Systematics and Epidemiology of Trichinella

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ABSTRACT

In this review, we describe the current knowledge on the systematics, ecology and epidemiology of *Trichinella* and trichinellosis, and the impact of recent research discoveries on the understanding of this zoonosis. The epidemiology of this zoonosis has experienced important changes over the past two decades, especially with regard to the importance of the sylvatic cycle and the sylvatic species. Outbreaks of trichinellosis due to *Trichinella spiralis* from domestic swine, while still frequent, increasingly are caused by other *Trichinella* spp. infecting hosts such as horses, dogs, wild boars, bears and walruses. The latter revelations have occurred as a result of a series of discoveries on the systematics of *Trichinella* spp., facilitated by new molecular tools. As a consequence, the genus is now composed of two clades, an encapsulated group (five species and three genotypes) and a non-encapsulated one (three species). This has sparked renewed investigations on the host range of these parasites and their epidemiological features. Most dramatic, perhaps, is the recognition that reptiles may also serve as hosts for certain species. This new knowledge base, in addition to having an important relevance for food safety policies and protection measures, is raising important questions on the phylogeny of *Trichinella* spp., the ecological characteristics of the species and their geographic histories. Answers to these questions may have great value for the understanding of the evolutionary biology for other parasitic helminths, and may increase the value of this genus as models for research on parasitism in general.

1. INTRODUCTION

Trichinellosis, the proper term for the human zoonotic disease also known as trichinosis or trichiniasis, is caused by a group of unusual nematodes belonging to the genus *Trichinella*. This zoonosis has had a long and eventful history of scientific investigation and discovery. Because of the existence of several excellent reviews by others of the history, biology and clinical aspects of trichinellosis (Gould, 1970;
Campbell, 1983a, 1991; Nelson 1988), this introduction will only summarize the major epochs that mark the history of the research on and control of Trichinella, but instead highlight those landmarks that have had a major impact on our understanding of these parasites and their important biological properties.

The unraveling of the nature of trichinellosis may reach back to antiquity as suggested by historical references to diseases that bear striking similarities to the clinical aspects of Trichinella sp. infection. The earliest such case involved a young Egyptian living along the Nile about 1200 BC (Gould, 1970; Campbell, 1983a). There is evidence of human infections even in prehistoric cultures (Owen et al., 2005). The modern history of trichinellosis, however, begins in 1835, with the discovery, by microscopy, of the larval stage of the parasite by James Paget and Richard Owen in London. It was Owen who coined its first name, Trichina spiralis (Owen, 1835). Over the next 60 years, new revelations on the parasite’s life cycle, epidemiology and clinical diagnosis resulted from research chiefly carried out in Germany. Several important discoveries stand out, perhaps the most important from a public health standpoint is the linkage of Trichinella spiralis infection with the human disease and mortality by Friedrich Zenker in 1860. His identification of the form of origin of a deceased 20-year-old housemaid who had died of T. spiralis infection and the subsequent epidemiological detective work he performed demonstrated that the source of infection was pork, the first clear evidence of transmission of T. spiralis from an animal to man (Nelson, 1988). This was hailed within the medical community as the most important medical advance of the time (Campbell, 1983a). The other related breakthrough was the discovery of the parasite’s life cycle, from the investigations of Rudolf Virchow, F. Zenker, Rudolf Leukart and others. Particularly important was the recognition that Trichinella sp. was primarily a parasite of animals, and that it existed in both a domestic cycle (e.g. pigs, rodents and pets) and a sylvatic cycle (wild animals) (Kozar, 1970).

Based on these advances in the understanding of the basic biology of T. spiralis, a second phase in the history of the zoonosis unfolded: the adoption of control strategies to prevent infection in humans. Because of the relative ease of detecting microscopically the larval stage (trichina) in selected muscles, the inspection of pork at slaughter
was declared feasible and introduced in 1863 into slaughterhouses, first locally in a few areas of Germany, and then, led by R. Virchow, nationally throughout the country in 1866. This ushered in the now worldwide practice of veterinary control over the slaughter of food animals to ensure food safety.

1.1. Trichinella as a Model for Basic Research

With the growing understanding of the epidemiology and clinical aspects of trichinellosis, and the ease of maintaining the parasite's life cycle in the laboratory, the study of Trichinella spp. throughout the 20th century expanded to basic investigations on its morphology, the physiology and biochemistry of its intracellular parasitic mode, and on the immunological aspects of infection (Larsh, 1975; Despommier, 1983; Stewart, 1983; Britov, 1994; Castro, 1997; Wakelin and Grencis, 1997; Appleton and Romaris, 2001; Romaris and Appleton, 2001; Bruschi, 2002; Wu et al., 2002). The understanding of the basic biology of Trichinella spp. has benefited also from the use of these parasites as a model for a broad range of investigations in parasitology on such general topics as immunology and physiology to the scientific interest in what an understanding of its intrinsic biological features might contribute to gaining important insights into parasitism in general.

1.2. History of Trichinella Taxonomy

The third major era in the history of trichinellosis might be fairly characterized as a revolution in the systematics of Trichinella spp. and the understanding of its ecology and epidemiology. Although Railliet (1896) revised the genus name to Trichinella, inasmuch as the designation Trichina had been employed for a genus of Diptera (Trichina clavipes Meigen, 1830) (Gould, 1970), important misunderstandings of its taxonomy, host range and epidemiology persisted until well into the 20th century. During the first 150 years of its scientific recognition, T. spiralis was considered the sole member of the genus, and as having a phenomenally wide host range, extending to more than 100 species of mammals (Campbell, 1983b). However, beginning in 1950s
and 1960s, scientists began reporting an increasing number of host-specificity peculiarities among different geographic isolates (Rausch et al., 1956; Nelson et al., 1966). Several investigators reported that isolates from some wild animals appeared to have poor infectivity in pigs and rats, the major hosts for the domestic cycle, leading to speculation that important geographic variability existed within the species. Among the earliest reports were those of Rausch et al. (1956) and Rausch (1970) that attempts to infect a pig with infected meat from an arctic fox (Alopex lagopus) in Alaska were unsuccessful. The breakthrough discovery, however, occurred in Kenya in the 1960s, when Nelson and coworkers convincingly demonstrated that an isolate from the bushpig (Potamochoerus porcus), which had been responsible for a human outbreak, had low infectivity in rats and pigs, in comparison to porcine isolates from Europe and the United States (Nelson et al., 1966). These comparative infection results sparked great interest among investigators and led to similar comparative studies with various geographic isolates of the parasite (reviewed by Rausch, 1970). These studies, conducted over the next 30 years, yielded a remarkable series of new revelations on the genetic diversity within the genus, and yielded finally a new Trichinella taxonomy encompassing eight species (see Table 1), along with a more complete zoogeographical and epidemiological knowledge base (Pozio et al., 1992a; Murrell et al., 2000; Pozio and Zarlenga, 2005). These advances and the insights they have provided are a focus of this review.

The epidemiological view of trichinellosis has, because of these new biological revelations, expanded from a primarily domestic cycle source to an increasingly sylvatic one (Dupouy-Camet, 2000; Murrell and Pozio, 2000; Pozio, 2000, 2001a). While the zoonosis is continuing to be brought under control in some regions (e.g. Europe and North America), its potential for rebounding, due to laxity in veterinary control, is great because of the parasite’s ability to exploit new opportunities for transmission presented by changes in demography, agriculture and wildlife habitat (Boireau et al., 2000; Pozio et al., 2001a, 2005a; Djordevic et al., 2003). Current trends in those factors that have the potential to perturb the ecology and epidemiology of Trichinella sp. and their potential consequences are discussed.
<table>
<thead>
<tr>
<th>Clade Species or genotype</th>
<th>Geographical distribution</th>
<th>Host range</th>
<th>Main source of infection for humans</th>
<th>Resistance of larvae in frozen muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encapsulated T. spiralis</td>
<td>Cosmopolitan</td>
<td>Domestic and sylvatic mammals</td>
<td>Domestic and sylvatic swine horse</td>
<td>No</td>
</tr>
<tr>
<td>T. nativa</td>
<td>Arctic and subarctic areas of the Nearctic and Palearctic regions</td>
<td>Sylvatic carnivores</td>
<td>Bear, walrus</td>
<td>Yes in carnivore muscles</td>
</tr>
<tr>
<td>Trichinella T6</td>
<td>Canada; Alaska, Rocky Mountains and Appalachian in the USA</td>
<td>Sylvatic carnivores</td>
<td>Carnivores</td>
<td>Yes in carnivore muscles</td>
</tr>
<tr>
<td>T. britovi</td>
<td>Temperate areas of the Palearctic region, Northern and Western Africa</td>
<td>Sylavtic mammals, seldom domestic pigs</td>
<td>Wild boar, domestic pig</td>
<td>Yes in carnivore muscles</td>
</tr>
<tr>
<td>Trichinella T8</td>
<td>South Africa and Namibia</td>
<td>Sylvatic carnivores</td>
<td>Non-documented</td>
<td>No</td>
</tr>
<tr>
<td>T. murrelli</td>
<td>USA and Southern Canada</td>
<td>Sylvatic carnivores</td>
<td>Bear, horse</td>
<td>No</td>
</tr>
<tr>
<td>Trichinella T9</td>
<td>Japan</td>
<td>Sylvatic carnivores</td>
<td>Non-documented</td>
<td>No</td>
</tr>
<tr>
<td>T. nelsoni</td>
<td>Eastern-Southern Africa</td>
<td>Sylvatic mammals</td>
<td>Warthog, bush pig</td>
<td>No</td>
</tr>
<tr>
<td>Non-encapsulated T. pseudospiralis</td>
<td>Cosmopolitan</td>
<td>Sylvatic mammals and birds, domestic pigs</td>
<td>Domestic and wild pigs</td>
<td>No</td>
</tr>
<tr>
<td>T. papuae</td>
<td>Papua New Guinea</td>
<td>Wild pig, saltwater crocodile</td>
<td>Wild pig</td>
<td>No</td>
</tr>
<tr>
<td>T. zimbabwensis</td>
<td>Zimbabwe, Mozambique, Ethiopia</td>
<td>Nile crocodile, monitor lizard</td>
<td>Non-documented</td>
<td>No</td>
</tr>
</tbody>
</table>
The genus *Trichinella* is the sole member of the Family: Trichinellidae Ward, 1907, Order: Trichocephalida, Class: Nematoda, Phylum: Nemathelemminthes. According to Blaxter *et al.* (1998), this Order is an archaic group, related to such basal nematodes as the free-living Mononchida, the plant parasitic Dorylamida and the entomophagous Mermithida. As mentioned above, it was eventually realized that different isolates of *T. spiralis* possessed important biological differences, even though no reliable morphological features could be identified. However, following the pioneer studies of Nelson and Mukundi (1963), Kozar and Kozar (1965), Perevertseva (1966), Britov (1969) and Rausch (1970), two different experimental approaches evolved for characterizing various isolates: (1) comparison of reproductive potentials, i.e. the reproductive capacity index or RCI, which is the ratio of the number of recovered larvae to the number of larvae administered to laboratory rodents (Kruger *et al.*, 1969; Arakawa and Todd, 1971), and (2) the ability of two different isolates to interbreed in laboratory mice (usually reciprocal parasite gender mating) (Britov, 1971). Based on the results from these experimental approaches, particularly interbreeding experiments, Britov and Boev (1972) described two new species of *Trichinella*: *T. nativa*, which was widespread among wildlife of the arctic and subarctic regions; and *T. nelsoni*, considered to be widely distributed in the temperate areas of the Palearctic region and in Africa. Simultaneously, a third new species, *T. pseudospiralis*, was described by Garkavi (1972) in the Caucasus from a raccoon (*Procyon lotor*), mainly on the basis of a lack of a collagen capsule around the larva in the muscle cell and the larva’s smaller size, a property lacking for the other species. The description of these species generated an intense debate over their taxonomic validity, however, because of the lack of clear morphological differences among these proposed species (Lichtenfels *et al.*, 1983). Soon, however, *T. pseudospiralis* (Garkavi’s strain) became widespread in laboratories throughout the world as a reference strain for this species, and for comparative studies, principally with *T. spiralis*, which generated considerable new comparative biological data that provided persuasive evidence for its species validity. Much of the controversy over these new
species revolved around questions of the validity of the interbreeding method for detecting genetic incompatibility, and the lack of a consensus on the definition of species (see Dick, 1983). Although there was little disagreement that there were important biological differences among various geographical and host species isolates, the debate revolved mainly around their appropriate taxonomic position (whether to classify them as ecotypes, subspecies or species).

2.1. Biochemical and Molecular Studies

The resolution of the taxonomic issues has only occurred in the last 15 years, facilitated by the adoption of new biochemical and molecular techniques for systematics research. Initial attempts to apply biochemical methods to investigate genetic variation among *Trichinella* isolates revealed consistent allozyme differences between certain isolates with different host and geographical origins (Flockhart et al., 1982). Allozyme patterns were then produced for a large number of isolates, confirming the existence of consistent genetic variability among certain isolates, even if the lack of formal types of the existed isolates prevented a general agreement and a clear comprehension of the taxonomic status of this nematode genus (Mydynski and Dick, 1985; Fukumoto et al., 1987, 1988; Murrell et al., 1987a; Pozio, 1987). Early molecular tools based on parasite DNA also readily distinguished these particular isolates, supporting the concept that the genus *Trichinella* was genetically complex, and that the genetic differences marked important biological variation (Chambers et al., 1986; Dame et al., 1987; Zarlenega and Barta, 1990; Zarlenega and Dame, 1992).

The first comparative analysis of biochemical differences among a large number of isolates (152) appeared in 1992, the results of which proved to be a landmark in the clarification of the systematics of *Trichinella* (La Rosa et al., 1992). The study, comparing 27 allozyme patterns of 152 isolates from various host species and geographical regions, identified eight distinct genotypes (with the code from T1 to T8), four of which represented the four previously proposed species (*T. spiralis*, *T. nativa*, *T. nelsoni* and *T. pseudospiralis*) (La Rosa et al., 1992). This study also compared seven biological and two
morphological characters of 40 of the isolates, and the results supported the recognition of the same eight genotypes (Pozio et al., 1992b). The meta-analysis of these data and data from the published literature, encompassing about 300 Trichinella isolates, formed the basis for a taxonomic revision of the genus, in which five sibling species were recognized: *T. spiralis*, *T. nativa* and *T. pseudospiralis*, as previously proposed, *T. nelsoni*, restricted to the isolates from tropical Africa, and *T. britovi* n. sp. for the Palearctic isolates previously misidentified as *T. nelsoni sensu stricto* (Britov and Boev, 1972). In addition, three genotypes, named *Trichinella* T5, T6 and T8, whose taxonomic rank was unclear, were also identified (Pozio et al., 1992a). This new taxonomic scheme, which has remained the paradigm up to the present, has only been modified to add new species (Table 1) (Murrell et al., 2000; Pozio and Zarlenza, 2005).

### 2.2. The Polymerase Chain Reaction Era

The introduction of polymerase chain reaction (PCR)-derived method has proved important in simplifying the identification of *Trichinella* isolates from different host species and geographical regions and has confirmed the current taxonomy of the genus (Table 1) (Dick et al., 1992; Bandi et al., 1993, 1995; Soule et al., 1993; Wang et al., 1995; Appleyard et al., 1999; Nagano et al., 1999; Zarlenza et al., 1999; Gasser et al., 1998, 2004; Wu et al., 1998, 1999, 2000; Rombout et al., 2001; Pozio and La Rosa, 2003). Its greater sensitivity has also permitted the opportunity to analyze single larvae, which revealed the occurrence of mixed species infections in the same host, a finding that has added important information on the potential for gene flow between sympatric species or genotypes (Pozio et al., 1995; 1997a; Malakauskas, 2002; Oivanen et al., 2002). In 1999, a new species, *T. papuae* was identified in Papua New Guinea on the basis of its molecular and biological characteristics (Pozio et al., 1999a). In 2000, the genotype *Trichinella* T5 was erected to the species level as *T. murrelli*, chiefly on the basis of interbreeding experiments (Pozio and La Rosa, 2000), attesting to the value that biological analyses can continue to have in resolving molecular uncertainties. In 2002, a new species, *T. zimbabwenis*, the first to be recognized as
infecting reptiles, was identified on the basis of biological, biochemical and molecular data (Pozio et al., 2002).

A major resource that provided vital parasite material and information for these studies is the International Trichinella Reference Centre (ITRC), established in 1990, and which now contains more than 1600 isolates from around the world. Each isolate includes, in addition to its molecular typing results, data on their host species, geographical origin, and other epidemiological information (Pozio et al., 1989, 2001b; www.iss.it/site/Trichinella/index.asp).

2.3. Current Methods for Trichinella spp. identification

Except for the existence of some size differential in T. pseudospiralis, all species and genotypes of the genus Trichinella are morphologically indistinguishable at all developmental stages (new born larvae, muscle larvae and adults). Consequently, only biochemical or molecular methods can be used reliably to identify the species or the genotype.

The use of allozymes as markers has been replaced over time by DNA methods because the latter requires so much less parasite material, and the reduced need to produce large amounts of parasite in laboratory animals, a practice that risks the loss of genetic variability. However, the allozyme analysis is still useful for phylogenetic studies (La Rosa et al., 2003a).

There are a large number of molecular methods useful for identifying species and genotypes, including (1) repetitive DNA probes (Klassen et al., 1986; Dame et al., 1987; La Rosa et al., 1994); (2) restriction fragment length polymorphism (RFLP) (Zarlenga et al., 1991; Nagano et al., 1999); and (3) random amplified polymorphic DNA (RAPD)-derived primers (Wu et al., 1998, 1999). Among PCR-derived methods the RAPD-PCR (Bandi et al., 1993, 1995) has demonstrated low reproducibility, even though it permits the detection and identification of a single muscle larva. Other methods with much higher sensitivity and specificity (multiplex PCR, PCR-RFLP, PCR-single strand conformational polymorphism and reverse line blot hybridization) are also capable of identifying a single larva and consequently are routinely used in laboratories throughout the world (Table 2).
3. THE TAXONOMY OF THE GENUS

Biological, biochemical and molecular data all support the existence of two main clades in the genus *Trichinella*; one that encompasses species which induce a nurse cell to form a thick collagen capsule around the larva in the host muscle tissue (encapsulated), and a second that includes non-encapsulated species which induce a very thin collagen capsule only visible by the electron microscope (Table 1) (Xu et al., 1997; Pozio et al., 2001c; Pozio et al., 2002; La Rosa et al., 2003a; Gasser et al., 2004; Zarlenga et al., 2004). Encapsulated species and genotypes are restricted to mammals, whereas the three non-encapsulated species are more diverse in host range: *T. pseudospiralis* infects both mammals and birds,
and *T. papuae* and *T. zimbabwensis* parasitise mammals and reptiles (Table 1) (Pozio *et al*., 2004a). Non-encapsulated species reportedly induce a lower immunopathological response in the mouse muscle than do encapsulated species as indicated by a lower number of infiltrating inflammatory cells surrounding the larvae (Stewart, 1989).

### 3.1. The Encapsulated Clade

Five species and three genotypes of undetermined taxonomic status belong to this mammal-infecting clade.

#### 3.1.1. *Trichinella spiralis* (*Owen, 1835*) (*Genotype T1*)

This is the first species discovered and the most characterized because of its importance both as a cause of human disease and as a model for basic biological research investigations, due in large part to its relatively high frequency in domestic and sylvatic animals and to its high infectivity for laboratory animals. This species probably originated in East Asia (see Section 4), where its genetic variability is greater than isolates from other regions (La Rosa G., personal communication). It has probably spread along with domestic pigs and, probably, the brown rat (*Rattus norvegicus*) by the migration of people throughout the globe. Dissemination of the parasite and its hosts was especially facilitated by the European colonization of North, Central and South America, New Zealand, Hawaii and Egypt (Figure 1) from the 16th to 20th centuries. Its low resistance to low environmental temperatures may have inhibited its spread among wildlife living in frigid zones. The current geographic distribution of *T. spiralis* (Figure 1) can be linked to three distinct life cycle patterns: (1) countries where *T. spiralis* is present in domestic, synanthropic and sylvatic animals (i.e. Europe, Egypt, China, Russia, Southeast Asia, Argentina, Chile, Mexico, New Zealand and the United States) (Mason, 1978; Barakat *et al*., 1982; Khamboonruang 1991; Mikhail *et al*., 1994; Schenone *et al*., 1994; Buncic, 1997; Correa *et al*., 1997; Gasser *et al*., 1998; Venturiello *et al*., 1998; Ortega Pierres *et al*., 2000; Takahashi *et al*., 2000; Pozio, 2001a, 2001b; Pozio and Zarlenaga, 2005; Ribicich *et al*., 2005); (2) countries
where *T. spiralis* was present in the past in domestic and synanthropic animals but is currently reported only in sylvatic animals (i.e. Austria, the Czech Republic, France, Germany, Hungary, the Slovak Republic, Sweden, the Netherlands and Canada) (Dick and Pozio, 2001; Pozio, 2001a); and (3) countries where *T. spiralis* was never introduced or has been eradicated, i.e. Italy, Portugal and Switzerland and all of Africa except Egypt (Gottstein et al., 1997; Pozio and La Rosa, 1998; Pozio 2001a; Pozio et al., 2005b).

The main biological features of *T. spiralis* in comparison to other species and genotypes are (1) highest RCI in rodents (mice and rats) and swine (both domestic and sylvatic) (Murrell et al., 1985; Pozio et al., 1992b; Yao et al., 1997; Kapel and Gamble, 2000; Kapel, 2001; Malakauskas and Kapel, 2003); (2) the length of the uterus is the longest in comparison to that of *T. nelsoni* and *T. nativa*. The length of uterus has a direct correlation with the infectivity index, i.e. the longer the uterus, more the intrauterine larval capacity and higher the infectivity index (Sukhdeo and Meerovitch, 1977); (3) highest number of newborn larvae produced by female worms (Sukhdeo and Meerovitch, 1977; Boev et al., 1979; Pozio et al., 1992b); (4) fastest development of

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*Figure 1* World map showing the distribution area of *Trichinella spiralis*. The distribution is strongly influenced by human activity, which probably passively introduced this zoonotic pathogen into North, Central and South America, New Zealand and Egypt. (Redrawn from www.iss.it/site/Trichinella/index.asp).
the nurse cell and collagen capsule (Pozio et al., 1992b); and (5) very low resistance of larvae to freezing in muscles of all host species studied (Smith, 1975; Pozio et al., 1992b; Malakauskas and Kapel, 2003). Although unpublished claims that *T. spiralis*, along with *T. britovi* and *T. pseudospiralis*, larvae can survive $-18 \, ^\circ\text{C}$ for four weeks in the muscles of ponies is provocative, these data require confirmation, because in another study, larvae of *T. spiralis* in a naturally infected horse did not survive freezing at $-15 \, ^\circ\text{C}$ for 24 hours (Pozio and Zarlenka, 2005).

Analysis of submissions to the ITRC indicated that *T. spiralis* was the species identified in 87% of all isolates from domestic pigs, 67% from wild boars (*Sus scrofa*), 88% from horses (*Equus caballus*), 79% from synanthropic rats and in the only two isolates from synanthropic armadillos (*Chaetophractus villosus*) (ITRC). In many regions of the world this species has been transmitted to wildlife hosts (e.g. badgers, *Meles meles*; red foxes, *Vulpes vulpes*; wolves, *Canis lupus*; bears, *Ursus americanus* and *Ursus arctos*; mountain lions, *Felis concolor*; bobcats, *Lynx rufus*; raccoon dogs, *Nyctereutes procyonoides*) through exposure to garbage dumps or foraging near human settlements, where pork scraps and offal from slaughtered animals may be scattered in the environment (see Dick and Pozio, 2001). In many countries of the Americas (e.g. Argentina, Chile, the United States), Europe (e.g. France, Germany, Ireland, Lithuania, Poland, Spain) and Asia (e.g. Thailand), *T. spiralis* is a parasite of wildlife maintained in nature by a sylvatic cycle (Worley et al., 1994; Pozio, 2000; 2001a; Pozio and Zarlenka, 2005; Rafter et al., 2005).

*T. spiralis* is the etiological agent of most of the *Trichinella* infections in human beings and deaths around the world, and the pathology it causes, appears higher than that of other species, probably due to the higher number of newborn larvae produced by the females; the level of the inflammatory response in host tissues, especially muscle, does not seem to be directly related to invasion of the tissue by newborn larvae; in fact, a higher inflammatory response has been observed around *T. spiralis* larvae in the muscle tissue, compared to that produced by larvae of other encapsulated species irrespective of the worm burden (Bruschi F., personal communication). However, a high larval production equates to more severe clinical pictures (Pozio et al., 1993; Bruschi et al., 1999; Gomez Morales et al., 2002).
3.1.2. Trichinella nativa Britov and Boev, 1972 (Genotype T2)

This species is usually characterized as the arctic or freeze-resistant species and is widespread among wildlife of the arctic and subarctic areas of the Holarctic region (i.e. Canada, Greenland, Alaska and New Hampshire in the United States, Byelorussia, Estonia, Finland, Latvia, Lithuania, Norway, Russia, Sweden, Siberia, China, Kazakhstan, Kyrgyzstan, and Tajikistan) (Figure 2). The southern distribution boundary has been tentatively identified between the isotherms −5°C and −4°C in January (Shaikenov and Boev, 1983; Shaikenov, 1992; Pozio et al., 1998a; Pozio and La Rosa, 2000). The main biological features of *T. nativa* are (1) the length of the uterus is the shortest in comparison to that of *T. spiralis* and *T. nelsoni* (Sukhdeo and Meerovitch, 1977); (2) a low RCI in laboratory rodents (Rausch, 1970; Dick, 1983; Pozio et al., 1992b) and in domestic and sylvatic swine (Rausch, 1970; Kapel and Gamble, 2000; Kapel, 2001); and (3) a high resistance to freezing in muscles of carnivores (up to 5 years) (Dick and Pozio, 2001); this biological character is lost, however, in frozen muscles of swine and rodents (Dick and Belosevic, 1978; Pozio et al., 1992b; 1994a; Kapel, 2000; Malakauskas and Kapel, 2003).

The common hosts are terrestrial and marine carnivores living in arctic and subarctic areas (several species of mustelids; artic fox; red fox; wolf; raccoon dog; domestic and sylvatic cats, *Felis silvestris*, *Felis euptilura*; lynx, *Lynx lynx*; Siberian tiger, *Panthera tigris*; black bear; brown bear; polar bear, *Ursus maritimus*; several species of seals; and walrus, *Odobenus rosmarus*). This species has rarely been detected in either domestic or wild swine (Pozio and Kapel, 1999). Since the advent of molecular methods for species confirmation, there have not been any documentations of natural infections of *T. nativa* in rodents or lagomorphs (Pozio, 2005); however, there are several reports of nematodes putatively identified as *Trichinella* sp. larvae in muscles of such wild hosts from arctic and subarctic regions (Rausch, 1970). The importance of sylvatic carnivores as reservoirs of *T. nativa* in nature is attested to by the finding that this parasite survives in these host’s musculature for at least 20 years (Kumar et al., 1990). *Trichinella nativa* is also the etiological agent of trichinellosis in human populations living in frigid zones, who acquire the infection from eating raw
Figure 2  World map showing the distribution areas of *Trichinella nativa* (Tna), *Trichinella britovi* (Tb), *Trichinella murrelli* (Tm), *Trichinella nelsoni* (Tne), *Trichinella T6* (T6), *Trichinella T8* (T8) and *Trichinella T9* (T9). In some regions the distribution areas of these encapsulated species and genotypes overlap. (Redrawn from www.iss.it/site/Trichinella/index.asp).
meat from reservoir hosts such as walruses, bears and other game animals (Rausch, 1970; Margolis et al., 1979; MacLean et al., 1989; Serhir et al., 2001; Schellenberg et al., 2003; Forbes, 2005; Moller et al., 2005).

3.1.3. Trichinella britovi Pozio et al., 1992 (Genotype T3)

Among sylvatic species, T. britovi has the widest geographical range, occurring in wildlife of the temperate areas of the Palearctic region, from the Iberian peninsula to the Far East (Pozio, 2000, 2001a) and extending southward to Northern and Western Africa (Nezri et al., 2006; Pozio et al., 2005b) (Figure 2). The northern geographic boundary appears to be determined by the isotherms $-6^\circ C$ to $-5^\circ C$ in January (Shaikenov and Boev, 1983; Shaikenov, 1992; Pozio et al., 1998a; Pozio, 2000). In Palearctic regions, this species is sympatric with T. nativa between the isotherms $-4^\circ C$ and $-6^\circ C$, and there are several reports of mixed infections in the same host from Estonia, Finland and Lithuania (Pozio et al., 1998a; Pozio, 2000; Malakauskas, 2002; Oivanen et al., 2002). This species is prevalent among sylvatic carnivores such as mustelids, viverridae (European genet, Genetta genetta; African palm civet, Nandina binotata; true civet, Viverra civetta), red foxes, jackals (Canis aureus), wolves and brown bears. In Europe, it has been identified in 83, 30 and 11% of isolates from red foxes, wild boars and domestic pigs, respectively (ITRC). This species was also detected in three horses that were the source of three outbreaks of human trichinellosis in Italy (Pozio and Zarlenga, 2005; International Commission on Trichinellosis, ICT). Infections in brown rats living in farms or garbage dumps has been reported in Italy and Estonia although larvae of this species have a very short survival time in this host (Pozio, 2000).

Certain important biological features of T. britovi can be considered intermediate between T. spiralis and T. nativa: (1) RCI in laboratory mice and rats and in domestic and sylvatic swine is lower than that of T. spiralis, but higher than that of T. nativa (Pozio et al., 1992b; Kapel and Gamble, 2000; Kapel, 2001) and (2) larvae of T. britovi survive in frozen muscles of swine up to three weeks and up
to 11 months in fox muscle (Dick and Pozio, 2001), whereas the survival in frozen muscles of mice and rats ranged from three to seven days according to the isolate and the freezing temperature (Pozio et al., 1992b, 1994a; Malakauskas and Kapel, 2003). This species can be transmitted to humans through the consumption of meat from wild boars, horses and domestic pigs (usually those raised in extensive grazing systems) and from sylvatic carnivores (e.g. red fox and jackal) (Pozio et al., 2001d; Nezri et al., 2006). The clinical picture is moderate or benign and no deaths have been documented to date, although this may be a consequence of infective dose size (Pozio et al., 2003).

3.1.4. Trichinella murrelli Pozio and La Rosa, 2000 (Genotype T5)

This is a sibling species of T. britovi, apparently restricted to North America (Pozio and La Rosa, 2000). It occurs in sylvatic carnivores (e.g. bob cat; black bear; coyote, Canis latrans; raccoon; and red fox) and domestic animals (e.g. domestic dog, horse, cat) across the United States (California, Connecticut, Georgia, Illinois, Indiana, Maryland, New Mexico, Pennsylvania, Virginia and Texas) and in the Vancouver area of Canada (Minchella et al., 1989; Snyder et al., 1993; Yao et al., 1997; Pozio and La Rosa, 2000; Pozio et al., 2001e; Gajadhar et al., 2004; ITRC) (Figure 2). The isotherm –6 °C in January may be a determinant of its northern border of distribution. The southern limit is unknown due to the lack of adequate survey data from Mexico and Central America. A mixed infection of T. murrelli and T. spiralis larvae was detected in a black bear in California (ITRC). This species has not been detected as a natural infection in swine. The main biological features of this species are (1) very low resistance to freezing (Pozio et al., 1992b, 1994a; Malakauskas and Kapel, 2003); (2) low infectivity for laboratory mice and rats (Pozio et al., 1992b; Pozio and La Rosa, 2000), and domestic and sylvatic swine (Kapel and Gamble, 2000; Kapel, 2001), but high infectivity for Peromyscus leucopus and Peromyscus maniculatus (Minchella et al., 1989; Yao et al., 1997); and (3) very slow development of the collagen capsule around the larva in mouse muscles (Pozio et al., 1992b).
species has been identified in human outbreaks due to the consumption of meat from black bears in the United States (Roy et al., 2003). A great deal of clinical information on this species was gained from a 1985 outbreak in France due to the consumption of horse meat imported from Connecticut (Ancelle et al., 1988; Ancelle, 1998). The clinical picture is typically moderate or benign, probably due to low numbers of larvae in ingested meat meals, but severe cases leading to death have been reported (Ancelle, 1998; Ancelle et al., 1988).

3.1.5. *Trichinella nelsoni* Britov and Boev, 1972 (sensu Pozio et al., 1992) (Genotype T7)

The documented distribution of this species is restricted to eastern Africa, from Kenya to South Africa (Pozio et al., 2005a), but this is based on only a few surveys and its range may be much broader (Figure 2). The host range includes Hyaenidae (spotted hyena, *Crocuta crocuta*, and striped hyena, *Hyaena hyaena*), Canidae (side-striped jackal, *Canis adustus*; black-backed jackal, *Canis mesomelas*; bat-eared fox, *Otocyon megalotis*; domestic dog), and Felidae (lion, *Panthera leo*; leopard, *Panthera pardus*; cheetah, *Acynonix jubatus*; and serval, *Felis serval*); it occurs at least occasionally in sylvatic suids (bush pig; wart-hog, *Phacochoerus aethiopicus*), some of which have been the source of human infections (Young and Kruger, 1967; Nelson, 1970; Sachs, 1970; Young and Whyte, 1975; Pozio et al., 1994b; 1997b; ITRC). In over 1000 rodents in Africa, *Trichinella* sp. larvae were detected in only one (*Mastomys natalensis*) from the Kruger National Park of South Africa (Young and Kruger, 1967); however, since this Park is endemic for both *T. nelsoni* and *Trichinella* T8 (Pozio et al., 2005b), the identity of the species cannot be assumed.

The main biological features of this species are (1) low infectivity to laboratory rodents (Nelson, 1970; Sukhdeo and Meerovitch, 1977; Pozio et al., 1992b) and swine (Nelson, 1970; Kapel and Gamble, 2000; Kapel, 2001) compared to *T. spiralis*, but higher than that of *T. nativa*; (2) very low resistance to freezing in host muscles (Pozio et al., 1992b; 1994a; Malakauskas and Kapel, 2003); and (3) the length of the uterus is intermediate between those of *T. spiralis* and *T. nativa* (Sukhdeo and Meerovitch, 1977).
Less than 100 human infections have been documented for this species in Kenya and Tanzania (Pozio et al., 1994b). The clinical picture ranges from benign to severe and several deaths have been documented (Nelson, 1970; Bura and Willett, 1977); however, the pathology due to *T. nelsoni* seems to be less than that for the other encapsulated species, because death was observed only in persons with more than 4000 larvae per gram of muscle, whereas those with 420–2800 larvae per gram recovered (Nelson, 1970; Bura and Willett, 1977). Other infections, including some deaths, attributed to *T. nelsoni* because of its geographic location and to the consumption of wild pigs, have been reported in Ethiopia (Perdomo Gonzalez et al., 1986; Kefenie et al., 1988; Kefenie and Bero, 1992; Gelnew, T., personal communication), although the species was never confirmed.

### 3.1.6. *Trichinella T6* Pozio et al., 1992 (*Genotype T6*)

This North American genotype (Pozio et al., 1992a) is widespread in carnivores (e.g. brown and black bears; wolves; gray fox, *Urocyon cinereoargenteus*; coyote; wolverine, *Gulo gulo*; fisher, *Martes pennanti*; mountain lion; bob cat). The distribution range is confined to the arctic and subarctic regions of the United States and Canada (Alaska, Idaho, Montana, Ohio, Pennsylvania, Wyoming and Ontario) (Worley et al., 1990; Weyermann et al., 1993; Pozio, 2000, 2001a; La Rosa et al., 2003b; Gajadhar et al., 2004) (Figure 2). This genotype is distinguished from *T. nativa* by biochemical and molecular characters, in spite of their ability to interbreed in both the laboratory (in two-way sex crosses) and naturally (hybrids have been found in wolves of Alaska) (La Rosa et al., 1992, 2003b). The genetic differences between these two genotypes are probably due, at least in part, by geographical fragmentation of *T. nativa* colonization and subsequent evolutionary divergence during the glacial periods (La Rosa et al., 2003b). Otherwise, *Trichinella T6* and *T. nativa* are very similar in biological features: (1) high freezing resistance of larvae in muscles of carnivores (Worley et al., 1986; Dick and Pozio, 2001); (2) low infectivity to laboratory mice and rats (Pozio et al., 1992b; Malakauskas and Kapel, 2003) and to domestic and sylvatic swine (Murrell et al., 1985; Kapel and Gamble, 2000; Kapel,
A few human infections have been documented, from the consumption of cougar and black bear meat in the United States (Idaho) and Canada (Ontario), respectively (Dworkin et al., 1996; ITRC). The clinical picture is benign or moderate and no death has been documented, probably due to low infection dose, a typical circumstance in its hosts (low larval density).

3.1.7. Trichinella T8 Pozio et al., 1992 (Genotype T8)

Trichinella T8 has been identified only from a lion of the Ethosa National Park of Namibia and a lion and a spotted hyena from the Kruger National Park of South Africa, where it lives in sympatry with T. nelsoni (Pozio et al., 1992a, 1994b, 2005b) (Figure 2). This genotype can be easily distinguished by certain biochemical and molecular characters from T. britovi (La Rosa et al., 1992, 2003a; Nagano et al., 1999; Wu et al., 1999; La Rosa and Pozio, 2000; Pozio et al., 2005b) although they share similar biological characters and can interbreed in two-way sex crosses (Pozio et al., 1992b; Pozio, E., unpublished data). The presence of this genotype in southern regions of Africa was proposed to be due to passive introduction from Europe during the European colonization, similar to that which has occurred for T. spiralis (La Rosa and Pozio, 2000). However, the recent finding of T. britovi in wildlife of West Africa suggests that Trichinella T8 is a geographic isolate (or subspecies) of T. britovi, which colonized Africa before T. britovi (Pozio et al., 2005b). No human cases caused due to this genotype have been documented.

3.1.8. Trichinella T9 Nagano et al., 1999 (Genotype T9)

Trichinella isolates originally identified as T. britovi from Japanese wildlife (raccoon dog; Japanese black bear, Ursus thibetanus japonicus) (Pozio et al., 1996a), have now been shown by molecular methods to differ from the European strains, and are provisionally designated Trichinella T9 (Nagano et al., 1999) (Figure 2). More recently, isolates from five red foxes of the Hokkaido Island, which were earlier erroneously identified as T. nativa (Yimam et al., 2001), are now recognized as Trichinella T9 (ITRC). Although Trichinella
T9 and *T. britovi* interbreed under experimental conditions in two-way sex crosses, a recent analysis of the former’s ITS2 sequence suggests that *Trichinella* T9 is genetically more closely related to *T. murrelli* than to *T. britovi* (Pozio and Zarlenga, 2005). No human cases caused due to this genotype have been documented.

### 3.2. The Non-Encapsulated Clade

Three species, infecting mammals and birds (one species) or reptiles (two species), compose this clade.

#### 3.2.1. Trichinella pseudospiralis *Garkavi, 1972* (Genotype T4)

For sometime after its discovery in 1972, *T. pseudospiralis* was considered an enigma (Dick, 1983), because only the initial isolate the “Garkavi’s isolate” (Garkavi, 1972) existed. Also, its ability to infect birds appeared to be an anomaly (see Bessonov *et al.*, 1978). Nematode larvae resembling *Trichinella* sp. had been detected previously in muscles of some birds from Alaska, Iowa, California and Spain, but none could be confirmed as *Trichinella* (Rausch *et al.*, 1956; Zimmermann and Hubbard, 1969; Calero *et al.*, 1978; Wheeldon *et al.*, 1983). Eventually, new isolations of non-encapsulated larvae of *Trichinella* were made from birds (ravens, *Corvus frugilegus*) and mammals (corsac fox, *Vulpes corsac*; and Indian mole rat, *Bandicota bengalensis*) from Kazakhstan and India, and identified as *T. pseudospiralis* by breeding experiments using the Garkavi’s isolate as a reference strain (Niphadkar, 1973; Shaikenov, 1980; Shaikenov and Boev, 1983). In the early 1990s, new foci of this parasite were discovered in Tasmania involving both marsupials and birds (Obendorf *et al.*, 1990; Obendorf and Clarke, 1992). Since then, this species has been reported in Asia, Europe and North America from domestic and sylvatic animals (Figure 3) (Shaikenov and Boev, 1983; Pozio *et al.*, 1992c; Lindsay *et al.*, 1995; Pozio *et al.*, 1999b, 2004b; Malakauskas, 2002; Oivanen *et al.*, 2002; van der Giessen *et al.*, 2004; Gamble *et al.*, 2005; Hurníkova* et al.*, 2005; Nöckler *et al.*, 2006). In total, this species has been found in 14 mammalian species and seven
Figure 3  World map showing the distribution areas of the three populations of *Trichinella pseudospiralis* from Ne-arctic (TpsN), Palearctic (TpsP) and Australian (TpsA) regions. *Trichinella papuae* (Tpa) and *Trichinella zimbabwensis* (Tz). (Redrawn from www.iss.it/site/Trichinella/index.asp).
avian species (Pozio, 2005); the higher number of reports from mammals than from birds is likely the result of a greater number of examinations of mammals than birds. With regard to mammals, *T. pseudospiralis* of North America and Europe has been detected almost exclusively in wild boars and only twice in a lynx and in a red fox. The main biological features of *T. pseudospiralis* are (1) lack of a collagen capsule detectable by light microscopy around larvae in muscles (Xu *et al*., 1997); (2) infective for both mammals and birds; (3) smaller size of newborn and muscle larvae and adults than that of all other *Trichinella* spp. and genotypes (Boev *et al*., 1979; Dick, 1983; Lichtenfels *et al*., 1983); (4) lower RCI in laboratory mice, rats and swine, both domestic and sylvatic, than that of *T. spiralis*, but higher than that of all other species and genotypes (Pozio *et al*., 1992a; Kapel and Gamble, 2000; Kapel, 2001; La Rosa *et al*., 2001); and (5) low resistance to freezing (Pozio *et al*., 1992b; Malakauskas and Kapel, 2003). The RCI in birds is lower than that in rodents (La Rosa *et al*., 2001); however, this difference may be due to selection pressure by the constant maintenance in laboratory mice for many years.

Three *T. pseudospiralis* geographical populations from the Paleartic, Nearctic and Australian (Tasmania) regions can be distinguished by molecular markers in the expansion segment five (ES5) (Zarlenga *et al*., 1996; La Rosa *et al*., 2001). At this locus, individual larvae from the Tasmanian isolate exhibits three distinct banding patterns characterized by either one band of 310 bp, two bands of 310 and 320 bp or 310 and 340 bp; individual larvae from Nearctic isolates exhibit two bands of 300 and 310 bp; whereas, larvae from the Palearctic show two bands of 290 and 300 bp (La Rosa *et al*., 2001). Biochemical analysis of 12 allozymes also reveals different patterns at the PGM locus between the Palearctic isolates and those from the Nearctic and Australian regions (La Rosa *et al*., 2001). In addition, a biochemical polymorphism has been detected at the PGM locus between two Palearctic isolates originating from Caucasus (the Garkavi’s strain) and one isolate from a domestic pig of the Slovak Republic (Hurníková *et al*., 2005).

A single human case, probably acquired in Tasmania, and three outbreaks involving 92 people, in Kamchatka, Thailand and France have been documented (Andrews *et al*., 1995; Britov, 1997; Jongwutiwes...
et al., 1998; Ranque et al., 2000). Infections in people range from clinically moderate to severe, with one death (Jongwutiwes et al., 1998).

3.2.2. Trichinella papuae Pozio et al., 1999 (Genotype T10)

Non-encapsulated larvae of Trichinella sp. were discovered in 1988 in the muscles of domestic sows and wild pigs (hybrids between Sus scrofa and Sus celebensis) of south-west Papua New Guinea (PNG), near the border with Irian Jaya (Owen et al., 2000). Biological and molecular studies demonstrated that these parasites were a new species, subsequently named as T. papuae (Pozio et al., 1999a). This species has now been detected in farmed saltwater crocodiles (Crocodilus porosus) of PNG (Pozio et al., 2005a) (Figure 3). In experimental infections, this species exhibits a high RCI in caimans and monitor lizards, but a very low RCI in turtles and pythons (Pozio et al., 2004a); its RCI in red foxes is similar or higher than that of T. spiralis (Webster et al., 2002). The main biological features are (1) lack of a collagen capsule detectable by light microscopy around the muscle larvae; (2) infectivity for both mammals and reptiles, but not birds; (3) lower RCI in laboratory mice and rats than that of T. spiralis, T. britovi and T. pseudospiralis, but higher than that of the other encapsulated species and genotypes (Pozio et al., 1999a); (4) muscle larva and adult sizes similar to that of encapsulated species and genotypes, but greater than that of T. pseudospiralis (Pozio et al., 1999a); and (5) low resistance to freezing (Webster et al., 2002).

Based on the ES5 sequence, two distinguishable populations have been identified so far in PNG (Pozio et al., 2005a). Although this parasite has never been isolated from humans, a high percentage of people living in regions where only this Trichinella spp. appears to exist among wild pigs have specific antibodies against Trichinella antigens (Owen et al., 2001, 2005). This discovery of a Trichinella spp. infecting both mammals and reptiles may provide an explanation for earlier reports of human outbreaks attributed to the consumption of turtle and brown lizard meat in Thailand (Khamboonruang, 1991).
3.2.3. Trichinella zimbabweensis Pozio et al., 2002 (Genotype T11)

This species is very similar to *T. papuae* with which it shares important biological features. However, *T. zimbabweensis* is distinguished from *T. papuae* by three diagnostic allozymes (GLD, MPI, PGM), differences in the ES5 sequence (88% similarity), the cytochrome oxidase I (91% similarity), and mt-lsrDNA (96% similarity) (Pozio et al., 2002).

This recently described species has been detected only in reptiles of Africa, although experimentally it is able to infect mice, rats, hamsters, foxes, pigs and monkeys (Mukaratirwa and Fogin, 1999; Mukaratirwa et al., 2001, 2003, 2005; Pozio et al., 2002; Hurníková et al., 2004). When first discovered in 1995, *T. zimbabweensis* larvae were detected in 256 (39.5%) farmed Nile crocodiles (*Crocodylus niloticus*) from 18 (62.1%) Zimbabwe crocodile farms. This parasite has also been detected in sylvatic monitor lizards (*Varanus niloticus*) from two localities in Zimbabwe, in sylvatic Nile crocodiles from Lake Cahora Bassa in Mozambique and in a farmed Nile crocodile from Lake Abaja in Ethiopia (Pozio and Zarlenka, 2005) (Figure 3). Human infections are yet to be reported.

4. PHYLOGENY

The current taxonomic scheme for the genus *Trichinella* originated from biochemical studies (allozymes) on 152 *Trichinella* isolates belonging to eight taxa (Pozio et al., 1992a) and led to the creation of the first extensive dendrograms (La Rosa et al., 1992). These were later corroborated by Bandi et al. (1995) who used RAPDs and allozymes to independently generate congruent trees. In 1997, Zarlenka used mitochondrial DNA data to produce a distance-based (UPGMA) tree that strongly supported the topology of Bandi et al. (1995). Independent trees were later generated from all 11 recognized species and genotypes of *Trichinella* by neighbour joining (NJ) and UPGMA using multilocus enzyme electrophoresis data (La Rosa et al., 2003a). Although the latter trees of La Rosa et al. (2003a) showed remarkable similarity with each other, they raised questions regarding the overall topology relative to
those of Bandi et al. (1995) and Zarlenga (1997) because they did not completely delineate non-encapsulated species as a monophyletic clade, but placed them at the base of the Trichinella tree (Pozio and Zarlenga, 2005). In 2004, Gasser et al. sequenced the D3 domain of the nuclear ribosomal DNA from all currently recognized species and genotypes of Trichinella and demonstrated strong bootstrap support for monophyly among T. spiralis and T. nelsoni, and among T. nativa and Trichinella T6 using maximum likelihood, parsimony and/or NJ methods. In this analysis, the non-encapsulated species clustered at the base of the tree and isolates of the bird-related species (T. pseudospiralis) clustered independent of those species related to reptiles (T. papuae and T. zimbabwensis).

In the recent past, scientists believed that extant, non-encapsulated species of Trichinella coevolved first with lower vertebrate classes (e.g. reptiles and birds), and then later in mammals, followed by the evolution of the encapsulated species, which was restricted to mammals only (Pozio et al., 2002, 2004a). More recently, Zarlenga et al. (2006) analyzed the phylogeny of Trichinella using the variation in three genes (nuclear SSU rDNA and ITS2; mitochondrial LSU rDNA and COI DNA) from all 11 recognized species and genotypes. The results showed that the extant species of Trichinella probably diversified only within the last 10–20 million years and coincided with the divergence of Suidae from the Tayassuidae (Bowen et al., 2002) in the Lower Miocene (Figure 4). In addition, T. spiralis, which anecdotally has been considered a crown species due to its strong association with domestic pigs, synanthropic rats and humans (Britov, 1982), appears in the most recent analysis to be basal to the encapsulated clade where the timeframe for divergence is constrained to the Lower Miocene (Zarlenga et al., 2006).

5. BIOGEOGRAPHY

The biogeographic history of the encapsulated species and genotypes of Trichinella has recently been thoroughly investigated for the first time by Zarlenga et al. (2006). Since T. spiralis is the basal species of the encapsulated clade and exhibits high genetic variability among localized populations in Eastern Asia (La Rosa, unpublished data),
these authors identify this geographical region as the probable origin of the encapsulated clade. In prior phenetic (Bandi et al., 1985; Zarlinga, 1997; La Rosa et al., 2003a) and phylogenetic (Gasser et al., 2004) trees, the placement of *T. nelsoni* has always occurred at or near the base of the encapsulated clade. A tree placing *T. nelsoni* basal to *T. spiralis* would support an “out-of-Africa” event in which *T. nelsoni* could have expanded into the Palearctic, and the introduction of *Trichinella* T8 and *T. britovi* into Africa would have occurred later. However, the data and tree put forth by Zarlinga et al. (2006) suggests that the occurrence of *Trichinella* T8 and *T. britovi*, as well as *T. nelsoni*, in Africa (Pozio et al., 2005b) is likely the result of

Figure 4  Phylogeny for species of *Trichinella* showing host associations and primary traits for life history reconstructed from variation in mtLSU and COX I mitochondrial genes and redrawn from Zarlinga et al. (2006). Bold tree = encapsulated clade infecting only mammals; Parallel line tree = non-encapsulated clade infecting both mammals and birds (*T. pseudospiralis*) or both mammals and reptiles (*T. papuae* and *T. zimbabwensis*). MYBP = million years before present.
three independent expansion events from Eurasia following the land connections that formed during upper Miocene and into the Pleistocene. The expansion of *T. britovi* into northern and western Africa is likely the most recent event given the biochemical congruence among these isolates and those from Western Europe (Pozio *et al.*, 2005b).

According to Zarlenga *et al.* (2006), ursids, canids and felids are principally responsible for the radiation of Holarctic species throughout Europe and into North America through Beringia where vicariance speciation during the Quaternary was likely the driving force for divergence among many of the crown species. The unclassified status for *Trichinella* T6 suggests that evolutionary forces driving the speciation of the freeze-resistant genotypes are both recent and unresolved; however, La Rosa *et al.* (2003b) have identified natural hybrids of these two genotypes in Alaskan wolves. The divergence of these two genotypes may have resulted from environmental factors causing a bifurcation of the freeze-resistant genotype (*T. nativa* and *Trichinella* T6). The limited amount of information on non-encapsulated species, due in part to their recent discovery, the lack of sufficient numbers of isolates, and the worldwide dissemination of *T. pseudospiralis*, presumably resulting from migratory avian hosts, does not permit a deep understanding of the biogeography of this clade at this time.

There are many instances of sympatry among the *Trichinella* spp. Some examples are *T. nativa* (and the closely related genotype *Trichinella* T6) and *T. murrelli* in the United States and Canada (Pozio and La Rosa, 2000; La Rosa *et al.*, 2003b); *T. nativa* and *T. britovi* in Europe and Asia (Shaikenov and Boev, 1983; Shaikenov, 1992; Pozio *et al.*, 1998a); and *T. nelsoni* and *Trichinella* T8 in South Africa (Pozio *et al.*, 2005b) (Figure 2).

The distribution area of *T. spiralis* (Figure 1), which has been passively disseminated by humans and their domestic and synanthropic animals, also overlaps with that of *T. nativa*, *T. britovi* and *T. murrelli* in many regions (Figure 2) (Shaikenov and Boev, 1983; Pozio, 1998; Pozio and La Rosa, 2000). The non-encapsulated species *T. pseudospiralis* (Figure 3) has been detected in the same regions where *T. spiralis*, *T. nativa*, *T. britovi* and *T. murrelli* occur. In some regions of Africa, the distribution area of the non-encapsulated species *T. zimbabwensis* may
overlap with those of *T. britovi*, *T. nelsoni* and *Trichinella* T8, but additional data is needed to confirm this. The overlapping of these distribution areas has occasionally resulted in mixed infections in natural hosts (*T. spiralis* with *T. nativa*, or *T. britovi*, or *T. murrelli*, or *T. pseudospiralis*; *T. nativa* with *T. britovi* or *Trichinella* T6; and *T. britovi* with *T. pseudospiralis*) (Pozio *et al.*, 1997a, 1998a; Malakauskas, 2002; Oivanen *et al.*, 2002; La Rosa *et al.*, 2003b; Nöckler *et al.*, 2006; ITRC).

The discovery of mixed species infections in some regions provides some insight into the frequency of infection of sylvatic hosts. Although in experimental conditions many hosts develop an acquired immunity to secondary infection, such immunity may not be strong enough to prevent reinfection under natural conditions. For example, immunity depends to a large degree on the dose (infection level); therefore, strong protection from a natural initial infection may not always result because the larval density in wild animals is usually quite low (Kazura, 1982; Wakelin and Denham, 1983; Murrell 1985; Marti and Murrell, 1986). Further, in frequent transmission situations, re-infection may occur before development of protective immunity from a primary infection. Multiple species infections suggest, then, that high levels of exposure exist in some circumstances. Repeated infections could also promote the gene flow between larvae belonging to the same species, even if this flow cannot be directly demonstrated because of adult worm mating between rapidly acquired infections (Zarlenga, 1994; Pozio *et al.*, 1997a). Another factor important in the development of any immune barrier to repeated infections is the nutritional status of the host, which in some seasons can be less than optimal due to restrictions in food availability. Nutritional component levels for protein, selenium and vitamin A are strongly linked to host cellular immunity and resistance to micro- and macro-parasites (Solomons and Scott, 1994; Pedersen and Murrell, 2001; Pedersen *et al.*, 2002).

6. EPIDEMIOLOGY

Although *T. spiralis* was first discovered in domestic animals (see historical review by Campbell, 1983a), all other species of this genus
are primarily parasites of wildlife. The importance of wildlife as reservoir hosts for all species of *Trichinella* is underscored by the parasite’s biomass, which is greater in wild than in domestic animals, unlike other nematode infections involving both sylvatic and domestic animals. When humans fail in the proper management of domestic animals and wildlife, *Trichinella* sp. (especially *T. spiralis*) infection is transmitted from the sylvatic environment to the domestic one, sometimes through synanthropic (intermediary between domestic and sylvatic) animals. In addition, some species can transfer in a reversible path from domestic animals to wildlife.

### 6.1. The Sylvatic Cycle

The sylvatic cycle occurs in all continents with the exception of Antarctica, where there is neither a record of this nematode nor evidence of any searches for it, especially in marine mammals. Differences in species cycles exist because of differences in reservoir host species and between regions, especially in areas where two or more species are present (Figure 2).

#### 6.1.1. Natural Hosts

Although natural *Trichinella* infections have been reported in more than 100 species of mammals belonging to 11 orders (Marsupialia, Insectivora, Edentata, Primates, Lagomorpha, Rodentia, Cetacea, Carnivora, Perissodactyla, Artiodactyla and Tylopoda), those from Insectivora, Lagomorpha, Cetacea, Tylopoda and infections in most of Rodentia, are problematic and need confirmation (Pozio, 2005). In experimental infections, these parasites are able to complete their life cycle in all species of mammals tested, but only a few of these appear to play an important role in the sylvatic and/or domestic cycle.

The transmission cycles of the different sylvatic species and genotypes are closely related to their host species ecologies. For example, in Europe, *T. spiralis* and *T. britovi* occur almost equally in wild boars (49% and 47%, respectively), with some differences related to the habitat characteristic and human behavior at the country level, whereas the same two species occur with quite different frequencies in red foxes and
other sylvatic carnivores (7% and 92%, respectively) (ITRC). A similar pattern occurs in North America, where *T. spiralis* and the sylvatic species *T. murrelli*, and *T. nativa*, and the genotype *Trichinella* T6, have been detected in 12% and in 87% of sylvatic carnivores, respectively (ITRC). These prevalence figures, based on data from over a thousand examinations, are not completely in agreement with data from experimental infections, which do not reveal great differences in the host susceptibilities (Kapel, 2000). Data from experimental infection with laboratory animals is not always in agreement with findings from natural populations. As a rule, wild animals encounter over time many pathogens (including helminths), which potentially could influence the immune response to *Trichinella* sp. Unlike the situation with wild animals, infections of clean, naïve laboratory animals which have very different encounters with other infectious agents and normally are on a high level nutritional plane could react quite differently than the same species reared under natural conditions. Further, the genetics of the host may have a dramatic effect on its immune response, especially when comparing rodents to animals such as swine (Murrell et al., 1987b). In addition, the biology of the different *Trichinella* spp. in host carrion cannot be easily evaluated in laboratory animals raised and maintained under controlled conditions, shielded from the influence of the natural habitat. Therefore, we believe that experimental data from laboratory animals, should be interpreted with caution.

The host range for the sylvatic cycle is determined by the potential host species available in the different regions (Pozio, 2000, 2001a). Because swine are not a suitable host for *T. nativa*, *T. murrelli* and *Trichinella* T6 (Kapel and Gamble, 2000; Kapel, 2001), these animals do not play a role as a reservoir for these pathogens in the regions of Eurasia and North America, although there is an occasional report of *T. nativa* in wild boars (Pozio and Kapel, 1999).

Among primates, only humans have been naturally infected with *Trichinella* spp., although experimental infections in monkeys with *T. spiralis* or *T. pseudospiralis* have demonstrated the high susceptibility of these hosts (McCoy, 1932; Kocieka et al., 1981); the ecological consequences of these infections are severe for the parasite since in the absence of cannibalism, they normally represent a dead end. Evidence suggests that the infection of horses (Perissodactyla), rodents
(especially rats) and edentata (armadillos) occurs most commonly where poor livestock rearing practices (exposure to infected meat) exists. The reservoir role of Marsupialia is also limited, it has only been documented in Tasmania (Obendorf et al., 1990), and in in opossums (Didelphis spp.) in North America, where the parasite is widespread among placental mammals; however, its role as a reservoir is unknown (Solomon and Warner, 1969; Zimmermann 1970; Schad et al., 1984; Murrell et al., 1985; Leiby et al., 1988). In spite of the potential broad host spectrum of Trichinella spp., the greatest biomass of these parasites occurs among the Carnivora (Campbell, 1983b; Pozio et al., 1997b, 2005b; Pozio and Dick, 2001) and the artiodactylid family Suidae (mainly domestic pigs, different races of wild pigs, wild boars, bush pigs and warthogs) (Nelson, 1970; Sachs 1970; Campbell, 1983b; Pozio et al., 1999a, 2005a; Pozio, 2005). Natural infections in other artiodactylid species, both sylvatic (reindeer and roe deer), and domestic (sheep, goat and cow) are sporadic (Murrell, 1994; Takahashi et al., 2000; Pozio, 2001a).

Seven species of birds are documented as hosts for T. pseudospiralis, and six other species suspected, but unconfirmed; these hosts belong to the orders Strigiformes (four species), Ciconiiformes (eight species) and Passeriformes (one species) (Shaikenov, 1980; Pozio et al., 1992c; Lindsay et al., 1995; Pozio, 2005; Garkavi, B.L., personal communication). The role of birds as reservoirs of this species needs evaluation; however, the low inter-regional genetic variability and the discontinuous distribution of T. pseudospiralis in the Nearctic and Palearctic regions could be explained by the transmission through birds because of the latter’s mobility and wide distribution patterns (La Rosa et al., 2001). The recentness of the discovery of T. papuae and T. zimbawensis infections in certain reptile species (Pozio et al., 2004a, 2005a) and because so few surveys for them have been conducted, it is impossible to speculate on the role of other vertebrates in the ecology of these species.

6.1.2. The Parasite’s Adaptation to the Environment

An important adaptation of the parasite, which facilitates its transmission, is the physiological mechanism utilized by muscle larvae to
promote its survival in decaying carcasses; the greater the persistence of larval viability, the higher the probability of being ingested by a scavenging host. In spite of the larva-induced angiogenic process that develops around the nurse cell after larval penetration of the muscle cell, larval metabolism is basically anaerobic (Despommier, 1990), which favors its survival in decaying tissues, probably longer for the encapsulated than for the non-encapsulated species (Stewart et al., 1990). The persistence of larvae in putrefying flesh is, of course, also determined by the environment: high humidity and low temperatures favor survival even when the muscle tissue is completely liquefied. This condition has been proposed as the environment of the “free-living” stage, resembling the egg stage of most of other nematode species (Madsen, 1974).

The importance of this stage in the natural cycle of the parasite is underscored by the survival of muscle larvae of species in frozen muscles of carrion for one (T. britovi) or more years (T. nativa and Trichinella T6) (Dick and Pozio, 2001). The anaerobic metabolism favoring the survival in putrefying flesh, along with the ability of larvae of some species to survive freezing, are two separate mechanisms that strongly increase the survival of the parasite in nature. Survival is greatest at temperatures between 0°C and –18°C. At lower temperatures, survival time is reduced, suggesting that the optimal temperature range for survival to freezing corresponds to the temperature under the snow.

6.1.3. The Human Influence

The sylvatic cycle may also be influenced by human actions. For example, the common habit of hunters to leave animal carcasses in the field after skinning, or removing and discarding the entrails, increases the probability of transmission to new hosts (Cironeanu, 1974; Madsen, 1974; Batkaev and Vakker, 1992; Worley et al., 1994; Pérez-Martín et al., 2000; Pozio et al., 2001f).

Epidemiological surveys carried out in Europe, North America and Africa have shown that Trichinella spp. are more prevalent in wild animals living in natural or undisturbed areas such as parks and
forests (Worley et al., 1994; Pozio et al., 1997b), protected areas and mountain regions (Pozio et al., 1996b, 2005b; Pozio, 1998) where the human activity has not strongly changed the habitat, even when suitable hosts are present in areas where human activity is strong. A possible explanation is that in undisturbed areas of these three continents, hosts of Trichinella spp. which have predominantly scavenger and cannibalistic behaviors, practice their natural trophism, whereas in habitats where human activity is high, the number of potential hosts is reduced or even if not, the animals have access to alternative food resources resulting from human activity (e.g. synanthropic and domestic animals, garbage). This is the case of several European countries, where the sylvatic cycle is nearly absent in areas characterized by a strong human influence, but present in mountain and/or protected areas (Pozio et al., 1996b; Pozio, 1998).

Since the prevalence of Trichinella infection increases with the host age, a question needing study is how the age structure of potential host populations affects transmission. This is important because in natural areas where the human impact is low or absent, animal populations generally have a wide age range, but in areas influenced by the human activity, these potential host populations tend to be skewed toward young individuals, most likely immigrants in search of a new home range (MacDonald, 1980).

Human-caused perturbations of the sylvatic environment may also affect the epidemiological patterns of human and animal trichinellosis, a phenomenon well documented for many animal pathogens (Daszak et al., 2000). A case in point is the consequences from the increase in forests and fallow land, concomitant with a decrease in farms, in Europe over the past 100 years, which has facilitated a great enlargement in the regions of wild boar populations and increased transmission of Trichinella spp. to animals and humans (Pozio et al., 1996b). In France, there was a nine-fold increase between 1975 and 2000 in wild boars, which is of concern to outdoor farming because of the risk of transmission of Trichinella spp. from wild boars to domesticated pigs (Dupouy-Camet, 2000). This risk should be of concern to public health authorities in the United States, where the wild pig populations (feral pigs, Eurasian wild boars and their hybrids) have grown dramatically since the recent decades, and now have
extended their range to 31 states and number between 4 and 5 million (Mayer and Brisbin, 1991; Frazier, 2005). Along with considerable environmental damage, the risk of transmission of *Trichinella* spp. to other game animals and outdoor pig farms has increased. Along with a potential effect on safety of domestic pig consumption, an impact on export of meat could occur, as happened in France in 1998, when vacuum-packed meat sold as wild boar imported from the United States caused an outbreak of trichinellosis in humans in Normandy (Dupouy-Camet, 2000), a food safety issue not unlike that for imported horse meat (see Section 6.2.2).

6.1.4. The Role of Micromammals

There is a lack of consensus among scientists on the role of micromammals (mainly rodents and insectivores) in the sylvatic cycle due to the usually low number of infections in their populations. Most records on infection in these hosts date back before *Trichinella* isolates could be confirmed and typed by biological, biochemical or molecular methods; therefore, the role these animals play is uncertain. Identifications in some reports of nematode larvae from these hosts, on purely morphological grounds, as *Trichinella* sp. needs verification and should probably be considered as rare events (Merkushev, 1970; Rausch, 1970; Zimmermann, 1971; Holliman and Meade, 1980; Bessonov, 1981; Pozio, 2005). Among the non-*Trichinella* nematode larvae that can be detected in muscles, are those from the gut, which may contaminate muscles during necroscopy. Only skilled and experienced microscopists can identify these larvae correctly.

Because the number of micromammals living on the home range of a single carnivore is, as a general rule, a thousand-fold greater than for carnivores, prevalence studies for *Trichinella* is difficult, given that worm burdens in sylvatic carnivores and omnivores is generally low (e.g. 1.0 larvae/g or less, in preferential muscles). Therefore, the chance that a micromammal of 10–20 g of body weight will ingest enough flesh of infected carrion to permit transmission and development of at least one male and one female larva is very low. Another issue is that 90% of micromammals live less than 10 months (Burton and Pearson, 1987),
reducing their role in transmission compared to longer-lived carnivores. This question on the role of micromammals can best be answered by large investigations of micromammals in areas where there is a relatively high prevalence of *Trichinella* sp. in carnivores.

6.1.5. *Trichinella* spp. in Lower Vertebrates and in Invertebrates

There is a single report of experimental infections of amphibians (frogs and axolotl) with *T. spiralis*, in which it was observed that the development of larvae in the muscles was incomplete (Gaugusch, 1950). Attempts to infect fish with either *T. spiralis*, *T. britovi*, *T. pseudospiralis*, *T. papuae* or *T. zimbabwensis* have also failed (Guevara Pozo and Contreras-Pena, 1966; Tomasovicova, 1981; Moretti *et al.*, 1997; Pozio and La Rosa, 2005).

In addition to vertebrates, there are older reports describing the identification of encapsulated larvae of *Trichinella* in the intestine of adult insects and fly maggots (*Musca domestica*) and their potential role as paratenic hosts for *T. spiralis* (Merkushev, 1955). Several species of insects were experimentally infected and infective, non-encapsulated larvae of *T. spiralis* were observed up to eight days p.i. (Merkushev, 1955). More recently, infective *T. spiralis* larvae were detected in maggots of *Sarcophaga argyrostoma* experimentally fed on *T. spiralis*-infected mouse carcasses after up to five days p.i. at 8°C and for shorter periods of time at higher temperatures (Maroli and Pozio, 2000).

Infection of a dog by feeding it 5000 amphipods that had been fed infected bear meat was reported by Fay (1968). Others have shown that seven species of crustaceans (amphipods and shrimp), trapped in the Arctic sea and fed on rat muscles infected with *T. spiralis* larvae, can retain larvae only up to 28 hours after infection in cold seawater (Hulebak, 1980). These results suggest that invertebrates play a very limited role, if any, in the dissemination of *Trichinella* sp. larvae in nature.

6.2. The Domestic Cycle

This cycle occurs where there are high-risk farming practices such as the intentional feeding of food waste, potentially containing pork
scraps (Gamble et al., 2000), or unintentionally through exposure to carcasses of dead swine (Hanbury et al., 1986), or infected wildlife, usually by unsecured free-range pasturing (Murrell et al., 1987a; Pozio, 2000). A comprehensive picture of the domestic cycle also includes certain other transmission sources: (1) pigs allowed to scavenge on garbage dumps (Campbell, 1983b); (2) feeding of wild game carcasses or scraps from hunting (Pozio et al., 2001f); (3) horses fed with pork scraps or with carcasses of fur animals (Pozio et al., 2001a; Murrell et al., 2004); (4) sled dogs fed with carcasses of other dogs or of game in the Arctic (Madsen, 1974); (5) the use of carcasses of slaughtered fur animals as food for other fur animals present at the farm (Madsen, 1974; Miller et al., 2006); (6) the use of meat of slaughtered crocodiles to feed other farmed crocodiles as observed in Zimbabwe (Pozio et al., 2002); and (7) the use of pork scraps to feed young crocodiles as recently demonstrated in Papua New Guinea (Pozio et al., 2005a).

The most common etiological agent of the domestic cycle is *T. spiralis*, which is very well adapted to swine and in which it exhibits a very high reproductive rate without inducing serious pathology except in very high level of infection (Gould, 1970; Kapel and Gamble, 2000; Kapel, 2001). Occasionally, *T. britovi* can be transmitted in the domestic cycle, when humans feed pigs with game meat scraps or “pasture” pigs in refuse dumps containing carcasses of sylvatic animals (Pozio et al., 2001d). *T. pseudospiralis* has been also transmitted to domestic pigs and rats on farms in Kamchatka, Russia and Slovak Republic (Pozio, 2001a; Hurníková et al., 2005). *Trichinella*-infected meat scraps or carcasses of domestic animals from villages, farms and garbage dumps can be the source of infection for both synanthropic and wild animals circulating in the local habitat favoring the increase of the parasite biomass in the sylvatic cycle (spillover).

6.2.1. The Role of Rats

In the domestic habitat, where *Trichinella* is circulating among domestic animals, the brown rat is frequently found to be infected with *T. spiralis* and infrequently with *T. britovi* (Pozio et al., 1996b) 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 to whether it is a true reservoir host (sustaining the infection in the habitat in the absence of introductions of the parasite by other host species) or functions primarily as a vector of Trichinella (because of accidental infection) to domestic hosts (Haydon et al., 2002). In the 19th century, Leuckart proposed a “Rat Theory”, which implicated 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 merely an indicator of Trichinella sp. exposure risk in the area and that the real source of infection for both pigs and rats was meat scraps and offal of infected pig carcasses.

Although T. spiralis infection in pigs is often associated with infection in rats living in abattoirs (Schenone et al., 1994; Bianli et al., 2001), farms (Smith and Kay, 1987; Leiby et al., 1990; Stojcevic et al., 2004) and garbage dumps (Robinson and Olsen, 1960; Zimmermann and Hubbard, 1969; Mikkonen et al., 2005), the results from various investigations are not conclusive (Schad et al., 1987; Stojcevic et al., 2004). There are no reports showing T. spiralis infection in brown rats where pig populations unequivocally have been found to be negative or in farms where pigs do not exist (Gomez Villafane et al., 2004), although Mikkonen et al. (2005) propose that the continuing presence of T. spiralis in dump rats in Finland is facilitated by rat-to-rat transmission through cannibalism. Regardless of their capability to act as a true reservoir, there is substantial evidence that they can play a role in transmitting T. spiralis to pigs and must be considered in any design of an on-farm control program (Schad et al., 1987; Gamble et al., 2000; Oivaneen et al., 2002). Rat-control campaigns and farm renovations, however, must be done with care and incorporate an area-wide approach, because while these actions may solve the local problem, it could force infected rats to migrate and spread the infection to neighboring farms and villages. Evidence for this was reported by Smith et al. (1976) in some swineherds of the Atlantic provinces of Canada. In addition, the use of rat pesticides can actually favor the transmission, because poisoned rats are easy prey for pigs. Their role of vector can be amplified if pigs are not adequately fed, forcing these animals to eat...
rats. 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 was implemented to control bacterial and viral infections (Hall, 1937).

### 6.2.2. Trichinella Infection in Horses

The emergence of equine trichinellosis is an intriguing story with still puzzling and unresolved aspects. As early as the late 19th century, there have been reports of both experimental infections with *Trichinella* sp. larvae in horses in Germany and Austria (Gerlach, 1873; Csokor, 1884) and a natural infection in Ohio (Thornbury, 1897); however, the potential role of horses in the transmission to humans was largely ignored until 1975, when an outbreak of trichinellosis occurred among 89 persons in Italy, who had eaten horse meat (Mantovani et al., 1980). In the same year, another outbreak occurred in France (Bouree et al., 1979), prompting the European Union to examine thousands of horses for the presence of *Trichinella* sp. larvae, adopting the method used to detect this infection in pigs (i.e. artificial digestion of 1.0 g of diaphragm pillar muscle) (Pozio et al., 2001a). No natural infection in horses was detected at that time. However, between 1975 and 2005, human outbreaks of trichinellosis have occurred in France (2296 persons in eight outbreaks) and Italy (1038 persons in seven outbreaks), from the consumption of meat from individual horses imported from Canada, the former Yugoslavia, Mexico, Poland and the United States (Pozio and Zarlenga, 2005; ITRC). Routine examinations at the slaughterhouse had revealed infection in only one of the 15 horses involved in these outbreaks; unfortunately, the meat from even that horse was erroneously placed on the market (Pozio et al., 1998b). The failure to detect infection in the other 14 horses was probably due to the examination of an inadequate quantity of muscle tissue (i.e. 1.0 g). Since then, the requirement for 5–100 g for testing was instituted, and *Trichinella* sp. larvae have been detected in a total of 18 horses bred in the former Yugoslavia, Mexico, Poland, Romania and Serbia (Pozio and Zarlenga, 2005). Worldwide, the prevalence of horse infection appears to be very low, with only 32 infections reported since 1975.
(horses that were the source of infection for human outbreaks and positive horses detected at the slaughterhouse). In this period of time, approximately 7 million horses have been consumed in the European Union; thus the 28 infected animals detected in Europe, represent a prevalence of only 4/1 million slaughtered horses. The fact that all of the infected horses were imported from countries with a high prevalence of *Trichinella* sp. infection in pigs and/or wildlife suggests that there is a close relationship between the infection in these animals and the horse infection (Pozio, 2001a; Murrell et al., 2004).

Epidemiological investigations of the five most recent human outbreaks have shown that they occurred because of inadequate veterinary controls at the slaughterhouse. Horse-meat outbreaks have important consequences for public health because of the high number of infected persons resulting from consumption of meat from a single horse and the very severe symptomatology, at times resulting in death. This has a high impact in terms of medical costs, horse-meat market economics, which collapses after each outbreak, and in legal and administrative terms related to the implementation of control measures at the national and international level (Ancelle, 1998).

In spite of several epidemiological surveys performed at the point of origin of the infected horses (Murrell et al., 2004; Pozio, E., unpublished data), more evidence on the epidemiology of equine trichinellosis is needed. Recent investigations have shown a relationship between *Trichinella* sp. infection in horses and the local prevalence of pig infections, and the feeding of animal meat scraps to improve horse condition prior to sale; although considered herbivores, 32% of horses tested ate meat when offered (Murrell et al., 2004). In the four cases where sylvatic species of *Trichinella* (*T. britovi* or *T. murrelli*) have been detected, an association between infection in horses and wildlife or fur-reared animals has also been postulated (Pozio, 2001a).

The feeding of animal products to horses is a practice that occurs in several countries, including those of origin of infected horses (e.g. Serbia) (Murrell et al., 2004). The increasing reports of human outbreaks of trichinellosis in France and Italy in the 1990s, and the detection of *Trichinella*-infected horses at slaughter occurred during a period in eastern European countries when there was a widespread breakdown in veterinary control services (Murrell and Pozio, 2000; Olteanu, 2001;
The presence of thin capsules around the larvae in muscle tissues of the horses slaughtered in January and the presence of thick capsules in the larvae from horses slaughtered in April and October seem to support the hypothesis that horses acquire this infection in late autumn or winter, i.e. when most of the backyard pigs are slaughtered at home, which in Europe is legal without any veterinary control if pork is for own consumption. Almost uniformly, infections in humans occur where there is either no veterinary control over meat hygiene or the service in place is inefficient (Olteanu, 2001; Djordjevic et al., 2003, 2005).

6.3. Trichinellosis in Humans

*Trichinella* sp. infections in humans are related to cultural food practices, which include dishes based on raw or undercooked meat of different animal origins (Table 3). The presence of the parasite in domestic and/or wild animals is not a sufficient risk in itself, however, for the infection to occur in the human population. For example, in Finland, where there is a high prevalence of infection in animals, no infection has been documented in humans, probably due to the practice of eating only well-cooked meat (Pozio, 1998). Similarly, in most African countries south of the Sahara, human infection is seldom documented in spite of the presence of sylvatic *Trichinella* sp., because about a third of all African populations are of the Bantu ethnic group, which rarely consumes meat.

Overall, the most important source of *Trichinella* sp. infection for humans remains pork and its related products from domestic pigs. Important foci of human trichinellosis from pork occur in Central (Mexico) and South America (Argentina and Chile) (Ortega Pierres *et al.*, 2000; Ribicich *et al.*, 2005), Asia (China, Laos, Myanmar, Thailand, Vietnam) (Takahashi *et al.*, 2000; Pozio, 2001b; Liu and Boireau, 2002) and Europe (Bosnia-Herzegovina, Bulgaria, Byelorussia, Croatia, Georgia, Latvia, Lithuania, Poland, Romania, Russia, Serbia, Spain and Ukraine) (Pozio, 2001a; Pozio and Zarlenga, 2005).

In conclusion, raw or undercooked meat from carnivore and omnivore mammals, birds and reptiles pose an important risk for
Table 3 Animals, besides domestic pigs, which were the source of trichinellosis

<table>
<thead>
<tr>
<th>Meat origin</th>
<th>Country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild boar (<em>Sus scrofa</em>)</td>
<td>Europe, North and South America, Asia</td>
<td>Nadzhimiddinov et al., 1965; Boev et al., 1970; Khamboonruang, 1991; Eisenman and Einat, 1992; Jongwutiwes et al., 1998; Pozio, 1998; Roy et al., 2003; Ribicich et al., 2005</td>
</tr>
<tr>
<td>Wild pig (hybrid between <em>Sus scrofa vittatus</em> and <em>Sus celebensis</em>)</td>
<td>Papua New Guinea</td>
<td>Owen et al., 2001, 2005</td>
</tr>
<tr>
<td>Warthog (<em>Phacochoerus africanus</em>)</td>
<td>Ethiopia, Senegal, Tanzania</td>
<td>Gretillat and Vassiliadis, 1967; Bura and Willett, 1977; Perdomo Gonzales et al., 1986; Kefenie et al., 1988; Kefenie and Bero, 1992; Pozio et al., 1994b</td>
</tr>
<tr>
<td>Bush pig (<em>Potamochoerus larvatus</em>)</td>
<td>Kenya</td>
<td>Nelson, 1970; Pozio et al., 1994b</td>
</tr>
<tr>
<td>Walrus (<em>Odobenus rosmarus</em>)</td>
<td>Canada and Greenland</td>
<td>Proulx et al., 2002; Serhir et al., 2001</td>
</tr>
<tr>
<td>Black bears (<em>Ursus americanus</em>)</td>
<td>USA, Canada</td>
<td>Nelson et al., 2003; Roy et al., 2003; Schellenberg et al., 2003</td>
</tr>
<tr>
<td>Brawn bears (<em>Ursus arctos</em>)</td>
<td>Alaska, Canada, China, Bulgaria, Russia, Siberia</td>
<td>CDC, 1981; Shiota et al., 1999; Peklo et al., 2002</td>
</tr>
<tr>
<td>Cougar (<em>Felis concolor</em>)</td>
<td>USA, Argentina</td>
<td>Dworkin et al., 1996; Ribicich et al., 2005</td>
</tr>
<tr>
<td>Badger (<em>Meles meles</em>)</td>
<td>Korea, Argentina</td>
<td>Suzdaltsev et al., 1999; Sohn et al., 2000; ProMed mail 2005</td>
</tr>
<tr>
<td>Red fox (<em>Vulpes vulpes</em>)</td>
<td>Italy</td>
<td>Pozio et al., 2001</td>
</tr>
<tr>
<td>Jackal (<em>Canis aureus</em>)</td>
<td>Thailand, Algeria</td>
<td>Khamboonruang, 1991; Nezri et al., 2005; Ribicich et al., 2005</td>
</tr>
<tr>
<td>Armadillo (<em>Chaetophractus villosus</em>)</td>
<td>Argentina</td>
<td></td>
</tr>
<tr>
<td>Squirrel</td>
<td>Thailand</td>
<td>Khamboonruang, 1991</td>
</tr>
<tr>
<td>Monitor lizard (<em>Varanus nebulosus</em>)</td>
<td>Thailand</td>
<td>Khamboonruang, 1991</td>
</tr>
<tr>
<td>Turtle</td>
<td>Thailand</td>
<td>Khamboonruang, 1991</td>
</tr>
<tr>
<td>Horse</td>
<td>France, Italy</td>
<td>Ancelle, 1998; Pozio and Zarlenga, 2005</td>
</tr>
<tr>
<td>Beef, mutton, goat*</td>
<td>China</td>
<td>Murrell, 1994; Takahashi et al., 2000</td>
</tr>
<tr>
<td>Dogs</td>
<td>China, Russia, Slovak Republic</td>
<td>Takahashi et al., 2000; Dubinsky et al., 2001; ProMed mail 2004</td>
</tr>
</tbody>
</table>

*Meat from these animals has been implicated as a source of infection for humans, even if experimental infections have shown that cattle, sheep and goats can acquire a transient infection (Campbell, 1983b; Smith et al., 1990; Reina et al., 1996; Theodoropoulos et al., 2000). It could be argued that sheep, goats and cattle could acquire *T. spiralis* infection when they are bred in areas that are highly endemic for *T. spiralis* infection in pigs, but this must be supported by further epidemiological investigations.
Trichinella sp. transmission to humans. Except for T. zimbabwensis, Trichinella T8 and T9, all species and genotypes of Trichinella have been detected in humans; based on animal experiment data, it is likely these exceptions are also infective for humans.

6.3.1. Changing Patterns of the Epidemiology of Human Infections

In the United States, Canada and European Union countries, human infections due to the consumption of pork from domestic pigs have nearly or completely disappeared because of both the improvement of pig-production facilities and practices and the improvement of detection technologies employed in the slaughterhouses. In these countries, the occasional infections with T. spiralis that occur are related to the consumption of pork from the so-called backyard pigs or pigs reared on organic farms (Pozio, 1998). However, a large biomass of the parasite continues to exist in developing countries of Central and South America, Europe and Asia, where an increase in human population movement to urban areas as occurring in China, or even to industrialized countries is seen. This important demographic factor has resulted in new and different patterns of human infections. For example, trichinellosis is emerging in some urban areas in China where affluence has increased the demand for pork, particularly, in dishes that traditionally may not be well-cooked (meat dumplings) (Wang et al., 1998).

The migration of persons from eastern Europe to the European Union (EU), mainly for work, has led to an increase in the quantity of pork products sent from these countries to the EU as Christmas gifts or brought back to the EU by expatriates visiting their country of origin for the holidays. This behavior has resulted in several human outbreaks of trichinellosis in Germany, Italy and the United Kingdom (Pozio and Marucci, 2003). Since human trichinellosis is quite rare in many EU countries, many local physicians are not familiar with the disease and experience problems in diagnosing it. Delays in diagnosis and treatment favor the establishment of larvae in muscles and the development of a collagen capsule, which render the larvae resistant to drugs (Pozio et al., 2001g, 2003; Dupouy-Camet et al., 2002). In
addition to constituting a risk to human health, imported pork products infected with *T. spiralis* threaten the pig industry in the EU, particularly for organic farms which typically practice free-range pasturing of pigs, and which may have a risk due to the spread in the environment of infected pork scraps. For example, *T. spiralis* is not present in domestic or wild animals of Italy or Switzerland (Pozio, 2001a), and an accidental exposure to infected products could introduce a new pathogen into the food supply of these countries.

In some cases, human migration has resulted in the introduction of new food practices and dishes based on raw or undercooked pork or pork products, which have led to trichinellosis outbreaks among the unaware immigrant communities in endemic countries, especially for migrants from Cambodia, Laos, Thailand and Vietnam; those in the United States and Israel, where the control for *Trichinella* infection in domestic pigs and wild boars is not compulsory, are especially at risk (Imperato *et al.*, 1974; Stehr-Green and Schantz, 1986; McAuley *et al.*, 1992; Graves *et al.*, 1996; Marva *et al.*, 2005).

In China, the “Western Region Development of China” strategy implemented in 1990s, elicited migration and settlement of large numbers of people from the central to the western areas, which led in turn to an increased quantity of pork products being taken from central to western regions, either commercially or privately. The areas of central China where potentially infected meat can be exported to other provinces have prevalence rates of *T. spiralis* infection in pigs of 6.8% in Hubei and 4.3% in Henan (Wang and Cui, 2001). In the new western communities which are likely to import pork, pigs are being reared under relatively primitive conditions in which the animals are exposed frequently to raw waste meat scraps, and are free to scavenge animal carcasses from both wild and domestic sources (Cui *et al.*, 2006). This has led to a dramatic increase in the size of the human population at risk for trichinellosis in the western areas of China (Cui *et al.*, 2006). In 1990, the incidence of *Trichinella* infection in examined pork samples from the markets in Xing City (Qinghai province, western China) was 0.1%, but was 15.9% in Huangyuan county in 1997 (Tibet Autonomous Prefecture, Qinghai province, western China), and 23.0% in Delingha city in 2004 (Qinghai Province, western China). These infection rates also pose a potential danger to the rapidly developing tourist
industry. In fact, the increasing demand for meat for tourist hotels and restaurants has led to a rapid increase in the number of small farms where pigs are often fed with swills containing raw pork scraps from the restaurants or hotels, thereby providing a mechanism for the amplification of the transmission cycle (Cui et al., 2006).

The increasing number of international travelers has resulted in many reports of tourists who acquired *Trichinella* sp. infections while traveling or hunting in endemic areas and later developed the clinical disease after their return to their home countries. In most instances, diagnosis was difficult because the infections appeared as isolated cases (McAuley et al., 1991; Dupouy-Camet et al., 1998; Shiota et al., 1999; Nakamura et al., 2003). Trichinellosis in travelers has occurred after the consumption of pork from a warthog in Africa (Dupouy-Camet et al., 1998); bear meat in Canada and Greenland (Nozais et al., 1996; Dupouy-Camet et al., 1998; Ancelle et al., 2005), pork in China, Egypt, Indonesia (Bali Island), Laos and Malaysia (Therizol et al., 1975; De Carneri and Di Matteo, 1989; Shiota et al., 1999; Kurup et al., 2000; ICT); and wild boar meat in Turkey and Algeria (Niquet et al., 1979; Michel et al., 1986).

In countries where most of the population follows the Muslim or Judaism religious law, the consumption of pork and the meat of carnivores is forbidden, therefore, *Trichinella* sp. infection is seldom documented in humans. However, the increasing secularism, demographic changes and the presence of populations with different religions in these countries, along with the increasing tourism they are experiencing, has stimulated an increase in pig production, and the consumption of game meat, which is not subject to veterinary control because officially “pigs” do not exist. In Turkey, three outbreaks of trichinellosis occurred from the consumption of pork in Antalya (more than 40 people), Bursa (seven people) and Izmir (more than 600 people) between 2003 and 2004 (Heper et al., 2005; Ozdemir et al., 2005; Pozio and Zarlenga, 2005; Turk et al., 2006). In Israel, six small outbreaks occurred mostly in the Christian Arab population from the consumption of pork from wild boars prior to 1997 (Eisenman and Einat, 1992; Marva et al., 2005). In Lebanon and Syria, large outbreaks have also occurred in Christian villages from the consumption of pork from wild boars (Olaison and Ljungstrom,
In Algeria, six small outbreaks (involving a total of 51 people) occurred from the consumption of a domestic pig (Gerard, 1946) and wild boars (Lanoire et al., 1963; Verdaguer et al., 1963; Mémin et al., 1968; Barabe et al., 1977; Michel et al., 1986), and a single case due to the consumption of meat from a jackal (Nezri et al., 2006). In Ethiopia, where the Coptic religion is professed by 35–40% of the inhabitants, trichinellosis has been frequently occurring from the consumption of warthog (Perdomo Gonzales et al., 1986; Kefenie et al., 1988; Kefenie and Bero, 1992; Gelnew, T., personal communication).

7. A NEW APPROACH: TRICHINELLA-FREE AREAS OR FARMS. IS IT POSSIBLE?

In recent years, debate has intensified over the validity of the concept of *Trichinella*-free areas (Pozio, 1998; European Food Safety Authority, 2005). This has great economic importance because of the high cost associated with testing of all slaughtered pigs from the area for *Trichinella*. In countries, where pig production is carried out using good rearing standards (Gamble et al., 2000) and humans do not consume raw pork products, *Trichinella* infection in either humans or pigs has not been documented for a long time (e.g. Denmark, The Netherlands). Unfortunately, the absence of reports of *Trichinella* infections in these regions tends to lead to complacency among consumers and producers, with a lessening of appreciation for the risk of this zoonosis and a relaxation of veterinary control measures at slaughter. As an example, until recently in Ireland no infections had been documented in either humans or animals for 34 years, suggesting that the country was *Trichinella*-free (Rafter et al., 2005). This in turn 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 in countries thought to be *Trichinella*-free for this period, clearly demonstrating that the sylvatic cycle can flourish 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. In Ireland, the high humidity and low temperatures during the hunting season, i.e. autumn and winter, favor the survival of larvae and allow for the transmission of *Trichinella* through a fox–fox cycle. It can be considered, that the lack of positive reports among domestic pigs may be related to the fact that random routine testing is conducted on relatively few pigs, rather than to the existence of a barrier between the sylvatic and the domestic cycle. The lack of human infections may simply be due to the fact that people of this region tend to cook pork well.

In New Zealand, where *T. spiralis* and its hosts such as pigs and rats were imported during the European colonization, the comprehensive control for *Trichinella* infection in pigs is only compulsory for exported meat and only a random sampling from 300 mature pigs is performed at slaughter for products destined for the domestic market. This sampling size is based statistically on the expectation that the inspection method would detect *Trichinella* at a prevalence of 0.5%. Clearly, this is a minimalist approach, and it may not prevent transmission of the infection to animals or humans. In fact, *Trichinella* infections have been repeatedly documented in domestic pigs in New Zealand in 1965, 1968, 1974, 1997 and 2001; brown rats in 1965 and 2001; cats in 1965, 1974 and 2001 (Buncic, 1997; Paterson *et al.*, 1997; E. Pozio, unpublished data); and humans up to 2001 (Liberona and MacDiarmid, 1988; ICT).

In Tasmania, *T. pseudospiralis* has been detected in marsupials and birds, beginning in 1990 (Obendorf *et al.*, 1990). Australia, however, has always been considered *Trichinella*-free, but this status is based not on any extensive epidemiological investigation on wildlife, but on limited investigations on synanthropic rats, and domestic cats and pigs (Waddell, 1969) and on examination of tongues from 45 dingoes and 22 red foxes from Victoria and New South Wales in 2001, all of which were negative by artificial digestion (Jenkins D. and Pozio, E., unpublished data). There is not a documented autochthonous case in humans. But because *T. papuae* is widespread in wild pigs and saltwater crocodiles of Papua New Guinea, which is very close to Australia (less than 180 km of sea separate the two countries) and the saltwater crocodile is also a marine animal, the status as *Trichinella*-free should be modified until more extensive searches have been conducted.
Mediterranean islands are also considered to be Trichinella-free for the lack of reports in animals or humans, but because these locations lack veterinary control at slaughter, the status of pigs is uncertain. To verify whether or not the island of Corsica can be considered as Trichinella-free, a survey of domestic pigs was conducted at slaughterhouses in 2004. Surprisingly, *T. britovi* was detected in pigs and in the surrounding wildlife (wild pigs and a fox) (Boireau and Vallée, 2004). In Sardinia, no control has been done at the slaughterhouse due to a conviction that the island was Trichinella-free, which was based on the examination of a very low number of domestic and sylvatic animals. However, in 2005, an outbreak of trichinellosis involving 11 people resulted from the consumption of raw pork infected with *T. britovi* from a free-range reared sow (Pozio et al., submitted). This proves the validity of the belief that the declaration as a Trichinella-free area must be based on extensive animal surveys and surveillance, preferably at slaughter.

These examples demonstrate how the lack of reports of infection among domestic animals and humans over an extended timeframe cannot be considered as a justification for area-wide Trichinella-free status. All self-declared Trichinella-free areas should institute a sustaining monitoring program, which includes not only domestic animals but also wildlife.

However, an alternative approach that awards certification to farms with sustained Trichinella-free status is being seriously studied in some countries, most notably in the United States and in the European Union (Pozio, 1998; Pyburn et al., 2005; Kapel, 2005). An extensive pilot program, The U.S. Trichinae Certification Program, under the supervision of the U.S. Department of Agriculture, involving over 450 pig farms, is in progress (Pyburn et al., 2005). These farms undergo a pre-certification site audit for risk factors and infection status of the pig herd. Subsequently, the farm is subjected to both scheduled and unscheduled audits and testing of pigs to ensure the herd remains Trichinella-free. Pork products from these certified herds can be marketed as such, with the anticipated commercial benefits associated with selling safe pork. The progress and results of this trial will be watched with interest.
It was the authors’ intent in undertaking this review of *Trichinella* to highlight aspects of the parasite’s biology and epidemiological features that are of importance to those with basic research interests and to public health efforts. Although trichinellosis has declined significantly as a zoonosis, due chiefly to a reduction of domestic trichinellosis in developed countries, it remains a potential risk because of the continuing presence of most species of *Trichinella* as a parasite of wild animals. Further, the strong opportunism associated with this parasite, with its broad reservoir host range and diverse transmission features makes the potential for its re-emergence whenever the food-safety barriers are weakened by socioeconomic events. Examples are seen in the equine trichinellosis outbreaks in Europe, and the reemergence of porcine trichinellosis in countries undergoing major political and economic change (e.g. former Yugoslavia, Romania and Argentina). It behooves veterinary and public health agencies to become well acquainted with the causes of the re-emergence of trichinellosis in countries where its formerly successful control measures for this zoonosis was placed in jeopardy or severely compromised by these political, economic and agricultural changes. It is important then, that the systematics and ecology of all species including both, the classical domestic *T. spiralis* and the sylvatic species, which are increasingly implicated as a cause of human trichinellosis or a significant risk, be well understood.

The emphasis placed in this review, therefore, is the knowledge gained in recent years on the basic biology, especially evolutionary biology, ecology, and biogeography of *Trichinella* spp. It should be apparent that our current knowledge of these aspects represents only the barest of essentials pertaining to these parasites. In a sense, we are only at the beginning of the journey to full knowledge, and that much more research is needed to be carried out before the gaps are filled. Those interested in pursuing these questions can look forward to a demanding task, but they can also take comfort in having a good platform to build upon the availability of molecular tools for genetic analysis and a large, available collection of isolates and biogeographic knowledge residing in the International *Trichinella* Reference Centre. If another review on the systematics and epidemiology of *Trichinella* is undertaken in a
decade or so, it will likely describe much new knowledge and perhaps provide a surprising new view of this species complex. We certainly hope so!

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