The trichinellosis cases in man were traced back to the following main food sources: pork consumed or other raw products played the most important role as the source of infection (Cuperlovic *et al.*, 2001; Marinculic *et al.*, 2001). Wild boar meat was detected as another important source for human trichinellosis in many countries (Ramisz *et al.*, 2001) and horse meat was firstly discovered as emerging *Trichinella* food-borne infection in Italy in 1975 (Boireau *et al.*, 2000). Finally, trichinellosis cases due to infection acquired abroad in third countries or imported food products, which may harbor *Trichinella* larvae, have to be considered.

Risk factors for human trichinellosis: from all the aspects mentioned above, different levels have to be considered for the specification of risk in acquiring a *Trichinella* infection: first of all it must be clear if a sylvatic or domestic cycle or both of them are present in the defined region or not. If the *Trichinella* species is present in the habitat of the kind of species, its characteristics (freezing tolerance, encapsulated or non-encapsulated forms) and presence in domestic and sylvatic host animals have to be identified (Pozio, 2000). In this respect the kind of pig farming has to be specified (indoor and outdoor). Sylvatic and domestic animals kept under poor hygienic conditions, *i.e.* feeding of untreated food, no rodent control or extensive husbandry allowing uncontrolled contact to sylvatic animals, will pose a high risk (Pozio, 2001). Every pig and wild boar has to be examined for trichinellosis. If the meat inspection is not applied at all or if it is not performed properly, trichinellosis can be introduced in the food chain and pose a risk for consumers. After the slaughter or hunting of animals, the kind of meat preparation may imply a risk. This especially relates to all raw or insufficiently cooked products (Gamble *et al.*, 2000). The consumers expose themselves to the risk of trichinellosis by the consumption of raw or insufficiently treated meat from improperly examined carriers of the *Trichinella* species (Nöckler *et al.*, 2003). Moreover, cultural behaviors such as hunter meals with undercooked roasted ribs from wild boars favored human infections (European Commission, 2001).

Consequently, a very high risk for human trichinellosis will exist if *Trichinella* species are endemic in the domestic and /or sylvatic cycle, if meat inspection is not at all or improperly performed and if the meat is consumed raw or under insufficiently processed conditions. The risk will significantly decrease when every infected carcass can be removed from the food chain by an efficient meat inspection (Gamble *et al.*, 2000; Nöckler *et al.*, 2000).

Trichinellosis is more common in temperate regions than in tropical regions. It occurs in North America, South America (Argentina and Chile), northern and Eastern Europe, Kenya, Egypt, Lebanon, China, Nepal, Thailand and Indonesia. Three species are important in Southeast Asia and the Pacific region, *T. spiralis*, *T. pseudospiralis*

and *T. papuae*. In the Pacific region, serological evidences of *Trichinella* spp. have been found in Fiji, Kiribati, Palau and Samoa, Solomon Islands. In Southeast Asia *T. spiralis* and *T. pseudospiralis* are found principally in China, Japan and Thailand (Takahashi *et al.*, 2000). For example the outbreak that occurred in Thailand, which affected 59 individuals who ate raw pork from a wild pig, was particularly severe with one death and individuals showed clinical signs of myalgia, muscular swelling and aesthesia that persisted for more than 4 months (Jongwutiwes *et al.*, 1998). The newest non-encapsulated species of *Trichinella*, *T. papuae*, was isolated from a wild pig in a remote part of southwestern Papua New Guinea and described in 1998 (Pozio *et al.*, 1999). Infection was identified in approximately 8.8% of village and wild pigs in the area (Owen *et al.*, 2000).

2.4.6 Diagnosis

The main sources of infection in humans are eaten pork, horse meat, bears and small wild carnivores. According to the “Manual of Standards for Diagnostic Tests and Vaccines” published by OIE (2004), two main methods are recommended for the diagnosis of trichinellosis: direct detection of first-stage larvae encysted or free in striated muscle tissue, and indirect detection of parasitism by tests for specific antibodies (Gamble, 1996).

Direct detection:

(a) Applications of the methods

The direct detection of *Trichinella* larvae in muscle samples is usually done at post-mortem inspection. In order to prevent human trichinellosis in many countries, the examination of muscle samples of pigs and of all other animal species, *e.g.*, horses, wild boars, etc., that may potentially serve as a source of this food-borne infection, is a part of routine slaughter inspection (Gamble, 1996). Direct detection is useful for epidemiological studies in wildlife, in which indicator animals, *e.g.*, foxes and raccoon dogs are examined for the presence of this nematode in order to

investigate the reservoir competence of the host and to evaluate its importance within the sylvatic and domestic cycles. Indicator animals provide an estimation of the prevalence of *Trichinella* in the environment (Forbes *et al.*, 1998).

(b) Factors important for direct detection

Direct methods for the detection of *Trichinella* larvae in muscle samples are designed to provide maximum sensitivity, but have limitations. Methods suitable for routine meat inspection are designed primarily to prevent clinical trichinellosis in humans and do not have the capacity to prevent infection entirely. The efficiency of the direct detection of *Trichinella* larvae depends on the methods used, the site sampled and the sample size. The correct choice of a suitable diagnostic method is necessary in order to obtain reliable results. *T. spiralis*, *T. nativa*, *T. britovi*, *T. nelsoni* and *T. murrelli* induce the formation of a nurse cell in the striated muscles of the host, whereas the non-encapsulating species *T. pseudospiralis* and *T. papuae* are characterized by the lack of a capsule around the muscle larva (Murrell *et al.*, 2000). Larvae of non-encapsulating species would be more difficult to detect by trichinoscopy. Therefore, all samples should be examined in conjunction with or by the digestion method because trichinoscopy cannot ensure the detection of all *Trichinella* species.

(c) Sample location

After the internal and migratory phases, larvae of *T. spiralis* reach the striated muscles where they become infective for a new host as early as 17 days post-infection. Larvae prefer sites in muscle tissues that are well supplied with blood. Predilection sites differ among animal species and may be dependent on the specific mobility behavior of the species (Kapel *et al.*, 1995). Identification of the predilection sites in an animal species will determine the choice of muscle to be tested for *Trichinella* larvae. In domestic pigs, the three main predilection sites for *T. spiralis* are the diaphragm, the tongue and the masseter muscle (Gamble, 1996; Forbes and Gajadhar, 1999). Wild boars infected with *T. spiralis*, *T. nativa*, *T. britovi*, *T.*

pseudospiralis, *T. murrelli*, *Trichinella* T6 and *T. nelsoni* harbored most larvae in the diaphragm and the tongue (Kapel, 2000). In horses infected with *T. spiralis*, the tongue and masseter were found to be typical predilection sites (Gamble *et al.*, 1996). In a naturally infected horse, most larvae of *T. spiralis* were detected in different muscles of the head. Infections with *T. pseudospiralis* in poultry (cock-broilers) demonstrated that the muscles of the head (*e.g.*, masseter and the neck) were typical predilection sites (Britov *et al.*, 1997).

(d) Sample size

The amount of sample to be used for the detection of *Trichinella* larvae must be chosen to provide an adequate level of sensitivity and an acceptable cost-benefit relationship. It is generally accepted that for routine meat inspection, to prevent clinical trichinellosis in humans, it is necessary to ensure a sensitivity of approximately 1–3 larvae/g (LPG) of tissue taken from the predilection site. Theoretically, a 1 g sample would be enough for the detection of at least 1 LPG of tissue, on the condition that there is a homogenous distribution of larvae in the tissue investigated. In practice, this is true for high larval densities, but in cases of a low level of infection, larvae are not distributed homogeneously.

For the routine slaughter inspection of pig carcasses and game meats, using the pooled sample digestion method, a minimum of a 1 g sample of tissue from a predilection site is recommended. For the same purposes, a minimum of 0.5 g sample and preferably more may be used for the inspection of individual pig carcasses by trichinosis (Gamble *et al.*, 2000). To ensure high sensitivity, horse meat is examined with the pooled sample digestion method using 5 g or preferably 10 g samples. If the muscles from predilection sites are not available for inspection, carcasses should be tested using larger amounts (up to 100 g samples) in order to achieve adequate sensitivity (Gamble *et al.*, 2000).

Concerning epidemiological studies in reservoir animals, the sample size should be adjusted upward to achieve a sensitivity of less than 1 LPG. Low larval densities

occur in the muscle tissues of wild carnivores infected with *Trichinella*. For this reason, the samples to be tested in such studies should have a weight of at least 5 g or more.

(e) Main characteristics and performance of direct methods of detection

Trichinoscopy is a simple but tedious method for the inspection of individual carcasses and requiring much time and labor. In contrast, the pooled sample digestion method allows testing of up to about 100 carcasses at the same time. The digestion method requires more technical equipment than trichinoscopy, but is cheaper and has become the method of choice for routine slaughter inspection in most industrialized countries. Because of the enhanced sensitivity of digestion tests, the use of trichinoscopy as a standard method of control is discouraged. OIE guidelines for tests of trichinellosis are available for examining pork, as well as wild boar and horse meat for muscle larvae, and should be adequate for preventing clinical trichinellosis in humans (Gamble, 1996, 1998). To ensure that tests are performed properly, all authorities conducting routine slaughter inspection should introduce and maintain a suitable quality control system.

A documented quality assurance system (QAS) that meets international standards will soon be essential for any test used in domestic or international trade. Complete data for the validation of a digestion test for pigs and horses are available (Forbes and Gajadhar, 1999).

International regulation of direct detection for meat inspection includes:

(f) Trichinoscopy (Compression method)

According to the OIE “Manual of Standards for Diagnostic Tests and Vaccines”, 28 small pieces of muscle of about 2 mm x 10 mm in size, with a total weight of about 0.5 g, should be taken from prescribed predilection sites (Gamble, 1996). The small

spindle shaped pieces along the muscle fibers are cut with a scissors. Then the muscle pieces are compressed between two glass plates until they become translucent, and then examined individually for *Trichinella* larvae, using a trichinoscope or a conventional stereo-microscope (15–40x magnification). All the samples are recovered for processing by artificial digestion test (Nöckler *et al.*, 2000).

(g) Artificial digestion (Pepsin fermentation)

The magnetic stirrer method described as follows:

Homogenize a maximum of 100 g of samples of muscle tissue 1 gram each from the prescribed predilection sites of the animals under inspection are pooled. The sample pool is digested using an artificial digestive fluid consisting of 2 liters of tap water, 10 g of 1% pepsin (1:10.000 NF= US National Formulary), and 16 ml of 25% HCl.

The digest is stirred for 30 min at a temperature of 44–46 °C in a 3-liter glass beaker using a hot plate magnetic stirrer. During this process, the trichinae are released from the muscle. The digestion fluid is then poured through a sieve (mesh size 180 mm), which keeps back any undigested tissues, but allows the passage of *Trichinella* larvae, into a 2-liter separation funnel. Larvae are allowed to settle for 30 min, and then a 40ml sample is quickly released into a 50 ml tube. After a further 10 minutes of sedimentation to clarify the suspension, 30 ml of supernatant is withdrawn. The remaining 10 ml of sediment is poured into a gridded petri dish. The 50 ml tube is rinsed with 10 ml of tap water, which is added to the petri dish. Subsequently, the sample in the petri dish is examined by a trichinoscope or stereomicroscope (15–40x magnification) for the presence of *Trichinella* larvae (Gamble *et al.*, 2000).

Indirect detection including serological and molecular studies

Serological study: Part of this review is directed at sero-diagnostic methods for the detection of *Trichinella* specific antibodies in different animal species, *i.e.*, antibody detection with larval E/S antigen AB-ELISA test. Classical methods of sero-

diagnosis such as the complement fixation test and immuno-fluorescence antibody test are reviewed and the characteristics and performance of the AB-ELISA are discussed. Factors dependent upon the animal species being tested or on components of the AB-ELISA test system are considered. Using serology, it has become possible to perform additional *Trichinella* control measures (Directive on Zoonoses EEC /117/92) to ensure consumer protection.

(a) Applications and characteristics of the methods

Serological methods are used mainly for ante-mortem and post-mortem examination of blood serum samples for *Trichinella*-specific antibodies, and under some conditions may have a higher sensitivity than methods of direct detection. Other uses include *in vivo* studies on immune responses in long-term infection and surveillance of live caught wild animals. Because serological methods allow testing of samples from living pigs as well as of samples obtained post-mortem, they may be useful for establishing *Trichinella*-free areas and reducing restrictions in international animal trade. The tissue fluids (meat juice) from slaughtered pigs or from hunted or other dead animals (*e.g.*, wild boars) may be suitable for serologic examinations using ELISA (Gamble and Patrascu, 1996; Gamble, 1999; Kapel *et al.*, 1998).

(b) Conventional serological methods

The immunofluorescence antibody test (IFAT), western blot analysis (WBA), complement fixation test (CFT) and haemagglutination test (HAT) are examples of conventional serological methods that are labor intensive and can not be used in an automated system. As a result, these methods are more expensive in comparison to the enzyme-linked immunosorbent assay (AB-ELISA) and are preferentially used in human medicine for the examination of individual samples (Nöckler *et al.*, 2000).

Enzyme-linked immunosorbent assay (AB-ELISA)

In comparison to the CFT, HAT, WBA and IFAT, the AB-ELISA is easy to conduct, can be automated and detects infection levels as low as one larva/100 g of tissue (Gamble, 1996). The AB-ELISA method is recommended for herd surveillance programs and is useful for detecting ongoing transmission of *Trichinella* at the farm level (Gamble, 1996). However, the AB-ELISA may fail to detect infected pigs during both the early and the very late phases of infection. It is for this reason that this serological method can not be used to replace digestion testing for the detection of *Trichinella* larvae at slaughter inspection, but it can be recommended for practical use in herd surveillance in pigs (Nöckler *et al.*, 1995; Gamble, 1996). The use of enzyme-linked immunosorbent assay (AB-ELISA) to detect the presence of parasite-specific antibodies provides a rapid method that can be performed on blood serum and tissue juices collected before or after slaughter. Several antigen preparations have been developed that provide a high degree of specificity for *Trichinella* infection in pigs and horses. In slaughterhouse testing, the AB-ELISA yielded less than 0.3% false-positive results and was nearly 100% sensitive in detecting infected pigs with more than one larva/gram of tissue. The *T. spiralis* excretory and secretory (E/S) antigens used in the AB-ELISA are conserved in all species/types of *Trichinella*, and therefore infection may be detected in pigs or other animals harbouring any of the seven species or types. Serological tests other than AB-ELISA (*e.g.*, indirect immunofluorescence tests) lack specificity and are not sensitive enough for detection of *Trichinella* infection.

Molecular studies

This method includes the PCR test, which enables differentiation between the different species of *Trichinella*. However such tests require the development and amplification of specific DNA-probes. DNA sequences have been identified and are specific for *T. spiralis* and other *Trichinella* species as well. This new generation of PCR test is not available, *i.e.*, specific primers are very costly and uneconomical in the diagnosis of meat in the human food chain.

2.4.7 Therapy and prevention

Therapy exists for humans if diagnosed in time. When the life of the patients is threatened by overwhelming infection, intensive care treatment with all available supportive therapies is mandated, *i.e.*, fluid replacement, steroids, treatment for shock and toxemia as well as circulatory and cardiac failure. Specific therapy for the parasites with various benzimidazoles (mebendazole or albendazole) is also necessary. Immunosuppressions due to steroids, although often a life-saving procedure, prolong the life of the adult parasites as well and result in further production of newborn larvae if unchecked. As already mentioned, patients may harbor adults shedding newborn larvae for several weeks during the acute phase of infection. Mebendazole (200mg/day for 5 days) or albendazole (400mg/day for 3 days) should be given to adults, as well as to children (5 mg/ kg of body weight per day for 4 days). Prednisolone at 40-60mg/ day alleviates the fever and the side effects of inflammation due to the cell damage that results from larval penetration into the tissues. They can also use engimidazoles over 14 days, effective in the muscle phase (Kociecka, 2000).

These symptoms usually disappear within days after the initial dose is given. Prolonged treatment with steroids is not recommended, although symptoms may recur when treatment is suspended. Long-lasting sequels must be treated symptomatically as they arise.

To prevent trichinosis in pigs at the farm level (main host at risk for human health in the region), measures involve the environmental control (requirement for *Trichinella*- free pig production) such as architectural and environmental barriers, feed and feed storage, rodent control and farm hygiene (dead animals are disposed of within 24 hours) and by sanitation means as well as no garbage dumps present within a 2 km radius of the farm (Craig *et al.*, 2002; EC, 2000).

No vaccines have been developed so far for the prevention of trichinellosis.

Prevention of trichinellosis for consumers or humans can be done by avoiding the consumption of raw or contaminated meat from game animals or pigs raised in situations that favor the existence of rodent population. These animals are the most frequent source of infection by any *Trichinella* species. In addition, trichinellosis can be prevented by either cooking meat thoroughly at 58.5°C for 10 min. or by deep-freezing it at -20 °C for 3 days. Consumer food safety education programs include cooking to an internal temperature of 71 °C (160°F), freezing solid (-15 °C or less) for 3 weeks (cut up to 15 cm in thickness) and freezing solid (-15 °C or less) for 4 weeks (cut up to 69 cm in thickness). Cooking using microwaves, curing, drying or smoking are not recommended (Gamble *et al.*, 2000; Bessonov *et al.*, 2000).

According to the OIE guideline and EC regulations for trichinellosis, the gold standard are subjected to a testing procedure for trichinellosis with negative results or have been processed to ensure the destruction of all the larvae of the parasite (OIE, 2004; Anonymous, 1994; Herenda *et al.*, 2000).

2.5 Global importance of porcine cysticercosis and trichinellosis

2.5.1 Porcine cysticercosis

Infection with *T. solium* cysticercosis is widely prevalent in human and pig hosts in many developing countries of Latin America, Africa, and Asia (Sarti *et al.*, 1992).

Asia: several reports of patients with cysticercosis from many countries in Asia such as India, China, Indonesia, Thailand, Korea, Taiwan and Nepal are a clear indicator of the wide prevalence of *T. solium* cysticercosis and taeniosis in these and other Asian countries. However, epidemiological data from community-based studies are sparse and available only for a few countries in Asia. Cysticercosis is the cause of epilepsy in up to 50% of Indian patients showing partial seizures. It is also a major cause of epilepsy in Bali (Indonesia), Vietnam and possibly China and Nepal. Sero-

prevalence studies indicate high rates of exposure to the parasite in several countries (Vietnam, China, Korea and Bali (Indonesia)) with rates ranging from 0.02 to 12.6%. Rates of taeniosis, as determined by stool examination for ova, have also been reported to range between 0.1 and 6% in the communities in India, Vietnam, China, and Bali (Indonesia) (Rajshekhar *et al.*, 2003).

Africa: in West Africa, *T. solium* cysticercosis in both pigs and man has been reported in Benin, Burkina-Faso, Ghana, Ivory Coast, Senegal and Togo, and although official data are lacking, *T. solium* is anticipated to be present in most of the pig-raising regions of other West African countries as well. In some regions of Nigeria, the prevalence of porcine cysticercosis and human taeniosis is quite high (20.5 and 8.6%, respectively). Surprisingly, however, no cases of human cysticercosis have been reported, although epilepsy is very common. Large epidemiological surveys have only been carried out in Togo and Benin, where the prevalence of human cysticercosis was 2.4 and 1.3%, respectively. In Central Africa, porcine and human cysticercoses are (hyper)-endemic in Rwanda, Burundi, the Democratic Republic of Congo and Cameroon. The parasite also has been reported in pigs in Chad and Angola. Cysticercosis has been shown to be one of the major causes of epilepsy in Cameroon with figures as high as 44.6% (Zoli *et al.*, 2003).

Europe: *T. solium* cysticercosis has been eradicated from European countries except for a few areas where sporadic human cases are reported (Overbosch *et al.*, 2002). However, it is unclear if these cases are due to the European *T. solium* or to foreign *T. solium* re-introduced by immigrants or travelers from other areas. It is possible that some isolates of European origin might remain in Spain/Portugal, Eastern Europe and Russia or the northern part of Mongolia where *T. solium* cysticercosis still exists (Ito *et al.*, 2003).

Latin America: During the last century information was based on autopsy findings, which clearly pointed to those countries where cysticercosis was considered afterwards a priority health problem: Brazil, Colombia, Mexico and Peru (Del-Brutto, 2000). Recent reports demonstrate the presence of *T. solium* also in other countries,

such as Guatemala (Flisser *et al.*, 2003; Allan *et al.*, 1996). The prevalence data of these countries are summarized in Table 2.

Table 2. Global prevalence data on porcine cysticercosis, taeniosis and human cysticercosis in some countries of the world

Countries	Human cysticercosis %	Taeniosis %	Porcine cysticercosis %	References
China	3-4	0.06-19	5.4 (0.8-40)	Rajshekhar <i>et al.</i> , 2003
Cambodia	NA	NA	10	Singh <i>et al.</i> , 2002
Vietnam	5-7	0.5-6	0.04-0.9	Rajshekhar <i>et al.</i> , 2003
India	NA	2	9.3	Rajshekhar <i>et al.</i> , 2003
Nepal	NA	10-50	32.5	Rajshekhar <i>et al.</i> , 2003
Tanzania	NA	NA	4.5-26.9	Boa, 2002
Zambia	NA	NA	8.2-20.8	Phiri <i>et al.</i> , 2002
South Africa	NA	NA	0-9.1	Phiri <i>et al.</i> , 2003
West Africa	1.3-2.4	NA	0.6-20.5	Zoli <i>et al.</i> , 2003
Latin America	3.7-22.6	0.3-2.8	1-38.9	Flisser <i>et al.</i> , 2003

NB: NA= No data available.

2.5.2 Trichinellosis

Human trichinellosis, a parasitic nematode infection, represents the most frequent widespread food-transmitted helminth zoonosis with worldwide distribution with both sylvatic and domestic spreading, and is caused by tissue-dwelling roundworms of the genus *Trichinella* (Pozio, 1998). At present, human and animal trichinosis is considered as an emerging infection due to the increase in its prevalence

in countries such as Bulgaria, Rumania, Yugoslavia, Croatia, Lithuania, Russia, China, Argentina, and Mexico (Pozio, 2001). It is also considered to be a re-emerging infection since epidemiological and biological research on its life cycle in the wild life have led to the description of new species in various regions with new patterns of transmission, which increase the risk of human infection from consumption of the meat of wild animals (*e.g.*, in Canada, United States, Russia), or domestic animals such as the horse (in France and Italy) or dog (in China) (García *et al.*, 2005; Pozio, 2001).

Unlike other parasite infections, it has been the main public health problem in advanced countries where there is a great amount of meat consumption such as European countries and USA. This nematode infection has been reported in all of the continents except Australia. Due to the wide spread of trichinosis in many Asian countries including China and Japan, it has been suspected to be prevalent in Korea. However, trichinosis had not been reported in Korea until 1997. In December 1997, the first human infection with *T. spiralis* confirmed by detecting encysted larvae in the biopsied muscle (Sohn *et al.*, 2000)

Trichinellosis study is introduced the presence of *Trichinella* spp. in Asia, especially historical review of *Trichinella* in Japan, the epidemiology of trichinellosis in China and in Thailand. There have been numerous outbreaks of trichinellosis in continental Asia including countries such as China and Thailand, but there have been very few records of outbreaks in the island countries. These island countries are geographically isolated from the Asian continent, which is known to have *Trichinella*.

Although, no data on *Trichinella* are available for Taiwan, the Philippines, Malaysia and Cambodia, it is likely that high rates of trichinellosis occur in various parts of Southeast Asia. It is known, for example, that the aborigines of Taiwan in the Wulai district commonly eat raw flesh and internal organs of pigs and other wild animals (Takahashi *et al.*, 2000).

Many provinces of Thailand near the Laotian and Cambodian borders were reported as foci of trichinellosis, but the number of cases was low, consisting of 1.4% of the total number of reported cases in Thailand. Likewise, only 0.6% of the cases were reported in the central part of Thailand (Takahashi *et al.*, 2000). The prevalence of trichinellosis in some countries is addressed in the Table 3.

Table 3. Global Prevalence data on trichinellosis in some countries of the world

Countries	Human cases/ year	Human trichinellosis %	Animal trichinellosis %	Reference
China	NA	4.01	0.021-7.3 (Pigs)	Liu <i>et al.</i> , 2002
Thailand	NA	NA	0.02 (Pigs) 11.4 (hilltibe pigs)	Takahashi <i>et al.</i> , 2000
North America	38 (91-96)	NA	0.013 (Pigs)	Mooread <i>et al.</i> , 1999
Mexico	1-19(90-97)	NA	1-2.5 (Pigs)	La Rosa <i>et al.</i> , 1998

NB: NA= No data available.