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Review Article

Trichinellosis: The World Wide Food Originated Zoonotic Disease

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Introduction

Infection of *Trichinella spp*. parasite is known as Trichinosis or Trichiniasis. Throughout much of the world, *Trichinella spp*. have been found to be the causative agents of human trichinellosis, a disease that not only is a public health hazard by affecting human patients but also represents an economic problem in porcine animal production and food safety. Infection by *Trichinella spp*. has been reported in domestic and wild animals in all the continents, including Antarctica. This parasite builds its own home in the infected muscles by secreting the proteins. The home is a capsule which is composed of a collagenous wall and cellular components [1].

The most important source of human infection worldwide is the domestic pig, but, e.g., in Europe, meats of horses and wild boars have played a significant role during outbreaks within the past three decades. Infection of humans occurs with the ingestion of *Trichinella* larvae that are encysted in muscle tissue of meat from domestic or wild animals [2].

Due to political and economic changes, recent increases in prevalence and incidence have been observed in many former eastern European countries. Such increases have been related mainly to a reduced efficacy of the veterinary control on susceptible production animals [3].

History

For the first time *Trichinella* was revealed by Sir James Paget. Sir Rechard Owen wrote up his findings and published them in the transactions of the Zoological Society of London on February 24, 1835 [4]. In 1942 Maplestone and Bhaduri first reported trichinellosis in India [5]. First recognized human case of *Trichinella* pseudospiralis was reported by Anisworth and his co-workers in 1994 from New Zealand [6]. The sample of diaphragm that Sir Rechard Owen had examined, and that diaphragm had been carefully protected by British Museum and it was destroyed in 1941 by air radiation.

Morphology

It is largest intracellular parasite. Males are smaller than females. Males measures 1.1 to 1.6mm while females are 1.5 to 3.3mm in length. Females are viviparous. Newly shed larva is cylindrical and measures 80-120µ long and 5 to 6 diameter. The body of male is slender and the oesophageal portion is smaller than the posterior part. The hind end of the body bear a pair of lateral flaps on either side of cloacal opening, with two pairs of papillae behind them. In body of female, vulva situated near the middle of the oesophageal region the egg measures by 40 by 30µm and contained fully developed embryo when present in the uterus of the mother (http://www.trichinella.org/) (Figure 1).

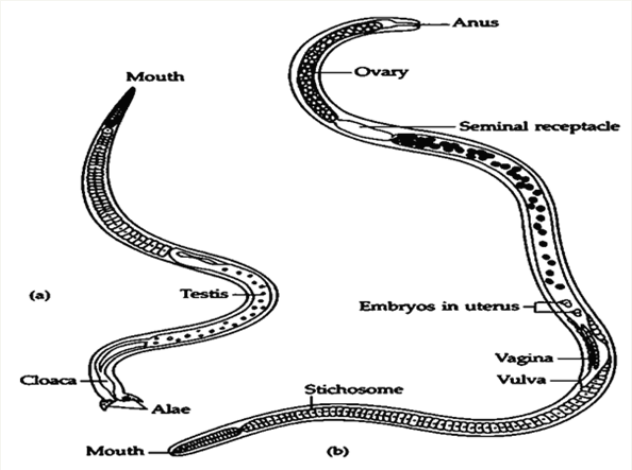

Figure 1

Classification:

Phylum	Nematoda
Class	Ahasmidea
Subclass	Adenophorea
Order	Enoplida
Superfamily	Trichuroidea
Family	Trichinellidae
Genus	*Trichinella*

Twelve genotypes of genus *Trichinella* have been identified, eight of which have been designated as species. Several other species of *Trichinella* have number of designations only [7].

i. *T. spiralis*, T1
ii. *T. nativa*, T2
iii. *T. britovi*, T3
iv. *T. pseudospiralis*, T4
v. T. murrelli, T5
vi. T6
vii. *T. nelsoni*, T7
viii. T8
ix. T9
x. *T. papuae*, T10
xi. T. zimbabwensis, T11
xii. T12

Epidemiology

Fifty percent infection in domestic animals as well as 47.9% in human is recorded in Europe [8]. During 1986-2009, total 64338 cases were reported in humans with 36 cases of death (www.cdc.gov, 2011). From remote areas of Uttarakhand, 42 humans cases were reported including 11 death (www.cdc.gov, 2012). Former Uttarakhand Chief Minister and BJP National Vice-President Ramesh Pokhriyal Nishank had been affected by the disease (www.dailypioneer.com, 2011) (Table 1 & 2).

Table 1:

Species	Geographic Distribution	Host	Infectivity	Clinical Aspects
T. spiralis	Cosmopolitan	Swine, wild boar, bear, horse, fox	High	Highly pathogenic Can be lethal Nurse cell formation in 16 days
T. nativa	Arctic, subarctic, holoarctic	Bear, horse	High	Moderate pathogenicity Long incubation time Prominent symptoms Nurse cell formation in 30 days
T. britovi	Temperate zone, Palearctic region	Wild boar, horse	Moderate	Moderate pathogenicity Long incubation period No gastrointestinal symptoms Low muscle larvae invasion Nurse cell formation in 42 days

Table 2:

Species	Geographic Distribution	Host	Infectivity	Clinical Aspects
T. pseudospiralis	Cosmopolita	Birds, omnivorous, mammals	Moderate	Incomplete information: only single human case reported; no nurse cell formation(Mumbai)
T. nelsoni	Tropical	Warthog	High	Low pathogenicity; not lethal; nurse cell formation in 40 days
T. papuae and T. zimbabwensis	Papua, Zimbabwe, Ethiopia	wild and domestic pigs and farmed crocodiles	High	

Summary of trichinellosis outbreaks reported from 2008 to at present (Tables 3-5):

Table 3:

Continent	Infections in Domestic Animals (%)	Infections in Humans (%)
Africa	5.7	13.2
America	13.5	13.5
Asia	20	40
Europe	50	47.9
Total	21.9	27.82

Table 4:

Continent	No. of cases reported (Human)	No. of death
Africa	28	1
America	7179	10
Asia	219	1
Europe	56912	24
Total	64338	36
(www.cdc.gov, 2011)		

Table 5: India (WHO, 19 Oct., 2011) (From Remote Area of Uttarakhand).

Cases	Male	Female	Death
42	30 (69%)	12 (31%)	11 (26.19%)

Source: (www.dailypioneer.com, 2011).

i. 28 Jul 2012 Trichinellosis - Argentina (02): (La Pampa) porcine

ii. 12 May 2012 Trichinellosis - Argentina: (NQ, CB)

iii. 23 Mar 2012 Trichinellosis - Chile: (CE)

iv. 23 Nov 2011 Trichinellosis - Chile : (LR)

v. 28 Oct 2011 Trichinellosis - Russia: (AL)

vi. 22 Oct 2011 Trichinellosis - Argentina (02): (CN) swine

vii. 19 Oct 2011 Trichinellosis - India: (UT)

viii. 21 Aug 2011 Trichinellosis - Argentina: (CB)

ix. 24 Jul 2011 Trichinellosis - Latvia: (DV)

x. 06 Mar 2011 Trichinellosis, fatal - Spain: (AR) wild boar meat

xi. 09 Jan 2011 Trichinellosis - Ukraine: (CV), RFI

xii. 06 Nov 2010 Trichinellosis - Argentina (02): (CB)

xiii. 24 Aug 2010 Trichinellosis - Chile: (VD)

xiv. 13 Aug 2010 Trichinellosis - Argentina: (ER)

xv. 05 Jul 2010 Trichinellosis - Mexico: (OA)

xvi. 15 Dec 2009 Trichinellosis - Belarus: (BR) wild boar meat

xvii. 05 Nov 2009 Trichinellosis - Russia (04): (VR) badger meat

xviii. 23 Oct 2009 Trichinellosis - Russia: (KE), bear meat

xix. 07 Oct 2009 Trichinellosis - France ex Canada: (NU)

xx. 27 Sep 2009 Trichinellosis - Lithuania: (VI) wild boar meat

xxi. 16 Jul 2009 Trichinellosis, dog meat, human - Russia: (ZB)

xxii. 21 May 2009 Trichinellosis, warthog ham - France ex Senegal

xxiii. 05 Apr 2009 Trichinellosis - Russia: (KX)

xxiv. 08 Mar 2009 Trichinellosis - China: background

xxv. 07 Mar 2009 Undiagnosed fatal illness - China (02): (YN) trichinellosis

xxvi. 23 Nov 2008 Trichinellosis, porcine - Germany: (Western Pomerania)

xxvii. 23 Nov 2008 Trichinellosis - Russia (06): (Kemerovo Region)

xxviii. 19 Sep 2008 Trichinellosis - Russia (05): (Chukchi Autonomous Region)

xxix. 07 Sep 2008 Trichinellosis - Russia (04): Magadan

xxx. 22 Jul 2008 Trichinellosis, salami - Argentina: (SFE)

xxxi. 06 Jul 2008 Trichinellosis - Russia (03): (Tomsk), bear meat

xxxii. 22 Jun 2008 Trichinellosis - Russia (02): (Zabaykalye, Tomsk)

xxxiii. 23 Feb 2008 Trichinellosis - Russia (Krasnodar)

Transmission

Figure 2

Transmission of *Trichinella spp*. to the humans can take place from both sylvatic as well as domestic animals. Sylvatic side it is transmitted from polar bear, wild boar, seal, walrus. Domestic side it is transmitted from pig, horse, dog, fur animals and rodents. So seal and walrus comes under marine animals (Figure 2).

Lifecycle

It is divided into two phases, Enteral phase and Parentral phase (Figure 3).

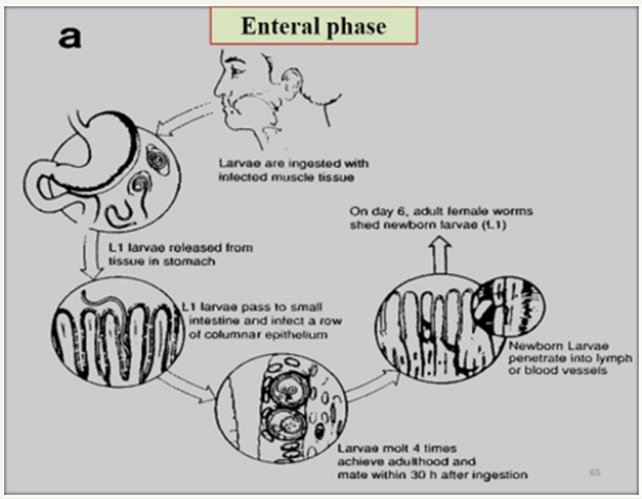

Figure 3

Enteral phase

Infection is initiated by ingesting raw or uncooked meat harboring the Nurse cell-larva complex. Larva are released from muscle tissue by digestive enzyme in the stomach and then locate to the upper two third of the small intestine. The outer most cuticular layer becomes partially digested. This enables the parasite to receive environmental cues and then to select the infection site within the small intestine. The immature parasite penetrates the columnar epithelium at the base of the villus. Larvae molts four times in rapid succession over a thirty hour period developing into adults. Copulation occur about 40 hours of infection, after copulation male dies and female penetrate into mucosa of small intestine and some may reach to the lymph space. Here they produce, over a period of several weeks, eggs that hatch inside the uterus of the female. Adult female worms lay the larvae (L1) [9].

Parenteral phase

New born larvae migrate through the body of the host via lymph and blood. New born larvae penetrate out of the blood vessel. 4 days after penetrating muscle cell nurse cell begins to form. 12 days after penetrating muscle cell nurse cell formation almost complete. Mature nurse cell- L1 larva complex is fully developed by 20 days after entering in muscle [9](Figure 4).

Pathogenesis

The most important pathogenic effect produced by the larvae is in the muscle is formation of nurse cell. The larvae enters in the striated muscle fibers and cause Loss of muscle proteins and enzymes (e.g. actin, myosin and creatinine kinase). No muscle contractile proteins can be detected beyond Day 8 after the parasite invades the muscle cell. Larva surrounded by a collagen capsule formed from the muscle fiber. There is modulation or re-differentiation in the structure of the muscle cell. It is termed as Nurse Cell. To nourish themselves in the nurse cell, the larvae stimulate angiogenesis leading to formation of a capillary rete around the invaded muscle cell [9] (Figure 5).

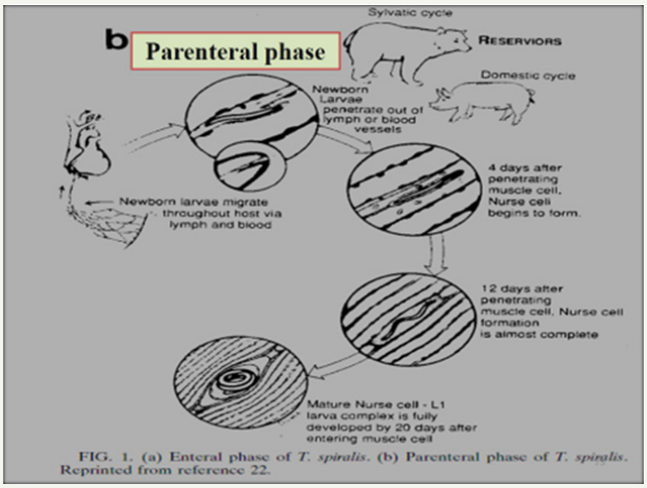

Figure 4

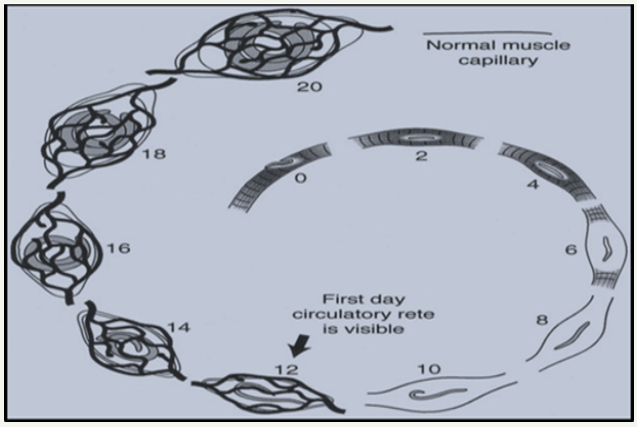

Figure 5

Public health hazard/importance

European commission had given guidelines, Meat containing at least 1 larva per gram is necessary to induce a clinical infection in man. The sudden occurrence of high fever, facial edema and myalgia in a group of persons suggests the presence of *Trichinell*a infection [10]. linical forms of Trichinellosis based on disease severity are severe, moderately severe, benign, abortive, and asymptomatic [2].

Different clinical forms having various incubation periods, more severe form having incubation period generally shorter, severe form having incubation period 1 week, moderately severe form having incubation period 2 weeks, benign and abortive form

having incubation period 3-4 weeks [2]. Most common initial symptoms are diarrhea, fewer, myalgia. Myalgia seen in 75% cases. Most common sites are masseter, diaphragm, and intercostal muscles. High percent reported in extremities and neck/shoulder girdle up to the severity, inability to ambulate or perform simple upper extremity or truncal tasks like feeding or sitting [2]. Main clinical signs are periorbital edema, facial edema, edema of bulbar conjunctiva, chemosis, myositis, splinter hemorrhage [2].

Diagnosis

There are two testing methods for the detection of *Trichinella* infection. One is direct method for detection and second is indirect method for detection [11]. The only recommended procedure for the detection of *Trichinella* larvae in muscle tissues is digestion assay. The International Commission on Trichinellosis (ICT) recommends this assay, which is documented standards in the European Union (EU), Canada and elsewhere in other countries [12]. Direct method can identify pigs, horses or other animals infected with *Trichinella spp*. as early as 17 days after exposure. Direct method is most sensitive on fresh samples because the number of larvae that can be recovered from samples, declines unpredictably after prolonged storage, putrefaction and freezing [7]. Muscles are taken from different predilection from different animals. In Pigs muscles are taken from the diaphragm, tongue, masseter muscles. In horses muscles are taken from tongue, masseter muscle. In wild animals predilection sites are unknown but tongue, diaphragm or masseter should be taken [7].

Digestion assay

Take meat sample and trimmed it to remove fat and fascia and make it 100g amount and add 50-100ml of the preheated Digestive solution (water/HCl solution) for a 100g sample. Chop the meat in a blender until it is homogeneous. Sprinkle 10g of pepsin into the homogenate, again add about 200ml of water/HCl solution and blend for about 5 seconds. Transfer the homogenized sample to a 3-litre beaker containing a stir bar. Allow the digestion to proceed for 30 minutes than filter through 177-180µm sieve. Allow the fluid undisturbed for 30 minutes to settle. Remove supernatant and collected sediment quickly transfer into a petridish and examine under stereomicroscope [7].

Polymerase chain reaction

This is the molecular method. It is used to detect the nucleic acid of larvae in the musculature. However, this method is not practical for routine testing of food animals. It is costly method. It is mainly uses for identification of the species or genotype of *Trichinella* recovered from muscle tissue is useful in understanding the epidemiology of the parasite in animals [13].

Trichinoscopy

This method involves the compression of multiple 2×10mm pieces of muscle tissue between two glass plates until they become translucent followed by examination using a microscopic technique. Trichinoscopy is not as sensitive as digestion assay, so it is not recommended by the ICT or EU for the routine examination of carcasses [11] (Table 6).

Table 6:

Larvae Per Gram	Technique
3	Trichinoscopy
1	Pooled sample digestion method
0.01	ELISA antibody detection
0.001	PCR methods

Serological tests

The ELISA is the only immunological assay endorsed by the ICT. It is only approved as an epidemiological surveillance tool to detect anti-*Trichinella* antibodies in pigs. It is not reliable for the detection of *Trichinella* infection in other animals. Disadvantage of this method also there. Low rate of false-negative results observed in infected animals. This is primarily due to detectable levels of antibody are not usually present in pigs until 3-5 weeks or more following exposure For this reason, serological methods are not recommended for individual carcass testing [14].

Prevention and Control

Following methods are uses for prevention and control of this disease.

Slaughter testing

After doing slaughter in slaughter house testing of each carcass can be done. Slaughter testing of swine can be done. Slaughter testing of horse can be done. Slaughter testing of game animal meats can be done. After doing testing of carcasses recommended actions taken when a positive test result is obtained. Develop quality assurance systems for digestion testing [15].

Processing methods to control trichinellosis

Cooking to inactivate *trichinella*: Guidelines set by the United States Department of Agriculture's Code of Federal Regulations (1990) (Appendix C) for treatment of meat to prevent human Trichinellosis. Meat must be cooked up to 71ºC. A change in color from pink to grey throughout, and a change in texture such that muscle fibers are easily separated from each other are indicators that meat has been rendered safe to eat [15].

Minimum internal temperature		Minimum time (h)
(F)	(C)	
120	49.0	21h
122	50.0	9.5h
124	51.1	4.5h
126	52.2	2.0h
128	53.4	1h
130	54.5	30min

132	55.6	15min
134	56.7	6min
136	57.8	3min
138	58.9	2min
140	60.0	1min
142	61.1	1min
144	62.2	Instant [15]

Freezing to inactivate *trichinella*: Guidelines set by the United States Department of Agriculture's Code of Federal Regulations (Appendix D) are acceptable for treatment of meat to prevent human Trichinellosis. Cuts of meat up to 15cm in thickness are frozen solid (at least -5 ºC) for no less than 3 weeks and Cuts of meat up to 69cm in thickness are frozen solid (at least -15ºC) for no less than 4 weeks [15]. *T. britovi* larvae in pork have survived up to 3 weeks at -20 °C, *T. spiralis* larvae in horse meat frozen at -18 °C (-0.4 °F) can survive up to 4 weeks [1].

Irradiation to inactivate *trichinella*: ICT considers irradiation, at levels proven to inactivate *Trichinella* (0.3kGy), to be an acceptable method for rendering meat safe for human consumption [15].

Consumer education: Educate the consumer for various methods of prevention. Cooking to an internal temperature of 71ºC (160 ºF). Freezing solid (-15 ºC or less) for 3 weeks (cuts up to 15cm in thickness). Freezing solid (-15 ºC or less) for 4 weeks (cuts up to 69cm in thickness). In areas where freeze-resistant *Trichinella* are endemic, consumers should be informed that freezing is not recommended. Methods for preparation of meats which are not considered secure include cooking using microwaves, curing, drying, or smoking [15].

On-Farm Control

On the pig farms requirements for *Trichinella* free pig production. Requirements for *Trichinella*-free production of horses. Certification of *Trichinella*-free livestock production, measures of Architectural and environmental barriers can be taken. Control of rodents can be done on the farm. Maintain hygienic condition on farm and good management of feed and feed storage.

Requirements for vaccines

There are no vaccines for Trichinellosis in food animals or game animals [7].

Trichinella organizations and reference laboratories:

International commission on trichinellosis (ICT): The principal functions of the ICT are:

i. The promotion and facilitation of studies relating to all phases of *Trichinella* infection in animals and humans

ii. Hosting of an International Conference on Trichinellosis every 4 years for presentation of reports on all aspects of *Trichinella* and trichinellosis. Organizing or participating in national or international congresses, symposia.

International reference laboratories: The OIE has established two reference laboratories for Trichinellosis

i. Centre for Foodborne and Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Canada

ii. Istituto Superiore di Sanita, Rome, Italy

Treatment

i. Albendazole is given @400mg twice daily for 8 to 14 days. Mebendazole is given @200 to 400mg three times a day for 3 days, followed by @400 to 500mg three times a day for 10 days [16] (Table 7).

Table 7:

Severity Code	Recommendation for Treatment
Severe and moderately severe diseases	Hospitalization is compulsory for severe forms and debatable for moderately severe forms
	Administration of anthelmintics (albendazole or mebendazole)
	Monitoring of the pharmacokinetics of anthelmintics (if possible)
	Administration of glucocorticosteroids (e.g., prednisolone), always with anthelmintics
	Compensation of fluid and electrolyte deficits
	Administration of pain killers
Benign, abortive, and asymptomatic diseases	Administration of anthelmintics (albendazole or mebendazole)
	Administration of nonsteroidal anti-inflammatory drugs if necessary

ii. They are contraindicated during pregnancy and not recommended in children aged <2 year [17].

iii. Steroids, e.g., prednisone, administered at a dose of 30mg/day to 60mg/day for 10 to 15 days for severe symptoms [18-20].

iv. Pyrantel is given in a single dose of 10 to 20mg/kg of body weight, repeated for 2 to 3 days, and may be used by pregnant women and children, but it is active only against worms in the gut, and it has no effect against newborn and muscle larvae [2].

Conclusion

i. Infection of *Trichinella* is known as Trichinosis or Trichiniasis

ii. Infection by *Trichinella spp*. has been reported in domestic and wild animals in all the continents, including Antarctica.

iii. For the first time *Trichinella* was revealed by Sir James Paget.

iv. Twelve genotypes of genus *Trichinella* have been identified.

v. During 1986 - 2009, total 64338 cases were reported in human with 36 cases of death

vi. During the year 2011 from Uttarakhand 42 human cases were reported including 11 human death.

vii. *Trichinella sp*p. infection in human was documented in 55 (27.8%) countries.

viii. Main clinical signs of *Trichinella* are facial edema, chemosis, splinter hemorrage, myositis, periorbital edema.

ix. Diagnosis of Trichinellosis can be done by using Digestion assay, PCR, Serological methods, Trichinoscopy.

x. Prevention and control of the disease can be done by Freezing, Cooking, Curing, Irradiation and by Consumer education.

xi. Treatment of the disease can be done by using Albendazole, Mebendazole, Glucocorticosteroides and pyrantel.

Future Prospects

i. Epidemiological studies are required urgently because the infection is likely to be under-diagnosed, and it is necessary to explore the existence of the parasite among wildlife reservoirs, pig, horse meat etc.

ii. There is a need for development of vaccine for Trichinellosis

iii. There is a need to educate the people about the infection of the disease with the help of the extension workers.

iv. Need to establish good laboratories that will perfectly diagnose the disease.

v. Certification program for *Trichinella*-free farms need to be establish.

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