Trichinellosis

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Learning Objectives

1. To make the reader aware of the various other *Trichinella* species which have zoonotic potential, apart from the well-characterized *T. spiralis*.
2. To understand epidemiological significance with the domestic and sylvatic cycles going on in parallel in nature.
3. To emphasize importance of serological diagnosis because of non-specific and protean clinical manifestations.

Introduction

Trichinellosis is a zoonotic disease, commonly of pig origin and other animals caused by the nematode *Trichinella spiralis*. The practice of eating undercooked pork, pork products or meat of wild or game animals predisposes to trichinellosis. Although serology is useful in the diagnosis of the condition, nevertheless, the definitive diagnosis of the condition is made by demonstration of the larva of the parasite in the muscle by biopsy.

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History

The encysted larval stages of *Trichinella*, causing trichinellosis or trichinosis, were first discovered in the muscles of infected man by Tidemann in 1821 (in Germany and by James Paget and Richard Owen in 1835 in London. Joseph Leidy in 1846 observed similar encysted larvae in the pork in Philadelphia during the year 1846. Leuckart in 1855 and Virchow in 1859 showed the development of infective larva to the adult worm in the intestine in experimental animal. They observed that the young larvae, produced by the female adult worm migrated through blood vessels to reach the muscle in which they became encysted. Zenker in 1860 implicated the parasite to be the causative agent of trichinellosis in humans. The larval stage of the parasite was first demonstrated in the human blood by Herrick and Janeway in 1909.

Taxonomy

T. spiralis Owen, 1835, Railliet, 1895 belongs to the genus *Trichinella*, family Trichinellidae, superfamily Trichuroidea Railliet, 1916; order, Enoplida Chitwood, 1933; subclass Adenophorea, and class Nematoda under the phylum Nemathelminths.

Until recently, *T. spiralis* was considered to be a single species of the genus *Trichinella*. However, various workers have recognized strain

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differences within the species. Thus, the larvae of Arctic strains remain infective even by freezing the meat, while those of temperate zone strains are killed by freezing. East African strains were reported to be less infective to rats compared to strains from other areas. Because of these differences, it was proposed to subdivide *T. spiralis* into three species: *T. spiralis* (man, domestic animals and pigs), *Trichinella nativa* and *Trichinella nelsoni*. A new species, *Trichinella pseudospiralis* which has a very weak cyst and unique characteristic to develop in birds, has been included as a fourth one in the list of *Trichinella* species. In recent years, molecular methods have been used to better delineate the members of the genus to the species level and genotypes.

Currently, the genus *Trichinella* consists of nine species and three genotypes. Two clades have been defined based on the presence or absence of collagen capsule around the muscle larva:

(a) **Encapsulated**: These are found only in mammals. It includes *T. spiralis, T. nativa, Trichinella britovi, T. nelsoni, Trichinella murrelli*, *Trichinella patagoniensis* and the genotypes *Trichinella* T6, T8, T9.
(b) **Non-encapsulated**: They have a much wider distribution among mammals, birds and reptiles. It includes *T. pseudospiralis, Trichinella papuae* and *Trichinella zimbabwensis*.

The International Trichinella Reference Centre contains detailed information of the various species and genotypes (www.iss.it/site/Trichinella/index.asp).

Genomics and Proteomics

In earlier studies, a genomic approach was used using Expressed Sequence Tags (ESTs) generated from three life stages of *T. spiralis,* namely adult worm, immature larva and mature larva in muscles. A total of 3262 unique genes were found out of 19,552 genes on analysis of more than 10,000 ESTs. The GC content of protein-coding exons was found to be 39%. The *Trichinella* genome has been compared with that of *Caenorhabditis elegans* and it has been shown that there is 56% homology between them as far as EST clusters are concerned. Species and phylum based analysis has revealed great phylogenetic distance of *T. spiralis* from other nematodes. As of now, the 64-Mb nuclear genome has been sequenced using whole-genome shotgun approach and hierarchal map–assisted sequencing and is estimated to contain 15,808 protein-coding genes. The GC content of the overall genome is 34%. The 15,808 protein-coding sequences occupy 26.6% of the genome. For a detailed report of the draft genome, the readers can refer to the article by Mitreva et al. (2011).

The characterization of excretory-secretory products (ESP) in parasites is an important step in understanding their roles in host-parasite interaction and future developments in diagnostic methods and vaccine development. By using a combination of protein sequence similarity and signal peptide prediction, 345 *T. spiralis* clusters have been identified as having homology with predicted secreted or membrane proteins. Identities have been given to 43 ESP peptide spots which represent 13 different proteins, signifying the presence of substantial protein isoforms in the ESP. Some of the important proteins identified so far include serine protease, cysteine protease, zinc dependent metalloprotease, 45 KD antigen, gp43 and two unidentified open reading frames.

The Parasite Morphology

The Adult Worm

The adult *T. spiralis* is the smallest nematode which infects man. The worm is minute, whitish, thread-like and just visible to the naked eye. The oesophagus fills up from one-third to one-half of the body and is covered by a single layer of large cells. The oesophagus joins the intestine, which extends posteriorly to end in the terminal anus (Fig. 1).

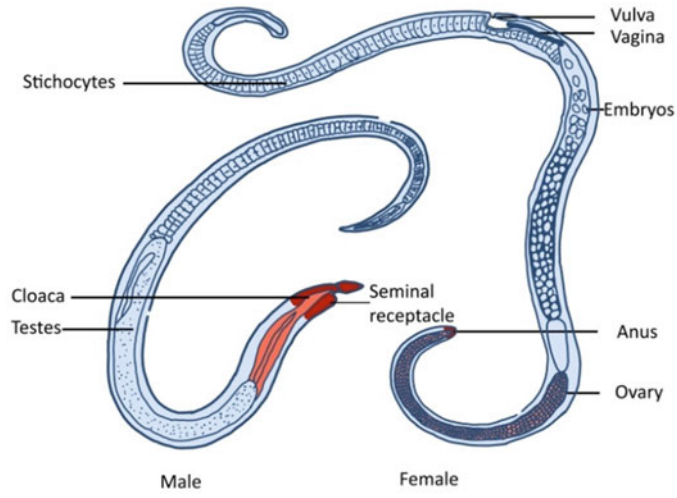

Fig. 1 Schematic diagrams of adult male and female *Trichinella spiralis*

Male worm measures 1.4–1.6 mm in length and 0.04 mm in diameter. The anterior end is delicate, filariform and possesses cephalic papillae. The posterior end is filled up with the testes and bears two conspicuous conical papillae, on either side of the cloacal orifice. The male dies usually after fertilizing the female or is excreted out along with the faeces.

The female is approximately twice as long as the male and measures 3–4 mm in length and 0.06 mm in diameter. The female genitalia consist of a single ovary, coiled uterine tube and vulva situated ventrally at the anterior fifth of the body near the middle of the oesophageal area. After fertilization by the male, the female starts producing the eggs which immediately develop into the larvae in the uterus. The females are viviparous, and by the sixth day of infection, they begin to lay motile larvae instead of eggs. Each female is capable of producing nearly 1000–10,000 larvae during its lifetime of 16 weeks. The larvae, but not the eggs, are excreted in the faeces.

Larva

The larvae measure 100 μm in length and 6 μm in breadth. They are deposited by the viviparous female in the intestine, from where they are carried by the systematic circulation and are deposited in various organs and tissues of the body. The larva becomes encysted only in the striated voluntary muscles, where it continues to develop, sexually differentiate, and attains a length of 1 mm, ten times its original size, inside the cyst. The anterior end of the fully grown mature larva is thin, while the posterior end is thick and rounded. The larva inside the encysted cyst is infective to other hosts and is viable for many years before it is calcified. The encysted cyst is lemon-shaped and lies parallel to the muscle fibres.

Cultivation of Parasites

T. spiralis is cultivated from the larval to the adult stage in artificial culture media containing 50% chick embryo extract in serum of rabbit, ox or chicken. A continuous-flow culture system with a gas phase of 85%N-5%CO_2–10%O_2 is necessary for cultivation. In a recent study, the newborn larvae were successfully cultured in 5% CO_2 at 37 °C for 18 h in the RPMI-1640 medium containing 10% fetal bovine serum.

Laboratory Animals

Laboratory animals are widely used to study the pathological and immunological reactions of the host against *T. spiralis* infection. Rat and mouse

are commonly employed in various studies. *Trichinella* is easily maintained in these animals and each developmental stage of its life cycle is recovered from these animals for in vitro studies. The pathological and immunological changes observed in experimental infections of these animals by *Trichinella* closely resemble those seen in humans.

Life Cycle of *Trichinella spiralis*

Hosts

Definitive Hosts

Pigs, rats, horses, humans

Intermediate Hosts

No intermediate hosts

Infective Stage

Larval form of *Trichinella*

Transmission of Infection

The life cycle of all species of *Trichinella* comprises of two generations, larval and adult, in the same host (Fig. 2). Although *Trichinella* has a wide host range of mammals, birds and reptiles, humans, pigs and horses are the ones most important from a public health point of view. The adult *T. spiralis* inhabits the small intestine of the pig, rat and human. The adult and larvae are the distinct stages of the parasite observed in the life cycle of the parasite.

Man acquires infection by ingestion of raw or inadequately cooked pork, infected with the larvae of *Trichinella*. On ingestion, the larvae are liberated in the stomach from the cyst by acid-peptic digestion. The larvae migrate down to the duodenum and jejunum, attach to the mucosa and grow to the adult worms by the third day of infection. The adult male and female worms mature sexually within 5–7 days and the female is then fertilized by the male, after which the male dies. The fertilized female lies deeply burrowed in the mucosa and discharges 1500–2000 larvae for a period of 5–7 weeks or till it is alive. The adult worm remains viable in the intestine for a few weeks, but may survive much longer in immunocompromised host. The larvae are carried by the portal blood circulation or lymphatics to reach the systemic circulation. These are then carried in the systemic circulation for deposition in striated muscles especially the diaphragm. Apart from the diaphragm, the intercostal muscles, muscles of the neck and other large voluntary muscles are commonly affected. The larvae inside the muscles burrow their way into individual muscle fibre causing myositis. The larva lies along the long axis of the muscle and grows rapidly over a period of 3 weeks to attain the size of 1 mm, about ten times its original length. Finally, a cyst wall develops and the larva remains locked up inside the cyst. The mature cyst measures 0.5 mm in length and 0.25 mm in breadth. In human muscle cells, the lifespan of the larva may extend for decades (up to 40 years). In other areas like the myocardium, the larvae do not encyst and die in a short time. After a variable period of time (6–-18 months) under the immune response, calcification occurs. The mature cyst when calcified can be found as fine granules in the muscle.

Trichinella infection in man is the dead end. The propagation of species however is maintained by infection in animals. Pig acquires infection by eating carcasses of other pigs (pig-to-pig) or rats (rat-to-pig) infected by *Trichinella* larvae. Rat gets an infection from an infected rat (rat-to-rat) and less commonly from a pig (pig-to-rat). Ingestion of raw flesh infected with the viable encysted larvae is responsible for the transmission of the disease to a new host.

Pathogenesis and Pathology

Pathogenesis of *Trichinella* infection is largely dependent upon the number of invading organisms and frequency of previous exposure. The larvae invade mucosal epithelium of the duodenum and jejunum and mature to the adult worms in the mucosa of the intestinal tract. The adults are responsible for the development of

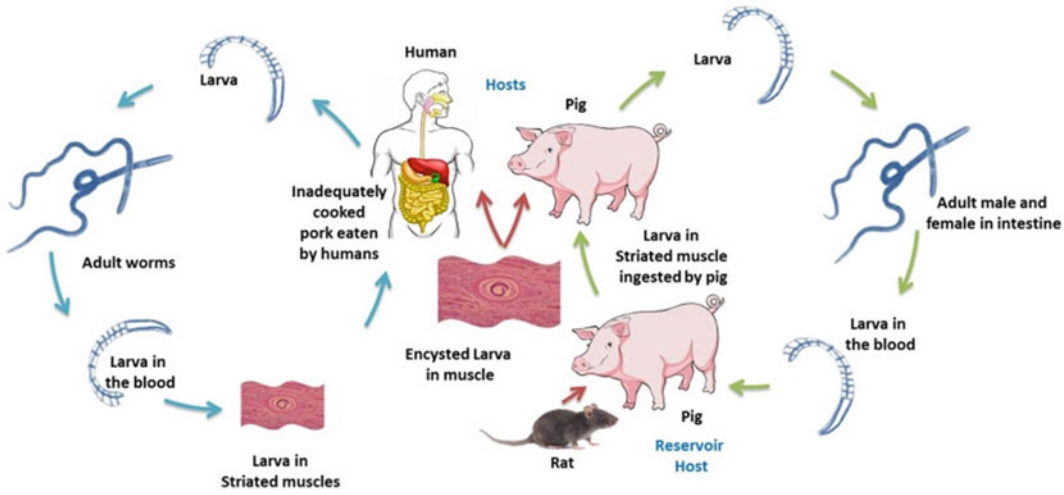

Fig. 2 Life cycle of *Trichinella spiralis*

gastrointestinal manifestations such as nausea, diarrhoea or abdominal cramps in man.

Migration of the larvae and reactions of the host to the encysted larvae within the striated muscles are responsible for the characteristic extra-intestinal manifestations of the disease such as myalgia, myositis, peri-orbital oedema, fever and prostration. The migrating larvae show predilection for their encystment in the striated muscles, particularly at the sites of attachment of these muscles to the tendons and bones. The diaphragm, tongue, larynx, intercostal, deltoid, gluteal and pectoral muscles are most commonly affected. The migrating larvae in their course evoke inflammatory reactions, which subside only after their encapsulation in the striated muscles. Encapsulation does not take place in the myocardium.

Various proteases found in parasites participate in host tissue and cell invasion in the intestinal tract and may also help in the moulting process. The transformation of the host muscle cell into the nurse cell is also under the influence of the hitherto unidentified secreted protein of the parasite. After penetration of the enterocytes, the larvae take residence in the striated muscle cells. Here, it invokes the transformation of muscle cell into "nurse cell," with the disappearance of sarcomere myofibril. Then, following encapsulation, a capillary network develops around the whole structure. The sarcoplasm becomes basophilic, the cell nucleus assumes a central position, and the nucleoli increase in number and size. Increased cell permeability leads to a release of muscle enzymes.

The intestine and the muscles are the common sites invaded by *T. spiralis*. An acute inflammatory response, predominantly neutrophilic, develops in the mucosa of the intestine around the adult worm and is usually associated with a mild and partial villous atrophy.

Migration of the larvae in various muscles provokes marked inflammatory reaction in the tissue. The muscle fibres are destroyed and an acute inflammatory reaction consisting primarily of lymphocytes and eosinophils appears in the muscles. The adjacent muscle fibres show hyaline degeneration (Fig. 3). The encapsulation of the larvae resulting in the formation of cysts of 1 mm or less in diameter takes place in the striated muscles. These cysts eventually calcify within a period of 6 months to 2 years along with the larvae. The growth or encapsulation of the larva does not take place in the cardiac muscles. In the myocardium the larva produces only inflammation, necrosis and fibrosis of the myocardial fibres. Eosinophilia is the hallmark of trichinosis and occurs after 2–4 weeks of infection by the infective larvae.

Diffuse leptomeningeal round cell infiltration and, less frequently, minute foci of gliosis around the capillaries are the major pathological changes

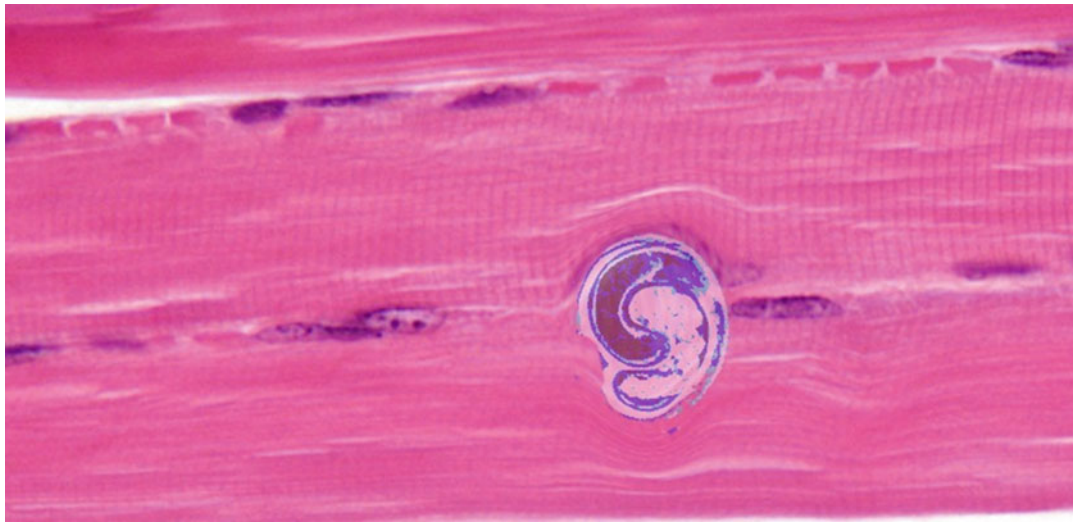

Fig. 3 *Trichinella spiralis* in skeletal muscle. (From: Rawla P, Sharma S. *Trichinella spiralis*. [Updated 2020 May 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538511/. Image courtesy S Bhimji MD)

caused by the infective larvae in the central nervous system.

Immunology

T. spiralis infection is always associated with the participation of both the humoral and cell-mediated immunities. The role of humoral and cellular responses in the development of resistance against *Trichinella* infection has been elucidated partially. The former is characterized by the elevated levels of serum IgG, IgM and IgA antibodies in experimentally infected mice after 4 weeks of infection. The parasite-specific IgG1, IgG2, and IgE antibodies are produced against the cuticula, while the IgA and IgM antibodies are produced against the membrane of the helminths. In man, *T. spiralis* infection is characterized by an increased level of circulating IgM, IgG and IgA antibodies after 10–25 days of the infection. The bulk of parasite-specific IgG1 (80%) recognize a highly immunogenic sugar, tyvelose, of parasite origin. This antibody response is associated with a strong TH2 response in the regional lymph nodes. It is to be noted that an increase in the level of IgE is not a constant feature in trichinellosis and co-relates with the degree of eosinophilia in the patients.

The cell-mediated immunity (CMI) showing delayed hypersensitivity reaction has also been demonstrated in the experimental infections of the animals. The T lymphocytes act as the regulator of the inflammatory response during muscle infection. In experimental animals, the concentration of IL-4 and the number of IL-4 producing cells increase, and it has been found that lymph nodes in infected animals produce IL-5, 10, 13 and IFN-γ. Similar findings have been noted in infected humans who demonstrated high levels of IL-5, 10 and IFN-γ. The CMI plays an important role in the development of acquired resistance against the parasite. The transfer of peritoneal exudate cells or lymph node cells from the immunized donor mice to the normal mice led to the transfer of protection against the parasite in the recipient animal. T cells are the effector cells in the development of resistance against the parasite. The gut immunity against *Trichinella* is also believed to be T cell dependent. The humoral antibodies probably play a less important role in the development of acquired resistance in

trichinellosis. No consistent correlation between levels of circulating antibodies and the number of larvae in the muscle or expulsion of adult worms from the gut has been demonstrated. The reaginic IgE antibodies are suggested to play an important role in the complex interactions between mast cells, eosinophils and helminths in the development of acquired resistance against the disease.

A discussion about the immune response in trichinellosis will be incomplete without the mention about the role of eosinophils. Eosinophilia is a prominent feature of *Trichinella* infection and the count may go up to >10,000/μL and the higher level co-relates with the degree of myalgia and central nervous system (CNS) complications. In the initial phases of infection, fall in the eosinophil count carries a grave prognosis and even death of the patient has been reported with count <1%. Eosinophils may act as double-edged swords by having a protective role together with tissue damage. On one hand they can protect the individual from severe infection by killing the newborn larvae by antibody-dependent cellular cytotoxicity phenomenon by releasing major basic proteins, peroxidase and eosinophilic cationic protein. At the same time, the release of histamine, serotonin, bradykinin, prostaglandins E2, D2, J2 and other products cause increased vascular permeability in the capillaries with leakage of fluid, electrolytes, and proteins in the surrounding tissue with resultant vascular damage. Chronic eosinophilia has been linked to severe tissue damage in muscle, myocardium and CNS.

Infection in Humans

Trichinella infection is asymptomatic in the majority of cases in humans. The clinical manifestations depend mainly on the number of larvae invading the intestine and frequency of previous exposures to *Trichinella* and may be divided broadly into (a) intestinal, (b) muscle invasion and (c) convalescence stages.

The clinical signs and symptoms of the intestinal phase are due to irritation of the gastrointestinal mucosa by the adult worm and are observed during the first week of infection. Nausea, vomiting, diarrhoea or constipation and abdominal cramps are the presenting features. In heavy infection, the patients may occasionally develop fulminant enteritis.

The symptoms in trichinellosis, due to muscle invasion by the larvae, are by far more common and are seen during the second week of infection. The condition is characterized frequently by manifestations of the peri-orbital oedema with or without subconjunctival haemorrhages and chemosis and the myositis developing in the intraocular muscles, masseters, neck muscles, limb flexors and lumbar muscles. Occasionally, a macular and petechial rash is observed. Marked peripheral eosinophilia even up to 70% is commonly seen. The patient may die because of myocarditis, encephalitis and other neurological complications. Myocarditis is seen in 5–20% of cases and presents as pericardial pain, tachycardia and electrocardiogram abnormalities. Neurological complications are rare in trichinellosis and may show multiple small cortical infarcts in magnetic resonance imaging.

The convalescence stage is marked by the beginning of the encapsulation of the larvae during the third week of infection. The systematic manifestations are usually absent in this stage; however malaise and weakness may be present for a few months. Myocarditis and less frequently bronchopneumonia, vascular thrombosis and encephalitis may be present as sequelae of the infection in this stage.

Mortality due to the condition is relatively low. Myocarditis is the commonest cause of the death. Bronchopneumonia, vascular thrombosis and encephalitis are the less frequent causes of death in this condition.

Different species of *Trichinella* may cause some variations in their clinical manifestations. It has been observed that *T. spiralis* may cause more severe infection compared to *T. britovi* possibly because females of the latter species are less prolific. *T. murrelli* may not cause peri-orbital or facial oedema but may provoke skin reactions. The non-encapsulated *T. pseudospiralis* causes more prolonged symptomatic disease.

Infection in Animals

Over 120 species of mammals including wild carnivores, felines, fur-bearing animals, rodents and insectivores are infected with *Trichinella* under natural conditions. The infection in domestic animals is rarely associated with any overt clinical manifestations. The predatory animals under natural conditions may die of trichinosis.

In rat, the infective larva shows more predilections for the diaphragm, while in pig, the masseters are most commonly affected. The skeletal muscle cysts are always round in the carnivores, round and elongated in rats and oval in pigs. The cyst undergoes calcification, the timing of which varies from host to host amongst animals. In rabbits and pigs, the onset of calcification is visible within 3–5 months and is completed by 7–9 months of infection. In mice, the process of calcification is much slower and takes a longer time of more than 1 year.

Most of the domestic and wild carnivorous animals are susceptible to infection but show no or minimal sign of infection, even though the parasite burden may be quite high. Interestingly, the household pigs and rats are resistant to the sylvatic species of *Trichinella*.

Epidemiology and Public Health

Human trichinellosis is distributed worldwide. It is most prevalent in the Northern hemisphere including the Arctic and parts of Africa and Asia. In South and Central America, autochthonous infections have been reported from Brazil, Uruguay and Chile. Recent outbreaks of trichinosis have been reported from Italy, Laos, Tanzania and France. As per the cumulative report from 55 countries, the total number of cases has been estimated to be 10,000 per year with 0.2% mortality.

Trichinella infection in humans is closely associated with the consumption of raw or undercooked meat; hence cultural and social factors play a role in the epidemiology of trichinellosis. Trichinellosis is rare in communities that consume fully cooked meat. Pork or pork products are the main source of infection especially when pigs are raised in the backyards or in the village community. Local meat consumption, particularly horse meat, has been associated with *Trichinella* infections in France and Italy. Dog meat has been incriminated as the source in China and the Slovak Republic. Human infections due to *T. nativa* has been documented in the Arctic region linked to the consumption of walrus or bear meat. Recently, *T. papuae* has been implicated in outbreaks of human trichinellosis in Thailand after eating wild boar. The first authentic case of a *Trichinella* infection in an animal was reported by Maplestone and Bhaduri in the diaphragm of a cat in the year 1942. Subsequently, it has been demonstrated in various animals like domestic pigs, rodents and wild animals like civet cats. The first human infection in India was documented in 1996 and till date at least nine case reports and one outbreak of trichinellosis have been reported. The outbreak was reported in 2014 in Tehri Garhwal district of Uttarakhand State in North India with 54 cases and one death and was associated with the consumption of raw or undercooked pork.

Nearly, 120 species of mammals and also reptiles and birds are found to be infected by *Trichinella* (Table 1). The natural cycle of *Trichinella* infection involves primarily the carnivores and is usually maintained by these carnivorous animals. The prevalence and spread of *Trichinella* infection is dependent upon the food habits of potential host species and environmental and climatic factors. In nature, *Trichinella* spp. exhibit two cycles: the domestic cycle and the sylvatic cycle.

Domestic Cycle: This is the classical well-known pig-to-pig transmission where humans get the infection by eating pork. In many parts of the world, cats, mice, dogs and various wild animals can also enter into the cycle. The domestic cycle is maintained by the pigs by consumption of meat scraps, rats or mice, dead pigs and other mammals, or ingestion of pig faeces. This type of cycle can be observed in small, local meat-producing communities or farms.

Table 1 Species and genotypes of *Trichinella* spp. and their distribution

Species (genotype)	Normal host	Sources of infection	Geographic distribution	Human cases reported	Countries from where reported
Trichinella spiralis (T1)	Pigs, rats, carnivores	Pork	Worldwide	Yes	Many countries
Trichinella nativa (T2)	Marine and terrestrial carnivores	Bear meat, walrus, dog	Arctic or Subarctic regions	Yes	Alaska in the USA, Russia, China
Trichinella britovi (T3)	Carnivores, pigs	Wild boar meat, dogs, jackals	Temperate regions, North and West Africa	Yes	Algeria, Turkey, Poland, France
Trichinella pseudospiralis (T4)	Mammals, birds	Wild boar	Worldwide	Yes	France, Thailand
Trichinella murrelli (T5)	Carnivores	Horse, bear	Temperate regions	Yes	USA, France
Trichinella papuae (T10)	Mammals, reptiles	Wild boar, soft shelled turtles	South East Asia	Yes	Thailand, Korea, Taiwan
Trichinella zimbabwensis (T11)	Mammals, reptiles	Not known	East Africa	No	–
Trichinella patagoniensis (T12)	Carnivores	Not known	Argentina	No	–
Others: *Trichinella nelsoni* (T7), T6, T8, T9	Carnivores	Not known	USA, Canada, Ethiopia, Japan, South Africa	No	–

Sylvatic Cycle: In this cycle, the carnivorous wild animals are primarily involved. Cannibalism, predation or scavenging habits of such wild animals maintain the cycle. Transmission of infection occurs by the consumption of fresh or decomposing carcasses. The species of *Trichinella* associated with the sylvatic cycle include mostly species other than *T. spiralis*. Humans and pigs normally do not enter this cycle, but they may become infected by eating infected meat of wild animals.

Diagnosis

Clinical diagnosis of trichinellosis is difficult due to protean manifestations of the disease. The condition needs to be differentiated from other similar conditions. The gastrointestinal symptoms may cause confusion with various other conditions of gastroenteritis, while systematic manifestations may mimic those of influenza, typhoid fever, sinusitis, glomerulonephritis or angioneurotic oedema. The presence of cardinal features of peri-orbital oedema, myositis, fever and high grade eosinophilia are highly suggestive of trichinosis. The history of ingestion of the inadequately or poorly cooked pork supports further the clinical diagnosis of the disease. The diagnosis of trichinellosis depends on (a) clinical findings (b) laboratory findings (Table 2) and (c) epidemiological investigation.

Microscopy

The definitive diagnosis is made by demonstration of free or encapsulated *Trichinella* larvae in the skeletal muscles (deltoid, biceps, gastrocnemius or pectoralis) at either autopsy or biopsy. In light or early infection, the larvae are difficult to show by these methods. Moreover it involves surgical invasive procedure and the amount of muscle taken for biopsy may influence the sensitivity. Sample should be collected from the muscle, free from fat or skin, weighing about 0.2–0.5 g. Examination of a muscle biopsy can be carried out by artificial digestion using 1%

Table 2 Laboratory diagnosis of Trichinellosis

Diagnostic approaches	Methods	Targets	Remarks
Microscopy	Histopathology of muscle biopsy sample	Larval forms; basophilic transformation of muscle cells	Not very sensitive
In vitro cultivation	Continuous-flow culture system	Transformation of larval form to adult	Complicated procedure; live larva needed
Serology	ELISA, indirect fluorescent antibody test (IFAT), bentonite flocculation test	TSL-1 antigen for ELISA; larva or infected muscle in IFA	IFAT shows cross-reactions with *Onchocerca* and *Schistosoma*
	Immunoblotting	Excretory-secretory antigens	Confirmatory test
Molecular diagnosis	PCR-RFLP	ITS-1 and ITS-2 of rRNA	Species and genotype identification
Other laboratory tests	Blood biochemistry and haematological investigations	Elevated serum levels of CPK, LDH and aldolase. Leucocytosis and eosinophilia	Detected from second to fifth week of infection

pepsin and 1% hydrochloric acid, or histological analysis by haematoxylin-eosin staining. Even in the absence of visualization of the larva, the basophilic transformation of muscle is an excellent indicator of trichinellosis. The larvae may be looked for, though difficult to find, in the faeces during the intestinal stage or in the blood, spinal fluid or milk during the migratory phase of the disease.

Serodiagnosis

A variety of immunological tests are currently available for diagnosis of the condition in individual cases and for epidemiological studies of trichinellosis.

The intradermal skin test using *Trichinella* antigen, used earlier, shows positive immediate hypersensitivity reaction after 11–16 days of infection and remains positive for many years.

During the acute stage of infection, IgE levels rise early in a majority of the cases, but the absence of raised IgE cannot rule out trichinellosis. Hence IgE detection is of no diagnostic importance. Circulating IgG antibodies appear 12–60 days after infection and the window period depends on the number of ingested larvae, the particular species involved and the individual's immune response. ELISA and the indirect fluorescent antibody test (IFAT) are the most commonly used approach at present, replacing the bentonite flocculation test, latex agglutination (LA) and indirect haemagglutination (IHA) employed earlier in the serodiagnosis of trichinellosis. The antigens used for IFAT include infected rodent muscle or free muscle larva. Cross-reactions have been seen with *Onchocerca* spp. and *Schistosoma mansoni* in IFAT. Immunoblotting using excretory/secretory antigens of muscle larvae of *T. spiralis* is used as a confirmatory test after initial screening by ELISA or IFAT.

Molecular Diagnosis

Molecular methods have been employed to identify the *Trichinella* species or genotype of the isolated larva. PCR-RFLP can be used for species differentiation and genotype determination. Database derived from ITS 1 and ITS 2 as well as from expansion segment V region of the rRNA repeat of various *Trichinella* species and genotypes are available for this purpose.

Other Laboratory Findings

Leucocytosis along with high-grade eosinophilia (even up to 70%), elevated serum creatinine phosphokinase (CPK) and lactic dehydrogenase (LDH) is the non-specific finding of *Trichinella* infection in humans, especially during the stage

of muscle invasion. Eosinophilia appears early during the second to the fifth week of infection prior to the onset of clinical signs and symptoms. During the same period, there is a rise of serum CPK, LDH and aldolase levels in 75–90% of infected persons and may persist for up to 4 months.

Epidemiological Diagnosis

For epidemiological purposes, the patient should be asked for the place from where he had bought the meat or meat products, the time and the mode of consumption (raw or undercooked). The triad of high fever, peri-orbital oedema and myalgia occurring in a cluster in a community or household points towards an outbreak of trichinellosis and needs appropriate investigation.

Diagnosis in Animals

Diagnosis of trichinellosis in pigs is routinely carried out by the use of "Trichinoscope" to detect *Trichinella* larvae in the muscles. Trichinoscopy is a reliable procedure to diagnose moderate to heavy infections but occasionally fails to detect light infection. The pooled sample digestion method and the immunoassays such as IFAT and ELISA to demonstrate *Trichinella* antibodies in the serum are the alternate methods recently followed to establish the diagnosis of trichinellosis in the swine. Molecular methods are also employed.

Treatment

The benzimidazole group of anthelmintics like albendazole remain the mainstay of treatment. However, although useful against adult worms and early larval stages, they are ineffective against the encapsulated larva in muscle cells. Hence, treatment should be directed against the adult worms or the migrating larval stages and should be initiated within the first 3 days of infection.

Albendazole is administered in a dose of 400 mg twice daily for 8–14 days; and for mebendazole, it is 200–400 mg three times a day for 3 days, followed by 400–500 mg thrice daily for 10 days. Pyrantel pamoate is safe in pregnancy and in children and given as a single dose of 10–20 mg/kg of body weight and repeated for 2–3 days. However, it acts only against the adult worms and not against the larval stages.

Corticosteroids are helpful in relief of symptoms which result from inflammatory and allergic reactions to the larvae. Caution should be exercised in prolonging the steroidal treatment because of the danger of an increased number of larvae in muscles and more extensive muscle invasion. The standard treatment for severe symptoms is with prednisone at a dose of 30–60 mg/day for 10–15 days.

It needs to be emphasized that delay in initiation of treatment increases the probability of establishment of viable larva in the muscle which is no longer amenable to medical treatment. In such a scenario, the larva will persist in the muscle causing persistent myalgia. Although prolonged therapy can be started in the late stages of infection, it has been observed that it is useless against long-term sequelae and chronic trichinellosis.

Prevention and Control

Thorough cooking, deep freezing at −20 °C or refrigeration at 4 °C for more than 20 days are the effective methods of killing *Trichinella* larvae in the pork. Smoking, curing and drying of meat are unreliable and are not effective procedures to kill the larvae.

Control of infection in the swine and destruction of *Trichinella* larvae in the pork will prevent transmission of infection to man. Avoidance of the habit of feeding raw, infected garbage to

swine and the boiling of garbage prior to feeding of pigs will help to significantly reduce trichinellosis in swine.

Case Study

A 30 years old male attended outpatients department with symptoms of fever, headache and pain in the left calf muscle with restricted knee movement. On examination, tenderness in the left gastrocnemius muscle with mild rise in local temperature was noted. He gave a history of regular intake of pork and pork products. The patient was admitted for further evaluation. Laboratory findings revealed: TLC: 26,000/dL; eosinophils: 10%; CPK: Elevated. With a clinical suspicion of trichinellosis, biopsy from gastrocnemius muscle was performed. Histopathological examination showed typical coiled larva surrounded by inflammatory cell and nurse cell-larva complex. A definitive diagnosis of trichinellosis was made and the patient was prescribed Albendazole 400 mg twice daily for 14 days and discharged. On follow-up after 3 weeks, there was complete subsidence of signs and symptoms.

1. What other parasites can cause musculoskeletal involvement?
2. How can you diagnose this case serologically?
3. Apart from pork, what other animal meat can transmit this infection?

Research Questions

1. What are the drugs that can kill the muscle larva? How the study of bioinformatics can help in drug design?
2. Which genus and species specific antigen can be utilized for immunoassays?
3. What are the protective antigens which can help in vaccine development?

Further Readings

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