Taeniasis

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Learning Objectives

1. To have a knowledge about the importance of *Taenia asiatica* as a new species and its clinical manifestation.
2. To explain the importance of the extraneural manifestations of cysticercosis, particularly cardiac and ocular involvement.
3. To review the various serological tests including EITB in the diagnosis of neurocysticercosis.

Introduction

Taeniasis and cysticercosis are defined as the parasitic infection of humans and animals caused by adult and larval stages of tapeworms (*Taenia solium, Taenia saginata* and *Taenia asiatica*) respectively. The disease has worldwide distribution especially in areas where cattle and pigs rearing are carried out extensively. Though the distribution of the disease varies across the continents, the prevalence rate is found to be high in developing countries. Effective control and prevention of the condition is achieved by measures that include prevention of cattle or pigs from grazing on faeces or sewage polluted grass, avoiding the use of untreated human faeces as manure for land and avoiding the eating of raw or undercooked meat and meat products.

History

T. saginata has been identified as an intestinal parasite infecting man since ancient times and has even been depicted in Charaka Samhita, an ancient Indian medical book. In the third century BC, Aristophanes and Aristotle first described the cysts in pigs, and later in 1550, Parunoli noticed this infection in humans. In 1782, Goez differentiated it from other species *T. solium.* The role of cattle as an intermediate host and the complete life cycle of *T. saginata* was described by Leuckart, in 1861. A case of neurocysticercosis was reported from a coolie in Madras, who died due to a seizure (Armstrong 1888). In 1912, Krishnaswamy was the first to report the cases of muscle pains and subcutaneous nodules with abundant cysticerci in muscles, heart and brain through autopsy.

T. asiatica was first identified in Taiwan and later in Korea and other Asian countries; hence it was named Asian *T. saginata*. In 1966, suspicion of aetiology other than conventional *T. saginata* was put forth by S.W. Huang, as the Taiwan aborigines hardly eat beef. The naming of the parasite as *T. saginata asiatica* was by a group

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of Taiwanese parasitologists namely P.C. Fan, C. Y. Lin, C.C. Chen and W.C. Chung.

Taxonomy

The genus *Taenia* belongs to the Phylum, Platyhelminthes; Class, Cestoda; Order, Cyclophyllidea; and Family, Taeniidae. *T. saginata, T. solium* and *T. asiatica* are three important species that cause infections in humans.

Genomics and Proteomics

The genome size of *T. solium* is 122,393,951 bp with 12,467 coding genes. The complete nucleotide sequence of the tapeworm *T. solium* mitochondrial DNA (mtDNA) has been determined. The sequence is 13,709 base pairs in length and contains 36 genes (12 for proteins involved in oxidative phosphorylation, 2 for ribosomal RNAs and 22 for transfer RNAs). The gene content and organization of the *T. solium* mtDNA are identical to the mtDNAs of the other two species. The size of the protein-coding genes of the three human *Taenia* tapeworms did not vary, except for *T. solium* nad1 (891 amino acids) and nad4 (1212 amino acids) and *T. asiatica* cox2 (576 amino acids).

Genomic analysis shows much larger assembly sizes of *T. saginata* and *T. asiatica* than *T. solium* (169 Mb and 168 Mb vs 131 Mb), though GC content remains similar (43.2% vs 43.5%). Coding genes in *T. solium* were estimated to be 11,902 only with gene density 90.9/Mb, and in *T. saginata*, 13,161 and 77.9/Mb, respectively. While mean exon length remains similar (237 bp) across the three species, introns are longer in *T. saginata* (864 bp) than *T. solium* (775 bp) and *T. asiatica* (831 bp). The mitochondrial genome shows comparable assembly sizes (13,700, 13,670 and 13,703 kb) with 70% genes coding for proteins like other cestodes. Nucleotide composition of the mitochondrial genome is asymmetrical with a positive GC-skew. *T. solium* differs by 12.3% and 12% from *T. saginata* and *T. asiatica*, respectively in cox1 nucleotide composition. The latter two differ only by 4.6%; in fact, 18S rRNA genes are 99.2% identical. Genomic features strongly revoke the sister relationship between *T. saginata* and *T. asiatica* with a higher evolutionary drive in mutation rate, heterozygosity and expansion of genes related to ion transporters and tegumental components in the latter. However, the absence of a significant intra-species genetic variation in *T. asiatica* suggests that it could be an endangered status. *T. saginata*, in comparison, shows high genetic polymorphism (0.2–0.8%). A striking discordance has been discovered between mitochondrial and nuclear DNA among a few isolates of *T. saginata* and *T. asiatica* from Taiwan and China, raising the possibility of hybrids.

Proteomic analysis of metacestodes, oncosphere, vesicular fluids and excretory-secretory products redefines host-parasite relationship, immunological responses, identification of the diagnostic antigens and vaccine candidates. Tandem mass spectrometry and BLAST studies identified important proteins including microtubule-based movement/tegumental proteins (paramyosin, H17g), chaperones (HSP90), metabolic proteins (elongation factor 1 alpha, GAPDH, malate dehydrogenase) and detoxification molecules (ferritin, glutathione S-transferase). GP50 and T24 are diagnostically important proteins.

The Parasite Morphology

The three morphological forms of the parasites are adult worm, egg and larva.

Adult Worm

T. saginata: The worm is long, flattened and ribbon-like (Fig. 1). The adult worm consists of a head (*scolex*), neck and strobila. The *scolex* has four cup-like muscular suckers (or acetabula) which help in attachment. Situated next to scolex is neck, which is the narrow growing region from which the proglottids arise. Neck is longer in

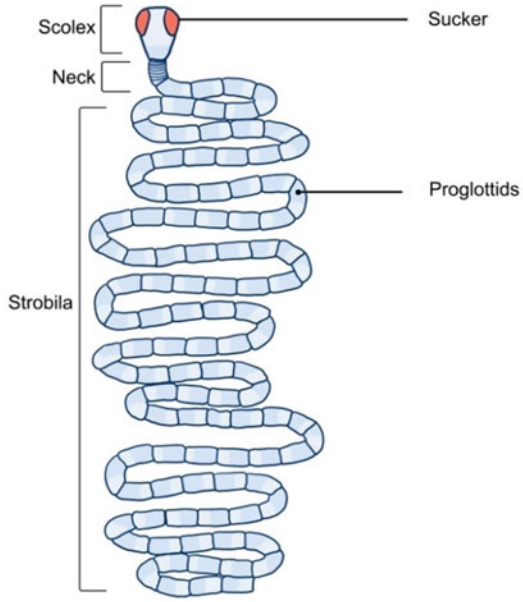

Fig. 1 Schematic diagram of *Taenia saginata* adult worm (5–10 m in length)

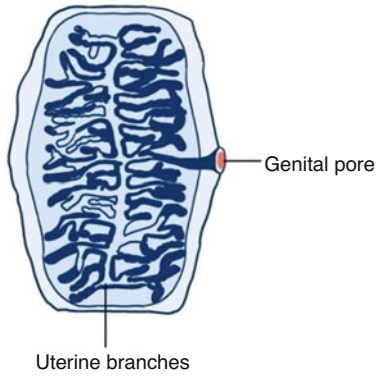

Fig. 2 Schematic diagram of *Taenia saginata* gravid proglottid

T. saginata than *T. solium*. The trunk or body is the *strobila* which consists of many segments or *proglottids*. Proglottids are further divided into immature, mature and gravid segments. The mature proglottids have both male and female reproductive organs. The female reproductive system includes ovary, a closed uterus with branches, ootype, single mass of vitelline gland and a genital pore which is laterally situated. Male organs consist of testes (follicles), vas deferens

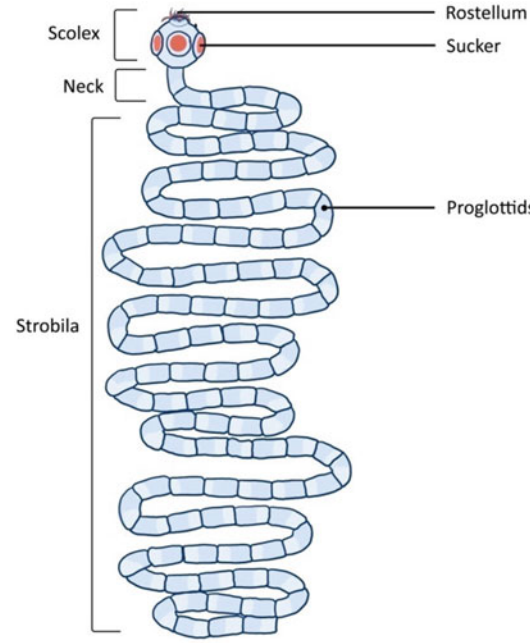

Fig. 3 Schematic diagram of *Taenia solium* adult worm (2–3 m in length)

and cirrus. The uterus in the gravid segment of *T. saginata* has 15–30 lateral branches as compared to *T. solium* (7–13 branches) (Fig. 2). The presence of a prominent vaginal sphincter and lack of accessory ovarian lobe differentiate it further from *T. solium*. The lateral wall of the segments has a common genital pore opening. Since there are no separate uterine openings, the gravid segments escape through the anal sphincter following which the eggs are released by the rupture of the uterine wall.

T. solium: The adult worm measures around 2–3 m in length. The shape of the scolex of *T. solium* appears globular, which has four large cup-like suckers and a rounded rostellum, armed with a double row of alternating round and small dagger-shaped hooks (Fig. 3). The neck is short and thick. The strobila is made up of proglottids numbering less than a thousand. Each gravid segment measures 12 mm by 6 mm, with a length twice that of breadth. There are around 150–200 follicles in the testes. The female reproductive system consists of a uterus which has thick lateral branches of about 5–10 (under 13) and an

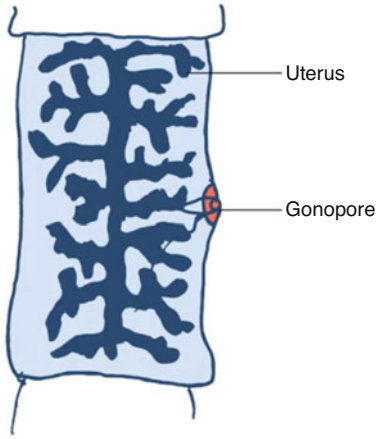

Fig. 4 Schematic diagram of *Taenia solium* gravid proglottid

accessory ovarian lobe. There is no vaginal sphincter. The genital pore is situated laterally and is alternate in position, appearing on the right and left sides of the adjoining segments (Fig. 4). In contrast to *T. saginata,* there is passive expulsion of short chains of the gravid proglottids. Rupture of the uterine wall releases the eggs.

T. asiatica: The worm measures about 350 cm in length and 1 cm in breadth and is divided into the anterior scolex and a short neck followed by strobila. The body cavity or digestive system is absent. The scolex bears four simple suckers and a distinct rostellum, which bears two rows of rudimentary hooklets, which distinguishes it from *T. saginata*. The strobila has more than 700 proglottids, but less than 1000, in comparison to *T. saginata* which have more than 1000 proglottids in the strobila. Similar to *T. saginata* the uterus has 13 lateral branches. The most defining and differentiating features of *T. asiatica* include a large number of uterine twigs in gravid proglottids and the presence of posterior protuberance.

Eggs

The eggs of the three *Taenia* species are morphologically similar to each other. Eggs are enclosed in the gravid proglottids. The spherical egg

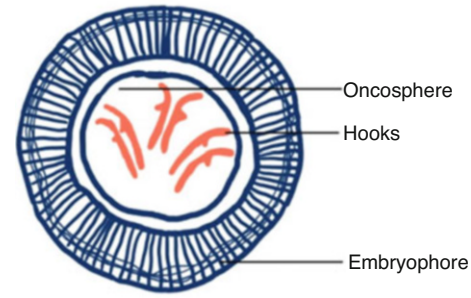

Fig. 5 Schematic diagram of *Taenia* egg

measures 30–40 μm in diameter with a thin hyaline embryonic membrane surrounding it. The radially striated inner embryophore is yellow-brown in colour due to bile staining. The fully developed embryo (oncosphere) consists of three pairs of hooklets (hexacanth embryo) in the centre (Fig. 5). *T. saginata* eggs are infective to cattle, while *T. solium* eggs are infective to both pigs and humans, but *T. asiatica* eggs are infective to pigs only.

Larva

T. saginata: *Cysticercus bovis* is the larval stage of *T. saginata*, which is infective to humans. It is small, measuring 6–9 mm size, round, greyish-white bladder-like worm containing opaque invaginated scolex without hooklets (bladder worm). The larva is commonly situated in the muscles of mastication, cardiac muscles, diaphragm and tongue of infected animals.

T. solium: Cysticercus cellulosae, the larval form of *T. solium*, is located in various organs of pigs as well as in humans. It is an oval-shaped milky-white structure which is about 5 mm by 10 mm in size. The invaginated scolex within the bladder appears as the larva along with its suckers which is visible as a thick white spot. It remains viable for several months.

T. asiatica: Cysticerci of *T. asiatica* appear morphologically similar to *T. saginata* except that they are smaller in size and are located primarily in the liver. They possess two rows of rudimentary hooks which are absent in *T. saginata.*

Cultivation of Parasites

In vitro culture of metacestodes of *T. saginata* is carried out in a biphasic medium consisting of solid phase made up of coagulated calf serum and fluid phase, consisting of buffered RPMI-1640 medium enriched with sodium pyruvate and foetal calf serum. The growing tapeworms show sexual organelles in the early developmental stage. In vitro culture of *T. solium* is done in a system of cell monolayer (HCT-8) without gas phase which induces most of the oncospheres of *T. solium* to enter post-oncospheral (PO) development. The larvae usually survive for up to 16 days. Experimental studies have shown that HCT−8 cell lines favour the formation of PO up to 32%, compared to other cell lines. The developmental forms can be visualized by an ordinary light microscope or electron microscope.

The cultivation of the parasite is useful since the changes occurring in the PO forms can explain the protection of the parasite from the host immune system and the changes observed in protein expression will aid in the development of new targets for vaccine production.

Laboratory Animals

Experimentally, immunosuppressed mice can be used to grow early larval stage and hamsters to grow the immature adult stage of *T. saginata*.

Experimental animal models for *T. solium* include hamsters, gerbils and chinchillas. Of these, chinchillas are the most successful experimental definitive model for adult *T. solium. Mesocestoides corti*, a cestode organism related to *T. solium,* was used for intracranial infection of mouse models to study pathogenesis and immune response associated with neurocysticercosis. There is also a report of a natural progression of innate, early induced and adaptive immune responses in infected mice.

Mice with severe combined immunodeficiency (SCID) when injected subcutaneously into the back with in-vitro-hatched oncospheres of *T. asiatica* developed into fully matured cysticerci. The morphology of the cyst was more advanced and bigger in size which suggested that SCID mice be valuable experimental animal models for studying human taeniid cestode infections.

Life Cycle of *Taenia saginata, Taenia solium and Taenia asiatica*

Hosts

Definitive Host

Man.

Intermediate Hosts

Cattle (*T. saginata*), pigs (*T. solium, T. asiatica*), wild boars and cattle (*T. asiatica*).

In the case of *T. solium,* humans can act as both definitive and intermediate hosts.

Infective Stage

Cysticercus (*Cysticercus cellulosae* and *Cysticercus saginata*), the larval stages of *T. solium* and *T. saginata*, respectively, are infective to humans, while eggs are infective to cattle or pigs and also to humans.

Transmission of Infection

Humans acquire the infection by (a) ingestion of undercooked/raw beef containing encysted larval stage (*T. saginata*), (b) ingestion of undercooked/raw pork containing encysted larval stage (*T. solium, T. asiatica*), and (c) ingesting food (mainly vegetables) or water contaminated with *Taenia* eggs (Fig. 6).

Following ingestion, the encysted larva is digested by the stomach gastric juice. The scolex evaginates out of the cysticercus in the small intestine and gets itself attached to the mucosa of the intestine and develops into the adult worm in a period of about two to three months by strobilization. Adult worms become sexually mature in about 10–14 weeks, fertilization occurs and eggs are formed and later released to the faeces. These eggs are infective to cattle, pigs and other animals.

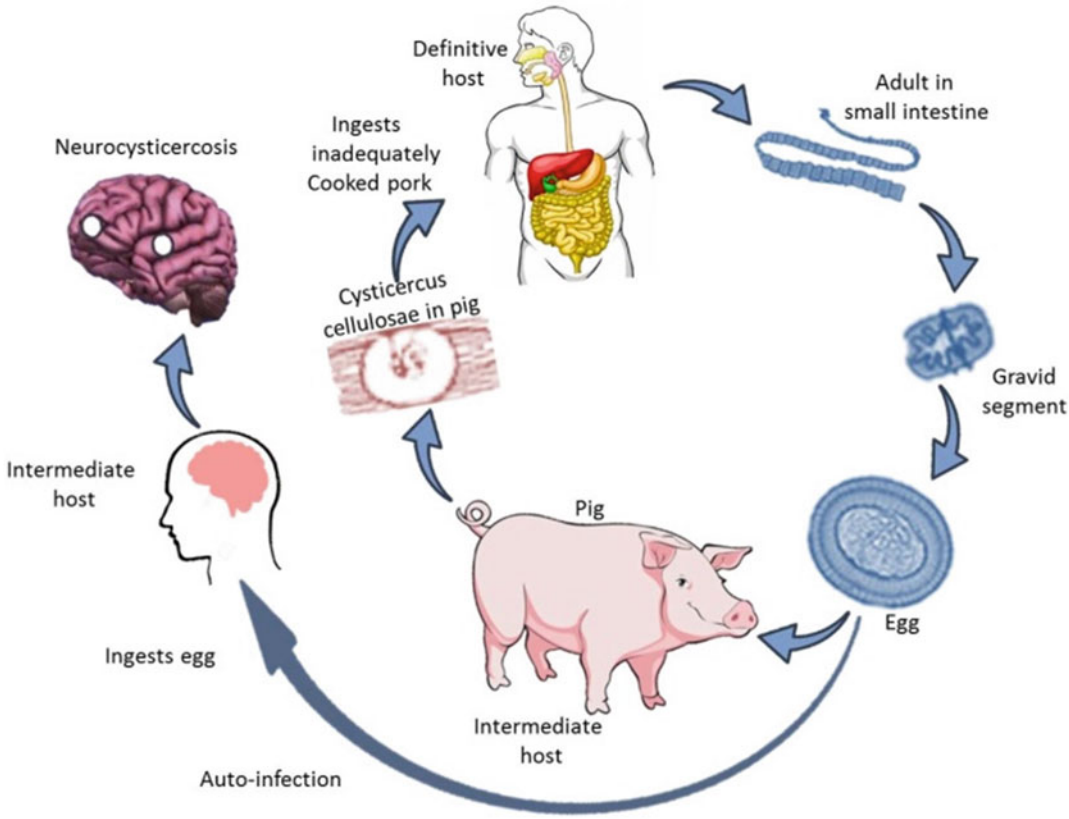

Fig. 6 Life cycle of *Taenia solium*

The animals are infected by the ingestion of eggs while grazing the field. In the small intestine, the oncosphere is released by rupture of the embryophore surrounding the egg. The oncospheres penetrate the intestine, with the help of hooklets and reach the skeletal muscle through the circulations, where they get transformed into bladder-like larvae. The larvae get encysted and deposited as cysts and this process is completed in 10–15 weeks of time. This larval stage of *T. saginata* is infective to man and causes intestinal taeniasis.

Humans also frequently act as intermediate hosts by ingesting eggs with contaminated food and water. In humans, the eggs develop in the same manner as in pigs. The onchosphere is released from the eggs in the first or second part of the small intestine and it penetrates the wall of the intestine to lie in the mesenteric venules or lymphatics. From here, they are carried to various tissues of the body by systemic circulation. These are mostly filtered in the muscle tissue where larval development takes place. Humans are dead-end hosts and the larvae die after variable periods of time.

Pathogenesis and Pathology

Due to aberrant migration of the segments of adult *Taenia* worms in the intestine, obstructive appendicitis or cholangitis can result. During the episodes of vomiting, the proglottids can obstruct the respiratory tract, enter the middle ear via the eustachian tube or tend to localize in adenoid tissues. The irritative action of the worm leads to inflammatory reactions. Moderate eosinophilia

is also frequently seen. In the intermediate host, *T. asiatica* causes hepatocyte degeneration and spotty necrosis of pig liver tissues. Following two months of infection, major changes noted around cysticercus include granulomatous reactions and focal liver fibrosis.

The migration of the *C. cellulosae* to extraintestinal sites such as bone muscle, skin, eye and bone presents a variety of pathology depending on the location of the cysts. The migration of the larva and its presence in the brain causes neurocysticercosis (NCC). The pathology in NCC depends upon the number, location, size and evolutionary stage of the parasites, as well as the presence and degree of the inflammatory response of the host. Parasitic larvae in the parenchyma of the brain tissue commonly present with seizures. The cyst which is viable in the due course of time goes into the involution process due to host immune response. Studies have proven the viability of the cyst for months even after treatment with antiparasitic agents. Initially the viable cysts appear as round vesicles of a membrane filled with clear fluid, containing a scolex. Following the attack by the host immune system, the cyst fluid becomes turbid accompanied by the degeneration of the parasitic membrane and scolex. The cysts gradually shrink and are replaced by hyaline and fibrotic tissue which later disappear or leave a residual calcified scar. It has been observed that cysts in the subarachnoid space tend to grow and infiltrate, which manifest as a space-occupying lesions and blocking the circulation of the cerebrospinal fluid with subsequent hydrocephalus. Unlike intraparenchymal NCC, subarachnoid disease is progressive and associated with significant mortality.

Immunology

Adult *Taenia* spp. are weakly immunogenic. They produce moderate eosinophilia with increased IgE levels. Acquired immunity against *Taenia* infections in humans following the elimination of infection has not been documented, whereas concomitant immunity plays a significant role. Animals once infected usually develop resistance to infection.

In contrast, the larval form of the parasite, *Cysticercus cellulosae* in tissues elicits an active immune response in humans with evasion and suppression of immunity. Viable *cysticerci* do not manifest any symptoms, but in contrast, the immune-mediated inflammatory response around degenerating cysts may precipitate symptomatic diseases. The mechanism behind the evasion of the host immune response by the parasite could be due to masking of *Cysticercus* antigens by host immunoglobulins, concomitant immunity, molecular mimicry and suppression or deviation of host responses. Predominant cellular components activated in the inflammatory response include plasma cells, lymphocytes, eosinophils and macrophages.

Among several immunoglobulin classes secreted against this parasite, IgG is found to be more frequent and are detected in the serum, cerebrospinal fluid and saliva. Antibodies are most frequent among cases with live or dying parasites and rarely in cases with calcified cysts. They engulf parasite remnants, leaving behind a gliotic scar with calcification. There is always a correlation between the presence of antibodies and the intensity of infection, as well as the viability of the parasite.

Increased levels of interleukins IL1, IL-6, IL-5 and Tumour necrosis factor-alpha have also been recorded in the CSF of patients with inflammatory neurocysticercosis, suggesting acute phase response. All these factors suggest a mixture of Th1 and Th2 responses in human brain granulomas caused by cysticerci.

Infection in Humans

The infections in humans are broadly of two types: intestinal taeniasis and cysticercosis.

Intestinal Taeniasis

Most patients with adult *Taenia* cause intestinal taeniasis which is mostly asymptomatic.

Clinical manifestations include mild abdominal pain, nausea, loss of appetite, weight loss, headache and change in bowel habits. In a few cases, proglottids may appear in the stools and even protrude from the anus. Patients may experience perianal discomfort or pruritus, when proglottids, which are often motile, are discharged. This may cause psychological disturbances in patients. Rarely, obstruction by the migrating proglottids can result in appendicitis or cholangitis.

Cysticercosis

Cysticercosis is defined as the tissue infection caused by larval stage of the tapeworm *T. solium*. This larval cyst has the tendency to develop in any part of the body such as CNS, skeletal and heart muscle, skin, subcutaneous tissues, the lungs, liver and other tissues. They can be classified based on the location into extra neural cysticercosis and neural cysticercosis (NCC).

Neurocysticercosis: T. solium cysticerci have a greater predilection to develop in the brain. The infection of the CNS by this parasite is termed neurocysticercosis. The nature and severity of infection depend upon the site, size and number of larvae in tissues and also on the immune response of the host. The symptoms of NCC include headaches, dizziness and seizures. Among these, seizures are the most common clinical manifestation and contribute to the prevalence of epilepsy in around 30% of cases in endemic regions. Other CNS manifestations include sensory deficits, involuntary movements and dysfunction of the brain stem.

NCC based on its location can be further classified into parenchymal and extraparenchymal disease. Parenchymal NCC is characterized by the development of cysticerci within the brain tissue, while the extraparenchymal NCC is characterized by cysts in subarachnoid space, meninges, ventricles and so on. *Racemose cysticercosis* is a rare and severe variant of cysticercosis, caused by an unusually large, multilobular, clustered *Cysticercus* that lacks scolex. The *Cysticercus* is usually located in extraparenchymal sites but mixed parenchymal and extraparenchymal infections can also occur. The condition poorly responds to treatment and is associated with increased morbidity and mortality.

Extraneural cysticercosis: Subcutaneous cysticercosis usually appears in the arms or chest as painless, small, mobile nodules. Muscular cysticercosis is an accidental finding in radiology which can appear dot-shaped or ellipsoidal calcifications in the thighs or arms muscle. About 5% of patients can have asymptomatic manifestations of cardiac cysticercosis.

Ocular cysticercosis: It is most commonly found in the vitreous humour or in the subretinal space as a free-floating cyst. Clinically, based on the degree of retinal tissue damage and development of chronic uveitis, it can present as a visual disturbance. Other sites of locations include the anterior chamber of the eye, conjunctiva or extraocular muscles.

Infection in Animals

Animals harbouring cysticerci usually are asymptomatic, but in massive and severe infections stiffness of muscles has been reported. The larval form, *C. bovis,* is not pathogenic for cattle, unless a vital organ such as the heart is massively infected. In order to prevent humans from acquiring infections from animals, whole carcasses need to be condemned at slaughterhouses, as there is always a risk of consuming improperly cooked meat. *C. cellulosae* infections in swine are usually asymptomatic except in cases when vital organs like the heart are massively infected. Muscle stiffness has also been documented in case of massive infections.

Epidemiology and Public Health

Incidence of taeniasis has been reported worldwide, especially in countries where raw or undercooked beef or pork is consumed. There is a dearth of knowledge on taeniasis and

cysticercosis in both developed and developing countries. Though some prevalence data are available, their accuracy remains questionable partly due to imperfect diagnostic tests. This has led to gross underestimation of the true prevalence of the disease. The prevalence of taeniasis ranges between 0.1% and 15%. Bovine, porcine and human cysticercosis prevalence varies from 0.03% to 80%, from 0.6% to 60% and from 1.3% to 40%, respectively.

An estimated 60–70 million people are considered to be carriers of *T. saginata* globally. In regions like Eastern Europe, eastern Africa, Latin America, Southeast Asia and Russia, where raw beef is consumed, *T. saginata* taeniasis has been reported. *T. saginata* infections are rare in Northern America, except in instances where cattle and humans live in close proximity and poor sanitary conditions prevail. A study on the prevalence of *T. saginata* in Asia indicated the highest incidence in the Philippines (33.7%), followed by Pakistan (7%), Vietnam (5.85%), Indonesia (4.68%), Nepal (4.37%) and India (3.84%) (Fig. 8). This may be in part due to traditional dishes containing raw beef, improper cooking of beef before consumption, co-habitation and poor sanitary conditions.

T. solium infections are more prevalent in communities with poor sanitation and where people eat raw or undercooked pork, like in Latin America, Eastern Europe, sub-Saharan Africa, India and Asia (Fig. 7). *T. solium* infections are on the rise in the United States largely due to immigrants from endemic areas like Latin America. A similar rise has also been witnessed in Europe due to immigration and increased travel to endemic areas. Neurocysticercosis had been described as endemic in north Portugal and the western provinces of Spain. According to World Health Organization (WHO), 30% of all epilepsy cases in endemic countries and 3% globally may be due to neurocysticercosis. In India, the disease is prevalent throughout the country but varies between the states. In the northern states of India, where pig rearing is common, the prevalence of taeniasis as high as 18.6% has been recorded.

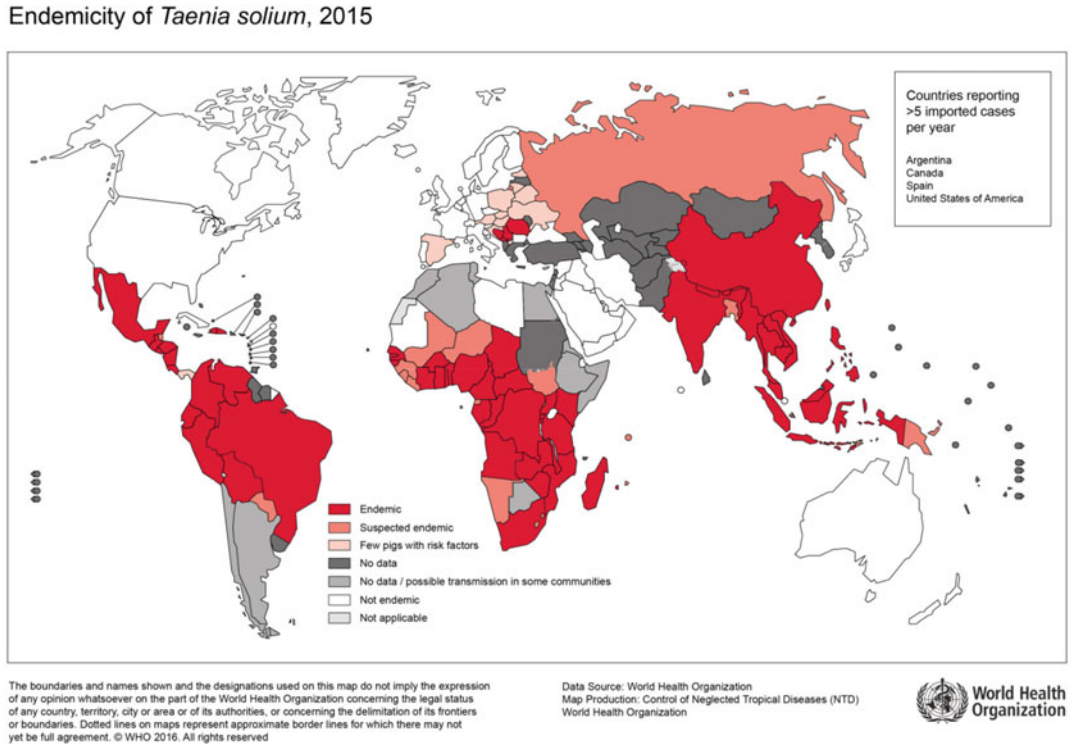

Fig. 7 Epidemiology of *Taenia solium* (Courtesy-WHO)

Table 1 Epidemiological features of *Taenia* spp.

Species	Definitive host	Intermediate host	Geographic distributions
Taenia saginata	Humans	Cattles	Africa, Latin America and Asia as well as in some Mediterranean countries
Taenia solium	Humans	Pigs, humans	Asia, Africa, Latin America
Taenia asiatica	Humans	Pigs, cattle, goats	Korea, China, Taiwan, Indonesia, Thailand, Japan, the Philippines, Vietnam, Nepal

T. asiatica is limited to Asia and cases have been reported from the Republic of Korea, China, Japan, Taiwan, Indonesia, Thailand and Nepal (Table 1). There is a lack of study on *T. asiatica* since it is difficult to identify carriers and needs expensive molecular diagnostic methods for species identification. Due to this, the true prevalence of *T. asiatica* taeniasis remains unknown.

In many countries of East, Southeast and South Asia, which are rich in cultural, ethnic and religious diversity, all three different human *Taenia* species have been shown to circulate in the region. A high prevalence of taeniasis and cysticercosis are reflective of deficient sanitation measures, below par health standards and poor food safety measures. Therefore, there is a need to improve local surveillance, sanitation, diagnosis and overall regulatory systems.

Diagnosis of Taeniasis

A wide variety of diagnostic tests are available, but there is wide variation in the detection levels and discriminating powers between the various *Taenia* species (Table 2).

Table 2 Laboratory diagnosis of taeniasis and cysticercosis

Diagnostic approach	Method	Target	Remarks
Microscopy	Stool examination	Eggs	Cannot distinguish between the species
		Proglottids	Can differentiate *Taenia solium* from *Taenia saginata. Taenia asiatica* has the same morphology as *Taenia saginata*
Antigen detection	CSF, Blood ELISA	HP10 (excretory/secretory glycoprotein of *Taenia saginata*), 87 kDa and 100 kDa of antigen from somatic extracts of adult *Taenia saginata,* 65 kDa antigen from excretory-secretory antigens of *Taenia saginata* cysticerci	Works better with CSF samples compared to serum in neurocysticercosis. Higher the number of viable cysts, the higher the antigen level
	Stool (Coproantigen) ELISA	Somatic antigens of the adult worm or excretory-secretory products	Genus specific. Some test specific for *Taenia solium*. Can detect immature tapeworm stages, before egg shedding
Antibody detection	ELISA	Antibody against Recombinant 50 kDa, Recombinant 24 kDa, Synthetic 8 kDa, Cathepsine L-like 53/25 kDa antigens	Cannot distinguish between active and inactive infection. Have a low positive predictive value in cases with viable cysticercosis
	EITB	GP50, GP42–39, GP24, GP21, GP18, GP14, and GP13 antigens	The presence of any one of the seven antibody bands is taken as positive. Test of choice
Molecular diagnosis	PCR, RFLP, RT-PCR, LAMP	pTsol9, HDP2, 12S rDNA	Highly specific and sensitive. Species specific diagnosis

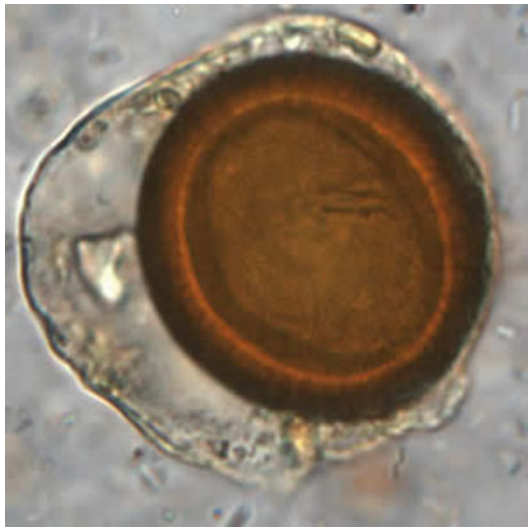

Fig. 8 Egg of *Taenia* spp. (wet mount, Iodine stained). (Courtesy: Oregon State Public Health Laboratory/CDC)

Microscopy

Direct microscopy of stool is performed to demonstrate *Taenia* eggs and proglottids in the diagnosis of intestinal taeniasis. *Taenia* eggs are round, 30–40 μm in size, bile-stained, with thick, brown, radially striated shell, and are embryonated, with a six-hooked oncosphere (Fig. 8). *Taenia* species eggs are morphologically similar to each other. Repeat stool examination and concentration methods such as formalin-ether sedimentation are frequently used to increase the detection rate.

Differentiation between *T. saginata* and *T. solium* is carried out by detection and identification of gravid proglottids containing uterine branches. In order to facilitate the counting of uterine branches, dyes such as carmine or Chinese ink are injected using a fine needle. Longitudinal histological sections stained with haematoxylin-eosin also allow more accurate counting of the branches.

T. asiatica is identified by the demonstration of rostellum on the scolex, presence of more than 57 uterine branches in the gravid proglottids, prominent protuberances in the posterior part of gravid proglottids and wart-like formation on the surface of the larvae.

Serodiagnosis

Demonstration of *Taenia* coproantigens in the stool is diagnostic of intestinal taeniasis and is carried out by a polyclonal antibody-based sandwich ELISA or dipstick ELISA. The tests have the advantages of increased sensitivity, no cross-reactions with other intestinal helminth infections such as *Ascaris*, *Trichuris* and *Hymenolepis* spp. and the ability to detect *Taenia* carriers However, this test is only genus specific and cannot differentiate between intestinal *T. solium* and *T. saginata* infections.

Molecular Diagnosis

The advantages of molecular methods include large-scale screening to detect worm carriers, to diagnose human intestinal taeniasis in animals and to differentiate between the three species.

CoproPCR is highly specific and sensitive to detect both mature and immature *Taenia* worms in stool, although the DNA extraction procedure is costly.

Several formats and targets have been utilized in the detection and differentiation of *Taenia* sp. in worm extracts. PCR coupled with the nucleotide sequencing of the amplified product is the most common approach. The markers used are mitochondrial (cox1, cob, nad1, 12S rRNA), nuclear (18S rRNA, 5.8S rRNA, 28S rRNA and ITS2), elongation factor-1-alpha (ef1) and ezrin/radixin/moesin-like protein (elp). Various approaches include (a) restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR), (b) random amplified polymorphic DNA-PCR (RAPD-PCR), (c) single strand conformation polymorphism (SSCP), (d) multiplex PCR and (e) loop mediated isothermal amplification (LAMP).

The RFLP-PCR, based on studying the restriction fragment length polymorphism (RFLP) of the nuclear ribosomal DNA (rDNA) or other genomic regions, including mitochondrial DNA, is carried out for differentiation of *Taenia* species. The target sequence studied is internal transcribed spacer 1 (ITS1) containing the 5.8S gene, mitochondrial cytochrome c oxidase subunit 1 (cox 1), mitochondrial 12S rDNA and so on.

RAPD-PCR is a relatively simple, rapid technique in which genomic DNA is amplified by PCR using a single oligonucleotide primer of arbitrary nucleotide sequence. However, the test is run along with other available DNA techniques to get a reliable result.

SSCP is a mutation scanning method with the potential to discriminate DNA sequences differing by a single nucleotide. The genes targeted to differentiate *Taenia* spp. in SSCP include mitochondrial cox 1 and NADH dehydrogenase subunit (nad1) genes. DNA sequencing of mitochondrial cox 1 and nad1, cytochrome b, 12S rDNA, nuclear 28S rDNA and ITS1/ITS2 rDNA genes are also valuable in differentiating mainly *T. saginata* from *T. solium*. The test has the advantage of the analysis of large numbers of samples in a short period.

Multiplex PCR employs genus-specific and species-specific primers used to differentiate *Taenia* species. Based on mitochondrial cox 1 as the target gene, specific amplicon size of 827 bp for *T. saginata* and 269 bp for *T. asiatica* have been observed. Specific amplicon size of 720 and 984 bp have been reported for *T. solium* American/African and Asian genotypes respectively. This technique is relatively easy and time saving, as it does not require DNA sequencing, hence most extensively used in the diagnosis of various forms of cysticercosis including neurocysticercosis.

The isothermal amplification methods such as LAMP is increasingly used for the diagnosis and differentiation of *Taenia* species and is found to be more sensitive and specific than the multiplex PCR. Among the two target genes for LAMP, primers targeting the *cox 1* differentiate the three species of *Taenia,* whereas the primers targeting *clp gene* failed to differentiate between *T. saginata* and *T. asiatica.*

Diagnosis of Cysticercosis

Microscopy

Microscopic examination of surgically resected lesions shows typical parasitic tegumental cytology in association with cholesterol crystals and calcareous corpuscles. Demonstration of invaginated scolex with the hooklets is diagnostic of *cysticerci*. Cysts can be in various stages of degeneration with higher intensity of inflammatory reactions in the later unviable stages. Fine-needle aspiration provides a cheaper alternative to open biopsy.

Serodiagnosis

Antibody-based serological tests such as ELISA and enzyme-linked immunoelectrotransfer blot (EITB) (Fig. 9) are most frequently used in the diagnosis of cysticercosis caused by *C. cellulosae*.

The ELISA platforms with the newer FAST-ELISA format use oncospheral peptides and crude antigen extracts to detect antibodies with suboptimal sensitivities and cross-reactions with hydatid disease and hymenolepiasis. EITB assays using lentil lectin purified glycoproteins (LLGP) like GP50, T24, 8 kDa proteins provide robust sensitivity but cannot distinguish the recent from a past infection. A newer EITB format using a recombinant antigen rT24H is showing promise with 94% sensitivity and 98% specificity. Western blot using the LLGPs provides highly specific results with 95% sensitivity.

The EITB, utilizing highly specific protein bands, helps in *Taenia* spp. differentiation. Immunoblot band of 21.5 kDa has been found to be highly specific for *T. asiatica*. In addition to it, there occur two other immunodominant candidate antigens which have been identified and expressed as recombinant molecules: *T. asiatica* Lactate dehydrogenase (rTaLDH) and the recombinant *T. asiatica* enolase (rTaENO). These proteins are found to be located in the tegument of adult *T. asiatica* and the embryonic membrane of the oncosphere.

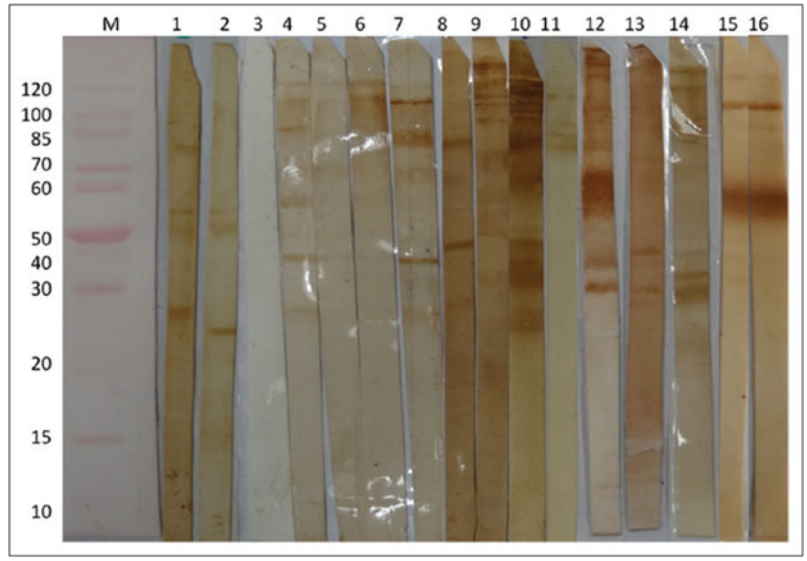

Fig. 9 Enzyme-linked immunoelectrotransfer blot for anticysticercal antibodies. Lane M: Ponceau stained protein ladder; lanes 1, 2, 4, 7–10, 12–16: positive serology for neurocysticercosis; lanes 3, 5, 6, 11: negative serology for neurocysticercosis. (Author's Collection)

The major drawback of the antibody-based serological tests including EITB is that these cannot differentiate between the present and past infection and antibodies persist even after.

Antigen-based tests are frequently used for demonstration of circulating *Cysticercus* antigens in the serum, saliva and urine for diagnosis of cysticercosis. The ELISA using monoclonal antibodies against HP10 antigen and excretory/secretory antigen B158/B60 is increasingly used for detection of *cysticercus* antigen in the serum as well as in the CSF for the diagnosis of neurocysticercosis. The detection of antigen in the serum and other body fluids is suggestive of recent and active cases of cysticercosis and is of prognostic value as antigen disappears from the circulation after clinical and parasitological cure of the cysticercosis.

Other Methods

Imaging methods such as X-ray, CT and MRI are used for the diagnosis of neurocysticercosis. Plain X-ray of skull and soft-tissue radiography is used to demonstrate calcified or dead cyst. CT and MRI are the currently used techniques to demonstrate the number, size, site and stage of cysticerci. CT is the method used for detecting dead, calcified and multiple cysts. MRI appears superior to CT in identifying non-calcified cysts and the cysts located in parenchymal and extraparenchymal tissues. The brain lesions are confused with tuberculoma and tumour on CT/MRI scans.

Any one of the absolute criteria (histological parasitic demonstration in the brain or spinal cord biopsy, CT/MRI showing scolex ("hole-with-dot") or fundoscopic demonstration of the subretinal parasite) provides a definitive diagnosis. Major criteria include highly suggestive lesions on neuroimaging, positive serum EITB assay, spontaneously resolving small single enhancing granuloma or resolution of an intracranial cystic lesion with albendazole or praziquantel therapy. Minor criteria consist of lesions compatible with NCC on neuroimaging, clinical manifestations suggestive of NCC, positive CSF ELISA for antigen or antibody and cysticercosis outside the central nervous system. One major criterion with two minor criteria constitutes a probable diagnosis of NCC. In unfulfillment of absolute criteria, meeting two major criteria and one minor criterion can provide a definitive diagnosis in conjunction with epidemiological criteria (endemic residence, travel history). Epidemiological criteria help in establishing a probable

diagnosis if combined with three minor criteria or one each from the major and the minor criteria.

Diagnosis of Ocular/Orbital Cysticercosis (OCC)

Ocular/orbital cysticercosis (OCC) is a common form of cysticercosis accounting for 75–80% of cysticercosis cases worldwide. Localization of the cysts can be extraocular (orbital tissues, muscles, lacrimal or subconjunctival) or intraocular. OCC can manifest in different ways in a patient depending upon the site of the cyst.

Depending on the cyst location, the outcome of an 8-point ophthalmological exam will vary. Visual acuity, pupils, external evaluation (proptosis, nodular mass, lid swelling, ophthalmoplegia), slit-lamp examination and funduscopic examinations are common in diagnosis.

CBC and anterior chamber paracentesis may reveal eosinophilia. FNAC can be used to aspirate the cyst for a confirmatory diagnosis. Histology can identify scolexes with hooklets within the cyst. The dying cyst presents with a fibrous cyst wall and granuloma with giant cell formation. ELISA can aid in the diagnosis, but negative test results do not rule out OCC (about 50% confirmed OCC patients test negative on ELISA).

MRI, CT and USG are far more reliable in diagnosing OCC than routine laboratory diagnostic methods. A B-scan ocular USG can reveal a well-defined cyst in the orbit with a hyperechoic scolex while an A-scan shows high amplitude spikes corresponding to calcification of cyst walls and scolex. CT may show a characteristic hypodense mass (non-enhancing lesion) with a central hyperdense scolex and adjacent soft-tissue inflammation. However, the scolex may not be identifiable if the cyst is dead and is occluded by surrounding oedema. MRI reveals a hypointense cyst and hyperintense scolex. Inflammation due to the cyst enhances CT and MRI signals.

PCR-based methods and DNA probes are also used to detect parasitic genomic materials in the tissue samples with high sensitivity and specificity.

Treatment

Praziquantel as a single oral dose of 5 or 10 mg/kg is recommended for the treatment of intestinal taeniasis caused by *T. saginata*. Niclosamide, administered in a single oral 2-g dose (50 mg/kg), is also effective. When no *Taenia* eggs are identified in faeces sample 1 and 3 months following treatment, the treatment can be considered successful.

Corticosteroids, antiseizure drugs and therapy by albendazole or praziquantel are recommended for neurocysticercosis. Albendazole is considered superior to praziquantel for NCC. The combination of albendazole plus praziquantel has been reported to result in a higher rate of radiologic resolution. Surgery may be necessary for obstructive hydrocephalus. Orbital cysticercosis is treated with albendazole and corticosteroids.

The prognosis of the disease largely depends on the site, stage of infection, patient's immunity status and surgical capabilities.

Prevention and Control

Intestinal taeniasis is prevented by adequate cooking of beef or pork viscera either by exposure to temperatures between 63 and 71 °C or by refrigeration or salting for long periods or freezing at −10 °C for 9 days. Effective disposal of faecal matter should be done to prevent cattle and pigs from getting infected. The various methods which can be adopted include the following.

Preventive measures require adequate sanitation, sewer treatment and pig corralling to prevent intermediate hosts from the exposure of the parasitic ova. Additionally, food hygiene like avoiding the use of the same chopping board for uncooked and cooked meat, hand hygiene and personal hygiene is necessary.

Meat hygiene is extremely important to prevent disease in humans; irradiation, salt pickling (12–24 h) and freezing at minus 24 °C for 24 h provide excellent results. Meat brought to a temperature between 60 and 65 °C until it loses its pink colour is effective. A strict market control of

infected pigs and cattle is difficult due to economic factors. Meat inspection detects heavy infections. Though the regulations vary globally, pigs are inspected for cysticerci in thigh muscles, diaphragm, heart, intercostals muscles and tongue. Masseters, ventricles of heart, liver and diaphragm of the cattle are the sites to look for. Targeted preventive therapy in specific human risk groups results in a sustained reduction in cases.

Vaccination and anthelmintic therapy of the cattle and pigs help reduce the burden. For pigs, SP3VAC and TSOL18 vaccines show high efficiency in combination with oxfendazole. The TSA9/TSA18 vaccine against *T. saginata* is promising in cattle. However, these vaccines cannot destroy the existing cysts.

Case Study

A 56-year-old man presented at a hospital with a history of passing segments of some worm in his stool of one-month duration. No other abdominal symptoms were present. Stool examination showed proglottides but no eggs. To ascertain the species, PCR amplification of mitochondrial cytochrome *c* oxidase subunit I gene and elongation factor-1 alpha was done with the segment. A 100% match with *T. saginata* was found with both markers. Repeat stool examination after treatment with praziquantel for 3 days showed the absence of any segments or ova.

Questions

1. What relevant dietary history would have been helpful in this case?
2. What tests other than PCR can be done here to arrive at a diagnosis?
3. What precautions should be taken to prevent the infection?

Research Questions

1. A simple diagnostic tool for species identification in taeniasis other than molecular methods in resource constraint settings has to be developed for effective management.
2. There exists a lacuna in defining the role of the immune mechanism during the course of this parasitic infection in humans and animals.
3. There is a lack of an appropriate animal model to study the pathogenesis and host-parasite relationship of these parasites.

Further Readings

Galán-Puchades MT, Fuentes MV. *Taenia asiatica*: The most neglected human *Taenia* and the possibility of cysticercosis. Korean J Parasitol. 2013;51:51–4.

Garcia HH, Del Brutto OH. *Taenia solium* cysticercosis. Infect Dis Clin N Am. 2000;14(1):97–119.

Garcia LS. Diagnostic Medical Parasitology. fifth ed. Washington, DC: ASM Press; 2007.

Gonzále LM, Bailo B, Ferrer E, et al. Characterization of the *Taenia* spp. HDP2 sequence and development of a novel PCR-based assay for discrimination of *Taenia saginata* from *Taenia asiatica*. Parasites Vectors. 2010;3:51.

Murrell KD. WHO/FAO/OIE guidelines for the surveillance, prevention and control of taeniasis/cysticercosis 2005;139.

Parija SC, Ponnambath DK. Laboratory diagnosis of *Taenia asiatica* in humans and animals. Trop Parasitol. 2013;3(2):120–4.