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Invited review

Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*

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Abstract

Since Owen first described *Trichinella* as a human pathogen in 1835, the number of organisms comprising this genus has grown dramatically. Where it was once thought to be a monospecific group, this genus is now comprised of eight species and three additional genotypic variants that have yet to be taxonomically defined. Along with the growth in the genus and description of the parasites has come a concomitant increase in our understanding of the epidemiology and geographical distribution of these organisms. Recent expansion of the non-encapsulated group to include three species biologically defined by their unique host ranges encompassing mammals, birds and reptiles, has raised substantial questions as to the term, '*Trichinella*-free' as it applies to geographical localities. A true appreciation of the adaptability of this genus to host and environmental selection factors, as well as its dissemination to the far reaches of the world can best be appreciated by reviewing what we know and what we hope to know about this ancient and elusive parasite. The review herein consolidates our current understanding of the taxonomy, epidemiology, and phylogeny of the genus *Trichinella*, and identifies areas where data are lacking and our knowledge requires additional clarification.

Keywords: *Trichinella*; Trichinellosis; Taxonomy; Epidemiology; Phylogeny; Systematics

1. Introduction

The etiological agents of human trichinellosis show virtually worldwide distribution in domestic and/or sylvatic animals, with the exception of Antarctica, where there is neither a record of this nematode nor evidence of any epidemiological study. This global distribution of *Trichinella* in conjunction with varying cultural eating habits, represent the main factor favouring human infections in industrialised and non-industrialised countries. Strong political and economic changes, revolutions and wars have contributed to an increase in prevalence among the human population (Murrell and Pozio, 2000; Bolpe and Boffi, 2001; Marinculic et al., 2001; Djordjevic et al., 2003). This is likely in response to breakdowns in veterinary services in charge of infection control, and to reductions in protein resources which in turn force select population groups to hunt and consume a broader spectrum of animals encompassing those known to be reservoirs of *Trichinella*. In the 1990 s, a dramatic increase of infections in domestic animals was observed in Eastern Europe favouring a concomitant elevation in human infections and the exportation of *Trichinella*-infected horses and pig products into Western Europe (Boireau et al., 2000; Murrell and Pozio, 2000; Pozio, 2001; Pozio and Marucci, 2003).

Based upon a sound understanding of the epidemiology of trichinellosis, it is well known that this zoonosis can be controlled by preventing parasite transmission at the farm level and by correctly applying standard hygiene procedures at the abattoir (Gamble et al., 2000). The success of these control measures is exemplified in the industrialised countries of North America and Europe, where no infections have been reported from highly industrialised pig farms in

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the last 50 years (Gamble and Bush, 1998; Pozio, 1998). However, at the global level, *Trichinella* infections derived from wildlife are still endemic in many countries including those, which constitute fully-developed sovereignties.

In the past 15 years, our knowledge and understanding of the taxonomy and epidemiology of parasites of the genus *Trichinella* has grown substantially (Murrell and Pozio, 2000; Murrell et al., 2000). However, since that time, new information has generated providing fresh insight on the infection potential of these nematodes and on the systematics of the genus. Herein, we present the current understanding of the taxonomy and systematics of this group of parasites as well as review up-to-date information on observations of their ever expanding geographical range.

2. Taxonomy

Today, two main clades are recognised in the genus *Trichinella*; one that encompasses species that encapsulate in host muscle tissue, and a second that does not encapsulate following muscle cell dedifferentiation (Pozio et al., 2001a; La Rosa et al., 2003a; Gasser et al., 2004; Zarlenga et al., 2004). The species and genotypes of the first clade parasitise only mammals, whereas of the three species that comprise the second clade, one infects mammals and birds and two parasitise mammals and reptiles (Pozio et al., 2004a). Except for the existence of a capsule and possibly some size differential in one of the non-encapsulated parasite groups, all species and genotypes of the genus *Trichinella* are morphologically indistinguishable at all developmental stages; consequently, only biochemical or molecular methods can be used reliably to identify the genotype of the parasite. Many methods have been developed for this purpose; however, today, the most widely used are those based on the polymerase chain reaction (PCR) of single larva (Zarlenga et al., 1999; Rombout et al., 2001; Pozio and La Rosa, 2003; Gasser et al., 2004).

2.1. Encapsulated clade

Five species comprise the encapsulated clade and three additional genotypes are yet to be defined taxonomically. This group of parasites induces the development of a collagen capsule around the larva following penetration of a striated muscle cell. A synopsis of each species and genotype is presented below (see Table 1 for the distribution at the world level).

2.1.1. *Trichinella spiralis* (Owen, 1835)

Trichinella spiralis shows a cosmopolitan distribution in temperate and equatorial climatic zones, because it has been passively imported into most continents due to its high infectivity to swine and synanthropic rats (Pozio, 2001) (Table 1). The predominant hosts 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 (Pozio, 2001; Dick and Pozio, 2001). Because of their herbivorous diet, horses are considered an unusual host; however, the potential for *Trichinella* to infect horses is an important human health consideration especially in the European Union (see below). The geographical distribution of this species in sylvatic mammals appears around current or past foci of infections originating from the domestic cycle i.e. pig transmission, but quickly infect the surrounding fauna. *Trichinella spiralis* is the etiological agent of most of the human infections and deaths around the world and its pathogenicity is higher than that of other species due to the higher number of newborn larvae produced by the females (Pozio et al., 1992a) and for the stronger immune reaction induced in humans relative to the other genotypes (Pozio et al., 1993; Bruschi et al., 1999; Gomez Morales et al., 2002).

2.1.2. *Trichinella nativa* (Britov and Boev, 1972) and unnamed genotype *Trichinella* T6 (Pozio et al., 1992b)

Trichinella nativa is the etiological agent of *Trichinella* infection in sylvatic carnivores living in frigid zones of Asia (China, Kazakhstan, Mongolia, Russia), North America (Canada and USA) and Europe (Estonia, Finland, Lithuania, Norway, Russia, Sweden) (Table 1). The isotherm −4 °C in January seems to be the southern border of distribution of this species. The main hosts are both terrestrial and marine carnivores (Kapel, 2000; Forbes, 2000; Dick and Pozio, 2001); however, there is one report identifying this species in two wild boars of Estonia and in one domestic pig of China (Pozio and Kapel, 1999). The main biological characteristic of this species is the ability of larvae to survive in frozen (−18 °C) muscles of carnivores for up to 5 years (Dick and Pozio, 2001). Human infections occur frequently among people living in frigid zones of Canada, Greenland, Siberia, and Kamchatcka (Serhir et al., 1999; Schellenberg et al., 2003; Nelson et al., 2003).

The genotype *Trichinella* T6 is strictly related to *T. nativa* as demonstrated by successful interbreeding in experimental conditions. This genotype has been detected in several regions of Canada (British Columbia, Ontario, Manitoba) and the United States (Alaska, Montana, Idaho, Pennsylvania) (La Rosa et al., 2003b). Natural hybrids between *Trichinella* T6 and *T. nativa* have been detected in nature, suggesting a recent separation between the two taxa (La Rosa et al., 2003b). Further, the larvae of this genotype can survive in frozen muscles of carnivores for long periods of time (up to 34 months in grizzly bear) (Dick and Pozio, 2001). Human infections have been documented (Dworkin et al., 1996). The genetically defined characters between *Trichinella* T6 and *T. nativa* contrasts with the sympatric nature of these parasites and their ability to interbreed. This enigma gives cause to reservations in assigning a taxonomic status to *Trichinella* T6 at this time.

Table 1
Documented infections with *Trichinella* in humans and animals by country

Country	Documented infections		*Trichinella* species[a]	
	Humans	Animals	Documented	Possible[b]
Africa				
Algeria, Senegal	Yes	Yes	Tb	–
Egypt	Yes	Yes	Ts	Tb
Ethiopia	Yes	Yes	Tz	Tb or Tne
Guinea	No	Yes	Tb	–
Kenya, Tanzania UR	Yes	Yes	Tne	Tz
Mozambique	No	Yes	Tz	Tne
Namibia	No	Yes	T8	Tz
South Africa	No	Yes	Tne, T8	Tz
Tunisia	No	Yes	–	Tb
Zimbabwe	No	Yes	Tz	Tne
America				
Argentina, Chile, Mexico	Yes	Yes	Ts	–
Bolivia	No	Yes	–	Ts
Canada, USA	Yes	Yes	Ts, Tna, Tps, Tm, T6	–
Asia				
Cambodia, Malaysia, Vietnam	Yes	Yes	–	Ts
China	Yes	Yes	Ts, Tna	Tb
Hong Kong, Laos	Yes	Yes	Ts	–
India	Yes	Yes	Tps	Ts, Tb
Indonesia	Yes	Yes	–	Ts, Tpa
Iran Islamic Rep	No	Yes	Ts, Tb	–
Japan	Yes	Yes	T9	–
Kazakhstan	Yes	Yes	Tna, Tb, Tps	–
Korea Rep (South)	Yes	Yes	Ts	Tb
Kyrgyzstan	No	Yes	Tna	Tb
Israel, Lebanon	Yes	Yes	–	Tb
Myanmar	No	Yes	–	Ts
Tajikistan Turkmenistan	No	Yes	Tb	–
Uzbekistan	Yes	Yes	Tb	–
Thailand	Yes	Yes	Ts, Tps	Tpa
Europe				
Azerbaijan, Denmark	No	Yes	–	Tb
Bosnia-Herzegovina, Greece	Yes	Yes	–	Tb
Macedonia, Portugal	No	Yes	Tb	–
Austria, Belarus, Bulgaria, Croatia, Germany, Latvia, Poland, Romania, Serbia-Montenegro	Yes	Yes	Ts, Tb	–
Belgium, Switzerland, Slovenia	No	Yes	Tb	–
Czech Rep	No	Yes	Tb	Ts
Estonia	Yes	Yes	Ts, Tna, Tb	–
Finland	No	Yes	Ts, Tna, Tb, Tps	–
Lithuania, Russia, Sweden	Yes	Yes	Ts, Tna, Tb, Tps	–
France, Georgia, Netherlands, Slovakia, Spain	Yes	Yes	Ts, Tb, Tps	–
Hungary	Yes	Yes	Tb	Ts
Ireland	Yes	Yes	Ts	–
Italy	Yes	Yes	Tb, Tps	–
Norway	No	Yes	Tna, Tb	–
Turkey	Yes	Yes	Tb	–
Ukraine	Yes	Yes	Tb	Ts, Tna
Australia				
Australia	No	Yes	Tps	Tpa
New Zealand	Yes	Yes	Ts	–
Papua New Guinea	Yes	Yes	Tpa	–

Imported cases of infections were not considered.

[a] *Trichinella spiralis*, Ts; *Trichinella nativa*, Tna; *Trichinella britovi*, Tb; *Trichinella pseudospiralis*, Tps; *Trichinella murrelli*, Tm; *Trichinella nelsoni*, Tne; *Trichinella papuae*, Tpa; *Trichinella zimbabwensis*, Tz; *Trichinella* T6, T6; *Trichinella* T8, T8; *Trichinella* T9, T9.

[b] Since *Trichinella pseudospiralis* can infect birds, it could be ubiquitous, consequently it has been not added as 'possible' species, but its presence cannot be excluded.

2.1.3. *Trichinella britovi* (Pozio et al., 1992b) and unnamed genotype *Trichinella* T8 (Pozio et al., 1992b)

Trichinella britovi is the etiological agent of infection of sylvatic carnivores living in temperate areas of the Palearctic region from the Iberian Peninsula to Kazakhstan, Iran and Turkey (Pozio, 2001; Ozdemir et al., in press). It is likely that the distribution area of this species encompasses other Asiatic countries for which no information is currently available (e.g. India and China). The isotherm −6 °C in January can arbitrarily be assigned as the northern border of distribution of this species (Pozio, 2001). Recently, this species has been detected in sylvatic carnivores in the Republic of Guinea (West Africa) (Pozio et al., 2005a). In addition, epidemiological and molecular data show that it is present also in wildlife of the Mediterranean countries of Africa (Nezri et al., in press). The larvae of *T. britovi* have been shown capable of surviving in frozen muscles of carnivores for up to 11 months and in frozen muscles of swine for up to 3 weeks (Dick and Pozio, 2001), albeit they survive a shorter time than larvae of *T. nativa*. Human infections caused by *T. britovi* from the consumption of free-ranging pigs, game and horse meat, have been documented in France, Italy, Spain and Turkey (Pozio et al., 2001b; Gari-Toussaint et al., 2004; Rodriguez de las Parras et al., 2004; Ozdemir et al., in press). Since females of *T. britovi* produce fewer number of newborn larvae than *T. spiralis*, the clinical course is benign and death has not been documented (Pozio et al., 2003).

The genotype *Trichinella* T8, is related to *T. britovi* as demonstrated by successful interbreeding under experimental conditions. *Trichinella* T8 has been detected only three times in sylvatic carnivores (lions and a spotted hyena) and only in South Africa and Namibia (Murrell et al., 2000). In the earlier reports from the Russian scientists Britov and Boev (1972), both *T. britovi* and *Trichinella* T8 were identified as *Trichinella nelsoni* (see Pozio et al., 1992b). Since 1992, *T. britovi*, *Trichinella* T8 and *T. nelsoni* have been taxonomically separated (Pozio et al., 1992b). However, as with the differences between *Trichinella* T6 and *T. nativa*, inconsistencies between genetic distinctiveness and ability to interbreed have caused the taxonomic status of *Trichinella* T8 to remain unresolved.

2.1.4. *Trichinella murrelli* (Pozio and La Rosa, 2000)

Trichinella murrelli is the etiological agent of infection in sylvatic carnivores living in temperate areas of the Nearctic region. Its northern most boundaries have been tentatively assigned to the isotherm of −6 °C in January, but its southern boundary is still unknown. It has been detected throughout continental United States (California, Connecticut, Georgia, Illinois, Indiana, New Mexico, Pennsylvania and Texas) and in Canada near the USA border (Pozio et al., 2001c; Pozio E., unpublished data). This species shows very low infectivity to swine and rats (Kapel and Gamble, 2000; Kapel, 2001; Malakauskas et al., 2001). Surprisingly, an infected horse imported from Connecticut to France was identified as the source of a large human outbreak, which caused two deaths in 1985 (Ancelle et al., 1988; Ancelle, 1998; Pozio et al., 2001d).

2.1.5. Genotype *Trichinella* T9 (Nagano et al., 1999)

Trichinella T9 has been detected in sylvatic carnivores of Japan and has been considered strictly related to *T. britovi* with which it interbred under experimental conditions; however, the study of the ITS2 sequence suggests that *Trichinella* T9 is genetically more closely related to *T. murrelli* than to *T. britovi* (D. Zarlenga, unpublished data). For an explanation of this relationship, see Section 3 below.

2.1.6. *Trichinella nelsoni* (Pozio et al., 1992b)

Trichinella nelsoni is the etiological agent of infection of sylvatic carnivores living in Eastern Africa from Kenya to South Africa (Pozio et al., 2005a). Occasionally, it has been identified in sylvatic swine and man. This species has the same name used by Russian scientists (Britov and Boev, 1972) to identify what we now know as *T. britovi* and *Trichinella* T8. In effect, the work presented by Britov and Boev (1972) did not describe parasites belonging to this African species (Pozio et al., 1992b). This species shows low pathogenicity in humans where deaths were documented only in persons with more than 4000 larvae per gram of muscle (Bura and Willett, 1977). As with some of the other species, *T. nelsoni* has a low infectivity for swine and rats (Kapel and Gamble, 2000; Kapel, 2001; Malakauskas et al., 2001). It has never been detected in domestic pigs, but it has been detected in bushpigs (*Potamochoerus porcus*) and warthogs (*Phacochoerus aethiopicus*) (Nelson, 1970; Sachs, 1970). This parasite seems to be easily transmitted among wildlife living in protected areas, whereas there are no reports in wildlife outside of these areas (Pozio et al., 1997).

2.2. Non-encapsulated clade

In addition to the eight genotypes that induce the development of a collagen capsule during the muscle phase of the infection, three other species do not induce capsule formation. The numbers of isolates from each of these gene pools are low, but current information suggests that these can be delineated genetically as well as by variations in host range. A summary of each species is provided below.

2.2.1. *Trichinella pseudospiralis* (Garkavi, 1972)

Trichinella pseudospiralis is a cosmopolitan species infecting both mammals and birds. Three populations, which can be distinguished on a molecular basis, have been detected in the Palearctic, Nearctic and Australian regions (Zarlenga et al., 1996; La Rosa et al., 2001). Additional information is provided as part of the discussion of other non-encapsulated species to follow in Section 4.2.

2.2.2. Trichinella papuae (Pozio et al., 1999a)

To this date, *T. papuae* has been detected in Papua New Guinea (PNG) only, and is able to infect both mammals and reptiles. The wild pig seems to be the most important reservoir of this species (Owen et al., 2000; Pozio et al., 2005b). Wild pigs infected with this species and used as feed have been implicated in its transmission to saltwater crocodiles (*Crocodilus porosus*) (Pozio et al., 2005b), and to humans (Owen et al., 2005). Based on a region within the large subunit ribosomal DNA, known as 'expansion segment V' (ESV), two distinguishable populations have been identified so far in PNG (Pozio et al., 2005b). Under laboratory conditions, *T. papuae* infects mice, rats and red foxes (Pozio et al., 1999a; Owen et al., 2000; Webster et al., 2002) but was unable to develop in equatorial freshwater fishes (Pozio and La Rosa, 2005) suggesting this food group is not part of the infection cycle. Additional information on *T. papuae* is provided in Sections 4.1 and 4.5 below.

2.2.3. Trichinella zimbabwensis (Pozio et al., 2002)

Trichinella zimbabwensis has been detected in farmed crocodiles (*Crocodilus niloticus*) of Zimbabwe (Pozio et al., 2002) and Ethiopia (T. Gelnew and E. Pozio, unpublished data), in sylvatic crocodiles of Mozambique and in monitor lizards of Zimbabwe (Pozio, 2005). Under laboratory conditions, this species can infect domestic pigs, monkeys, rats, mice and foxes (Mukaratirwa and Foggin, 1999; Pozio et al., 2002; Hurníková et al., 2004; Mukaratirwa S., personal communication), suggesting that mammals are a suitable host even if no naturally-infected mammals have been detected thus far. As with *T. papuae*, *T. zimbabwensis* was unable to develop in equatorial freshwater fishes (Pozio and La Rosa, 2005). For additional information see Section 4.1 below.

3. Phylogeny

Recently, the National Human Genome Research Institute (NHGRI), one of the National Institutes of Health, announced that the Large-Scale Sequencing Research Network received financial support for sequencing the entire genome of *T. spiralis*, predicated upon the following facts: (i) parasites of this genus are among the select few that reside at the base of the Nematoda tree, are readily available, and members of a clade that is poorly represented in the DNA sequencing pool; (ii) they can be maintained in laboratory animals and provide easily obtainable and pure DNA; and most importantly (iii) they remain a zoonotic concern worldwide. Yet, amidst choosing *T. spiralis* as a representative of clade I parasites, the phylogeny of this genus remains in flux. Except for the delineation of encapsulated from non-encapsulated groups and some length limitations of *T. pseudospiralis*, parasites of the genus *Trichinella* are morphologically indistinguishable (Dick, 1983; Lichtenfels et al., 1983; Pozio et al., 1992b). As a result, beginning in earnest in the late 1980 s, numerous researchers started to evaluate the taxonomy and phylogeny of this genus based initially upon biological markers. Using these as a basis, some have maintained that the genus is best delineated by two clades only represented by encapsulated and non-encapsulated species, where the overlapping nature of measurable, biological characters warrant assigning encapsulated sylvatic genotypes subspecific classification under *T. spiralis* (Bessonov, 1998). Lack of consistency among some biological characters prompted the need to analyse and then classify these parasites predominantly through the use of genetic and/or biochemical methods. However, to date, virtually all work performed on parasite classification within this genus has been done using unweighted pair group method using arithmetic averages (UPGMA)-based methods. Distance methods rely upon clustering and similarity of sequences resulting from shared ancestral characters not shared derived characters. Methods employing parsimony, on the other hand, look for shared evolutionary characters where species with the most shared derived characters will group together. These two methods of data analysis do not necessarily coincide although oftentimes distance methods and parsimony do provide similar results.

Among the first extensive dendrograms, was that generated by La Rosa et al. (1992) based upon allozyme data from 27 enzymes and 152 *Trichinella* isolates. Eight gene pools were identified that maintain reasonable agreement with our current knowledge of the genus except for the placement of *Trichinella* T6; however, in this study the number of isolates of *Trichinella* T6 examined was quite small. Similar findings were generated by Bandi et al. (1995) using random amplified polymorphic DNA (RAPD) and allozymes, where congruence was observed among the independently constructed trees. The PCR banding patterns, though not identical among isolates of the same genotype, nonetheless clustered into taxonomic groups in a manner similar to that produced with the allozymes. The use of RAPDs, however, was quickly abandoned because of substantial discrepancies among other RAPD based trees (see Campbell et al., 1994), the probability of incorrectly interpreting data, and the effect that DNA integrity can have on PCR fragment banding patterns (Pozio et al., 1999b).

In 1997, Zarlenga used preliminary mitochondrial DNA data to produce a UPGMA tree that also showed strong support for the topology produced by Bandi et al. (1995) with variation occurring predominantly in the placement of the Palearctic and Nearctic sylvatic genotypes. More recently, La Rosa et al. (2003) generated independent trees using multilocus enzyme electrophoresis data analysed by neighbour-joining (NJ) and UPGMA. The two trees showed remarkable congruence but raised questions regarding the overall topology relative to that previously predicted, where UPGMA did not completely delineate non-encapsulated species as a monophyletic clade but placed them at the base of the tree. Further, in the tree constructed

using the NJ algorithm, *T. nelsoni* was basal to *T. spiralis*, which differed from the UPGMA tree and prior UPGMA-based topologies. Most striking however, was the prediction by both analyses that biochemical differentiation among the Arctic genotypes, *T. nativa* and *Trichinella* T6, was equivalent to the more ancestral species even amidst evidence showing gene-flow among these sympatric genotypes (La Rosa et al., 2003b). One possibility for the discrepancies in tree topologies is that genetic information can be lost in isoenzyme data analysis where banding patterns result from an average change in amino acid sequence over the length of the peptide. Thus, isoenzyme data are very good for identifying groups or changes within a taxon but are not as strong in estimating the magnitude of the changes or in clarifying the branching process.

To date, only one recent publication has attempted maximum likelihood, parsimony and/or NJ methods to address the issue of *Trichinella* phylogeny. In this regard, Gasser et al. (2004) sequenced the D3 domain of the nuclear ribosomal DNA (371–399 bp) from all currently recognised species and genotypes of *Trichinella* (Fig. 1). All trees showed congruence with that depicted in Fig. 1. There was strong bootstrap support for monophyly among *T. spiralis* and *T. nelsoni*, and also among *T. nativa* and *Trichinella* T6. Furthermore, as with the UPGMA trees, non-encapsulated species clustered at the base of the tree but in this analysis, isolates of *T. pseudospiralis* clustered independent of *T. papuae* and *T. zimbabwensis*.

From the evaluation of 15 years of data, several facts have consistently emerged in most *Trichinella* trees. First, the initial biologically-based conclusions that *T. nativa* and *T. nelsoni* (Britov and Boev, 1972), *T. britovi* (Britov and Boev, 1972; Pozio et al., 1992b), *T. spiralis* (Owen, 1835) and *T. pseudospiralis* (Garkavi, 1972) are unique taxonomic groups were supported. Second, there are clear biological and molecular distinctions between encapsulated and non-encapsulated species (Pozio et al., 2001a; La Rosa et al., 2003a; Gasser et al., 2004; Zarlenga et al., 2004), though monophyly of the group is unresolved. Third, either *T. spiralis* or *T. nelsoni* are likely candidates to form the basal clade of the encapsulated species. Fourth, all other sylvatic genotypes cluster centrally or at the crown of the phylogenetic (Gasser et al., 2004) and most phenetic trees, but these are inconsistently resolved with respect to their relative positioning.

Placement of *T. nelsoni* and *T. spiralis* within the broader context of the *Trichinella* tree as well as the issue of monophyly among the non-encapsulated parasites brings up

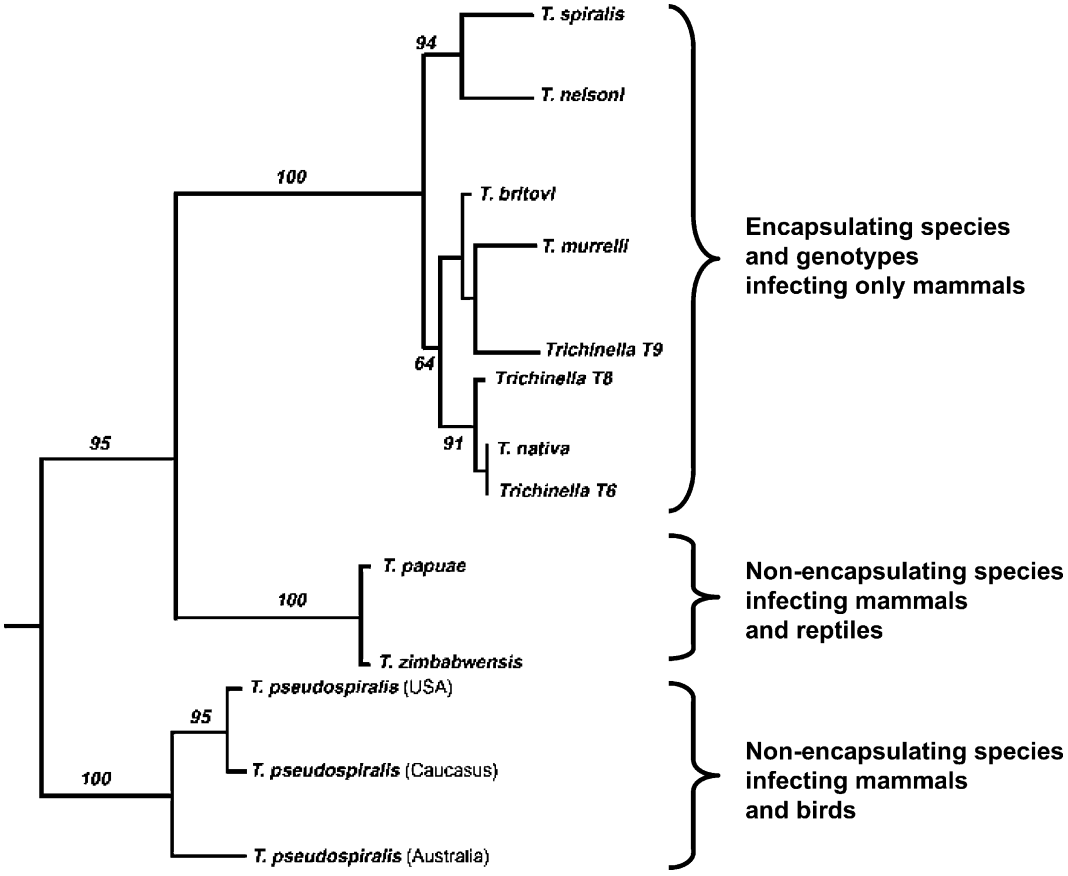

Fig. 1. Neighbour-joining tree of all currently available *Trichinella* genotypes redrawn from Gasser et al. (2004) and derived from sequence analysis of the D3 domain of the nuclear ribosomal DNA. Numbers at each node represent bootstrap values. Included in the tree are all genotypic variants of *Trichinella pseudospiralis*.

an important consideration as to the biogeography and evolutionary history of this genus. Inasmuch as *Trichinella* resides within clade I and at the base of the phylum Nematoda (Blaxter et al., 1998), a long history for initial divergence of this lineage is supported; however, the absence of morphological subdivision and the overlapping nature of many biological characters among encapsulated species may suggest more recent evolutionary events. Thus, the localisation of *T. spiralis* or *T. nelsoni* at the base of the encapsulated clade provides valuable information as to the origins of this group of organisms. Because of the absence of fossil record for nematodes, substantial emphasis has to be placed on the host-parasite assemblages in understanding the history of their geographic dissemination and host adaptation. Given that contemporary predatory guilds are believed to have originated in the Palearctic, their role in the radial distribution of *T. spiralis* among sylvatic hosts is consistent with this region and *T. spiralis* as a likely origin of the encapsulated species. However, fossil remains of extinct carnivores in southern Africa, presumably occurring prior to the spatial separation of Africa and South America, in conjunction with the exclusive presence of *T. nelsoni* in Africa, supports a tree in which *T. nelsoni* is basal to the encapsulated species. In-depth analyses of the systematics of this genus, based upon shared evolutionary characters, will require a more robust genetic analysis of the genus, and one that includes all ecologically and genetically recognised parasite forms.

4. Epidemiology

4.1. Trichinella in poikilotermic vertebrates

The detection of individual *Trichinella* species infecting both mammals and reptiles has opened new scenarios in the epidemiology of this parasite group by increasing the food sources, which carry potentially infective stages. Our present knowledge suggests that most infections in reptiles are due to an improper management of farmed crocodiles. In Zimbabwe, of the 648 crocodiles (*C. niloticus*) examined in 1995, 166 (39.5%) were found to be infected, and of the 29 farms existing in Zimbabwe at that time, 18 (62.1%) were positive for *Trichinella*. A more recent survey performed in 2002 indicated that 11 (40.7%) of the 27 crocodile farms in Zimbabwe had infected animals. Epidemiological investigations suggested that a farm located near Victoria Falls was among the first to be infected, and if so, was the likely source of the infections observed throughout Zimbabwe (Pozio et al., 2002).

Transmission at the farm level occurs by feeding meat from slaughtered crocodiles to the other crocodiles, whereas the spread of infection between crocodile farms is probably related to the translocation of infected, bred crocodiles. This may also explain the presence of *Trichinella* infection on a crocodile farm at Lake Abaja in Ethiopia (T. Gelnew and E. Pozio, unpublished data). However, natural infections have been identified in Nile crocodiles from Mozambique and in monitor lizards (*Varanus niloticus*) from Chiredzi, south-west of Zimbabwe (Pozio, 2005). These data may suggest that epidemiological profiles observed in the dissemination of *Trichinella* between farmed pigs and local fauna may be mimicked in the transmission characteristics among reptiles and their surrounding fauna as well.

The presence of *T. papuae* in saltwater crocodiles on a farm in Papua New Guinea is related to the practice of collecting and confining small, wild crocodiles, then feeding them market-bought, wild pig meat for several months prior to sending them for finishing at the crocodile farm. This mode of transmission has been confirmed by identical molecular markers found in both *T. papuae* from infected, wild pig meat purchased at the local market, and in *Trichinella* from farmed crocodiles (Pozio et al., 2005b). This finding clearly shows that *T. papuae* is capable of a transmission route from homoiothermic to poikilothermic animals.

Experimental infections of all known *Trichinella* species in four reptile species belonging to the three orders Loricata (caimans, *Caiman crocodilus*), Squamata (savannah monitors, *Varanus exanthematicus*; pythons, *Python molurus bivittatus*) and Chelonide (African helmeted turtles, *Pelomedusa subrufa*) have shown that only *T. papuae* and *T. zimbabwensis* can reproduce in these cold-blooded animals; however, in contrast to the very high reproductive rates observed in monitor lizards and caimans, reproductive rates in snakes and turtles were negligible. The lack of clinical signs in monitor lizards and caimans together with the high levels of infection are consistent with the hypothesis that these animals may act as reservoirs of *T. papuae* and *T. zimbabwensis*. Reptiles belonging to the Varanidae and Crocodilidae families are carnivores with scavenger behaviour, which is the most important biological and ecological character of animals known to act as *Trichinella* reservoirs (Campbell, 1988). In the light of these findings, reptile meat has potential as source of human trichinellosis (Pozio et al., 2004a).

Despite very high infecting doses, the very low numbers of *T. papuae* and *T. zimbabwensis* larvae detected in experimentally infected pythons and turtles suggest that these species do not play an important role in the epidemiology of *Trichinella* (Pozio et al., 2004a). This is consistent with the diet of pythons and turtles, which most likely does not include *Trichinella* carriers.

The demand for the skin and meat of crocodiles, caimans, and alligators in many areas of the world has increased dramatically. This has resulted in the development of national breeding programs in more than 30 countries in North and South America, Africa, Asia, and the Australian region, and produced an income of about US$60 million in 1998 for meat alone (Pozio et al., 2005b). As a result of this increased demand, the infection of reptiles with *Trichinella* species that are potentially infective for humans takes on

great importance. Learning to control *Trichinella* infection in these hosts will help further advance their use as sources of food and other marketable products.

4.2. Trichinella pseudospiralis: an unrecognised or spreading species?

After its discovery in a raccoon from Caucasus in 1972, *T. pseudospiralis* was considered more a scientific curiosity than an animal pathogen (Dick, 1983). In the 18 years that followed, very few reports were documented in Asia (Shaikenov and Boev, 1983). However, in 1990–1992, new foci of this parasite were discovered in Tasmania involving both marsupials and birds (Obendorf et al., 1990; Obendorf and Clark, 1992). In the following years, a growing number of reports of *T. pseudospiralis* in domestic and sylvatic animals (Pozio, 2001; 2005; La Rosa et al., 2001; Oivanen et al., 2002; Pozio et al., 2004b; Hurníková et al., 2005; Gamble et al., 2005) and in humans (Pozio, 2001) documented its presence in Asia, the Australian region, Europe and North America (Table 1). Today, this parasite has been found in 14 mammalian species and 13 avian species (Pozio, 2005), where the number of reports in mammals is much higher than that in birds. This is likely the result of a bias towards the examination of mammals for this parasite relative to the number of birds examined. The growing reports of *T. pseudospiralis* in domestic and sylvatic swine in Europe as well as other regions of the world, e.g. the USA and Thailand, and the potential to infect humans, suggests that this species is a more serious human health concern than originally believed. This epidemiological data forced a revision of older European Union (EU) legislation where the recommendation to use trichinelloscopy for detecting *Trichinella* parasites in slaughterhouses was deemed unsuitable because of difficulties identifying non-encapsulated larvae within the muscle fibres.

4.3. The role of synanthropic rats: reservoir, vector or victim?

Among synanthropic animals, the brown rat (*Rattus norvegicus*) is the most widespread species found to be infected with *T. spiralis,* primarily when surveyed in domestic habitats (e.g. pig farms and garbage dumps). Only seldom does one find the brown rat infected with *T. britovi* (Pozio et al., 1996) or *T. pseudospiralis* (Britov, 1997; Oivanen et al., 2002; Hurníková et al., 2005). The role of this animal in the epidemiology of *Trichinella* continues to be debated as either a reservoir or an accidental host functioning as a vector of *T. spiralis*. In the 19th Century, Leuckart, proposed a 'Rat Theory', implicating rats as a major reservoir of *T. spiralis* infection for domestic pigs. Zenker (1871) on the other hand, suggested that the infection in rats was more a marker of the infection in pigs, and that the real source of infection for both animals was scrap and offal of pig carcasses.

Although *T. spiralis* infection in pigs is often associated with infection in rats living in abattoirs, farms, and garbage dumps, there are no reports showing *T. spiralis* infection in brown rats where pig populations have been found to be negative. This indicates that brown rats alone, without external introduction of *T. spiralis* into their population, cannot maintain the infection. However, transmission of the parasite on a pig farm may involve rats as an important source of infection when this synanthropic animal is exposed to pork scraps or cannibalism under unique circumstances such as high population pressure.

A survey carried out in an endemic area in the Republic of Croatia showed a significant correlation between farms with low sanitation (i.e. with widespread pork scraps, offal and *Trichinella*-infected pigs) and the presence of infected rats. On the other hand, no infection was observed in rats from farms with better sanitation, i.e. without pork scraps, offal and infected pigs (Stojcevic et al., 2004). This finding, that infected rats were only found in the presence of infected pigs, is consistent with the absence of reports of *T. spiralis* infection in brown rats collected on farms where the pig population was negative. In a study carried out in Pennsylvania (USA), *T. spiralis*-free pigs placed onto a farm with low level of sanitation (i.e. infected pigs and synanthropic rats) acquired the infection within 3–4 months. The prevalence of infection in these newly introduced pigs was related to their level of exposure to rats (Schad et al., 1987) though the authors rightfully debated whether or not the presence of infected pigs was necessary for establishing *T. spiralis* infection in a surrounding rat population. This conclusion could only have been reached if positive rats were found in the presence of negative pigs, which was not the case.

The spread of infected pork scraps in the environment by humans seems always to be important for *T. spiralis* infection in rats. Rat-control campaigns and farm renovations may solve the local problem but can force rats to migrate and spread the infection to neighbouring farms and villages. The use of rat pesticides can actually favour transmission because poisoned rats are easy prey for pigs (Stojcevic et al., 2004). The role of the brown rat as a vector of *T. spiralis* was clearly shown by Smith et al. (1976) in some swine herds of the Atlantic provinces of Canada. In these herds, a control program forced rats to migrate from *Trichinella*-positive herds to *Trichinella*-negative herds where several months later the *Trichinella*-negative herds became positive. Thus, infected rats represent an offshoot of the domestic cycle, being recipients of infection from that cycle (Campbell, 1983). This is consistent with findings in the United States that the occurrence of *T. spiralis* infection in domestic pigs greatly decreased when feeding with uncooked garbage and offal was terminated, which is usually done to control bacterial and viral infections (Hall, 1937).

Table 2
Outbreaks of human trichinellosis caused by infected horse-meat in France (Fr) and Italy (It)

Year	Locality (country)	No. of human infections/ deaths	Country of origin of the horse	*Trichinella* species
1975	Bagnolo in Piano (It)	89/0	Former Yugoslavia	*T. britovi*
1975	Chatenary-Malabry (Fr)	125/0	East Europe	n.d.[a]
1984	Varese (It)	13/0	Former Yugoslavia	n.d.
1985	Paris and Melun (Fr)	431/2	Connecticut (USA)	*T. murrelli*
1985	Paris and 10 other foci (Fr)	642/3	Poland	*T. spiralis*
1986	Salsomaggiore (It)	300/0	Former Yugoslavia	*T. britovi*
1990	Barletta (It)	500/0	East Europe	*T. spiralis*
1991	Clermont-Ferrand (Fr)	21/0	USA	n.d.
1993	Paris and 3 other foci (Fr)	538/0	Canada	*T. spiralis*
1994	Provins (Fr)	7/0	Mexico	*T. spiralis*
1998	Haute Garonne (Fr)	128/0	Serbia	*T. spiralis*
1998	Piacenza (It)[b]	93/0	Poland	*T. spiralis*
1998	Toulouse (Fr)	404/0	Serbia	*T. spiralis*
2000	Bitonto (It)	36/0	Romania or Poland	*T. spiralis*

[a] n.d., not determined.
[b] The source of infection was a horse, which was found infected at the slaughterhouse in Brescia (Italy) in 1998 (see Table 3).

4.4. Trichinella infection in horses

In spite of thousands of human infections caused by the consumption of horse meat in France (2296 cases in eight outbreaks) and Italy (1031 cases in six outbreaks) (Table 2) (Ancelle, 1998; Boireau et al., 2000; Pozio, 2001; Pozio et al., 2001d), no one has been able to demonstrate how horses acquire an infection in nature. This is in spite of numerous epidemiological surveys being performed at the point of origin of the infected horses (Murrell et al., 2004; E. Pozio, unpublished data). However, information has shown a relationship between *Trichinella* infection in horses and that in pigs (Murrell et al., 2004). In the three cases where sylvatic species of *Trichinella* (*T. britovi* or *T. murrelli*) were detected, an association between infection in horses and wildlife or fur reared animals has been postulated (Pozio, 2001).

The feeding of animal products to horses is a practice that occurs in several countries, including those with infected horses (e.g. Poland, Romania, Serbia). The increasing numbers of reports of human outbreaks of trichinellosis in France and Italy in the 1990 s, and the detection of *Trichinella*-infected horses at slaughterhouses seem to overlap with the peak of *Trichinella*-infection in domestic pigs. This occurred in the same time period in eastern European countries following the breakdown of the veterinary services (Djordjevic et al., 2003). The statement made by Boireau et al. (2000) indicating "a low frequency infection with high human risk" clearly explains the epidemiological impact of *Trichinella* infection in horses, in which the prevalence of infection is lower than 0.001%. To date, only 18 *Trichinella*-infected horses have been detected at slaughter (Table 3).

Up to now, freezing of horse meat has been considered an acceptable method to kill *Trichinella* larvae present in muscles of horses (Gamble et al., 2000); however, recent experimental data from ponies infected with *T. spiralis*, *T. britovi* or *T. pseudospiralis*, suggest that *Trichinella* larvae survive freezing for several weeks and retain their infectivity (C. Kapel, personal communication). Though interesting, these data require confirmation, because others suggest that larvae of *T. spiralis* present in a naturally-infected horse did not survive freezing at −15 °C for 24 h (E. Pozio, unpublished data).

4.5. Do Trichinella-free areas actually exist?

As a general rule, parasites of the genus *Trichinella* are considered cosmopolitan even if there are many countries in which these pathogens have never been documented. The Australian region was one such geographical locality where even though the number of surveys was limited, it was nonetheless considered *Trichinella*-free, except for *T. pseudospiralis* in Tasmania (Obendorf et al., 1990).

In New Zealand, *T. spiralis* has been imported passively by humans from Europe. *Trichinella* infections in man, domestic (pigs and cats) and synanthropic (brown rats) animals have been documented since 1964 (Cairns, 1966). In this country, inspection for *Trichinella* in pigs is compulsory for exported meat; however, only random sampling of products destined to be sold in local markets from mature age pigs is performed at slaughter. This sampling number is based on a statistical expectation that a *Trichinella* prevalence of 0.5% would be detected. Clearly, this is a minimalist approach that does not prevent transmission of the infection to the consumer. In fact, an outbreak occurred in 2001 on a farm located on the Whangamata, Coromandel Peninsula, North Island, in which domestic pigs, synanthropic brown rats and a domestic cat were found infected with *T. spiralis* (E. Pozio, unpublished data).

A high prevalence of *Trichinella* (153/1536, 10%) has been recently recorded among hunters and horticulturalists living in the Morehead District of Papua New Guinea where *T. papuae* has been detected in 11% of wild pigs (Owen et al., 2005). The infection rate related significantly with the distance between the villages and the hunting area, where

Table 3
Natural *Trichinella* infections detected in horses at the slaughterhouse during surveys (in Italy between 1988 and 1989; in Mexico in 1994) and during routine examinations or in meat samples confiscated after human outbreaks

Year	No. of infected horses	No. of larvae/g (examined muscle)	Locality where the infection was detected (country or month)	Country of origin of the horse	*Trichinella* species
1988	1	0.02 (biceps brachii)	Brescia (Italy)	Poland	n.d.[a]
1989	1	0.26 (diaphragm)	Brescia (Italy)	Former Yugoslavia	n.d.
1994	4	0.8, 1.0, 1.6 and 1.8 (diaphragm)	State of Mexico (Mexico)	Mexico	*T. spiralis*
1996	2	0.01, 0.02 (tongue)	Bordeaux (France)	Poland	n.d.
1996	1	11.0 (tongue)	Barletta (Italy)	Romania	*T. spiralis*
1998	1[b]	256.0 (diaphragm)	Brescia (Italy)	Poland	*T. spiralis*
1998	1	615.0 (tongue)	Poggio Imperiale (Italy)	Serbia	*T. spiralis*
1998	1[c]	<0.2 in roast meat	France (February)	Serbia	*T. spiralis*
1998	1[c]	27 in steak	France (October)	Serbia	*T. spiralis*
1999	1	433 (tongue median apex) 626 (tongue apex)	France (October)	Poland	*T. spiralis*
2001	1	486 (tongue median apex)	France (March)	Serbia	*T. spiralis*
2001	1	12.5 (diaphragm)	Turin (Italy)	Romania	*T. spiralis*
2002	1	1221 (diaphragm)	Serbia	Serbia	*T. spiralis*
2003	1	2.1 (diaphragm)	Turin (Italy)	Serbia	*T. spiralis*

[a] n.d., not determined.
[b] This is the same horse, which was the source of infection for the human outbreak that occurred in Piacenza (Italy) in 1998.
[c] The meat was collected after the human outbreak.

the prevalence of positive samples increased as this distance decreased (Owen et al., 2005).

Similar findings have been documented even in frigid zones (Serhir et al., 1999; Proulx et al., 2002; Schellenberg et al., 2003), where the seroprevalence in hunters and fishermen, reached 22% among Inuits in Greenland (Bohm and van Knapen, 1989). In these areas, the main source of infection is walrus (*Odobenus rosmarus*) meat (igunaq) where the prevalence of *T. nativa* can exceed 60%. Infection in seals has also been documented but at a much lower rate (1%); consequently, the consumption of seal meat is unlikely to represent a serious health threat (Leclair et al., 2004; Forbes, 2005) even if experimental infections have shown a high reproductive capacity of *T. nativa* in this host (Kapel et al., 2003).

In countries, where pig production is carried out according to good farming practices and humans do not consume raw pork products, no infections in either humans or pigs have been documented for a long time. Unfortunately, the absence of reports of *Trichinella* infections in these countries results in consumers and producers becoming oblivious to the risk of this zoonosis, and a relaxation of control measures at slaughterhouses. As an example, in Ireland, no infections have been documented in either humans or animals in the last 34 years, suggesting that this country is *Trichinella*-free (Rafter et al., 2005). This resulted in a reduction in slaughterhouse control measures where the pig testing rate dropped to 20%. However, an epidemiological survey carried out on red foxes revealed the presence of *T. spiralis*-infected animals (3.1–4.2% prevalence) in counties thought to be *Trichinella*-free, clearly demonstrated that the sylvatic cycle flourished independent of the domestic cycle (Rafter et al., 2005). The long-term survival of *Trichinella* in foxes of Ireland may be explained in part by hunters leaving the carcasses in the field after skinning. The high humidity and low temperatures during the hunting season i.e. autumn and winter, favour the survival of larvae and allow for the transmission of *Trichinella* through a fox–fox cycle. The lack of human infections may simply be due to the fact that people of this region tend to cook pork well.

4.6. The problem of basing assumption on infection risk according to country religious laws

Trichinellosis is considered a rare or unknown disease in countries where the Muslim religion is predominantly practised, but outbreaks do occur in people who eat pork. In Turkey, one small-scale outbreak of trichinellosis (13 infected people) was documented in Kumkapi (Istanbul) from the consumption of wild boar meat from the Kastamonu region (Merdivenci et al., 1977). In the same country, two family outbreaks involving about 40 people have been documented in Bursa and Antalya in 2002 and 2004 (Akkoc N., personal communication). In addition, a single case was documented in a French tourist, who acquired the infection after eating pork in Turkey, but did not develop symptoms of the disease until returning to France (Dupouy-Camet et al., 1998). In late 2003 and early 2004, a large outbreak of trichinellosis infecting more than 600 persons, occurred in Izmir from the consumption of raw veal meatballs, which contained, unknowingly, pork

(Ozdemir et al., in press). *Trichinella* larvae isolated from both human biopsies and from a raw meatball were both identified as *T. britovi*. The occurrence of these outbreaks shows how the consumption of pork in non-commercialised regions, which lack proper control measures can result in serious health and economic problems even when religious law restricts consumption.

4.7. Immigrants, tourists and the illegal importation of infected meat

In the past decade, *T. spiralis* infection has been found widespread among domestic pigs in most countries of central and eastern Europe (i.e. Bulgaria, Belorussia, Croatia, Georgia, Latvia, Lithuania, Moldavia, Romania, Russia, and Serbia), with prevalence reaching 0.16% at the national level and as high as 50% in some villages (Murrell and Pozio, 2000; Pozio, 2001). For economic and political reasons many people have migrated from Eastern Europe to the EU. This has led to an increase in the large-scale illegal importation of pork products from these countries to the EU either as Christmas gifts, or personal acquisitions by migrants returning back to the EU. This behaviour has resulted in several human outbreaks of trichinellosis in Germany, Italy and the United Kingdom (Pozio and Marucci, 2003).

There are also many reports of tourists who acquired *Trichinella* infections while travelling in endemic areas such as Africa, Canada, China, Egypt, Greenland, Indonesia, Laos, Malaysia and Turkey, and develop the clinical disease after their return to their home countries. In these cases, diagnosis has been difficult because the infections appeared as isolated cases (Michel et al., 1986; De Carneri and Di Matteo, 1989; McAuley et al., 1991; Nozais et al., 1996; Dupouy-Camet et al., 1998; Shiota et al., 1999; Kurup et al., 2000; Nakamura et al., 2003).

5. Conclusions

This review shows how the fascinating world of *Trichinella* continuously gives us new discoveries in different fields. But in spite of the growth in our knowledge, these parasites persist as an important concern for human health because of dwindling economic resources to control this infection. The discovery of a high serological prevalence in humans who are hunters and horticulturalists (Owen et al., 2005) suggests that this infection likely has been associated with humans from his first appearance to the present day. The origin of the domestic cycle of *Trichinella* is probably related to the domestication of swine, when *Homo sapiens* went from being hunter-gatherers to farmer-breeders following the last Ice Age (i.e. after the 11th millennium BC).

There are still many countries where epidemiological information on animal and human infections is lacking e.g. India, many countries of Africa, Central and Southern America and Asia; consequently, we stress the need to investigate the local fauna, focusing not only on carnivore and omnivore mammals, but also carnivore and omnivore birds and reptiles. Finally, we should always keep in mind that the potential for recrudescence and the risk of human infection will continue to increase dramatically and concomitantly with an increase in government complacency to control a zoonotic organism with the vast host range and worldwide distribution as *Trichinella*.

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