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**Paper No. : 08 Biology of Parasitism**

**Module : 26 Morphology, Life Cycle, Pathogenicity and Prophylaxis of *Trichinella***



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## 1. Learning Outcomes

This unit will help to:

- Understand the medical importance of *Trichinella spiralis*.
- Identify the male and female worm from its morphological characteristics.
- Explain the importance of hosts in the life cycle of *Trichinella spiralis*.
- Diagnose the symptoms of the disease caused by the parasite.
- Understand the genomics and proteomics of the parasite to be able to design more accurate diagnostic, preventive, curative measures.
- Suggest various methods for the prevention and control of the parasite.

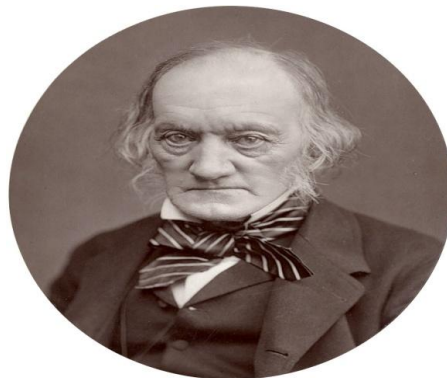
***Trichinella spiralis***  
(Pork worm)

### Classification

<b>Kingdom:</b>	Animalia
<b>Phylum:</b>	Nematoda
<b>Class:</b>	Adenophorea
<b>Order:</b>	Trichocephalida
<b>Superfamily:</b>	Trichinelloidea
<b>Genus:</b>	<i>Trichinella</i>
<b>Species:</b>	<i>spiralis</i>

## 2. History

*Trichinella spiralis* which causes trichinosis was first observed in 1821 in the muscles of a patient at autopsy by James Paget. Richard Owen in 1835 described the encysted larval form in muscles and named it *Trichina spiralis*. Virchow discovered its life cycle in 1859. Name *Trichinella spiralis* was given by Railliet in 1895. (Trichos- hair, ella- diminutive, spiralis refers to the spirally coiled appearance of larvae in muscles). It occurs in humans both as an adult in the intestine and as a larva in the tissues (usually muscles).



**Figure 1: Richard Owen**

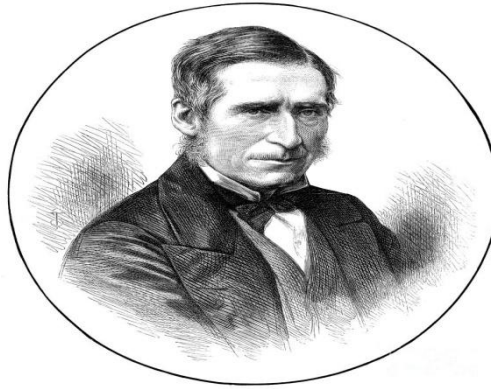


Figure 2: James Paget

### 3. Geographical Distribution

Species of *Trichinella* inhabit a broad geographic range from the arctic to the tropics; however, distributions of individual species are more restricted, with encapsulated species generally demonstrating adaptation to colder climates than non-encapsulated species. With the exception of some non-encapsulated species, noted major host groups of *Trichinella* spp. are domestic and sylvatic swine (*Sus scrofa*), synanthropic animals such as the brown rat, the armadillo, cats, dogs, and a broad range of sylvatic carnivores. It has been passively imported into most continents due to its high infectivity to swine and synanthropic rats. *T. spiralis* shows a cosmopolitan distribution in temperate and equatorial climatic zones. Several recent reviews summarize the presence of trichinellosis in individual countries, such as Argentina Hungary, China, Mexico and Greece. Furthermore, due to political and economic changes in southeastern Europe, a re-emergence of trichinellosis has been reported in countries of this region, and recent human outbreaks have been reported in Germany, Italy and United Kingdom (Figure 3). Human trichinosis had not been reported from India till 1996 when the first case was reported in Punjab.

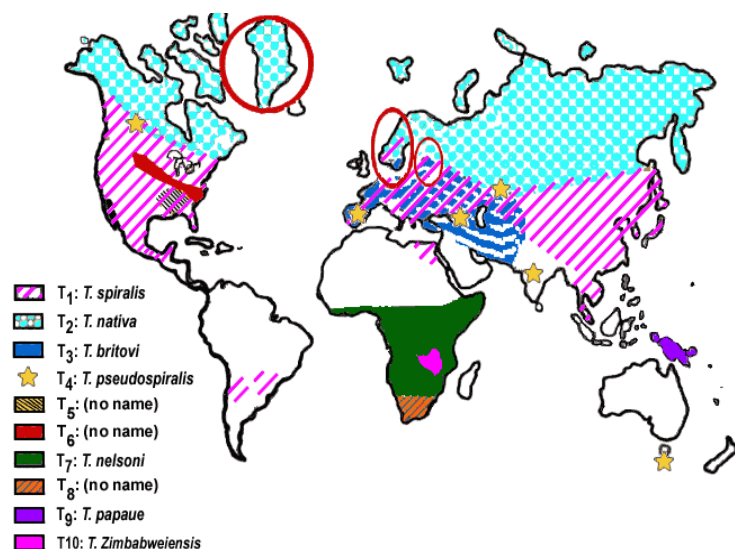


Figure 3: World map showing geographical distribution of *Trichinella spiralis* and other nematodes

## 4. Habit and Habitat

Adult worms remain buried in the duodenal or jejunal mucosa of pig, rat and human. The encysted larvae are present in the striated muscles of these hosts (Figure 4) due to the fertilized females discharging embryos into the circulating blood and reaching the muscles where they ultimately remain encysted.

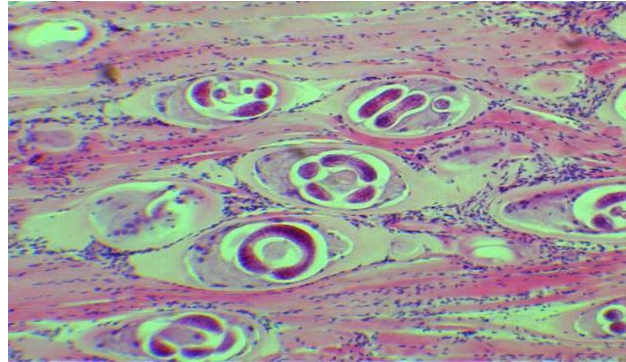


Figure 4:- *Trichinella spiralis* in human muscle

## 5. Morphology

### 5.1. Adult

It is one of the smallest nematodes infecting man and is white in colour. Male is 1.4 - 1.6 mm in length and 0.04 mm in diameter and female is much longer measuring 3- 4 mm in length and 0.06 mm in diameter. In males the copulatory sheath and spicule are absent but on the either side of tip of the tail there are two conspicuous conical papillae termed claspers that it uses to hold on to the female worm during mating. The females are viviparous and release first stage larvae into the intestinal mucosa (Figure 5).

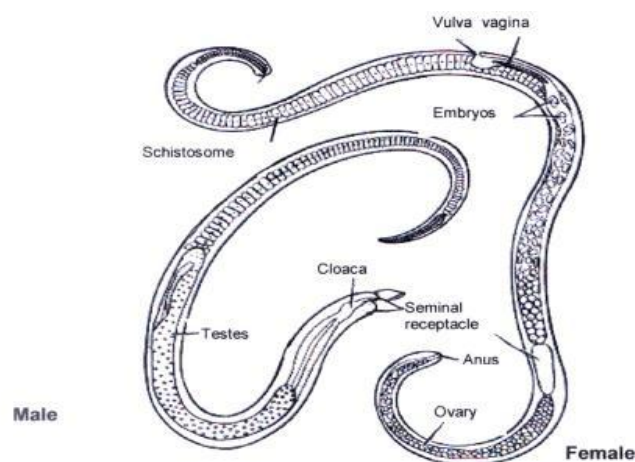


Figure 5: Male and female *Trichinella spiralis*

## 5.2. Larva

They measure 100 $\mu$ m in length and 6  $\mu$ m in diameter and remain encysted in striated muscles of the host (Figure 6).



**Figure 6- Larva of *Trichinella spiralis***

It then grows inside the cyst till sexual differentiation, becomes 10 times its original size from 100  $\mu$ m to 1000  $\mu$ m (1mm). Reaches maximum size by 35<sup>th</sup> day and normally only one larva is present in one cyst. Encapsulation of larva starts by about day 21 and completed in 3 months. An ellipsoidal lemon shaped sheath (0.4 by 0.25 mm) develops as a result of host-tissue interaction around the tightly coiled larva. The long axis of the capsule is parallel to the muscle fibre (Figure 7). Calcification occurs in 6-18 months. The muscles which are heavily parasitized are the diaphragm, intercostal, pectoralis major, deltoid, biceps and gastrocnemius muscles. There can be 1000 *Trichinella* per gm of muscle.

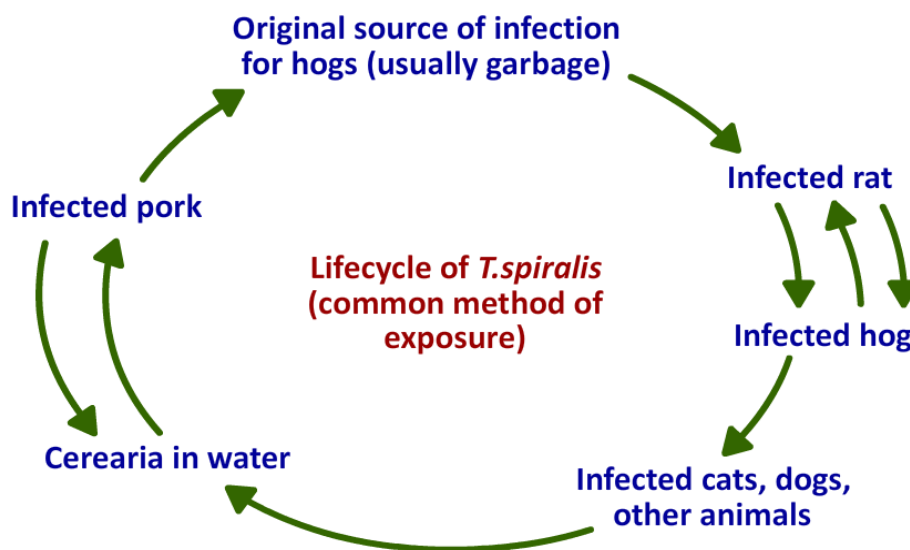
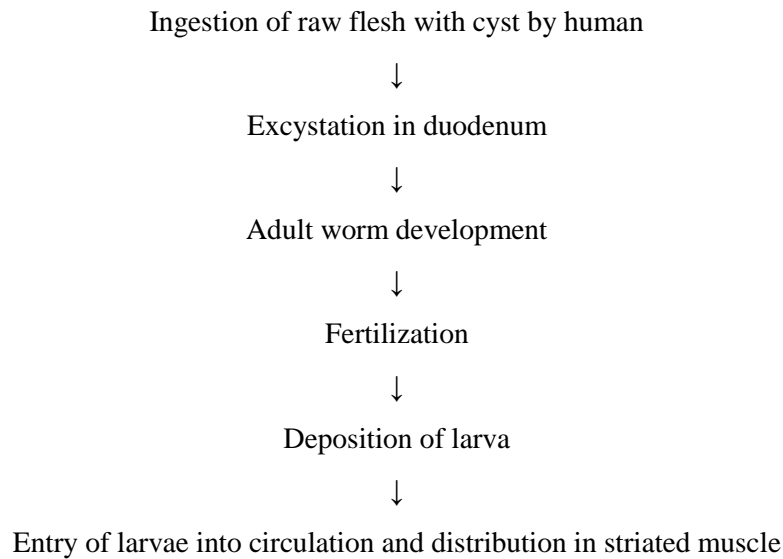


**Figure 7: Young cyst in striated muscle**

Life span of adult worm is very short. The male after fertilizing the female dies about one week after infection. The female lives for about 16 weeks. Majority of the larvae in muscle die within 6 months but some may survive upto 10 to 31 years.

## 6. Life Cycle

### Life cycle of *Trichinella spiralis* (flow chart)



**Figure 8: Life cycle of *Trichinella spiralis***

The entire life cycle is passed in one animal (pig, rat or man) but transference of the host is required for the preservation of the species from extinction.

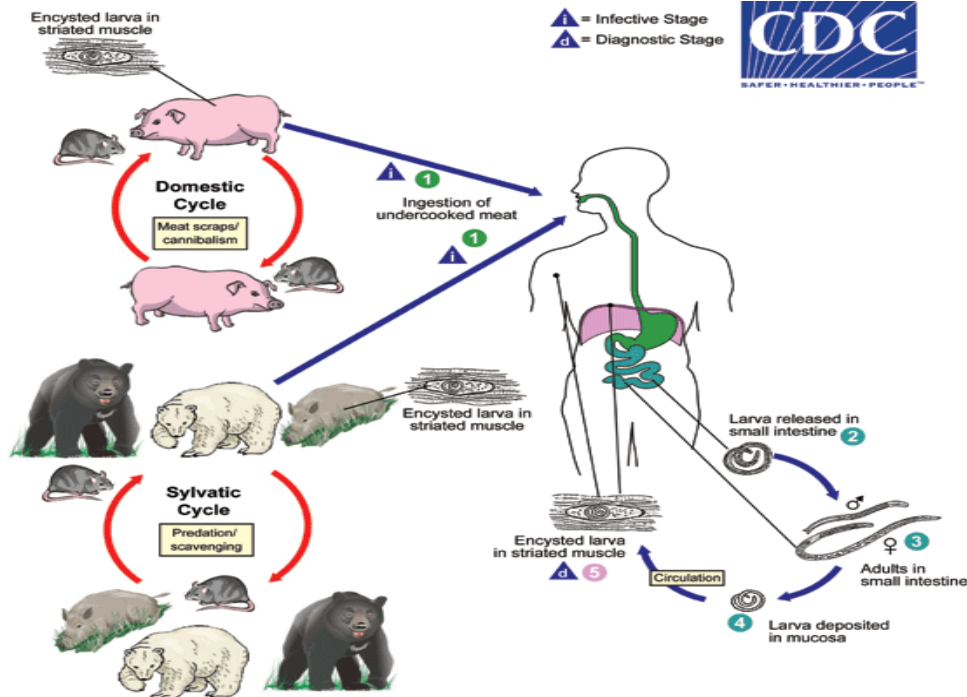


Figure 9: Life cycle of *Trichinella spiralis*

Although one individual animal serves both as definitive and intermediate host, two hosts are needed to complete the life cycle (Figure 9). The parasite entering man is unable to complete the life cycle. The continuance is maintained by other animal. The primary host of *Trichinella spiralis* is pig which serves as a reservoir host for man. Infection of a new host is always brought about by ingestion of raw flesh of the trichinosed animal.

## 7. Pathogenicity

The disease is called trichinosis or less commonly trichinelliasis or trichiniasis. Manifestation varies from asymptomatic infection to an acute fatal illness (rare). The clinical feature classified according to the stage of the life cycle of worm are-

### 1. Stage of Intestinal Invasion (Enteric Phase) (Incubation)

This occurs in the early stage of infection (5-7 days) during which the larvae grow into adult worms and the female begins to discharge larvae into the circulation. Symptoms are gastrointestinal nausea, diarrhoea, abdominal cramps and sometimes vomiting. This is diagnosed as acute food poisoning particularly when it occurs in groups of people who have taken the same food. In some constipation is seen instead of diarrhoea. The onset of illness may be from 2 - 30 hrs of ingestion of infective food.

### 2. Stage of Larval Migration (Muscle Invasion or Migratory Phase)

This occurs during the release of larvae, their migration, deposition and encapsulation in muscles. This occurs from 7<sup>th</sup> to 10<sup>th</sup> day and covers a period of 4 - 16 weeks. The chief symptoms are fever,



oedema of face, swelling and weakness of affected muscles. Eosinophilia is a constant feature. Myocarditis and encephalitis are serious and potentially fatal complications of toxanemia.

### 3. Stage of Encapsulation (Encystment Phase)

This occurs only in striated muscles while in other tissues they degenerate and are absorbed. This lasts from 1-8 months after infection, and fever and other symptoms have subsided. After this stage the cysts begin to calcify and may have permanent injury.

The clinically disease is self -limited and usually lasts 2-3 weeks in light and 2-3 months in heavy infections.

#### 7.1. Diagnosis

Clinically diagnosis is helped by the history of consumption of inadequately cooked pork or other meat. Correct diagnosis can only be achieved by demonstrating the muscle obtained either from biopsy or autopsy. The following measures can be adopted.

- (i) Stool examination - presence of adult worm in faeces rarely possible.
- (ii) Blood examination - for leucocytosis with an ascending eosinophilia.
- (iii) Serological tests - such as complement fixation, precipitin and bentonite.
- (iv) Muscle biopsy (by 3<sup>rd</sup> or 4<sup>th</sup> week of infection) suitable sites for biopsy are the tendinous insertions of deltoid or gastrocnemius muscle.
- (v) Skin test - Intradermal injection of 0.1 ml of 1 in 10000 dilution of Bachman's antigen causes an immediate erythematous patch.
- (vi) X-ray examination - if the cysts are calcified.

#### 7.2. Treatment

Thiabendazole is effective if treatment is started soon after infection. Mebendazole also may be useful. Corticosteroids have been found to be helpful in alleviating clinical symptoms.

#### 7.3. Prophylaxis

The best safeguard against human infection is proper cooking of pork and other meat likely to be infected. When pork and pork products are to be eaten raw they should be adequately processed. Smoking, salting and drying of meat may not ensure killing of infective trichina larvae.

Strains of *T. spiralis* tend to show differences in susceptibility to refrigeration and freezing.

The most effective method of control is to stop the practice of feeding pigs with raw garbage.

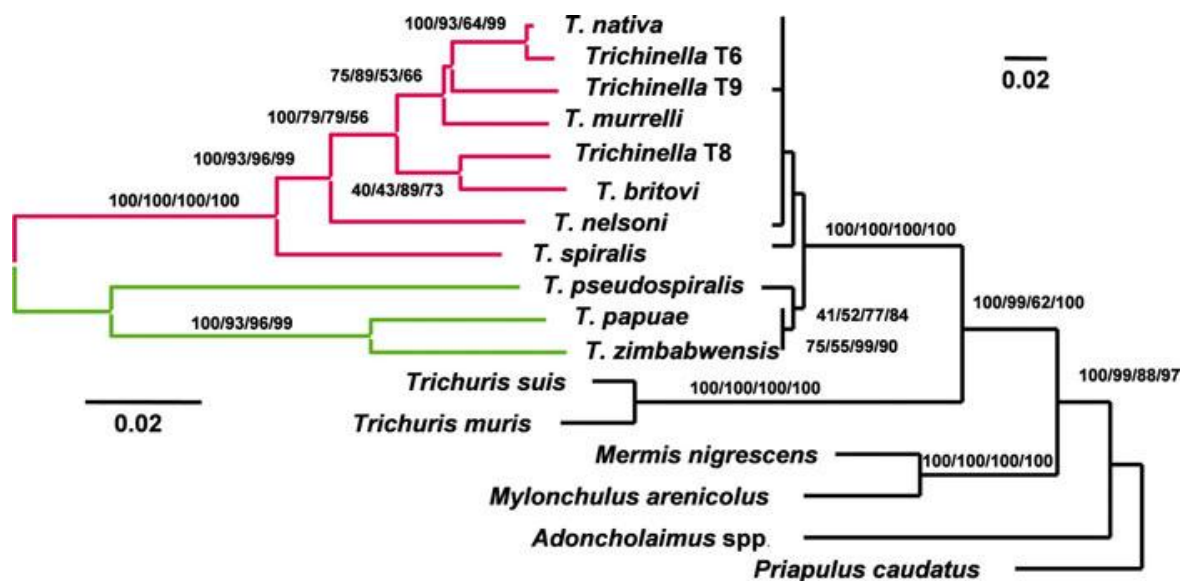
Extermination of rats from pig farms limits the spread of infection.

## 8. Phylogentic Postion of *Trichinella* within the Phylum Nematoda

Molecular phylogenetics has defined three major nematode classes which can be further divided into five Clades Dorylaimia, Enoplia and Chromadorea (including Spirurina-Clade III, Tylenchina-Clade IV, and Rhabditina-Clade V). Parasitism has arisen multiple times during nematode evolution and all

major clades include parasitic species. The model free-living nematode, *C. elegans*, is a member of Rhabditina (Clade V) and *T. spiralis* is a member of Dorylaimia (Clade I) making these examples some of the most distant species within the Nematoda.

The first robust and comprehensive analysis of the phylogeny and biogeographic history of *Trichinella* was recently reported, based on variation in several genetic loci (Zarlenga et al., 2006). The basal Dorylaimia lineage (nematode Clade I) which contains *Trichinella* also contains the free-living Mononchida, the plant parasitic Dorylaimida and the entomophagous Mermithida. These nematodes share features of early embryogenesis (Voronov et al., 1998) and small-subunit (SSU) rDNA sequences (Blaxter et al., 1998). The genus *Trichinella* is a monophyletic lineage in the Trichinellidae, which diverged 275 MY (Permian) from the putative sister Trichuridae. The 11 known species of this genus diverged into 2 distinct clades. Agreement for that divergence comes from both biological considerations, *Trichinella* clade I are encapsulated and Clade II are nonencapsulated, and genetic data (Figure 10).



**Figure 10: Midpoint rooted minimum evolution trees reconstructed from all known encapsulated (red) and nonencapsulated (green) species and genotypes of *Trichinella* based on the variation in mitochondrial LSU and COI DNA (on the left) and SSU rDNA (on the right).**

## 9. Genomics of *Trichinella*

There are currently eight recognized species or genotypes that comprise this genus. The species display diverse biological characteristics, the evolutionary significance of which has been recently extensively clarified. Some of that diversity translates into variable importance as zoonotic pathogens, with *Trichinella spiralis* having the highest significance. Trichinosis has re-emerged as an important zoonotic infection in various parts of the world, reminding us that control of this infection depends on persistent vigilance. *Trichinella* species display unique and biologically interesting complexity in interactions with host cells that they inhabit. Significant progress has been made towards understanding details of these interactions. Progress on transcriptomics, proteomics and now genomics offers exciting prospects for accelerating advances in future research.

The *T. spiralis* genome sequence and the accompanying genome-mining analysis address four key issues. First, details of genomic diversity that were deduced among species have outlined molecular determinants, where the magnitude of change likely reflects molecular elements that have figured decisively in both the lineage and species evolution of Nematoda. It has been argued that such drastic differences can be related to functional diversification, speciation and species adaptation. Given the modest number of nematode species with available genomes, we fully expect that as additional nematode genome sequences become available, a much greater resolution of differences will occur.

Washington University Genome sequencing Centre (GSC) was assigned to sequence the genome of *Trichinella spiralis*. The sequencing plan called for BAC fingerprint map, BAP end sequencing, 8-fold sequencing coverage in plasmids, end sequencing of a fosmid library at point 3-fold coverage followed by two rounds of directed improvement (pre-finishing). The PCAP package was used to perform an initial assembly. Preliminary analysis used Caenorhabditis species genes. (*C. elegans*, *C. brigssae*, *C. remanei*) and all parasitic nematode originated ESTs identified 6,845 unique loci within the 6,262 supercontigs of the *T. spiralis* assembly (at least 80 bits for a match to be considered as a significant). Of these, 38% (2,575/6,845) got matched by free-living and parasitic nematodes, and 41% (2,813/6,845) loci were identified only by the available *T. spiralis* ESTs. Furthermore, 21% (1,457/6,845) of the loci were identified only by parasitic nematode originated sequences i.e. putative parasitism genes. Several nematode genomes are completed or underway; however the majority of the available sequencing data from parasitic nematodes are ESTs therefore the parasitic nematode ESTs were used to identify putative parasitism genes.

## 10. Proteomics of *Trichinella*

Proteomics tools are used to analyze excretory and secretory (ES) products in parasites. ES proteins are believed to have roles in formation of the host-parasite complex and inducing changes in the host cells (Kwan-Lim et al., 1989). Early studies involved surface labeling of proteins on three stages of *T. spiralis* with iodine and examination by 1-DE (Clark et al., 1982), or use of 2-DE for identifying ES proteins in infected muscle (Jasmer, 1990), or muscle larvae (Dea-Ayuela et al., 2001; Wu et al., 1999). The attempts resulted in analyzing only few ES proteins in terms of their biological activity, due largely to problems in identifying individual proteins. Furthermore, two proteins found in the ES fraction collected from muscle larvae cultivated in vitro lacked a typical N-terminal signal sequence, while secretion appeared to be mediated through the classical ER/Golgi secretory pathway (Kuratli et al., 2001). Later studies used 2-DE and proteomic analysis to identify ES proteins from *T. spiralis*. PMF data obtained from MALDI-TOF mass spectrometric analysis of ES peptide spots excised from two-dimensional gels was used to confirm the existence of 2 members of a family of nematode-specific proteins that have N-terminal signal peptides (Gare et al., 2004). Expressed sequence tags (ESTs) generated from three life stages of *T. spiralis* (adult, mature muscle larvae, immature L1 larvae) improved the technical capacity for proteomic research on *Trichinella* spp. (Mitrevva et al., 2005 and section 4). By coupling protein sequence similarity with signal peptide prediction, 345 *T. spiralis* clusters were identified that had homology with predicted secreted or membrane proteins. The EST clusters supported interpretation of peptide mass fingerprint data obtained from 2DE analysis of muscle larvae ES proteins (Robinson et al., 2005). More recent 2DE electrophoresis of ES proteins

was coupled with MALDI-TOF- and LC-MS/MS enabling the most comprehensive identification of peptide spots from *T. spiralis* performed thus far (Robinson et al., 2005). Identities were assigned to 43 out of 52 ES peptide spots analyzed based on either the PMF data or de novo peptide data derived from LC-MS/MS. Interestingly, the 43 spots represented only 13 different proteins indicating that there are multiple protein isoforms present in the ES, the most prominent of which are a serine protease, the 45-kDa antigen, gp 43 and 2 unidentified open reading frames (Robinson and Connolly, 2005).

## 11. Summary

- *Trichinella* commonly known as pork worm is a nematode parasite able to infest rodents, pigs, horses, bears and humans.
- Causes the disease called trichinosis.
- World's largest intestinal parasite.
- Cosmopolitan distribution
- Female larger than male
- Has a direct life cycle i.e. completes all stages of development in one host
- Humans become infected when they eat improperly cooked *Trichinella* infected pork or meat
- Life cycle -- ingestion of raw flesh with cyst by human → encystations in duodenum → adult worm development → fertilization → deposition of larva → entry of larva into circulation → distribution in striated muscles
- Trichinosis is characterized by abdominal discomfort, diarrhea, nausea, muscle ache, itching, fever, chills, joint pain, splinter hemorrhage under fingernails and eye inflammation.
- Can be treated with thiabenzodole, albenzedole, mebendazole and corticosteroids
- Infections can be prevented by proper cooking of pork or freezing
- The genome size of *Trichinella spiralis* is 58.55 mbp with an estimated 16,549 genes substantially smaller than that of the prototypical nematode, *C. elegans*
- Proteomic tools are used to analyze excretory and secretory (ES) products in the parasites.