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Present status of trichinellosis—a neglected zoonosis in India

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ABSTRACT

Trichinellosis is a meat-borne helminthic zoonosis, caused by different species of the genus *Trichinella*. The disease is cosmopolitan in distribution and affects around 10,000 people annually around the globe. Based on genetic, biochemical and biological variability, 9 valid species (*T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*, *T. papuae*, *T. patagoniensis*, *T. pseudospiralis*, *T. spiralis* and *T. zimbabwensis*) and 3 genotypes (*Trichinella* T6, T8 and T9) of the parasite have been recognized. These species infect around 100 mammalian species including domestic and wild pigs, horses, game animals and wild carnivores. The infection starts with consumption of raw or undercooked meat or meat products containing encysted muscle larvae of the parasite. Most of the infections or outbreaks have been associated with the consumption of meat or meat products of pigs, wild boars, horses, crocodiles, walruses and dogs. Trichinoscopy is used in the veterinary inspection of pork in slaughterhouses and meat-packing facilities in many countries. It is a rapid process, but low in sensitivity and fails to detect mild infection. The muscle digestion method using HCl-pepsin is more sensitive and thus preferred. Recent outbreak of trichinellosis in Uttarakhand state opened up issues related to rapid diagnosis and lack of consumer awareness regarding safe cooking habits of meat of pig origin. This status report is an attempt to compile the information on *Trichinella* spp. infection in animals and humans in India at one place to draw the attention of medical and veterinary personnels involved in disease investigation and active research on zoonotic diseases.

Key words: Animal reservoirs, Human infections, Prevention, Risk factors, *Trichinella* spp.

Trichinellosis, also known as trichinosis, is an important food-borne helminthic zoonosis, caused by *Trichinella* spp. The disease is cosmopolitan in distribution and affects about 10000 people annually around the globe with a mortality rate of about 0.2% (Pozio 2007). It is exclusively transmitted through ingestion of infected meat of mainly pigs and wild boars. Meat of horses, crocodiles, bears, dogs and walrus are the other sources of infection (Gottstein *et al.* 2009). Depending upon the species of *Trichinella* involved and infective dose, clinical manifestation in humans may vary from transient myalgia and fever to fatal consequences. Animal are the reservoirs and generally do not suffer from clinical disease. Initially, all *Trichinella* infections occurring in animals and humans were considered to be solely caused by the type species, *T. spiralis* (Owen 1835, Railliet 1895). However, based on molecular, biochemical, biological variability and geographical distribution; it is now established that there are 9 valid species (*T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*, *T. papuae*, *T. patagoniensis*, *T. pseudospiralis*, *T. spiralis* and *T. zimbabwensis*) and 3 genotypes (*Trichinella* T6, T8 and T9) (Krivokapich *et al.* 2012, Pozio and Zarlenga 2013). These species and genotypes have further been categorized under two clades (Pozio and Murrell 2006) based upon their capability of encapsulation in the muscles of the host, viz. the encapsulated clade (*T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*, *T. patagoniensis*, *T. spiralis* and 3 genotypes T6, T8 and T9) and the non-encapsulated clade (*T. papuae*, *T. pseudospiralis* and *T. zimbabwensis*). *Trichinella* spp. infect around 100 mammalian species including domestic and wild pigs, horses, game animals and wild carnivores worldwide (Pozio 2005). A passel of literature is available on *Trichinella* infection around the globe. Unfortunately, there is meager information on this parasite from India. Apart from sporadic reports of the parasite in wild civet cats (Schad and Chowdhury 1967, Parmeter *et al.* 1968), rodents (Niphadkar 1973), domestic pigs (Niphadkar *et al.* 1979) and humans (Alipuria *et al.* 1996, Sethi *et al.* 2010) in India, there is no report on identification of the parasite at species level. In all the previous literature, the parasite recovered from muscles of animals or humans has been designated as *Trichinella spiralis*. This may not be the case as revealed in the initial findings of our laboratory (Anonymous 2016, Ram *et al.* 2017, Kumar *et al.* 2017), indicating presence of species other than *Trichinella spiralis*.

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Life cycle

Trichinella spp. are autoheteroxenous parasites, i.e. they

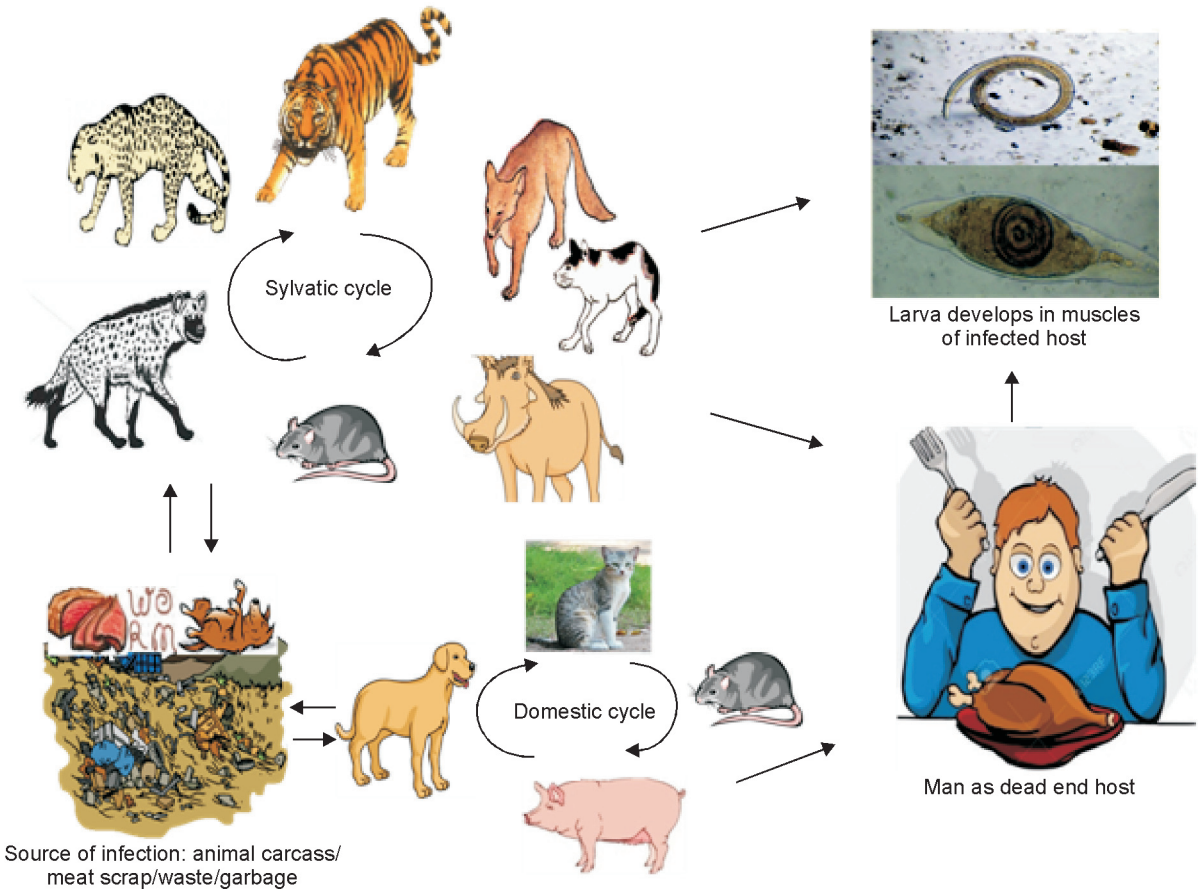

Fig. 1. Life-cycle of *Trichinella* spp.

utilize the same host as their definitive host as well as intermediate host. In other words, adult and larval development take place in the same host. All species of *Trichinella* can develop in mammalian hosts, but *T. pseudospiralis* can also develop in birds (Kapel and Gamble 2000), while *T. papuae* and *T. zimbabwensis* also occur in some reptiles (Pozio 2007).

Trichinella species infection starts with consumption of raw or undercooked meat or meat products containing encysted larvae of the parasite (Fig. 1). First stage larvae (L1) are released from the muscles following digestion and reach the duodenum. Subsequently, they penetrate the intestinal mucosa and within two to three days undergo four moults and develop to the adult stage. Female worms are about twice the length of male (1.4–1.6 mm) with cylindrical and tapering anterior and posterior ends. Adult parasites copulate in the intestine, and gravid females start producing larvae 6–7 days post infection. Larviposition continues for one to two weeks or more depending upon the immune status of the host, under the influence of which adult worms are expelled from the body. In case of pigs, a female worm produces larvae for several weeks before expulsion. Accordingly, newly laid larvae are distributed to different skeletal muscles, penetrate the muscle cell and modify it to become a cyst in case of encapsulated group of *Trichinella* spp. These modified muscle cells are called as 'nurse cells' as they provide nourishment and handle the excreta of the larva. These cysts mature in due course of time (17–21 days) and become source of infection for the next host. These cysts are resistant to dessication, salting and smoking of the meat (OIE 2012). In decaying tissues, larvae can survive up to 4 months. However, in some cases, the larvae die off and cysts become calcified.

Trichinella spp. infection in the meat of food and game animals are important because of the risk of infection to humans (OIE 2004). It is estimated that minimum infective dose of *Trichinella spiralis* to human is 70–150 ingested larvae (EFSA 2005). More than 500 larvae can prove to be fatal in humans (Murrell 1985, Battelli *et al.* 1994, Oivanen 2005). Transplacental transmission has also been reported in mouse (Boulos *et al.* 2005) and humans (Bowmen *et al.* 2003). *Trichinella* infection in humans can be divided into an intestinal (enteral) phase and a muscular (parenteral) phase, with clinical manifestations ranging from asymptomatic infection to fatal disease depending upon the number of larvae ingested. The enteral phase of infection with about 70–150 larvae can cause gastroenteritis with diarrhoea and abdominal pain approximately 2 days after infection (Gottstein *et al.* 2009), whereas parenteral phase is characterized by fever, myalgia, headache, periorbital oedema, eosinophilia and increased level of muscle enzymes (Clausen *et al.* 1996, Watt and Silachamroon 2004). Death generally is ascribed to myocarditis, meningeoencephalitis or pneumonitis (Pozio *et al.* 2003).

Respiratory failure due to specific muscle involvement, especially diaphragm may lead to fatal consequences (Compton *et al.* 1993, Watt *et al.* 2000).

Geographical distribution

Trichinella spp. infection is one of the most widespread zoonotic diseases in the world. This infection has been detected in domestic and wild animals of almost all continents except Antarctica (Pozio and Murrell 2006). This global distribution of *Trichinella*, together with different social and cultural food habits, represents the main factor favouring human infections in many countries. Major political and economic changes, revolutions and wars can further contribute to an increase in prevalence among the human population (Djordjevic *et al.* 2003). However, reliable epidemiological information on animal and human infections is not uniformly available from different parts of the world including India. Only a limited number of countries have implemented an official recording system for human and/or animal infections in the last 50 years. In most countries, reporting of infection has been and continues to be on a voluntary basis and relies on physicians, biologists, zoologists, veterinarians or epidemiologists who are working on these parasites.

Trichinella spp. have been found in domestic and wild animals in 66 countries, whereas human trichinellosis has been documented in 55 countries, particularly those with well-established food behaviour that includes consuming dishes with raw or undercooked meat or pork preparations. A detailed account of distribution of *Trichinella* in animals and humans have been reviewed by Pozio (2007) and Murrell and Pozio (2011). During 1986 to 2009, 65,818 cases and 42 deaths due to trichinellosis have been reported from 41 countries. Of these, European region alone accounted for 86% of cases (56,912); of which almost 50% cases occurred in Romania alone. Important foci of porcine and human trichinellosis occur in Central (Mexico) and South America (Argentina and Chile) (Ortega Pierres *et al.* 2000, Ribicich *et al.* 2005), Asia (the People's Republic of China, Laos, Myanmar, Thailand, Vietnam) (Takahashi *et al.* 2000, Liu and Boireau 2002), and Europe (Bosnia-Herzegovina, Bulgaria, Byelorussia, Croatia, Georgia, Latvia, Lithuania, Poland, Romania, Russia, Serbia, and the Ukraine) (Pozio and Zarlenga 2005). Geographical distribution, main reservoir hosts and type of cycle followed by different *Trichinella* spp. is presented in Table 1.

Outbreaks of *Trichinella* spp. infection have also been reported from South East Asian countries like China, Thailand, Laos, Vietnam, and in countries where refugees have settled (Green and Schantz 1986). In China, trichinellosis has been considered as most important food-borne parasitic infection. In some part of the China, anti-trichinella antibody has been detected in more than 10% of the local human population (Takahashi *et al.* 2000). *Trichinella* spp. infection in pig population of central China was up to 6.8%. *T. nativa* and *Trichinella* T9 have been reported from red foxes, raccoons, Japanese black bears, brown bears and domestic dog from Japan (Kanai *et al.* 2006). However, there is no information on the occurrence of these parasites in pigs. In Thialand also, *T. spiralis* infection has been reported frequently in domestic animals (pigs and dogs) and humans in northern mountain minority tribes. Since 1962, more than 7,500 infections with 97 deaths in about 130 outbreaks (morbidity rate 0.04 per 100,000 persons) has been reported (Kaewpitoon *et al.* 2006).

Risk factors

In nature, trichinellosis is maintained through two types of transmission cycles, viz. sylvatic and synanthropic. The sylvatic cycle continues in wild animals, mainly due to predation, without any interference of humans, where as synanthropic cycle involves different domestic animals (mainly pigs, horses and dogs) and rats. Humans become infected by ingesting meat originating from the synanthropic cycle as well as from the sylvatic cycle. Transmission from wild to domestic animals and subsequently to man is rare and unpredictable, but not impossible. For transmission to

Table 1. Distribution, major host species and type of cycle of different *Trichinella* spp.

Species/genotype	Cycle	Main hosts	Distribution
T. britovi	Sylvatic, seldom domestic	Carnivores, seldom swine	Temperate areas of Palearctic region, North and West Africa, India
T. murrelli	Sylvatic	Carnivores	Temperate areas of Nearctic region
T. nativa	Sylvatic	Terrestrial and marine carnivores	Arctic and subarctic areas of Holoarctic region
T. nelsoni	Sylvatic	Carnivores, seldom swine	Ethiopic region, India
*T. papuae**	Sylvatic, seldom domestic	Mammals and reptiles	Papua New Guinea
T. patagoniensis	Sylvatic	Carnivores	Argentina
*T. pseudospiralis**	Sylvatic, seldom domestic	Mammals and birds	Cosmopolitan
T. spiralis	Domestic and sylvatic	Swine, rats and carnivores	Cosmopolitan
*T. zimbabwensis**	Sylvatic	Mammals and reptiles	Ethiopia, Mozambic, Zimbabwe
Trichinella T6	Sylvatic	Carnivores	Canada, USA
Trichinella T8	Sylvatic	Carnivores	South Africa
Trichinella T9	Sylvatic	Carnivores	Japan

*non-capsulated species. Adopted and modified from Pozio *et al.* (2009).

man, pig meat is the main source in most of the countries (Murrell and Pozio 2011), but meat of horses (Dupouy-Camet 2000), bear (Dick and Pozio 2001) and crocodiles (Pozio *et al.* 2004) are other potential source, where meat of these animals is consumed on large scale. Improperly cooked wild game meat continues to be a source of infection for hunters and others who eat wildlife (Foreyt 2013). Pigs acquire *Trichinella* spp. infection mainly by eating the flesh of dead pigs coming through slaughter waste and kitchen waste. Pigs also get infection by eating dead rats while scavenging. Natural infections of *Trichinella* spp. in pigs are usually asymptomatic although heavy infection may cause death (Dick and Pozio 2001).

International tourists acquire *Trichinella* infections while travelling or hunting in endemic areas and later develop the clinical disease after their return to their home countries. For example, trichinellosis in travelers has occurred after the consumption of pork from a warthog (*Phacochoerus aethiopicus*) in Africa, bear meat in Canada and Greenland, pork from domestic pigs in the People's Republic of China, Egypt, Indonesia (Bali Island), Laos and Malaysia, and pork from wild boar (*Sus scrofa*) in Turkey and Algeria (Pozio and Murrell 2006).

It is very clear from the disease outbreaks/epidemics and reporting that causative agent of Trichinellosis, i.e. *Trichinella* spp. have wide geographical distribution and broad host range. Maximum infections or outbreaks have been associated with the consumption of meat or meat products from pigs, wild boars, horses, crocodiles, walruses and dogs.

Diagnosis

In animals: Diagnosis of *Trichinella* spp. infection in animals particularly in pigs is made generally by direct methods such as trichinoscopy (Gamble 1996, Forbes *et al.* 2003) and muscle digestion (Forbes and Gajadhar 1999, Gamble *et al.* 2000, Nöckler *et al.* 2000). Trichinoscopy is used in the veterinary inspection of pork in slaughterhouses and meat-packing facilities in many countries. It is a rapid process, but it is low in sensitivity and fails to detect mild infection. Experts opine that trichinoscopy can detect the infection only when there are three to five larvae per gram of muscles (Webster *et al.* 2006). Others say that number of larvae per gram of meat should be 15 or more for trichinoscopic detection (Vignau *et al.* 1997). Outbreaks involving several hundred cases had been reported in past following consumption of pork and pork products that had even passed the trichinoscopic examinations procedures in Sweden (1961) and Germany (1967). The muscle digestion method using HCl-pepsin is more sensitive (Van Knappen *et al.* 1980, Kohler and Pfeiffer 1983, Acha and Szyfres 1989) and thus it is the internationally accepted method (OIE 2008). Selected muscle pieces (diaphragm, masseters, tongue) are digested in acid-pepsin solution and the released muscle larvae may be demonstrated under low magnification of a compound microscope. Its sensitivity is primarily attributed to the use of a sample that is 50 to 100 times larger than that used in trichinoscopy.

Serological tests such as indirect immunofluorescence (IFAT) (Cui *et al.* 1999), ELISA (Gamble *et al.* 1983, Murrell *et al.* 1986, Gamble1998, Ribicich *et al.* 2000) and Western blot (Pozio *et al.* 2002, Yera *et al.* 2003, Bahn 2009, Frey *et al.* 2009, Nöckler *et al.* 2009) and molecular biology based techniques such as PCR (Uparanukraw and Morakote 1997, Golab *et al.* 2009, Li *et al.* 2010) and random amplified polymorphic DNA analysis (RAPD) (Rodríguez *et al.* 1996) have been developed for the diagnosis of *Trichinella* spp. infection and are now in use in many laboratories. Unlike the human infection, in which early diagnosis is needed, only a sensitive diagnosis is needed in swine because the larvae do not become infective until after the 16th day of infection. The serological tests like ELISA and IFAT have advantage of rapid detection of *T. spiralis* infection and also the pre-slaughter diagnosis in mass/herd scale is possible. The disadvantages of such tests being the lower sensitivity in early detection, cross reactivity with other parasites, and also the non-availability of diagnostic reagents, i.e. specific excretory/secretory (ES) antigens of *Trichinella* spp., which requires the parasite maintenance. Despite the robustness and sensitivity issues, ELISA assay has been recommended for surveillance programmes that monitor the transmission of *T. spiralis* infection within swine herds (OIE 2013). The PCR-based assays have very high sensitivity (may be 100%) but the specificity ranged between 88% to 100% for detection of a single larva of the parasite (Pozio *et al.* 1999).

In humans: Diagnosis of *Trichinella* spp. infection in humans can be made on the basis of clinical signs, parasitological observations and by immunological testing. The clinical signs include high eosinophilia, orbital oedema, muscle pain, fever and digestive disturbance following consumption of pork (Kociecka 2000, Capo and Despommier 1996, Sethi *et al.* 2010, Murrell and Pozio 2011, Sharma *et al.* 2014). The elevated eosinophilia level exists for 2–5 weeks, after which it stabilizes and slow reduction in level starts 9th week onwards. The disease must be differentiated from typhoid fever and influenza. In parasitological observations, one must recover the larvae of parasites in muscle biopsy. However, suitable time for biopsy examination is 4th week post infection. The most preferred muscles are the biceps or gastrocnemius and optimum quantity would be 1 gram. Half of this can be used for compression study and the remaining half may be used for histological examination. Digestion of several pooled muscle samples can be made in 1% pepsin containing 1% HCl at 46–48ºC for 30 min (Webster *et al.* 2006). The liberated larvae are examined under the low power magnification in a microscope. Occasionally, adult and larvae can also be seen in stool of the patient and larvae alone in the blood during 2–4 week post infection. Immunological technique, especially ELISA, is useful for detection of infection as early as day 12 post infection onwards in human beings.

Trichinellosis in India

In animals: Trichinella sp. infection has been reported sporadically from India in domestic and wild animals including civet cats, bandicoots and shrews (Table 2) (Maplestone and Bhaduri 1942, Kalapesi and Rao 1954, Schad and Chowdhury 1967, Parmeter *et al*. 1968, Niphadkar 1973) and twice in domestic pigs from Deonar Abattoir, Mumbai (Niphadkar *et al.* 1979, Kumar *et al.* 2015). *Trichinella* spp. was also recorded recently in pigs slaughtered at Nagpur (Dr. Vilas M. Vaidya, personal communication). Non-encapsulated species of *T. pseudospiralis* was also once reported in Indian mole rat (Shaikenov and Boev 1983). *Trichinella* sp. infection was detected in the carcasses of a wild pig, a leopard and a tigress in the laboratory of the first author at the Division of Parasitology, ICAR-IVRI, Izatnagar (TOI, June 2, 2016; TOI, Nov 4, 2016). Now *Trichinella* sp. infection in wild carnivores (big cats) and omnivores (pigs) in the country is very much alarming (Kundave *et al*. 2017) due to maintenance of this parasite in variety of animal species that may easily become source of infection to humans. Hitherto, there is no authentic report on the species of *Trichinella* circulating in India. Therefore, molecular biology techniques were used for species level identification of *Trichinella* sp. recovered from the carcasses of the wild animals by adopting the protocols of multiplex PCR, recommended by European Union Reference Laboratory for Parasites. Based on multiplex PCR results as well as the nucleotide sequence analysis of the mitochondrial large sub unit ribosomal DNA (Mt-lsr) and 5S intergenic spacer region DNA fragment (5S ISR), mixed infection of *T. britovi* and *T. nelsoni* was confirmed in all the three cases. This is the maiden attempt in this direction from the country.

Anti-parasitic activity of the herbal plants, *Gynura angulosa* (Asteraceae) and *Lasia spinosa* (Araceae) against *Trichinella spiralis* (strain code ISS 1597) was studied by Yadav and Temjenmongla (2006, 2012) in experimentally infected BALB/c mice. They recorded significant level of efficacy against all the stages of *T. spiralis* using the leaf extract of *G. angulosa*.

In humans: Literature mining on the occurrence of trichinellosis revealed sporadic reports including multiple small outbreaks that have been documented recently from the remote areas of Uttarakhand (India). However, trichinellosis has now emerged as new threat in few isolated pockets in India (Banerjee 2016) and reason for getting such parasitic infection in human could be the social and cultural practices, absence of hygienic standards in slaughter houses, improper meat inspection of food animals and consumption of contaminated meat (Ram *et al.* 2016).

Alipuria *et al.* (1996) recorded a case of trichinosis, in a 37 year old female for the first time from India. The patient was admitted to the Government Medical College, Rajendra Hospital, Patiala (Punjab) with the complaints of fever, generalized muscle and joint pain, swelling over face and feet and breathlessness on exertion for the last 15 days. She was a resident of Tehri Garhwal district of Uttar Pradesh (now part of Uttarakhand) and had the history of eating pork 15 days prior to the onset of symptoms. Initially, the case was diagnosed as polymyositis but later on presence of encysted *Trichinella* larvae in the muscle biopsy of vastus lateralis muscles confirmed trichinosis in the patient. After that, Mohan *et al.* (2002) incidentally discovered *T. spiralis* infection in a man, while dressing an abscess in the psoas

Table 2. Host-wise and state-wise report of *Trichinella* spp. infection in India

Host	Location and state	Diagnosis	References
Cat	Calcutta, West Bengal	Larval detection	Maplestone and Bhaduri (1942)
Cat	Bombay*, Maharashtra	Larval detection	Kalapesi and Rao (1954)
Civet cat	Calcutta, West Bengal	Larval detection	Schad and Chowdhury (1967)
Civet cat	Calcutta, West Bengal	Larval detection	Parmeter *et al.* (1968)
Bandicoot	Bombay*, Maharashtra	Adults in intestine and muscle larvae	Niphadkar (1973)
Domestic pig	Bombay*, Maharashtra	Acid pepsin digestion	Niphadkar *et al.* (1979)
Human	Tehri Garhwal, UP**	Muscle biopsy	Alipuria *et al.* (1996)
Human	Garhwal hills, UP**	Muscle biopsy	Handa *et al.* (2003)
Human (outbreak)	Tehri Garhwal and Pauri Garhwal, Uttarakhand	Muscle biopsy	Sethi *et al.* (2010, 2013) Sharma *et al.* (2014)
Human	PGIMER, Chandigargh	Serology and muscle biopsy	Dubey *et al.* (2011)
Human	Gangatic belt (North India)	Muscle biopsy	Pebam *et al.* (2012)
Human	-	Muscle biopsy	Joshi *et al.* (2014)
Human	Uttarakhand	Muscle biopsy	Shirazi *et al.* (2015)
Human	Karnataka	Muscle biopsy	Alva *et al.* (2015)
Domestic pig	Mumbai, Maharashtra	Muscle digestion	Kumar *et al.* (2015)
Field rat	Mumbai, Maharashtra	Muscle digestion	Dr. Vaidya personal communication
Wild pig	Pilibhit, Uttar Pradesh	Muscle press and Muscle digestion	ICAR-IVRI (Annual Report 2016)
Leopard	Bahraich, Uttar Pradesh	Muscle press and Muscle digestion	ICAR-IVRI (Annual Report 2016)
Tiger	Kheri, Uttar Pradesh	Muscle press and Muscle digestion	TOI (November 4, 2016)

*Now known as Mumbai, ** Now in Uttarakhand.

muscle. Handa *et al.* (2003) reported proximal muscle weakness due to the presence of the larvae of *T. spiralis*, in a 31 year old female patient hailing from Garhwal hills of Uttarakhand.

Sethi *et al.* (2010) reported small multiple outbreaks of human trichinosis in the remote areas of Garhwal, Uttarakhand. Epidemiological, clinical and laboratory findings of the patients (n=18) were analyzed retrospectively and prospectively. All the victims consumed improperly cooked meat of wild boar. Clinical investigations revealed generalized weakness in all the patients (18) followed by myalgia (17), fever (16), headache (12), gastrointestinal symptoms (12) and periorbital oedema (9). Sub-conjuctival haemorrhages, muscle atrophy, stiffness and breathlessness were also observed in some of the patients. Biochemical investigation revealed eosinophilia, leukocytosis, elevated creatine phosphokinase (CPK), lactate dehydrogenase (LDH), SGPT and SGOT levels. Muscle biopsy involving gastrocnemius muscles of 5 patients confirmed the presence of *Trichinella* larvae surrounded by inflammatory cells as well as atrophy and degeneration of adjacent muscle fibers. Another outbreak of trichinosis with high mortality rate was again reported by Sethi *et al.* (2013) after analyzing the medical records of 42 trichinosis patients (2008–2011) of Uttarakhand. Study confirmed death of 11 patients as a result of late diagnosis and/or treatment. Sharma *et al.* (2014) reported the detailed clinical and biochemical profile of this outbreak. In this study, a total of 54 people were investigated, all of whom participated in a community feast in the village. The type of pork consumed included uncooked in 24% people (n=13), open fire roasted in 39% (n=21) and fired in 37% people (n=20). Predominant clinical symptoms, viz. fever with chills and myalgia were noticed 2–3 week after consumption of uncooked or open fire roasted pork in 34 patients (100%). Other symptoms included periorbital oedema in 24 patients followed by difficult breathing in 3 cases, difficulty in swallowing in 1 and itching all over the body in a patient were also observed. Laboratory parameters studied in the patients revealed eosinophilia in 90% cases (n=41), increased ESR in 98% (n=45) and elevated creatinine phosphokinase (CPK) level in 85% cases. All symptomatic patients were treated with a short course of oral steroids and albendazole therapy. However, atypical case of trichinellosis with myositis of thigh muscles and osteomyelitis of the femur but without any eosinophilia and facial or periorbital edema has been placed on record by Dubey *et al.* (2011).

Pebam *et al.* (2012) reported a rare case of human trichinellosis, which predisposed to pyomyositis and secondary osteomyelitis. The patient was 12 year of age and presented to a tertiary center institute with state of the art laboratory facilities and isolation techniques (PGIMER, Chandigargh) with the complaint of fever and pain in right thigh since last 3 weeks. History of hamburger consumption in last one month, and thereafter episodes of diarrhoea, dyspepsia and myalgia for 4 days with self improvement without any medication was explained by the patient. Different laboratory investigations including X-ray, ultrasound, MRI, microbial culture, histopathology, hematology and serology based testing were carried out for confirmative diagnosis. Elevated WBC count (13700/mm^3) with more than 10% eosinophilia, positive results for IgM antibodies to *Trichinella* species and histological examination of muscle biopsy ultimately led to the conclusion of an unusual manifestation of trichinellosis associated with pyomyositis and secondary osteomyelitis in the patient. The medical interventions including surgical drainage and debridement of affected regions and mebendazole therapy (600 mg/day) in divided doses for 14 days resulted in improvement in general condition of the patient. A case of miscarriage associated with trichinosis in a 27-year old women suffering from intercostals pain and swelling for last 5 months was reported by Joshi *et al.* (2014). Biopsy showed a tiny calcified cyst surrounding a coiled thread like worm. Patient was initially given pyrantel for the 3 days to remove the worms from gut with no effect against newborn and muscle larvae. Abortion occurred in the patient at 16 weeks of pregnancy. The patient was advised bed rest and treated with albendazole, analgesics and steroids. Following treatment, the patient recovered.

Association of *Trichinella spiralis* larvae in squamous cell carcinoma in a 37 year old male patient suffering from painless ulcer in the right buccal mucosa and presented to the Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand was reported (Shirazi *et al.* 2015). Presence of encysted larvae was observed in tissue biopsy. The presence of *Trichinella spiralis* larvae in the parotid gland of a 32 year old patient was also reported (Alva *et al.* 2015). Initially the patient had swelling and redness extending from the angle of the mouth up to the targus of the ear. Swelling was painful in the beginning but later became painless. He underwent different levels of investigation including hematology, ultrasound, CT scan, FNAC, serology and urine analysis to rule out provisional diagnosis of parotid gland neoplasm. Finally, histopathology of cystic mass revealed presence of encircled, coiled, thread like worm in the parenchyma of the parotid gland, confirming the diagnosis of trichinellosis. No attempt has been made to identify the parasite at species level in any of the above mentioned human cases.

Future perspectives

Epidemiology of *Trichinella* sp. infection is not known in many countries including India. It is surprising that outbreaks of *Trichinella* sp. are occurring in many South East Asian countries like China, Thailand, Laos, Vietnam etc. even then no systematic research work in India had been undertaken on this important zoonotic disease. Further, inadequate meat inspection procedure before consumers reach is another big challenge, though it is time consuming and expensive too. Detection of recent *Trichinella* sp. infection in different wildlife species in the country, multiple small outbreaks in humans in remote areas of Uttarakhand

(India) along with many individual cases with other disease conditions of humans are the tocsins for the public health specialists and need to be addressed in prospective and retrospective manner. Many developed countries have legislations for the prevention and control of trichinellosis, but most of the developing countries like India do not have such legislations. Veterinary control over the slaughter of food animals to ensure food safety, particularly meat inspection is an essential intervention to prevent meat-borne infections. Enormous cost of meat inspection is one of the reasons for such laxity in developing countries. In the European Union, the estimated annual cost incurred for meat inspection of 167 million pigs ranges from • 25 million to 400 million. Considering the huge number of pigs slaughtered in either modern abattoirs or in the backyards of farmers, the cost is considerable. Thus, a comprehensive approach needs to be adopted to address this very important life-threatening disease of human.

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