



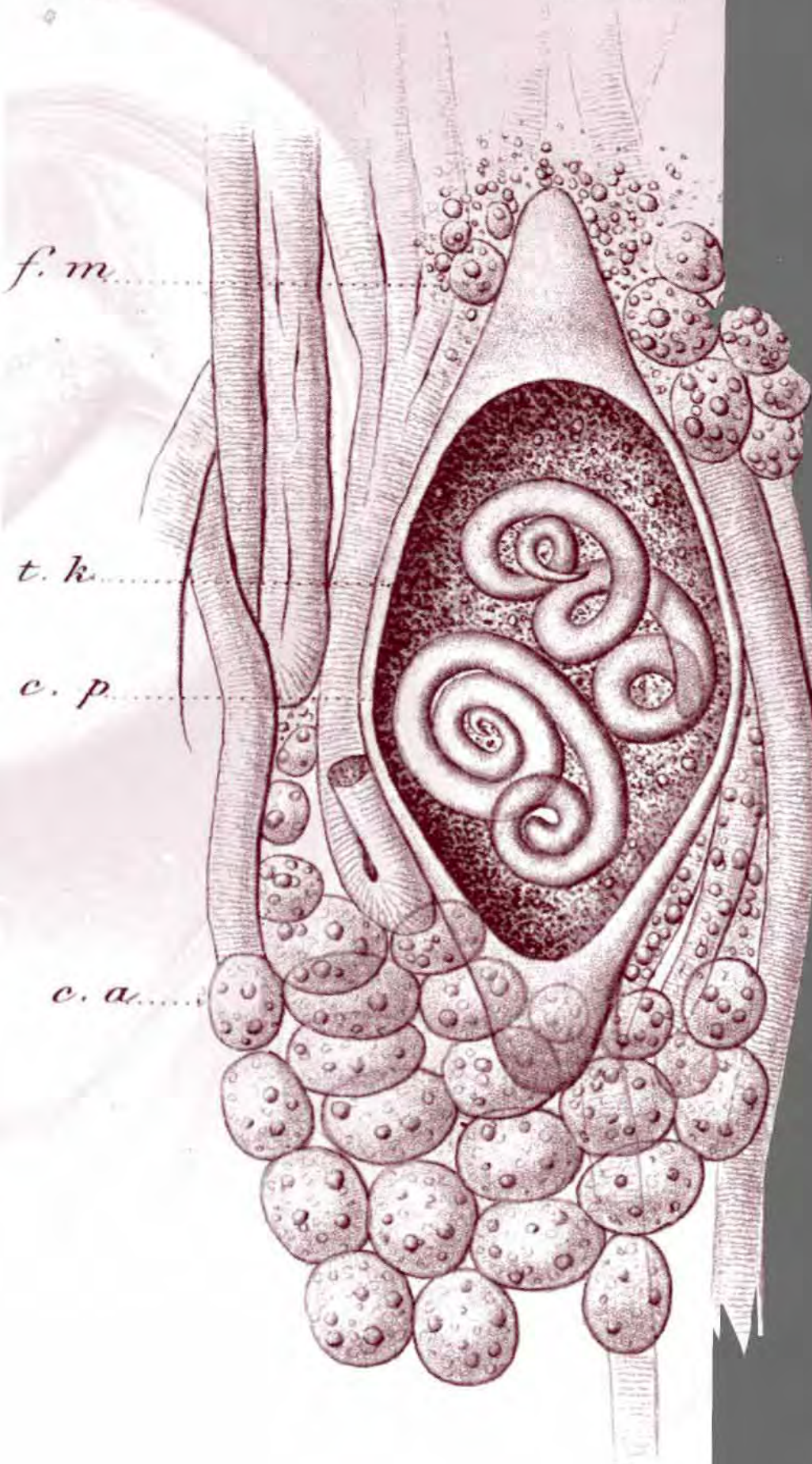
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**FAO/WHO/OIE
Guidelines
for the
surveillance,
management,
prevention
and control
of
TRICHINELLOSIS**

Editors:
J. Dupouy-Camet & K.D. Murrell

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Cover photograph: engraving from '*La trichine et la trichinose*', Joannes Chatin, Paris, 1883 (t.k.: cystic tissue; c.p.: parietal layer; f-m.: muscle fiber; c.a.: adipose cell)

FOREWORD

Trichinellosis is a parasitic disease that in the past has not always been recognised for its importance. However, it is becoming increasingly clear that greater priority should be given to this zoonosis because of its health and economic impact, particularly in resource-poor countries. It is now also recognised as a re-emergent problem in Latin America, eastern Europe and Asia. As is the case for all zoonoses, the control of trichinellosis requires the very close collaboration between both Veterinary and Medical public health Services at a national level. It was with the aim of assisting those responsible for trichinellosis control and prevention that these *Guidelines* were prepared and jointly published by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organisation for Animal Health (OIE). This joint publication is a reflection of the importance the three organisations place on working together to assist their Member Countries in designing, implementing and standardising control strategies against zoonoses on both national and international levels. These *Guidelines* are an example of the outcome of the long-term collaboration by FAO, WHO and OIE to educate and organise the surveillance and control of parasitic diseases worldwide.

These *FAO/WHO/OIE Guidelines for the Surveillance, Prevention, and Control of Trichinellosis* are a compilation of the accumulated knowledge and valuable expertise of many internationally recognised experts on this zoonosis. Without this knowledge base, effective and proven recommendations for diagnosis, treatment, prevention and control would not be possible. We would like to express our profound gratitude to them for their contributions and for those able to share their knowledge and advice to the authors. It is our hope that the book achieves the success it deserves.

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INTRODUCTION

The terms trichinellosis, trichinosis and trichiniasis all refer to an infection with the larval and adult stages of a parasitic nematode belonging to the genus *Trichinella*. The important features of this infection are that it is zoonotic and that the infective larvae are meatborne (typically pork, but increasingly other animal meat). Uniquely, the vertebrate host serves as both an intermediate and definitive host for the parasite; an infection in which both the reproducing adult worms and the infective larvae develop in the same host. Humans and a wide range of other animals acquire the infection by eating infected muscle (meat) that is not adequately cooked.

Although the classical paradigm of human trichinellosis involves man as a 'blind alley' host in which infection by *Trichinella spiralis* is acquired through the ingestion of infected domestic pork, in recent years this well-known epidemiology has greatly changed. It is now recognised that there are at least eight *Trichinella* species, many of which have as natural or reservoir hosts sylvatic animals. Humans, then, may acquire infection from a variety of meat sources, including, notably horses, wild game, birds, and even from reptile meat. Because of these factors, trichinellosis is a cosmopolitan zoonoses with a highly diverse source of potential avenues of transmission (meat sources). While the 'domestic cycle' (involving domestic pigs) is still a major cause of trichinellosis, the role of both sylvatic and non-pig domestic animal sources has become more important world-wide, and this has required a reassessment of traditional trichinellosis surveillance and control approaches.

Until the 1980s, there was a general consensus that trichinellosis was finally on the wane as a result of increasingly effective control in most regions through the widespread adoption of meat inspection, and improvements in animal husbandry practices for swine. Its persistence had become typically associated with poverty and poor sanitation and animal rearing habits. However, many regions, even those which had reduced trichinellosis to an infrequent occurrence, began to experience in the 1980s and the 1990s a resurgence of trichinellosis. Perhaps the most dramatic were outbreaks in Europe stemming from consumption of infected horse meat. It is now recognised that many of these outbreaks were traceable to Eastern Europe, where beginning in the late 1980s and early 1990s, tremendous social, political and economic changes occurred. These changes often caused serious disruptions in veterinary control over meat production, resulting in laxity of inspections and, for economic reasons, increased high risk animal production practices. These events illustrated the consequences of breakdowns in food security systems and failure to appreciate the ability of *Trichinella* to exploit gaps in food safety nets. Similar social-economic influences on the occurrence of trichinellosis were also seen in Latin America and Asia where hundreds and even thousands of human cases (some lethal) began to be reported.

In addition to the importance of this foodborne zoonosis as a cause of morbidity and mortality, it is also understood as a cause of income loss for farmers and meat processors because its persistence in the domestic food supply (e.g. commercially produced pork) imposes a stigma that burdens consumption and farmer profitability. In the European Union, which has both mandatory meat inspection for *Trichinella* and a very low prevalence in swine, the public cost to prevent transmission through commercial meat amounts to hundreds of millions of euros per year. Because trichinellosis is a concern for public health authorities world-wide, the importance of international networks such as the International Commission on Trichinellosis (ICT) has increased. The ICT members include scientists, veterinarians and physicians expert in this field who are communicating regularly to make recommendations to improve the management and prevention of this potentially lethal disease, and to encourage research.

To effectively establish or re-establish prevention and control programmes, awareness on the part of the public sector of the modern understanding of the complex epidemiology of trichinellosis must be increased, and especially the need to adopt up-to-date diagnostic tools, clinical management procedures, high standards of meat hygiene and greater veterinary control over pig husbandry and slaughter practices. In order to undertake these tasks, food safety personnel, especially those with limited experience with trichinellosis, must have access to the most useful and up-to-date information available. In recognition of this need FAO, WHO and OIE instigated the development of a new set of Guidelines that summarises the collective knowledge of experts in this field. These Guidelines on Surveillance, Prevention and Control of Trichinellosis are the successor to the earlier 1988 guidelines which had an important impact on efforts to focus attention on the problem of this zoonosis. It also stimulated research by identifying important

knowledge gaps. Since that time, much has been accomplished in this field: greater understanding of the epidemiology and effective control designs, the development of new and better serological and molecular (DNA based) diagnostic technologies; the discovery of several new species which has yielded marked advances in *Trichinella* systematics, and the ability to type the specific causative agent in outbreaks and animal epidemiological investigations; and advances in clinical management and treatment, especially in drug intervention. It is the objective of authors of this Guidelines to bring these advances together, in a practical format, to help not only those who undertake research, but also those with the responsibilities for patient care, prevention and control of this zoonosis.

The contents of the Guidelines cover biology, systematics, epidemiology, diagnosis, patient management, prevention and control. The chapters emphasise methods and procedures, including new concepts such as *Trichinella*-free certification and *Trichinella*-free areas. The Guidelines also provide advice and recommendations on programme planning, monitoring and evaluation. Special reference is made to inter-sector coordination and collaboration, particularly between medical and veterinary services, a partnership crucial for effective surveillance and control efforts. The authors of these Guidelines are confident that the material provided will prove useful and a satisfying resource for the detection and treatment of infection, and for the development of prevention and control programmes. We further hope that the information and guidance presented will enhance the progress towards the objectives of FAO, WHO and OIE.

Darwin Murrell & Jean Dupouy-Camet

CHAPTER 1

TAXONOMY, BIOLOGY AND EPIDEMIOLOGY OF *TRICHINELLA* PARASITES

E Pozio

Summary

Nematode worms belonging to the genus *Trichinella* are the etiological agent of a zoonotic disease. These parasites are widespread in wildlife on all continents but Antarctica, and in domestic pigs of many countries, with carnivores and omnivores representing the most important reservoirs. Infections occur in humans where cultural food practices include dishes based on raw or undercooked meat and meat products of different animal origins (e.g. pork, horse, game). At present, eight species and three genotypes are recognised in the genus, namely *Trichinella spiralis*, *T. nativa* and its related genotype *Trichinella* T6, *T. britovi* and its related genotype *Trichinella* T8, *T. pseudospiralis*, *T. murrelli* and its related genotype *Trichinella* T9, *T. nelsoni*, *T. papuae*, and *T. zimbabwensis*. All species can develop in mammals. *T. pseudospiralis* develops also in birds and *T. papuae* and *T. zimbabwensis* also infect some reptile species. Although no clearcut morphological difference exists between species and genotypes, they can be distinguished by biochemical or molecular analyses. The infective stage is a small larva present in the cell of striated muscles. This larva can survive for years in the muscle cell of the host and for weeks to some months in decaying tissues of dead hosts, depending upon the environmental temperature and moisture. Muscle larvae of some species can survive freezing for long periods of time depending on the species of the host tissue. The adult stage parasitizes the small intestine for weeks or months according to the host species and their immunological response in the mucosa. The average yearly incidence of the disease in humans worldwide is over ten thousand cases with a mortality rate of about 0.2%.

Keywords

Carnivore - Horse - Nematode - Omnivore - Pig -Raw meat - Scavenger - *Trichinella* - *Trichinellosis* -*Trichinosis* - Wildlife - Zoonosis.

INTRODUCTION

Nematode worms belonging to the genus *Trichinella* are the etiological agent of a zoonosis named trichinellosis. These parasites are widespread in wildlife on all continents but Antarctica, and in domestic pigs of many countries (Pozio and Murrell, 2006). Infections occur in humans where cultural food practices include dishes based on raw or undercooked meat and meat products of different animal origins (e.g. pork, horse, game). At present, eight species and three genotypes are recognised in the genus *Trichinella*, namely *Trichinella spiralis*, *T. nativa* and its related genotype *Trichinella* T6, *T. britovi* and its related genotype *Trichinella* T8, *T. pseudospiralis*, *T. murrelli* and its related genotype *Trichinella* T9, *T. nelsoni*, *T. papuae*, and *T. zimbabwensis* (Table I). The parasites are perpetuated in life-cycles with carnivorous and omnivorous animals representing the most important reservoir. All species can develop in mammals, but *T. pseudospiralis* can also develop in birds, and *T. papuae* and *T. zimbabwensis* also occur in some reptile species. No morphological differences exist between species and genotypes, and they are most reliably distinguished by biochemical or molecular analyses (see below).

Table I - Principal features of *Trichinella* species and genotypes

<i>Trichinella</i> species genotype	Distribution	Cycle	Hosts	Collagen capsule	Freezing resistance ^(f)
<i>T. spiralis</i> T1	Cosmopolitan ^(a)	Domestic and sylvatic	Swine, rats, carnivores	Yes	No
<i>T. nativa</i> T2	Arctic and subarctic areas of Holarctic region ^(b)	Sylvatic	Terrestrial and marine carnivores	Yes	Yes
<i>Trichinella</i> T6	Canada, USA ^(c)	Sylvatic	Carnivores	Yes	Yes
<i>T. britovi</i> T3	Temperate areas of Palearctic region ^(d) , North and West Africa	Sylvatic, seldom domestic	Carnivores, seldom swine	Yes	Yes
<i>Trichinella</i> T8	South Africa	Sylvatic	Carnivores	Yes	No
<i>T. pseudospiralis</i> T4	Cosmopolitan ^(e)	Sylvatic, seldom domestic	Mammals and birds	No	No
<i>T. murrelli</i> T5	Temperate areas of Nearctic region	Sylvatic	Carnivores	Yes	No
<i>Trichinella</i> T9	Japan	Sylvatic	Carnivores	Yes	Unknown
<i>T. nelsoni</i> T7	Ethiopic region	Sylvatic	Carnivores, seldom swine	Yes	No
<i>T. papuae</i> T10	Papua New Guinea	Sylvatic, seldom domestic	Mammals and reptiles	No	No
<i>T. zimbabwensis</i> T11	Ethiopia, Mozambique, Zimbabwe	Sylvatic	Mammals and reptiles	No	No

a) *This species has not been detected in arctic regions*

b) *The isotherm -5°C in January is the southern limit of distribution*

c) *Alaska, Idaho, Montana, Pennsylvania*

d) *The isotherm -6°C in January is the northern limit of distribution*

e) *Three different populations have been identified in the Nearctic region (Alabama and Texas), Palearctic region (many foci), and in the Australian region (Tasmania)*

f) *In muscles of carnivorous mammals*

TERMINOLOGY

Some particular terms are commonly used to indicate the parasite stages and the infected muscle cell. They are:

- 1) newborn larva (NBL) = the larva produced by the female (80 µm to 120 µm in length, 5µm to 7 µm in width)
- 2) muscle larva = a L1 (first stage) larva living in the muscle cell, i.e. the infective stage (0.65 mm to 1.45 mm in length, 0.026 mm to 0.040 mm in width)

- 3) nurse cell = the parasitised muscle cell, in which the larva induces strong morphological and physiological modifications; the nurse cell is present in both encapsulated and non-encapsulated species
- 4) capsule = a collagen structure surrounding the larval-nurse cell complex of encapsulated species infecting only mammals.

THE CYCLE IN THE HOST

A peculiarity of the cycle of nematodes of the genus *Trichinella* is the development of two generations in the same host (Figure 1). *Trichinella* NBL migrate from gravid female worms embedded in the intestinal mucosa directly into the lymphatic vessels, then enter the blood vessels which deliver them to striated muscle cells, in which they actively penetrate by a stiletto apparatus and lytic enzymes. The NBL develops in the muscle cell to the L1 infective stage (the muscle larva) in about 15 days. In the muscle cells, larvae can survive for years waiting to be ingested by a new host (over 20 years in polar bears (*Ursus maritimus*) and up to 40 years in humans). When a new host ingests infected muscle tissue, the larvae are released from the muscle cells in the stomach by digestion; when they reach the duodenum, they penetrate into the villa and within two days undergo four moults, developing to the adult stage. Males and females copulate, and six to seven days post infection (d.p.i), the females begin to produce NBL, i.e. the new generation. NBL production continues for at least one to two weeks or more according to the immune response of the host at the gut level, which usually develops and results in expulsion of adult worms.

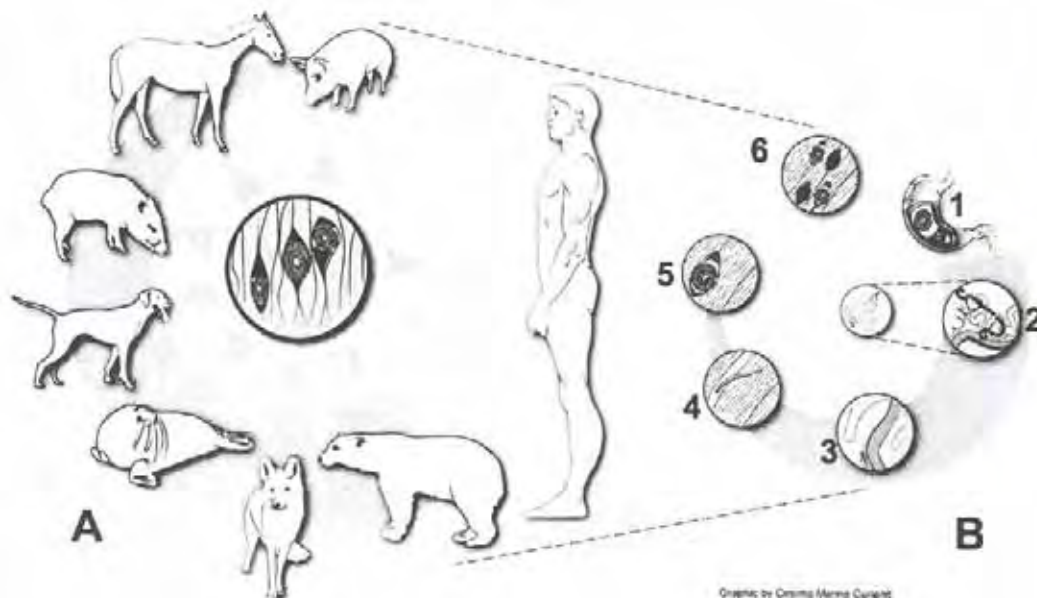


Figure 1 - *Trichinella* sp. life cycle

A: main sources of Trichinella spp. infections for humans

B: Trichinella spp. cycle in the host body. Enteral phase - 1: muscle tissues are digested in the stomach and larvae are released; 2: larvae penetrate the intestinal mucosa of the small intestine, reach the adult stage within 48 h post infection, male and female mate; 3: female worm releases newborn larvae in the lymphatic vessels (from the fifth day post infection onwards; the length of newborn production, from one to several weeks, is under the influence of the host immunity). Parenteral phase; 4: the newborn larva reach the striated muscle and actively penetrate in the muscle cell; 5: the larva grows to the infective stage in the nurse cell (the former muscle cell); 6: after a period of time (weeks, months or years) a calcification process occurs (redrawn from www.iss.it/site/Trichinella/index.asp)

The stage, which can be easily recognised in an infected host, is the larva parasitising the muscle cell. Adult worms can be collected from the gut only from experimentally infected animals; the opportunity to detect adult worms in the gut or in faecal samples of naturally infected hosts is rare. It is also very difficult to detect NBL in blood of naturally infected hosts.

GENERAL MORPHOLOGY

Muscle larvae

Muscle larvae (Figure 2) are L1 larvae because developmental moults occur only after their penetration in the gut mucosa of a new host. The following morphological characters can sexually distinguish male and female muscle larvae.

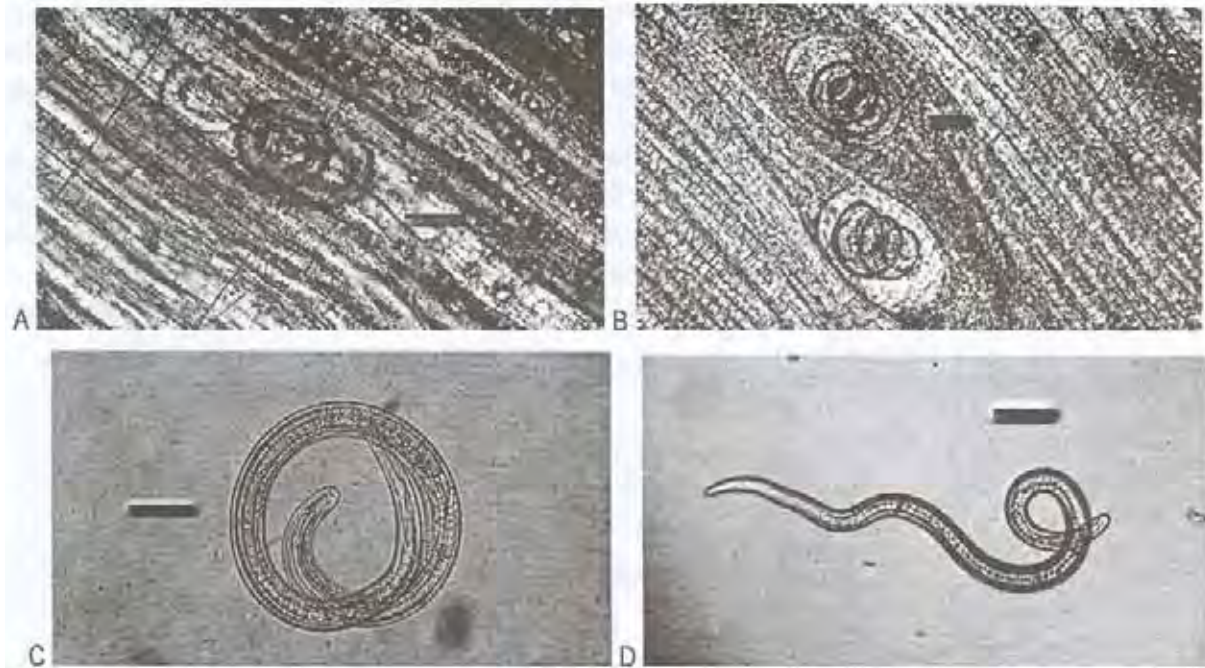


Figure 2 - *Trichinella* spp. larvae

A: Non encapsulated larvae of Trichinella pseudospiralis in muscle tissue

B: Encapsulated larvae of Trichinella britovi in muscle tissue

C: Coiled larva of Trichinella britovi after pepsin-HCl digestion

D: Extended larva of Trichinella britovi after pepsin-HCl digestion

Scale bars 100 μ m (redrawn from www.iss.it/site/Trichinella/index.asp)

Male

- Total length 0.65 mm to 1.07 mm, width 26 μ m to 38 μ m
- intestinal bulb generally close to the convex surface; in some larvae close to the concave surface
- intestine crossing the gonad from the convex to the concave surface; in some larvae, crossing the gonad from the concave to the convex surface and then re-crossing to the concave surface
- length of rectum of about 40 μ m to 50 μ m.

Female

- Total length 0.71 mm to 1.09 mm, width 25 μ m to 40 μ m
- intestinal bulb generally close to the concave surface
- intestine on the concave surface; in some larvae, intestine crossing the gonad from the concave to the convex surface and then re-crossing to the concave surface
- length of rectum of about 20 μ m to 30 μ m
- presence of a thickened subcuticular layer in the region of vulva primordium, i.e. on the convex surface at about 2/3 of the way along the stichosome.

Adults

The following morphological characters can distinguish male and female adult worms, both of which are colourless:

Male

- Total length 0.62 mm to 1.58 mm, width 25 μ m to 33 μ m.
- The cuticle is smooth, but exhibits pseudo segmentation and is periodically interrupted by dorsal and ventral pairs of hypodermal gland cells.
- Genital terminal consists of a pair of flattened copulatory appendages and accessory papillae.
- The alimentary tract consists of an oral cavity, capillary oesophagus, midgut with brush border and hind gut.
- The stichosome (45 to 55 specialised cells, named stichocytes), lies in the anterior portion of the worm.
- The reproductive tract consists of a single testis.

Female

- Total length 1.26 mm to 3.35 mm, width 29 μ m to 38 μ m.
- The cuticle is similar to that of male, but there are no copulatory appendages.
- The vulva is present in the posterior end of the stichosome.

Newborn larvae

Newborn larvae (average 110 μ m in length, 7 μ m in width) show rudimentary morphological features. They are L1 larvae as the muscle larvae. At this stage, the sex cannot be identified.

TAXONOMY

The genus *Trichinella* comprises a monophyletic lineage in the Family Trichinellidae, the putative sister to the Trichuridae (Capillariinae, Trichurinae and Trichosomoidinae). The superfamily Trichinelloidea to which *Trichinella* belongs is phylogenetically diagnosed by the stichosome, a region of the glandular oesophagus, and the bacillary bands, an assembly of structural characters unknown among the other nematodes (Zarlenga *et al.*, 2006). Today, two main clades are recognised in the genus *Trichinella*, one that encompasses species that encapsulate in host muscle tissue, and a second that does not encapsulate after muscle cell dedifferentiation (Pozio and Murrell, 2006).

The encapsulated clade

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

Trichinella spiralis (genotype T1)

This is the first species discovered and the most characterised 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 both domestic and sylvatic animals and to its high infectivity for laboratory animals. Dissemination of the parasite and its hosts was especially facilitated by the European colonisation of North, Central and South America, New Zealand, Hawaii, and Egypt from the 16th to 20th Centuries (Figure 3). Its low resistance to low environmental temperatures may have inhibited its spread among wildlife living in frigid zones. *Trichinella spiralis* is the species identified in 87% of all isolates from domestic pigs, in 67% from wild boars, in 88% from domestic horses, in 79% from synanthropic rats and in the only two isolates from synanthropic armadillos (*Chaetophractus villosus*) (Pozio and Murrell, 2006). In many regions of the world this species has been transmitted to wildlife hosts (e.g. badgers [*Meles meles*], foxes [*Pseudolopex gracilis*, *Urocyon cinereoargentatus*, *Vulpes vulpes*], wolves [*Canis lupus*], black and brown bears [*Ursus americanus* and *Ursus arctos*], mountain lions [*Puma 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 were scattered in the environment (Pozio and Murrell, 2006). In countries of the Americas, Europe and Asia, *T. spiralis* is also a parasite of wildlife maintained in nature by a sylvatic cycle (Pozio and Zarlenga, 2005). This species is the etiological agent of most of *Trichinella* infections in the human beings and deaths around the world (Table I).

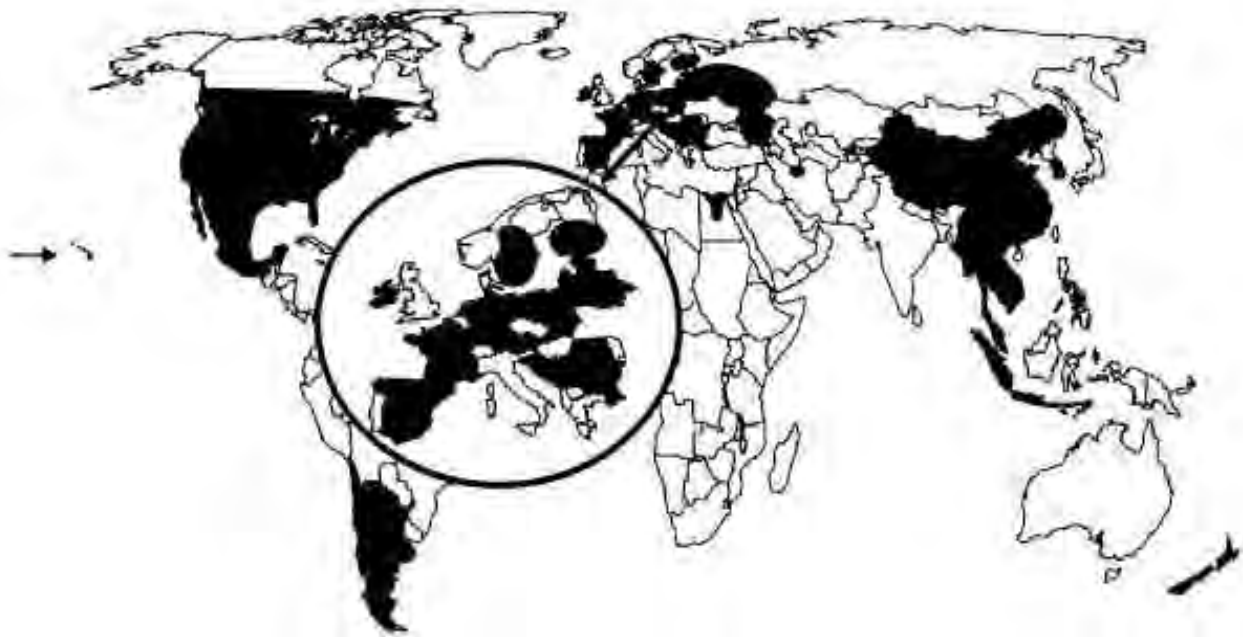


Figure 3 - Distribution of *Trichinella spiralis*

It should be stressed that T. spiralis has been never detected in Africa outside of Egypt, where it has been introduced by humans (redrawn from www.iss.it/site/Trichinella/index.asp)

Trichinella nativa (genotype T2)

This species is usually named as the arctic or freeze resistant species and is widespread among wildlife of the arctic and subarctic areas of America, Europe and Asia (Figure 4). The southern distribution boundary

has been tentatively identified between the isotherms -5°C to -4°C in January (Pozio and Zarlenga, 2005). The main biological features of *T. nativa* are a low reproductive capacity index (RCI) in laboratory rodents and in domestic and sylvatic swine and a high resistance to freezing in muscles of carnivores (Pozio and Murrell, 2006). The common hosts are terrestrial and marine carnivores living in arctic and subarctic areas (several species of mustelids [*Martes pennant*, *Martes martes*, *Martes zibellina*, *Meles metes*, *Gulo gulo*, *Musteta erminea*, *Mustela nivalis*]; arctic fox [*Alopex lagopus*]; red fox [*Vulpes vulpes*]; wolf [*Canis lupus*]; raccoon dog [*Nyctereutes procyonoides*]; domestic and sylvatic cats [*Felis silvestris*, *Felis euphilura*], lynx [*Lynx lynx*], Siberian tiger [*Panthera tigris*], black bear [*Ursus americanus*], brown bear [*Ursus arctos*], polar bear [*Ursus maritimus*], walrus [*Odobenus rosmarus*] and several species of seals [*Phoca groenlandica*, *Phoca fasciata*, *Erignathus barbatus*, *Pusa hispida*]). This species has rarely been detected in either domestic or wild swine. The importance of sylvatic carnivores as reservoirs of *T. nativa* in nature is attested by the finding that this parasite survives in these hosts's musculature for at least 20 years. Human populations living in frigid zones acquire *T. nativa* infection by eating raw meat from walruses (*Odobenus rosmarus*), bears and other game animals (Pozio and Murrell, 2006).

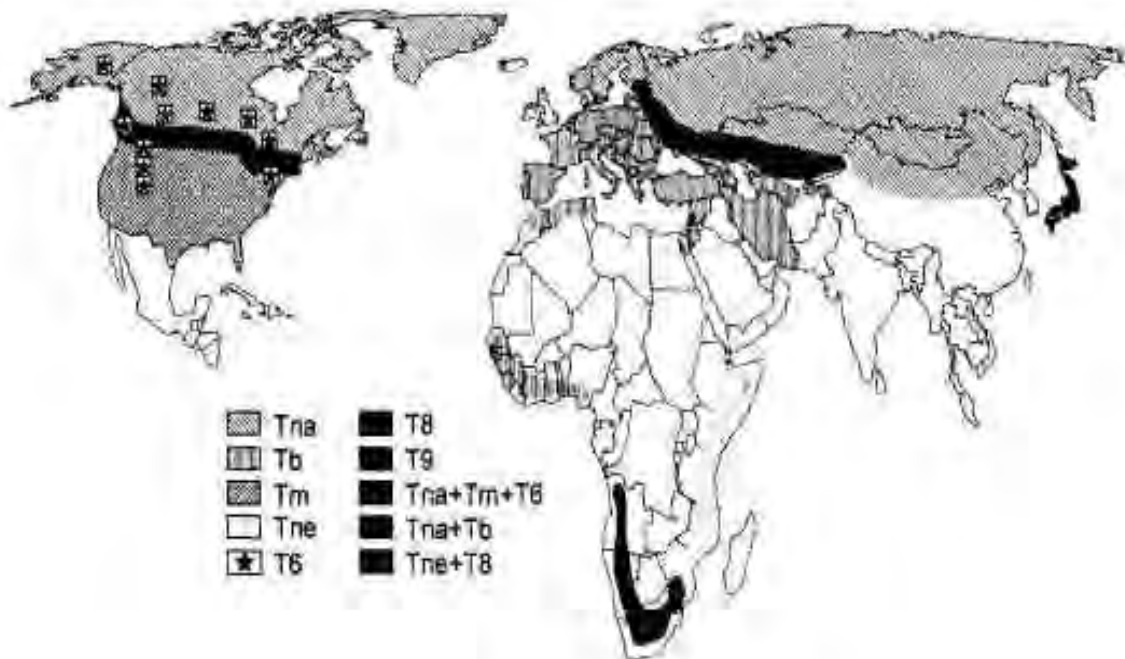


Figure 4 - Distribution of *Trichinella nativa* (Tna), *Trichinella britovi* (Tb), *Trichinella murrelli* (Tm), *Trichinella nelsoni* (Tne), *Trichinella T6* (T6), *Trichinella 18* (18) and *Trichinella T9* (T9)

It may be difficult to distinguish between areas in which both Tna and Tb coexist, from areas where Tna, Tm and T6 coexist (both areas are black), however, these two areas can be distinguished because Tm and T6 are present only in North America and Tb only in Eurasia (redrawn from [www. iss. it/site/Trichinella/index.asp](http://www.iss.it/site/Trichinella/index.asp))

Trichinella britovi (genotype T3)

European and Asian isolates of this species were previously named *T. nelsoni* by Russian scientists (Britov and Boev, 1972; Shaikenov and Boev, 1983; Pozio *et al.*, 1992). Among sylvatic species, *T. britovi* has the widest geographical range, occurring in wildlife of the temperate areas of the Europe and Asia, from the Iberian peninsula to the Far East and extending southward to Northern and Western Africa (Figure 4). The northern geographic boundary appears to be determined by the isotherms -6°C to -5°C in January. This species is sympatric with *T. nativa* between the isotherms -4°C and -6°C , and there are several reports of mixed infections in the same host from Estonia, Finland and Lithuania (Pozio and Murrell, 2006). This species is prevalent among sylvatic carnivores such as mustelids (*Meles meles*, *Martes foina*, *Martes martes*, *Lutra lutra*), viverridae (*Nandinia binotata*, *Viverra civetta*), red fox (*Vulpes vulpes*), jackal (*Canis*

aureus), wolf (*Canis lupus*) and brown bear (*Ursus arctos*). In Europe, it has been identified in 83%, 30% and 11% of isolates from red foxes (*Vulpes vulpes*), wild boars (*Sus scrofa*) and domestic pigs, respectively. Infections in brown rats (*Rattus norvegicus*) living in farms or garbage dumps have been reported in Italy and Estonia although larvae of this species have a very short survival time in this host. This species can be transmitted to humans through the consumption of meat from wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*), jackal (*Canis aureus*), horse and domestic pigs from extensive grazing systems or feed with scraps from sylvatic carnivores (Pozio and Murrell, 2006).

***Trichinella murrelli* (genotype T5)**

This species is spread among sylvatic carnivores (e.g. bobcat [*Lynx rufus*], black bear [*Ursus americanus*], coyote [*Canis latrans*], raccoon [*Procyon lotor*], pine marten [*Martes americana*] and red fox [*Vulpes vulpes*]) and domestic animals (e.g. domestic dog; horse, cat) across the United States of America (USA) (California, Connecticut, Georgia, Illinois, Indiana, Maryland, New Mexico, Pennsylvania, Virginia, Wisconsin and Texas) and in the Vancouver area of Canada (Pozio and Murrell, 2006) (Figure 4). 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 (*Ursus americanus*) in California. This species has not been detected in naturally infected swine. This species is a causative agent of infection in humans especially from consumption of meat from black bears (*Ursus americanus*), and horse meat. 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, 1998).

***Trichinella nelsoni* (genotype T7)**

The distribution area of this species is eastern Africa, from Kenya to South Africa, but this is based on only few surveys and its range may be much broader (Figure 4). The host range includes striped and spotted hyena (*Hyena hyena* and *Crocuta crocuta*), side-striped jackal (*Canis adustus*), black-backed jackal (*Canis mesomelas*), bat-eared fox (*Otocyon megalotis*), domestic dog, lion (*Panthera leo*), leopard (*Panthera pardus*), cheetah (*Acinonyx jubatus*), and serval (*Leptailurus serval*); it occurs at least occasionally in bush pigs (*Potamochoerus larvatus*) and warthogs (*Phacochoerus aethiopicus*), some of which have been the source of infection for humans (Nelson, 1970). Parasites belonging to this species show a low infectivity to laboratory rodents and swine (Nelson *et al.*, 1966; Kapel and Gamble, 2000) compared to *T. spiralis*, but higher than that of *T. nativa*. Less than 100 human infections have been documented for this species in Kenya and Tanzania.

***Trichinella* T6 (genotype T6)**

This genotype is widespread in carnivores (brown and black bears [*Ursus americanus*; and *Ursus arctos*]; wolf [*Canis lupus*]; grey fox [*Urocyon cinereoargenteus*]; coyote [*Canis latrans*]; wolverine [*Gulo gulo*]; fisher [*Martes pennanti*]; mountain lion [*Puma concolor*]; bobcat [*Lynx rufus*]) of the arctic and subarctic regions of Canada and along the Rocky Mountains and Appalachians in the USA (Alaska, Idaho, Montana, Ohio, Pennsylvania, Wyoming and Ontario) (Pozio and Murrell, 2006) (Figure 4). This genotype is distinguished from *T. nativa* by biochemical and molecular characters, in spite of their ability to interbreed in both the laboratory and in nature (hybrids have been found in wolves [*Canis lupus*] of Alaska) (La Rosa *et al.*, 2003). *Trichinella* T6 and *T. nativa* are very similar in their biological features: high freezing resistance of larvae in muscles of carnivores; low infectivity to laboratory mice and rats and to domestic and sylvatic swine (Kapel and Gamble, 2000).

***Trichinella* T8 (genotype T8)**

Trichinella T8 has been identified only from a lion (*Panthera leo*) of the Etosha National Park of Namibia and a lion (*Panthera leo*) and a spotted hyena (*Crocuta crocuta*) from the Kruger National Park of South Africa, where it lives in sympatry with *T. nelsoni* (Figure 4). This genotype can be easily distinguished by certain biochemical and molecular characters from *T. britovi* (Pozio *et al.*, 1992). No human cases due to this genotype have been documented.

***Trichinella* T9 (genotype T9)**

Trichinella isolates originally identified as *T. britovi* from Japanese wildlife (raccoon dog [*Nyctereutes procyonoides*]; Japanese black bear [*Ursus thibetanus*]) have now been shown by molecular methods to differ from the European strains, and are designated *Trichinella* T9 (Figure 4). Muscle larvae of this genotype have been also detected in red foxes (*Vulpes vulpes*) of the Hokkaido Island, where they live in sympatry with *T. nativa*. No human cases due to this genotype have been documented (Pozio and Murrell, 2006).

The non-encapsulated clade

Three species, infecting mammals and birds (one species) or reptiles (two species), compose this clade. The main biological features of these parasites in comparison to those of the previous clade are the lack of a collagen capsule and their infectivity to other vertebrates in addition to mammals (Table I).

***Trichinella pseudospiralis* (genotype T4)**

Trichinella pseudospiralis is a cosmopolitan species infecting both mammals and birds. Three populations, which can be distinguished on a molecular basis, have been detected in the Palaearctic, Nearctic and Australian (Tasmania) regions (Figure 5) (La Rosa *et al.*, 2001). This parasite has been found in 14 mammalian species and 13 avian species (Pozio, 2005), where the number of reports in mammals is much higher than that in birds. This is likely the result of a bias toward the examination of mammals for this parasite relative to the number of birds examined. A single human case, probably acquired in Tasmania, and three outbreaks involving 92 people, in Kamchatka, Thailand and France have been documented (Pozio and Murrell, 2006).

***Trichinella papuae* (genotype T10)**

This species has been detected in domestic sows, wild pigs (*Sus scrota*) and farmed saltwater crocodiles (*Crocodylus porosus*) of Papua New Guinea feed with flesh from wild pigs (*Sus scrota*) (Pozio and Murrell, 2006) (Figure 5). In experimental infections, this species exhibits a high reproductive capacity (RC) in crocodiles and monitor lizards (*Varanus exanthematicus*, *Caiman crocodylus*), but a very low RC in turtles and pythons (*Python molurus*, *Pelomedusa subrufa*). The discovery of a *Trichinella* species 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 (*Varanus nebulosus*) in Thailand (Khamboonruang, 1991).

***Trichinella zimbabwensis* (genotype T11)**

This species is very similar to *T. papuae* with which it shares important biological features such as the infectivity to both mammals and reptiles (*Python molurus*, *Pelomedusa subrufa*, *Varanus exanthematicus*, *Caiman crocodylus*). This species has been detected only in wild and farmed reptiles (*Crocodylus niloticus* and *Varanus niloticus*) of Africa (Zimbabwe, Mozambique and Ethiopia) (Figure 5) although experimentally it is able to infect mice, rats, hamsters, foxes (*Vulpes vulpes*), pigs and monkeys (*Papio* spp.,

Cercopithecus aethiops) (Pozio and Murrell, 2006; Pozio *et al.*, 2007). When first discovered in 1995, *T. zimbabwensis* larvae were detected in 256 (39.5%) of farmed Nile crocodiles (*Crocodylus niloticus*) from 18 (62.1%) Zimbabwe crocodile farms. Human infections have yet to be reported.

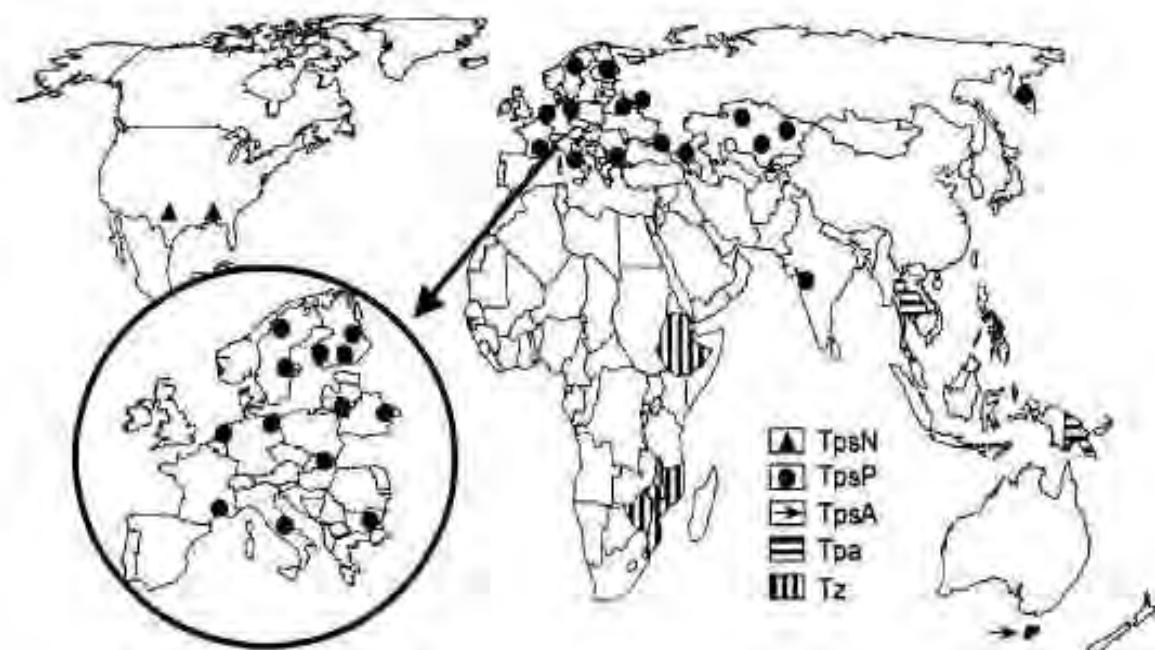


Figure 5 - Distribution of *Trichinella pseudospiralis* (from Nearctic [TpsN], Palearctic [TpsP] and Australian [TpsA] regions), *Trichinella papuae* (Tpa) and of *Trichinella zimbabwensis* (Tz)

(Redrawn from www.iss.it/site/Trichinella/index.asp)

PHYLOGENY

The phylogeny of species and genotypes of the genus *Trichinella* was recently studied using the variation in three genes (nuclear SSU rDNA and ITS2; mitochondrial LSU rDNA and COI DNA) (Zarlenga *et al.*, 2006). The results showed that the extant species of *Trichinella* diversified within the last 10 to 20 million years, which coincided with the divergence of Suidae from the Tayassuidae in the Lower Miocene. *Trichinella spiralis*, which anecdotally has been considered a crown species due to its strong association with domestic pigs, synanthropic rats and humans, appears to be the oldest of the encapsulated clade (Zarlenga *et al.*, 2006).

BIOLOGY

Biogeography

The biogeographic history of the encapsulated species and genotypes of *Trichinella* suggests an origin of this clade in Eastern Asia. The introduction of *T. nelsoni*, *Trichinella* T8 and *T. britovi* into Africa are the result of 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.*, 2005a). 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. 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.

Sympatry

There are many instances of sympatry between the *Trichinella* species. Some examples are: *T. nativa* (and the closely related genotype *Trichinella* 16) and *T. murrelli* in the USA and Canada; *T. nativa* and *T. britovi* in Europe and Asia; and *T. nelsoni* and *Trichinella* T8 in South Africa. The distribution area of *T. spiralis*, 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. The non-encapsulated species *T. pseudospiralis* 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; *T. britovi* with *T. pseudospiralis*). Multiple species infections suggest, then, that high levels of exposure exist in some circumstances (Pozio and Murrell, 2006).

The 'free-living' stage

An important adaptation of the parasite, which facilitates its transmission, is the physiological mechanism utilised 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 favours its survival in decaying tissues, probably longer for the encapsulated than for the non-encapsulated species. The persistence of larvae in putrefying flesh is, of course, also determined by the environment: high humidity and low temperatures favour survival of encapsulated larvae 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 (Pozio and Murrell, 2006).

Resistance to freezing

The importance of this stage in the natural cycle of the parasite is underscored by the survival of muscle larvae in frozen muscles of carrion for one (*T. britovi*) or more years (*T. nativa* and *Trichinella* T6) (Pozio and Murrell, 2006). The anaerobic metabolism favouring 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 (Table II). At lower temperatures, the survival time is quickly reduced. It is important to stress that the survival of muscle larvae to freezing occurs mainly when these larvae parasitise striated muscles of carnivores (bears, wolves [*Canis lupus*], foxes [*Vulpes* spp.], etc.), whereas the survival time to freezing is strongly reduced to a few days or weeks when muscle larvae of the same strain parasitise other mammalian hosts such as swine or rodents.

EPIDEMIOLOGY

Although *T. spiralis* was first discovered in domestic animals, the other species of this genus are primarily parasites of wildlife. When humans fail in the proper management of domestic animals and wildlife, some *Trichinella* species (*T. spiralis*, *T. britovi*, *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis*) are transmitted from the sylvatic environment to the domestic one, sometimes through synanthropic animals.

In addition, some species (e.g. *T. spiralis*) can transfer in a reversible path from domestic animals to wildlife.

Table II - Resistance to freezing of different species and genotypes of *Trichinella* in muscles of several hosts according to different experiments

<i>Trichinella</i> species or genotype	Host	Temperature below zero	Time of survival to freezing
<i>T. nativa</i>	Arctic fox (<i>Alopex lagopus</i>)	-18°C	4 years
	Arctic fox (<i>Alopex lagopus</i>)	-18°C	12 to 14 months
	Arctic fox (<i>Alopex lagopus</i>)	-18°C	3 years
	Polar bear (<i>Ursus maritimus</i>)	-18°C	5 years
	Polar bear (<i>Ursus maritimus</i>)	-18°C	6 months
	Raccoon (<i>Procyon lotor</i>)	-18°C	9 months
	Laboratory mice	-10°C	8 to 22 days
<i>Trichinella</i> T6	Grizzly bear (<i>Ursus arctos horribilis</i>)	-6 5°C to -20°C	34 months
	Laboratory mice	-10°C	5 to 13 days
<i>T. britovi</i>	Wolf (<i>Canis lupus</i>)	-20°C	6 months
	Fox (<i>Vulpes vulpes</i>)	-15°C	11 months
	Wild boar (<i>Sus scrofa</i>)	-20°C	3 weeks
<i>Trichinella</i> T5	Laboratory mice	-10°C	4 to 7 days
	Laboratory mice	-10°C	12 h to 108 h
<i>T. spiralis</i>	Laboratory mice	-10°C	12 h to 48 h
<i>T. nelsoni</i>	Laboratory mice	-10°C	No survival
<i>T. pseudospiralis</i>	Laboratory mice	-10°C	No survival

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 in marine mammals and birds.

Mammals

Although natural *Trichinella* spp. 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 play an important role in the natural 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 (*Sus scrota*) (49% and 47%, respectively), with some differences related to the habitat characteristic and human behaviour at the country level, whereas the same two species occur with quite different frequencies in red foxes (*Vulpes vulpes*) and other sylvatic carnivores (7% and 92%, respectively). A similar pattern occurs in North America, where the domestic species (*T. spiralis*) and the sylvatic species (*T. murrelli*,

T. nativa and the genotype *Trichinella* T6), have been detected in 12% and in 87% of sylvatic carnivores, respectively (Pozio and Murrell, 2006).

The host range for the sylvatic cycle is determined by the potential host species available in the different regions. Because swine are not a suitable host for *T. nativa*, *T. murrelli* and *Trichinella* T6, these animals do not play a role as a reservoir for these pathogens in the regions of Eurasia and North America.

Among primates, only humans have been naturally infected with *Trichinella*. Evidence suggests that the infection of horses (Perissodactyla), rodents (especially rats) and Edentata (armadillos [*Chaetophractus villosus*]) 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, and in opossums (*Didelphis virginiana*) in North America, where the parasite is widespread among placental mammals. In spite of the potential broad host spectrum of *Trichinella* spp., the greatest biomass of these parasites occurs amongst the Carnivora and the artiodactylid family Suidae (mainly domestic pigs, different races of feral pigs, wild boars [*Sus scrofa*], bush pigs [*Potamochoerus* spp.], and warthogs [*Phacochoerus aethiopicus*]) (Pozio and Murrell, 2006). Natural infections in other artiodactylid species, both sylvatic (reindeer [*Rangifer tarandus*] and roe deer [*Capreolus capreolus*]) and domestic (sheep, goat, and cow) are sporadic and need further investigation, since there is no scientific report showing the detection of nematode larvae, identified by molecular analysis, as belonging to the genus *Trichinella*, in these domestic and wild herbivores (Takahashi *et al*, 2000; Pozio, 2001a). Experimental infections have shown that cattle, sheep and goats can acquire only a transient infection. It could be argued that sheep, goats and cattle could acquire *Trichinella* infection, when they are bred in areas that are highly endemic for this parasite in pigs, but this must be supported by further epidemiological investigations.

The role of micromammals (mainly rodents and insectivores) in the sylvatic cycle is still uncertain due to the usually low number of infections in their populations and the lack of sufficient surveys on a large number of these mammals (Pozio and Murrell, 2006). 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.

Birds

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, Ciconiiformes and Passeriformes (Pozio, 2005).

Reptiles

Only three species of reptiles have been detected naturally infected in Africa (Nile crocodile [*Crocodylus niloticus*], Nile monitor lizard [*Varanus niloticus*]) and in Papua New Guinea (saltwater crocodile [*Crocodylus porosus*]) (Pozio *et al.*, 2005b; 2007). In addition, meat from a clouded monitor (*Varanus nebulosus*) and a turtle has been implicated as the sources of infection of two trichinellosis outbreaks, which occurred in Thailand (Kambounruang, 1991).

Amphibians and fish

There is a single report of an experimental infection of amphibians (frogs and axolotls) with *T. spiralis*, in which it was observed that the development of larvae in the muscles was incomplete. Attempts to infect fish with *T. spiralis*, *T. britovi*, *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis* have also failed (Pozio and Murrell, 2006).

Invertebrates

The role of invertebrates as paratenic hosts of *Trichinella* species has been investigated in adult and larval stages of several species of insects and amphipods. The survival of *Trichinella* larvae in these paratenic hosts is under the influence of the environmental temperature, lower the temperature, longer the survival. These results suggest that invertebrates play a very limited role, if any, in the dissemination of *Trichinella* larvae in nature (Pozio and Murrell, 2006).

Relationship between the sylvatic cycle and humans

The sylvatic cycle may also be influenced by human actions such as the common habit of hunters of leaving animal carcasses in the field after skinning, or removing and discarding the entrails, which increases the probability of transmission to new hosts (Pozio and Murrell, 2006). In addition, epidemiological surveys carried out in Europe, North America and Africa have shown that *Trichinella* is more prevalent in wild animals living in natural or undisturbed areas such as parks and forests, protected areas and mountain regions (Pozio, 1998) where human activity has not strongly changed the habitat. A possible explanation is that in undisturbed areas on these three continents, hosts of *Trichinella* have predominantly scavenger and cannibalistic behaviours, whereas in habitats where human activity is high, the number of potential hosts is reduced or even when not, animals have access to alternative food resources resulting from human activity (e.g. synanthropic and domestic animals predation or scavenging on carcasses, garbage). This is the case for several European countries, where the sylvatic cycle is nearly absent in areas characterised by a strong human influence, but present in mountain and/or protected areas (Pozio, 1998).

Human caused perturbations of the sylvatic environment may also affect the epidemiological patterns of the sylvatic cycle. The increase in forests and fallow land, concomitant with a decrease in farms, in Europe over the past 100 years has facilitated a great expansion of wild boar (*Sus scrofa*) populations in some regions and increased transmission of *Trichinella* to animals and humans.

The domestic cycle and the most important risk factors

This cycle occurs where there are high risks farming practices such as:

- 1) the intentional feeding of food waste containing pork scraps (Gamble *et al.*, 2000), or intentional or unintentional exposure to carcasses of dead swine, or wildlife; these risks are usually encountered in free-range pasturing (Pozio and Murrell, 2006)
- 2) pigs allowed to scavenge on garbage dumps
- 3) feeding of wild game carcasses or scraps from hunting
- 4) horses fed with pork scraps or with carcasses of fur animals
- 5) sled dogs fed with carcasses of other dogs or of game in the arctic
- 6) the use of carcasses of slaughtered fur animals as food for other fur-animals present at the farm
- 7) the use of meat of slaughtered crocodiles to feed other farmed crocodiles as observed in Zimbabwe
- 8) the use of pork scraps to feed young crocodiles as demonstrated in Papua New Guinea.

The most common etiological agent of the domestic cycle is *T. spiralis*, which is very well adapted to swine and synanthropic hosts, in which it exhibits a very high reproductive rate without inducing serious pathology except in very high level of infection. Occasionally, *T. britovi* has been 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 and Murrell, 2006). *Trichinella pseudospiralis* has been also transmitted to domestic pigs and rats on farms in Kamchatka, Russia and Slovak Republic. Both *T. papuae* and *T. zimbabwensis* are transmitted to saltwater crocodiles (*Crocodylus porosus*) in the domestic habitat and to Nile crocodiles (*Crocodylus niloticus*) at the farm level, respectively.

The role of rats

In the domestic habitat, where *Trichinella* is circulating among domestic animals, the brown rat (*Rattus norvegicus*) is frequently found to be infected with *T. spiralis* and infrequently with *T. britovi* or *T. pseudospiralis* (Pozio and Zarlenga, 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. In the 19th Century, Leuckart proposed a 'Rat Theory', which implicated rats as a major reservoir of *T. spiralis* infection for domestic pigs. In 1871, Zenker, on the other hand, suggested that the infection in rats was merely an indicator of *Trichinella* 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.

There are no reports showing *T. spiralis* infection in brown rats (*Rattus norvegicus*) where pig populations unequivocally have been found to be negative or in farms where pigs do not exist. 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 programme. 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 neighbouring farms and villages. Evidence for this was reported by in some swine herds of the Atlantic provinces of Canada. In addition, the use of rat pesticides can actually favour 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 USA 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 (Pozio and Murrell, 2006).

***Trichinella* sp. infections in horses**

Between 1975 and 2005, human outbreaks of trichinellosis have occurred in France (2,296 infected people in eight outbreaks) and Italy (1,038 infected people in seven outbreaks), from the consumption of meat from individual horses imported from Canada, the Former Yugoslavia, Mexico, Poland, and the USA (Pozio and Murrell, 2006). The failure to detect infection in horses, which were the source of infection for humans, was probably due to the examination of an inadequate quantity of muscle tissue (i.e. 1.0 g). Since then, the requirement for 5 g to 100 g for testing was instituted in countries of the European Union (EU), and *Trichinella* larvae have been detected in a total of 18 horses bred in the Former Yugoslavia, Mexico, Poland, and Romania (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 seven million horses have been consumed in the EU; 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* infection in pigs and/or wildlife suggests that there is 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. 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 more evidence on the epidemiology of equine trichinellosis is needed. Although considered herbivores, 32% of horses tested ate meat when offered. 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 (Djordjevic *et al.*, 2003; Cuperlovic *et al.*, 2005).

***Trichinella* sp, infections in sea mammals**

Trichinella nativa in marine mammals has a circumpolar arctic distribution and a narrow range of host species. It is commonly found in polar bears (*Ursus maritimus*), and increasingly in walrus (*Odobenus rosmarus*) where it presents a significant zoonotic hazard. This has resulted in the implementation of food safety programmes in some arctic communities to test harvested walrus meat for *Trichinella* larvae prior to consumption (Proulx *et al.*, 2002). *Trichinella* sp. has been reported infrequently in bearded seals (*Erignathus barbatus*) and ringed seals (*Phoca hispida*), and was once observed in a Beluga whale (*Delphinapterus leucas*). Arctic carnivores such as polar bears (*Ursus maritimus*), arctic foxes (*Alopex lagopus*) and domestic dogs have a high prevalence of *T. nativa* in the arctic and the carcasses of these animals are frequently deposited in the ocean. Scavenging of these carcasses by walrus probably occurs, but may not account for the high prevalence the parasite seen in this host species. Predation, carrion feeding and cannibalism have been documented for walrus and a sylvatic cycle similar to that of bears may exist in walrus populations. Seals and whales are likely infected through infrequent exposure to infected carcasses, either directly by scavenging or indirectly by consuming amphipods that have fed on infected carcasses.

TRICHINELLOSIS IN HUMANS

Trichinella infections in humans are related to cultural food practices, which include dishes based on raw or undercooked meat of different animal origins (from carnivorous and omnivorous mammals, birds and reptiles) (Table III). The presence of the parasite in domestic and or wild animals is not a sufficient risk in itself for transmission to human populations. For example, in most of European countries *Trichinella* spp. frequently occur in wildlife and in some countries it occurs also in the domestic pig populations; however, no infection has been documented in humans of these countries (e.g. Finland, Sweden, Switzerland), probably due to the practice of eating only well cooked meat (Poizio, 1998). Similarly, in most African countries south of the Sahara, human infection is seldom documented in spite of the presence of sylvatic

Trichinella species, because about a third of all African populations are of the Bantu ethnic group, which rarely consumes meat.

In Muslim countries, the number of human infections with *Trichinella* sp. is very limited due to the Muslim laws, which forbid the consumption of pork and meat of carnivorous animals (i.e. the main host of *Trichinella* sp.). Unfortunately, in these countries, the lack of any control at slaughtering or on game has resulted in a number of outbreaks (Poizio and Murrell, 2006).

The average yearly incidence of the disease in humans worldwide is probably close to ten-thousand cases with a mortality rate of about 0.2%; however, the number of infections is underreported in many countries due to the lack of appropriate serological tests and a lack of knowledge of the disease on the part of physicians. Since human trichinellosis is quite infrequent in many EU countries, many local physicians are not familiar with the disease and experience problems in diagnosing it. Delays in diagnosis and treatment favour the establishment of larvae in muscles and the development of a collagen capsule, which render the larvae resistant to drugs (Dupouy-Camet *et al.*, 2002).

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

Table III - Animals, besides domestic pigs, which were the source of trichinellosis

Meat origin	Continent or country^(b)
Wild boar (<i>Sus scrofa</i>)	Asia, Europe, North and South America
Feral pig (<i>Sus scrofa</i>)	Papua New Guinea, Thailand
Warthog (<i>Phacochoerus aethiopicus</i>)	Ethiopia Senegal, Tanzania
Bush pig (<i>Potamochoerus</i> spp.)	Kenya
Horse	France, Italy
Walrus (<i>Odobenus rosmarus</i>)	Canada and Greenland
Black bear (<i>Ursus americanus</i>)	Canada, United States of America
Brown bear (<i>Ursus arctos</i>)	Alaska, Canada, the People's Republic of China, Bulgaria, Kazakhstan, Romania Russia, Siberia
Japanese black bear (<i>Ursus thibetanus</i>)	Japan
Polar bear (<i>Ursus maritimus</i>)	Alaska, Canada, Greenland Siberia
Cougar (<i>Puma concolor</i>)	United States of America, Argentina
Badger (<i>Meles meles</i>)	Korea, Bulgaria, Russia
Red fox (<i>Vulpes vulpes</i>)	Italy
Jackal (<i>Canis aureus</i>)	Thailand, Algeria
Mink (<i>Mustela lutreola</i>)	Switzerland
Dog	The People's Republic of China, Greenland Russia, Slovak Republic, Switzerland
Armadillo (<i>Chaetophractus villosus</i>)	Argentina
Squirrel	Thailand
Monitor lizard (<i>Varanus nebulosus</i>)	Thailand
Turtle	Thailand
Beef ^(a) , mutton ^(a) , goat ^(a)	The People's Republic of China

a) These reports need further investigation before to be confirmed

b) In many cases, infected meat was imported from other countries where it caused outbreaks of trichinellosis

Trichinellosis is emerging in some urban areas in the People's Republic of China where affluence has increased the demand for pork, particularly in dishes that traditionally may not be well-cooked (meat dumplings). The migration of persons from Eastern Europe to the 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 behaviour has resulted in several human outbreaks of trichinellosis in Germany, Italy and the United Kingdom (UK) (Pozio and Zarlenga, 2006).

Human migration may result 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 USA and Israel, where the control for *Trichinella* sp. infection in domestic pigs and wild boars (*Sus scrofa*) is not compulsory, are especially at risk (Pozio and Murrell, 2006).

The increasing number of international travellers has resulted in many reports of tourists who acquired *Trichinella* infections while travelling 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 (Pozio and Murrell, 2006). For example, trichinellosis in travellers has occurred after the consumption of pork from a warthog (*Phacochoerus aethiopicus*) in Africa, bear meat in Canada and Greenland, pork from domestic pigs in the People's Republic of China, Egypt, Indonesia (Bali Island), Laos and Malaysia, and pork from wild boar (*Sus scrofa*) meat in Turkey and Algeria (Pozio and Murrell, 2006).

TRICHINELLA SPP. INFECTIONS IN ANIMALS AND HUMANS BY CONTINENT AND COUNTRY

Africa

Algeria

Six small human outbreaks occurred among French expatriates from the consumption of pork from a domestic pig and wild boars (*Sus scrofa*), and a single case in an Algerian man was due to the consumption of meat from a jackal (*Canis aureus*) infected with *T. britovi* (Pozio and Murrell, 2006). These reports suggest the occurrence of *T. britovi* infection among wildlife of Algeria, although no surveys have been conducted.

Congo DR

Only a single report of *Trichinella* sp. larvae in muscles of a spotted hyena (*Crocuta crocuta*) has been reported (Pozio and Murrell, 2006). This infection was not documented in domestic animals.

Egypt

A human outbreak of trichinellosis occurred among French tourists after the consumption of pork from a domestic pig in 1975. At that time, a prevalence of 4.5% was detected in domestic pigs slaughtered at the Cairo abattoir. In 2000, the prevalence dropped to 1.7%. High prevalence rates of infection (up to 13.3%) have been detected among synanthropic rats of Alexandria abattoirs. *Trichinella spiralis* has been documented in two stray dogs and in domestic pigs. *Trichinella* sp. larvae have been also detected in wolves [*Canis lupus*] from Sinai, which may have been *T. britovi*. These epidemiological data suggest that both a domestic and a sylvatic cycle occurs in Egypt (Pozio and Murrell, 2006).

Ethiopia

Trichinellosis in humans has been documented for the consumption of pork from warthogs (*Phacochoerus aethiopicus*) (Pozio and Murrell, 2006). Non-encapsulated larvae of *Trichinella* sp., probably *T. zimbabwensis*, have been detected in a farmed Nile crocodile (*Crocodylus niloticus*) from Lake Abaja (Pozio *et al.*, 2007).

Guinea

Trichinella britovi infection has been documented in Viverridae from the Fouta Djallon Massif. No other data are available for either humans or domestic animals (Pozio and Murrell, 2006).

Kenya

There are a number of reports on *Trichinella* infections in humans and in wildlife, since 1961. Less than 50 cases with one death were documented in five outbreaks, which occurred between 1959 and 1987 and in a single case of a Japanese tourist. The main source of infection was pork from Red river hogs (*Potamochoerus porcus*). The etiological agent, *T. nelsoni*, has been detected in a wide spectrum of carnivorous mammals. The domestic cycle has never been documented (Pozio and Murrell, 2006).

Mozambique

The only report is the detection of *T. zimbabwensis* in 20% of wild Nile crocodiles (*Crocodylus niloticus*) of the lake Cahora Bassa (Pozio *et al.*, 2007),

Namibia

The genotype *Trichinella* T8 has been detected in a lion (*Panthera leo*) of the Etosha park (Pozio and Murrell, 2006). This infection has been never documented in humans and domestic animals.

Senegal

An outbreak of human trichinellosis occurred among nine Europeans in Dakar from the consumption of pork from a warthog (*Phacochoerus aethiopicus*). *Trichinella* sp. infection, probably *T. britovi*, has been detected in jackals (*Canis aureus*) and warthogs [*Phacochoerus aethiopicus*] (Pozio and Murrell, 2006); there is no report of infection in domestic animals.

South Africa

Most of the epidemiological investigations have been carried out in wildlife of the Kruger National Park, where both *T. nelsoni* and *Trichinella* T8 have been documented in a spotted hyena (*Crocuta crocuta*) and in a lion (*Panthera leo*) (Pozio and Murrell, 2006). There is also a single report of *Trichinella* sp. infection in one wild rodent. This infection has been never documented in humans and domestic animals.

Tanzania UR

A human outbreak, with some deaths, occurred from the consumption of a warthog (*Phacochoerus aethiopicus*). *Trichinella nelsoni* is widespread among wild carnivores (Pozio and Murrell, 2006). This infection has never been documented in domestic animals.

Tunisia

In Tunisia, encapsulated larvae of *Trichinella* sp. (probably *T. britovi*) have been detected in sylvatic carnivores (genets [*Genetta genetta*], jackal [*Canis aureus*] and mongoose [*Herpestes ichneumon*]) (Fassbender and Mayer, 1974). This infection has been never documented in humans and domestic animals.

Zimbabwe

Trichinella zimbabwensis has been documented in a great number of farmed Nile crocodiles (*Crocodylus niloticus*) (Pozio *et al.*, 2002) and in wild Nile monitors (*Varanus niloticus*) (Pozio *et al.*, 2007). This infection has been never documented in humans or domestic animals.

America

Argentina

Trichinella spiralis is widespread in domestic and wild animals of Argentina and human outbreaks occur every year (Ribicich *et al.*, 2005). The central provinces (Buenos Aires, Cordoba and Santa Fe) are those with the highest prevalence in domestic pigs. Recently, the number of human infections increased up to one thousand per year. This parasite has been detected also in wild boars (*Sus scrofa*), foxes (*Pseudalopex gracilis*) and pumas (*Puma concolor*) and in synanthropic brown rats (*Rattus norvegicus*) and armadillos [*Chaetophractus villosus*] (Ribicich *et al.*, 2005).

Bolivia

Trichinella sp. larvae have never been reported in animals or humans of this country; however, anti-*Trichinella* antibodies have been detected in sera of domestic pigs from different country regions (Bjorland *et al.*, 1993; Brown *et al.*, 1996) and in the sera of three asymptomatic people.

Canada

Trichinella spp. is widespread in Canadian wildlife (both terrestrial and marine), but varies considerably from one province to another. Most human infections occur from the consumption of game meat from bears and walrus (*Odobenus rosmarus*). In the last ten years, there has been no documented report of *Trichinella* sp. infection in domestic pigs (Appleyard *et al.*, 2002). *Trichinella nativa* and *Trichinella* T6 are predominant in wildlife, whereas *T. murrelli* has been detected only in the Vancouver area close to the border with the USA. *Trichinella spiralis* has been detected in domestic pigs and occasionally in wildlife. Non-encapsulated larvae have been detected in birds.

Chile

Trichinella spiralis is widespread in domestic and wild animals and human outbreaks occur every year involving hundred of people. The main source of infection is pork from domestic pigs, which have a prevalence from 0% to 0.6%. A single case of trichinellosis has been recently documented for the consumption of pork from a wild boar (*Sus scrofa*). *Trichinella spiralis* has been frequently detected in synanthropic rats, in cats and dogs (Ortega-Pierres *et al.*, 2000).

Mexico

Although *T. spiralis* infections frequently occur in domestic pigs and humans, there is no information on the presence of this parasite in wildlife (Ortega-Pierres *et al.*, 2000). *Trichinella* sp. infection has been

detected in domestic and stray dogs, in cats and in synanthropic rats living nearby foci of infection in domestic pigs, *Trichinella spiralis* has been also detected in slaughtered horses in the State of Mexico.

United States of America

Trichinella spp. infections are widespread in wildlife, but vary considerably from one state to another. Most human infections occur from the consumption of game meat from black bears (*Ursus americanus*) or other game animals and very few cases are now related to pork from domestic pigs (Roy *et al.*, 2003). The domestic cycle is very rare. *Trichinella murrelli* is the main etiological agent of infection in wildlife, although *T. spiralis* is also transmitted among wildlife, evidence of the existence of the domestic cycle in the past. The freeze resistant species *T. nativa* and *Trichinella* T6 are present in wildlife of states close to the Canadian border; however, *Trichinella* T6 has been also detected in wildlife of the Rocky Mountains and Appalachians regions (Pozio and Murrell, 2006).

Asia

Afghanistan

Trichinella sp. infection has been detected in many wildlife species (e.g. lynx [*Lynx lynx*], wolf [*Canis lupus*], jackal [*Canis aureus*], red fox [*Vulpes vulpes*], mongoose [*Herpestes ichneumon*]). No infection has been documented in humans and domestic animals.

Cambodia

The only available information on this zoonosis is the detection of anti-*Trichinella* antibodies in a rural population (Pozio, 2001b).

The People's Republic of China

Trichinellosis is one of the most important foodborne parasitic zoonoses of the People's Republic of China and many outbreaks with deaths are reported each year. Most of the outbreaks occur in ten provinces, including Yunnan, Henan, Tibet, Hubei, Sichuan, Guangxi, Jilin, Liaoning, Helongjiang, Neimonggol and Jiangxi, where the domestic cycle occurs, with swine prevalence of *T. spiralis* up to 50%. The most important source of infection is pork from domestic pigs, but infections are also related to the consumption of bear, dog and goat meat (Takahashi *et al.*, 2000; Liu and Boireau, 2002). *Trichinella nativa* has been detected in dogs from northern regions of the People's Republic of China. The 'Western Region Development of China' strategy implemented in the 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 the central People's Republic of 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. This has led to a dramatic increase in the size of the human population at risk for trichinellosis in the western areas of the People's Republic of China (Wang *et al.*, 2006).

India

Trichinella infection has rarely been documented in India. A few reports showed the presence of encapsulated larvae of *Trichinella* sp. in domestic cats, in a wild toddy cat (*Paradoxurus hermaphroditus*), in a wild civet cat (*Viverricula indica*), and in domestic pigs. The non-encapsulated species *T. pseudospiralis* has been detected in an Indian mole rat (*Bandicota bengaiensis*) (Shaikenov and Boev,

1983). Trichinellosis in humans has been documented only once (Alipuria *et al.*, 1996), but there is probably under recognition of this infection in humans.

Indonesia

Though most people are Muslims and do not eat pork, the island of Bali is one of the few areas of the country where the majority of people are Hindus. In this island, anti- *Trichinella* antibodies have been detected by serology in 19.5% of young people. There are two reports in 1962 and 1972 on *T. spiralis* infections in domestic swine from Tapanuli, the northern region of the island of Sumatra, where the local custom of cooking or roasting meat greatly hinders the transmission to humans.

Iran Islamic Republic

Trichinella sp. infection has been documented in jackals (*Canis aureus*), red foxes (*Vulpes vulpes*), stray dogs, brown bears (*Ursus arctos*), wild cats (*Felis silvestris*) and wild boars (*Sus scrofa*) of the Caspian region. Both *T. spiralis* and *T. britovi* have been documented (Shaikenov and Boev, 1983). A single human infection from the consumption of pork from a wild boar (*Sus scrofa*) was also reported.

Israel

Trichinella sp. (most probably *T. britovi*) infection has been documented in wild boars (*Sus scrofa*) and in six human outbreaks, mostly in the Christian Arab population and in immigrants from Thailand, from the consumption of pork from wild boars (*Sus scrofa*) (Shimshony, 1997; Pozio and Murrell, 2006).

Japan

Both *T. nativa* and *Trichinella* T9 have been identified in red foxes (*Vulpes vulpes*), raccoon dogs (*Nyctereutes procyonoides*), Japanese black bears (*Ursus thibetanus*) and in a domestic dog. Trichinellosis in humans occurs sporadically from the consumption of game meat (Takahashi *et al.*, 2000).

Kazakhstan

Trichinella nativa and *T. britovi* circulate among red and corsac foxes (*Vulpes vulpes* and *Vulpes corsac*), wolves (*Canis lupus*), jackals (*Canis aureus*), martens (*Martes martes*), wild cats (*Felis silvestris*), lynxes (*Lynx lynx*) and wild boars (*Sus scrofa*). *Trichinella pseudospiralis* has been documented in a corsac fox (*Vulpes corsac*), in crows (*Corvus frugilegus*), and in an eagle (*Aquila rapax*) (Shaikenov and Boev, 1983). Trichinellosis in humans have been documented for the consumption of pork from wild boars (*Sus scrofa*).

Korea Republic (South)

Trichinellosis in humans has been documented for the consumption of meat from a badger (*Meles meles*) infected with *T. spiralis*. This *Trichinella* species has also been found in a wild boar (*Sus scrofa*) (Pozio and Murrell, 2006).

Kyrgyzstan

Trichinella sp. infection has been documented in wildlife (Shaikenov and Boev, 1983).

Laos

Trichinella spiralis should be quite common in domestic pigs; outbreaks have been documented in humans for the consumption of pork from domestic pigs (Sayasone *et al.*, 2006). *Trichinella spiralis* has been documented in both a human biopsy and in pork from a domestic pig.

Lebanon

Large outbreaks have occurred in Christian villages from the consumption of pork from wild boars (*Sus scrofa*) (Olaison and Ljungstrom, 1992).

Malaysia

There is no report of *Trichinella* infection in animals or humans of this country; however, there is evidence that this parasite occurs among local swine, because 84 students and teachers in Singapore, who had visited a neighbouring Malaysian island in 1998, acquired trichinellosis (Pozio, 2001b).

Myanmar (Burma)

Trichinella infection occurs in domestic pigs of Myanmar (Watt *et al.*, 2000), but the prevalence of infection is unknown.

Syrian AR

Small trichinellosis outbreaks have occurred in Christian villages from the consumption of pork from wild boars (*Sus scrofa*) (Pozio and Murrell, 2006).

Tajikistan

Trichinella sp. infection has been documented in wildlife (Shaikenov and Boev, 1983).

Thailand

Trichinella spiralis infection occurs frequently in domestic animals (pigs and dogs) and humans (more than 6,000 infections since 1962, with some lethal cases), primarily in northern mountain minority tribes. Approximately 200 to 600 human infections occur annually in northern Thailand during communal feasts celebrating the Thai New Year, though few of them have been documented. This parasite sometimes occur in synanthropic rats and wild pigs (*Sus scrofa*) and has been also reported in a black bear (*Ursus thibetanus*) (Khamboonruang, 1991). A human outbreak from the consumption of pork from a wild pig (*Sus scrofa*) was reported to have been caused by *T. pseudospiralis*. Two outbreaks caused by the consumption of meat from a monitor lizard (*Varanus nebulosus*) and a turtle have been also reported, suggesting the presence of another etiological agent (probably *T. papuae*) infecting both mammals and reptiles (Khamboonruang, 1991).

Turkey

Trichinella britovi is present in wildlife in Turkey. Recently, three outbreaks of trichinellosis occurred between 2003 and 2004 from the consumption of pork from domestic pigs and wild boars in Antalya, Bursa and Izmir, involving more than 500 people (Pozio and Murrell, 2006).

Turkmenistan

Trichinella infection has been documented in wildlife (Shaikenov and Boev, 1983).

Uzbekistan

Trichinella infection has been found in wildlife (Shaikenov and Boev, 1983). A large outbreak of trichinellosis has been reported to be due to the consumption of pork from a wild boar (*Sus scrota*) (Nadzhimiddinov *et al.*, 1965).

Vietnam

A trichinellosis outbreak involving 20 people who consumed raw pork from a domestic pig has been documented in Quai To (Tuan Giao district, Dien Bien province) suggesting the occurrence of the domestic cycle in the northern areas of the country (Nhan and Van De, 2004).

Europe

Austria

The last human outbreak of trichinellosis occurred in 1970 following consumption of a pig from a family farm fed with the flesh of an infected fox (*Vulpes* spp.). Between 1952 and 1982, *Trichinella* infection was detected in 12 backyard or free-ranging pigs (Hinaidy 1978; 1983). No infected pigs reared in Austria have been detected in the last 24 years. Both *T. spiralis* and *T. britovi* infections occur in wild boars (*Sus scrofa*) and red foxes (*Vulpes vulpes*).

Azerbaijan

Trichinella infection has been documented among wildlife (Shaikenov and Boev, 1983).

Belarus

Both domestic and sylvatic cycles occur in this country. *Trichinella* sp. infection has been detected mostly in carnivores (red fox [*Vulpes vulpes*], wolf [*Canis lupus*], raccoon dog [*Nyctereutes procyonoides*], black polecat [*Mustela putorius*], marten [*Martes martes*]) but also in wild boars (*Sus scrofa*) and in yellow-necked mice (*Apodemus flavicollis*). In the 1980s, the prevalence of infection in domestic pigs was 0.0005% (Skripova *et al.*, 1992). Between 1980 and 1989, the prevalence of human infections was 0.55 per 100,000 inhabitants (Skripova and Kovchur, 1994).

Belgium

The sylvatic cycle seems to be rare in this country, but recently *T. britovi* larvae have been detected in a wild boar (*Sus scrota*) (Schynts *et al.*, 2006). One small human outbreak of trichinellosis occurred following the consumption of pork from a wild boar (*Sus scrofa*) in 1979.

Bosnia-Herzegovina

Trichinella sp. infection is common among wildlife and in free roaming and backyards pigs. Human trichinellosis occurs quite frequently from the consumption of pork from wild boars (*Sus scrofa*) and pigs (*Sus scrofa*), with a yearly incidence of 12 cases per 100,000 inhabitants in 1998 and the occurrence of more than 51 outbreaks (775 infected people) in the last 14 years with some deaths (Ravlija *et al.*, 2006).

Bulgaria

Both *T. spiralis* and *T. britovi* are widespread in wildlife and in free roaming and backyards pigs (Kurdova *et al.*, 2004). Hundreds of infections in humans occur yearly from the consumption of pork from wild boars (*Sus scrofa*) and pigs (*Sus scrofa*).

Croatia

There is a high prevalence of both *T. spiralis* and *T. britovi* in wildlife. Recently, a control programme has strongly reduced prevalence in swine from 3.6% to 0.0001%. About 30 to 50 cases of human trichinellosis are detected every year (Marinculic *et al.*, 2001).

Czech Republic

No human infection has been documented in this country in the last 50 years, nor have infected pigs been detected. *Trichinella britovi* has been detected in red fox (*Vulpes vulpes*) and wild boar (*Sus scrofa*).

Denmark

There is no report of *Trichinella* sp. infection in pigs since 1930. Occasionally, single nematode larvae have been detected after HCl-pepsin digestion at the slaughterhouse, however none have been identified as belonging to the genus *Trichinella*. In 1996, a prevalence of 0.1% has been detected in red foxes (*Vulpes vulpes*) from a small area of Jutland, but the few recovered larvae were not identified at the species level (Enemark *et al.*, 2000).

Estonia

There is a high prevalence of infection of both *T. nativa* and *T. britovi* in wildlife. However, infection in domestic pigs and humans is rare. From 1992 to 1999, *T. britovi* infection has been documented in six backyard pigs from small farms, one of which was the source of infection for three patients (Jarvis *et al.*, 2001).

Finland

The domestic cycle is still endemic in this country although the yearly incidence in swine is very low. The number of *Trichinella*-positive swine and swine herds increased from the beginning of the 1980s until mid-1990s. Recently, there has been a reduction in the number of cases, and during the last few years the condemnation rate has been around 0.0001%. A high prevalence (up to 53%) of *T. spiralis*, *T. nativa*, *T. britovi*, and *T. pseudospiralis* has been detected in wild boars (*Sus scrofa*), lynxes (*Lynx lynx*), wolves (*Canis lupus*), brown bears (*Ursus arctos*), raccoon dogs (*Nyctereutes procyonoides*), red foxes (*Vulpes vulpes*) and badgers (*Meles meles*) (Oivanen *et al.*, 2002). Infections in humans have not been documented in the last 50 years.

France

Both *T. spiralis* and *T. britovi* circulate in red foxes (*Vulpes vulpes*) and wild boars (*Sus scrofa*). In 2004, *T. britovi* was detected in free-ranging pigs and wildlife of a remote area of Corsica. Human infections occur yearly from the consumption of pork from wild boars (*Sus scrofa*), but the most important source of infection for the French population has been horse meat imported from abroad (2,296 cases from 1975 to 1999) (Boireau *et al.*, 2000). The etiological agent of a human outbreak from the consumption of pork from a wild boar in the Camargue was identified as *T. pseudospiralis* (Ranque *et al.*, 2000).

Georgia

Trichinella sp. infection has been documented in 1970 in stone martens (*Martes foina*), jackals (*Canis aureus*), red foxes (*Vulpes vulpes*) and corsac foxes (*Vulpes corsac*) and domestic cats as well as in humans (Kurashvili *et al.*, 1970). Recently, trichinellosis in humans is increasing with hundreds of infections per year.

Germany

Today, only the sylvatic cycle (*T. spiralis*, *T. britovi*, *T. pseudospiralis*) in wildlife exists, with a prevalence of 0.08% to 0.22% for red foxes (*Vulpes vulpes*), 5% for raccoon dogs (*Nyctereutes procyonoides*), and 0.009% for wild boar (*Sus scrofa*). Sporadic infections occur among people from the consumption of pork from wild boars (*Sus scrofa*) and backyard pigs the infection of which originate always from the sylvatic cycle (Liftman *et al.*, 2006).

Greece

Trichinella sp. infection occurs only in wildlife, stray dogs and seldom in backyard or free-ranging pigs. Very few human cases have been documented since World War II, the last outbreak occurred in 1982 (Sotiraki *et al.*, 2001).

Hungary

Today, only the sylvatic cycle (*T. britovi*) occurs among red foxes (*Vulpes vulpes*) (Sreter *et al.*, 2003) and seldom in backyard or free-ranging pigs. Human trichinellosis has not been documented since the 1980s.

Ireland

Trichinella spiralis infection occurs only in red foxes (*Vulpes vulpes*). No infection has been documented in domestic pigs or humans in the last 38 years (Rafter *et al.*, 2005).

Italy

Only the sylvatic cycle (*T. britovi*) occurs among wildlife (e.g. red fox [*Vulpes vulpes*], wolf [*Canis lupus*], badger [*Meles meles*], marten [*Martes martes*], wild boar [*Sus scrofa*]) and the parasite is seldom transmitted to backyard or free-ranging pigs. In the last 58 years, 13 backyard pigs were detected positive at the abattoir or were the source of infection for humans. *Trichinella pseudospiralis* has been documented once in two birds. Infections in humans have been documented from the consumption of pork from wild boar (*Sus scrofa*) or backyard and free-ranging pigs and meat from the red fox (*Vulpes vulpes*) (Pozio *et al.*, 2001); however, the most important source of infection was horse meat imported from abroad (1,038 cases between 1975 and 2005) (Pozio and Murrell, 2006).

Latvia

Both the domestic and sylvatic cycles have been documented. A high prevalence of the infection was found in wildlife (e.g. in 16% to 60% of red foxes [*Vulpes vulpes*]), where *T. spiralis*, *T. nativa* and *T. britovi* have been detected. A significant increase of *T. spiralis* infection was observed in domestic pigs from 1984 to 1990. Human trichinellosis also increased dramatically during late 1980s and early 1990s, with hundreds of human infections and fatal cases. This was primarily caused by pork from backyard or free-ranging pigs and wild boars (*Sus scrofa*) (Viksna *et al.*, 2002).

Lithuania

Both the domestic and sylvatic cycles have been documented. Significant increases in *Trichinella* sp. infection in pigs, from 0.0027% during 1981-1985 to 0.1% in 1993 were documented. Today, the prevalence in domestic pigs is 0.001%. There is a high prevalence of infection (*T. spiralis*, *T. britovi*, *T. nativa*) in red foxes (*Vulpes vulpes*), raccoon dogs (*Nyctereutes procyonoides*), wolves (*Canis lupus*), martens (*Martes martes*) and wild boars (*Sus scrofa*). Human trichinellosis increased dramatically during the late 1980s and early 1990s, with hundreds of human infections and at least 14 fatal cases. It was primarily caused by pork, mostly from backyard pigs not inspected for *Trichinella*. The number of human infections significantly decreased in recent years to an average of 30 to 50 cases per year (Bartuliene *et al.*, 2005).

Macedonia

Trichinella britovi infections occur among wildlife and free roaming pigs. Human infections from the consumption of pork from wild boars (*Sus scrofa*), free-roaming and backyard pigs are sporadic.

The Netherlands

The sylvatic cycle (*T. spiralis*, *T. britovi* and *T. pseudospiralis*) occurs with a low prevalence in the red fox (*Vulpes vulpes*) and wild boar (*Sus scrofa*) populations. *Trichinella* sp. infection has not occurred in the Dutch pig population since 1979 (van der Giessen *et al.*, 2001). There are no documented cases of human trichinellosis in The Netherlands from the consumption of pork or pork products from pigs reared in this country, nor for the consumption of game meat.

Norway

Both *T. nativa* and *T. britovi* circulate among wildlife (4.8% in red foxes [*Vulpes vulpes*], 11% in minks [*Mustela lutreola*]) (Davidson *et al.*, 2006). Infections have been also documented in dogs and cats. No human case was documented since 1940.

Poland

Both the domestic and the sylvatic cycles occur. The yearly incidence of *T. spiralis* in domestic pigs ranges from 0.0032% to 0.00036% (i.e. from 66 to 465 infected pigs per year) (Ramisz *et al.*, 2001). In wildlife, a high prevalence of *T. spiralis* and *T. britovi* has been detected in the red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*) and wild boar (*Sus scrofa*). Prevalence in wild boars (*Sus scrofa*) is reported to be 0.25%. Human trichinellosis due to the consumption of backyard pigs occurs every year (from 15 to about 100 cases/year) (Ramisz *et al.*, 2001). From 1993 to 2000, a total of 1,178 human cases have been detected, linked to the consumption of pork from pigs and wild boars (*Sus scrofa*).

Portugal

The sylvatic cycle (*T. britovi*) is present in wildlife (red fox [*Vulpes vulpes*], wolf [*Canis lupus*]). In the last 50 years, *Trichinella* sp. infection was detected only in a single backyard pig from Montalegre in 1966. There are only two reports of human trichinellosis (five people in 1962 and one single person in 1967) caused by the consumption of pork from two backyard pigs (Vieira, 1983).

Romania

Both the sylvatic (*T. britovi*) and the domestic (*T. spiralis*) cycles occur. Between 1990 and 1999, the yearly incidence of trichinellosis in humans was 5.5 cases/10⁵ inhabitants, whereas in animals the incidence was 0.15% and 1.3% in domestic pigs and wild boars (*Sus scrofa*), respectively (Olteanu, 2001).

Russia

Trichinella spiralis, *T. nativa*, *T. britovi* and *T. pseudospiralis* infections are widespread in both wildlife and free-roaming and backyard pigs, with prevalence varying by region and host species. Human trichinellosis is also quite common, with 4,920 documented cases between 1996 and 2002. The main source of infection was pork from domestic pigs and wild boars (*Sus scrofa*), meat from badgers [*Meles meles*], jackal (*Canis aureus*), bears (*Ursus arctos*) and domestic and stray dogs (Ozeretskorskaya et al., 2005).

Serbia

A dozen cases of *Trichinella* sp. infections in pigs and 30 to 50 human cases occur each year, a reduction from earlier years (for example, in 2002 human trichinellosis cases were 232). Both *T. spiralis* and *T. britovi* have been detected in red foxes (*Vulpes vulpes*) and wild boars (*Sus scrofa*) (Cuperlovic et al., 2005).

Slovakia

Human trichinellosis is quite rare in this country. Between 1962 and 1999, only one outbreak was related to the consumption of pork from a pig fed with offal of wild animals and a large *T. britovi* outbreak from the consumption of dog meat (Dubinsky et al., 2001). No infected pig has been detected in spite of the routine examination of slaughtered animals. Recently, *T. pseudospiralis* was detected during routine examination at the slaughterhouse in four swine from a farm in eastern Slovakia (Hurnikova et al., 2005). *Trichinella britovi* is prevalent in red foxes (*Vulpes vulpes*), whereas, *T. spiralis* has been detected only twice in red foxes (*Vulpes vulpes*). *Trichinella pseudospiralis* has been found only in pigs, synanthropic rats and in a cat of the farm in eastern Slovakia (Hurnikova et al., 2005).

Slovenia

No human trichinellosis case, nor *Trichinella* infection in domestic pigs, have been documented in the last 50 years. Red foxes (*Vulpes vulpes*), wild boars (*Sus scrofa*), bears (*Ursus arctos*) and badgers (*Meles meles*) have been found infected with *T. britovi* or seldom with *T. spiralis*.

Spain

Human outbreaks (about 50 to 100 cases per year) caused by both *T. spiralis* and *T. britovi*, occur every year due to the consumption of pork or pork products from backyard pigs or pigs from organic farms. From 1990 to 2001, seven outbreaks occurred due to the consumption of autochthonous pigs; whereas from 1995 to 1998, a total of 192 cases were linked to the consumption of wild boar meat (*Sus scrofa*). In 1998-1999, *Trichineila-Infected* raw pork of unknown Spanish origin, imported to Germany, caused a human outbreak with 52 cases. *Trichinella* sp. infection in swine is still present in many regions of Spain, and a dozen cases are detected every year in backyard or free-ranging pigs. In wildlife, *T. spiralis* and *T. britovi* are highly prevalent and have been found in 3% of red foxes (*Vulpes vulpes*) and 0.003% of wild boars [*Sus scrofa*].

Sweden

Ten outbreaks of trichinellosis in humans, involving a total of 504 persons, were described between 1917 and 1969 and a few sporadic cases have also been reported until 1978. Most outbreaks were caused by the consumption of pork or pork products (sausages) from small family farms of Sweden, and only one was due to the consumption of wild boar (*Sus scrofa*) meat. In the years 1985-1994, 27 (0.000041%) domestic pigs from family farms were found infected with *Trichinella* larvae. *Trichinella spiralis*, *T. nativa* and *T. britovi* have been detected in red foxes (*Vulpes vulpes*) and *T. pseudospiralis* in a lynx (*Lynx lynx*) and wild boars (*Sus scrofa*).

Switzerland

Trichinella britovi infection has been detected in 0.9% of red foxes (*Vulpes vulpes*). This parasite species has also been detected in lynxes (*Lynx lynx*) and in a wolf (*Canis lupus*) which has recently colonised this country. No infection has been documented in humans or domestic pigs in the last 50 years.

United Kingdom

No documented case of human trichinellosis in the UK due to the consumption of pork or pork products from pigs reared in this country has been reported for the last 50 years. The last outbreak caused by the consumption of a local pig occurred in Liverpool in 1953. In addition, in the last 50 years *Trichinella* sp. infection has not been detected in swine reared in this country, nor in wildlife as well; however, only a very limited number of surveys has been performed.

Ukraine

Both *T. britovi* and *T. nativa* have been documented in wildlife and *T. spiralis* in free-roaming and backyard pigs. Human outbreaks occur quite frequently, but there is no a recent report on its prevalence.

Pacific

Australia

Trichinella pseudospiralis has been detected in marsupials and birds of Tasmania (Pozio and Murrell, 2006). The mainland of Australia, however, has always been considered *Trichinella-free*, but this status is not based on any extensive epidemiological investigation on wildlife, but on limited investigations on synanthropic rats, domestic cats, pigs, dingoes and red foxes (*Vulpes vulpes*) from Victoria and New South Wales (Pozio and Murrell, 2006).

New Zealand

Trichinella spiralis has repeatedly been documented in domestic pigs in 1965, 1968, 1974, 1997 and in 2001; in brown rats (*Rattus norvegicus*) in 1965 and 2001; in cats in 1965, 1974 and 2001; and in humans up to 2001 (Pozio and Murrell, 2006).

Papua New Guinea

Trichinella papuae has been documented among wild and domestic pigs and farmed saltwater crocodiles (*Crocodylus porosus*) (Pozio *et al.*, 2005b). A high serological prevalence for anti-*Trichinella* antibodies has been detected among humans living in the Western region of Papua New Guinea (Owen *et al.*, 2005).

'Non infected' countries and islands

No information is available on *Trichinella* sp. infection in humans and domestic or wild animals in a lot of countries in the world. However, the lack of information for a country does not mean that these zoonotic parasites are absent, but instead may reflect the lack of investigations. We can argue that *Trichinella* spp. could be ubiquitous in wildlife (mammals, birds and reptiles), whereas their presence in domestic animals and humans is relatively rare and, in most instances, where it is not reported, it exists but it does not represent a serious public health problem.

Trichinella spp. infections have been never documented in the islands reported below, but we can exclude that these parasites are present, unless it will be passively introduced by humans:

- Africa: Cape Verde, Comoros, Madagascar, Mauritius, Sao Tomé-Principe, Seychelles
- America: the Caribbean islands Antigua-Barbuda, Bahamas, Barbados, Cuba, Dominica, Dominican Republic, Grenada, Haiti, Jamaica, Netherlands Antilles, St Kitts-Nevis, St Lucia, St Vincent-Grenadines, Trinidad-Tobago
- Asia: East Timor, Maldives, Philippines, Singapore, Sri Lanka, Taiwan
- Europe: Cyprus, Malta
- Oceania: Fiji, Kiribati, Marshall Islands, Micronesia FS, Nauru, Palau, Samoa, Solomon Islands, Tokelau, Tonga, Tuvalu, Vanuatu.

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CHAPTER 2

MANAGEMENT AND DIAGNOSIS OF HUMAN TRICHINELLOSIS

J. Dupouy-Camet & F. Bruschi

Summary

The clinical diagnosis of trichinellosis is difficult because there are no pathognomonic signs or symptoms, therefore, epidemiological data are of great importance in diagnosing the infection. Trichinellosis is associated with eyelid or facial oedema and myalgia during the acute stage and can be complicated by myocarditis, thromboembolic disease and encephalitis. High eosinophilia and increased creatine phosphokinase (CPK) activity in the serum are the most frequently observed laboratory features. The parasitological examination of a muscle biopsy and the detection of specific circulating antibodies will confirm the diagnosis. The medical treatment includes anthelmintics (mebendazole or albendazole) and glucocorticosteroids. Mebendazole is usually administered at a daily dose of 5 mg/kg but higher doses (up to 20mg/kg/day to 25mg/kg/day) are recommended in some countries. Albendazole is used at 800mg/day (15 mg/kg/day) administered in two doses. These drugs should be taken for 10 to 15 days. The use of mebendazole or albendazole is contraindicated during pregnancy and not recommended in children aged < 2 years. The most commonly used steroid is prednisolone, which may alleviate the general symptoms of the disease. It is administered at a dose of 30 mg/day to 60 mg/day for 10 to 15 days.

Keywords

Albendazole - Diagnosis - Eosinophilia - Mebendazole - Trichinellosis - Trichinella.

INTRODUCTION

Trichinellosis usually begins with a sensation of general discomfort and headache, increasing fever, chills and sometimes diarrhoea and/or abdominal pain. Pyrexia, eyelid or facial oedema and myalgia represent the principal syndrome of the acute stage, which can be complicated by myocarditis, thromboembolic disease and encephalitis. Although human infection has not been reported for certain genotypes, all *Trichinella* species are pathogenic for humans, although differences have been observed among the different species or genotypes in terms of the signs, symptoms, and clinical course of infection in humans. The clinical signs & symptoms are directly related to the parasitic cycle in the human host.

PATHOLOGY

The parasitic cycle can be divided into two phases: an intestinal (or enteral) phase and a muscular (or parenteral or systemic) phase, which can co-exist for a period lasting from a few days to weeks (Figure 1). For more details, the reader is referred to the Biology section of these Guidelines. Table I is a glossary of terms used to define these different phases.

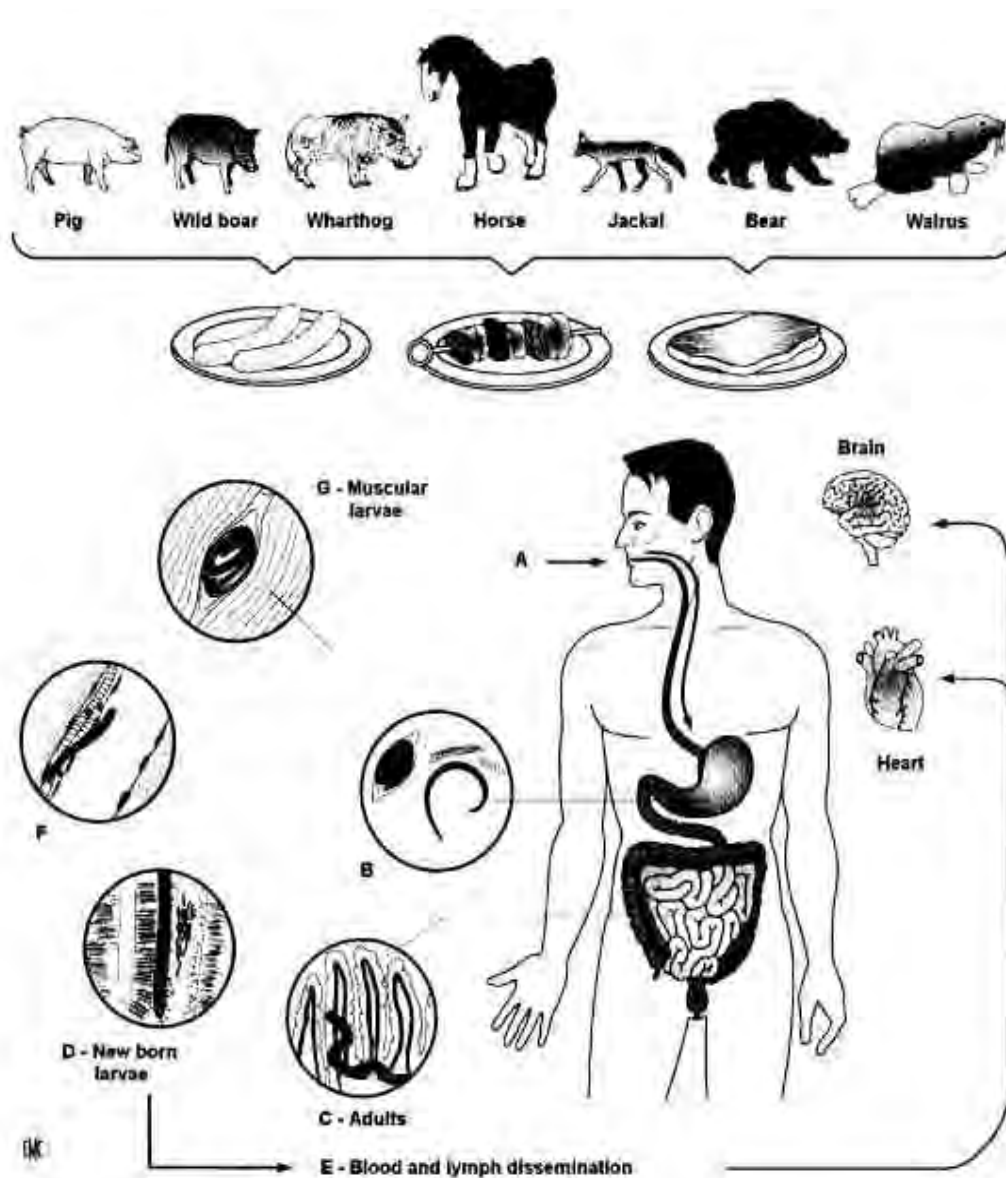


Figure 1 - Transmission and biological cycle of *Trichinella* in humans

Intestinal phase

After the gastric digestion of the infected meat (i.e. meat containing muscle larvae), the larvae are released in the stomach and move into the small intestine; there they penetrate the mucosa of the small intestine, and by 4 d.p.i. to 5 d.p.i. mature into adult worms. The larval penetration of the intestinal mucosa causes modifications in the cells of the epithelium, specifically, the brush border of villi, the lamina propria, and the smooth muscles of the jejunum. After mating in the intestine, adult females shed the so-called NBL into the blood and lymphatic vessels. Mature females release NBL for three to four weeks; although this estimate was based only on experimental data from pigs, it has been confirmed by the observation of a *Trichinella* female containing embryos on a duodenal section of a person infected three to four weeks earlier and presenting with fever, myalgia and high eosinophilia (Basset *et al.*, 1996). The females then die or are expelled by smooth muscle hypercontractility elicited by the immune response. The prolonged diarrhoea observed in the Inuit population in the Canadian Arctic outbreaks (Viallet *et al.*, 1986; McLean *et*

al., 1989) suggests the persistence of adult worms in the intestine of people frequently exposed to infection, showing that in humans immunity plays a crucial role in host protection. A similar status of pre-munition could occur in hunters of Papua-New Guinea who are practically asymptomatic, irrespective of high seroprevalence, evaluated with specific tests (Owen *et al.*, 2005).

Table I - Glossary for *Trichinella* infection and trichinellosis in humans

Chronic phase	Period during which live larvae are present in the striated muscles
Chronic trichinellosis	Refers to persons which acquired <i>Trichinella</i> infection months or even years before and still having signs or symptoms
Convalescent phase	Period following the acute phase during which signs or symptoms are due to the presence of muscle larvae but during which migrating larvae are no longer present
Incubation period	Period during-which infective larvae develop into adults in the intestine without any signs or symptoms
Intestinal (enteral) phase	Period during which the infective larvae develop into adults in the intestine; signs or symptoms present
Muscular (parenteral or systemic) phase	Period during which newborn larvae migrate throughout the circulatory system to striated muscles; signs or symptoms present
Sequelae	Definitive lesions of organs which occurred in the acute phase (e.g. brain, cardiovascular system or neuromuscular disturbances etc.)
<i>Trichinella</i> infection	Infection with <i>Trichinella</i> spp. with or without signs or symptoms
Trichinellosis	Infection with <i>Trichinella</i> with signs or symptoms

Systemic and muscular phase

The larvae released in the intestinal mucosa migrate to the blood vessels, by means of which they spread throughout the body until reaching their final location (i.e. the cells of the striated skeletal muscles). The migration of *Trichinella* larvae in the different organs provokes an immediate reaction, which causes immunological, pathological and metabolic disturbances and the various clinical phenomena observed during the acute stage of the infection (Murrell & Bruschi, 1994; Capo & Despommier, 1996; Kociecka, 2000). The immunological reaction consists of the production of inflammatory cells (i.e. mast cells, eosinophils, monocytes, and T and B lymphocytes), of cytokines and antibodies, which are important components of the cell- and humoral mediated immune responses to infection. The penetration and permanent presence of larvae in the striated skeletal muscle cells cause three major cell modifications:

- a) the acquisition by the cell of a new phenotype called 'nurse cell', accompanied by the disappearance of sarcomere myofibrils
- b) the encapsulation of the larvae (in the case of encapsulated species)
- c) the development of a capillary network surrounding the infected cell (Capo *et al.*, 1998).

Encapsulation consists of the production of a collagen capsule around the larva approximately 18 d.p.i. to 20 d.p.i. (Polvere *et al.*, 1993); this occurs in all species and genotypes, except for *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis* (these species are referred to as 'non-encapsulated'). In addition to these three major modifications, the sarcoplasm becomes basophilic, the cell nucleus is displaced to the centre of the cell, and the nucleoli increase in both number and size. The cell becomes more permeable, resulting in an increased release of muscle enzymes.

The parenteral or muscular phase is associated with inflammatory and allergic responses caused by invasion of the skeletal muscle cells by the migrating larvae. This invasion can directly damage the muscle cells, or indirectly stimulate the infiltration of inflammatory cells, primarily eosinophils. A correlation between the eosinophil levels and serum muscle enzymes such as lactate dehydrogenase (LDH) and creatine phosphokinase (CPK), has been observed in trichinellosis patients, suggesting that muscle damage may be mediated indirectly by these activated granulocytes (Ferraccioli *et al.*, 1988). Although *Trichinella* larvae do not mature or become encapsulated in heart-muscle tissue, their transitory passage can lead to morphological alterations, which consist of focal cellular infiltrates of eosinophils and mononuclear cells. The presence of larvae in the central nervous system causes vasculitis and perivasculitis, with diffuse or focal lesions. The NBL tend to wander, causing tissue damage before reentering the bloodstream, or remain trapped and destroyed by the following granulomatous reaction. Neural cells may also be damaged by eosinophil degranulation products such as eosinophil-derived neurotoxin and major basic protein (Durack *et al.*, 1979). Moreover, heart and brain lesions are often associated and could result from the simultaneous intervention of local prothrombotic effects of eosinophil activation and vascular injury caused by the migrating larvae (Fourestié *et al.*, 1993). Myocarditis is triggered initially by invasion of the migrating larvae, then from immunopathologic processes such as activated eosinophil infiltration and mast cell degranulation, according to experimental results in rats (Paolocci *et al.*, 1998). The length of survival of the nurse-cell parasite complex in the host is known to vary greatly, depending on many factors related to both parasite and host. It seems to vary from approximately one to two years to an undetermined number of years, although survival for up to 30 years has been reported (Fröscher *et al.*, 1988).

MANAGEMENT OF ACUTE TRICHINELLOSIS

Identification of the disease: clinical signs

In most persons, the acute stage begins with the sudden appearance of general discomfort and severe headaches, an increase in fever, chills and excessive sweating. The major syndrome of the acute stage consists of persistent fever, facial oedema (characteristically periorbital), muscle pain, and severe asthenia, lasting for several weeks. Transient dizziness and nausea can also occur. Though less common, diarrhoea and conjunctival and sub-ungual haemorrhages are also observed. This is the stage during which the adults and the migrating larvae provoke the signs and symptoms of the disease. The physician has to search for clinical typical signs.

Fever

Fever is one of the earliest and most common signs of trichinellosis. Body temperature increases rapidly, usually stabilising at 39°C to 40°C. The fever usually lasts from eight to ten days, although it can persist for up to three weeks when the disease is severe.

Periorbital and facial oedema

Oedemas are very typical signs of trichinellosis, although their intensity varies depending upon the intensity of the reaction to the infection (Figure 2). In the severe form of trichinellosis, oedema extends to the upper and lower extremities, The oedema is symmetrical. It usually vanishes rapidly following treatment (i.e. within 5 d.p.i. to 7 d.p.i), particularly when glucocorticosteroids are used.



Figure 2 - Facial oedema (left). The same lady after recovery (right)

Myalgia

Muscle pain affects various muscle groups, and its intensity is related to the severity of the disease. It most frequently affects the muscles of the cervix, trunk, and upper and lower extremities; it also affects the masseters, although less frequently. The pain usually appears upon exertion, although most persons with severe trichinellosis or phlebitis associated with trichinellosis also experience myalgia at rest. Some persons with severe disease become disabled with a profound muscle weakness as a result of pronounced angiomyositis-type lesions and neuro-muscular disturbances. The restriction of movement due to the pain associated with exertion leads to contractures of the upper and lower limbs, nuchal pseudoridity, and occasionally trismus. Severe myalgia generally lasts for two to three weeks.

Intestinal signs

The most common intestinal signs and symptoms are diarrhoea (from loose stools to as many as 10 to 15 stools per day, frequently containing mucus but free of blood) and abdominal pain. These signs and symptoms usually precede fever and myalgia by three to four days, and they disappear in less than one week. It has been observed that the shorter the duration between infection and the appearance of diarrhoea and fever, the longer the duration of both fever and facial oedema (Dupouy-Camet *et al.*, 1985).

Others

Conjunctival and subungual haemorrhagic lesions are caused by vasculitis, the leading pathological process of trichinellosis. In addition, maculo-papular rash (after the onset of muscular pain) and formication have been reported for a small proportion of persons.

Identification of the disease: non specific laboratory signs

Eosinophilia

Eosinophilia has been observed in practically every case of trichinellosis, with few exceptions. It appears early, before the development of the general syndrome of clinical signs and symptoms, and it increases between the second and the fifth week of infection. Eosinophilia occurs in various degrees: low

(<1,000/mm³ or 1G/l), moderate (1,000/mm³ to 3,000/mm³ or 1 G/l to 3 G/l), and high (> 3,000/mm³ or 3G/l); up to 19,000 cells per mm³ (19 G/l) have been reported. It regresses slowly and can remain at lower levels for a period of several weeks to three months. The level of eosinophilia is correlated with the degree of myalgia (Ferraccioli *et al.*, 1988) and is significantly higher in persons with neurological complications (Fourestié *et al.*, 1993). During the acute stage of infection, a massive decrease of eosinophils in persons with severe trichinellosis can be considered as a predictor of a severe outcome. The mechanism underlying this decrease has not been fully understood, though it could be related to modifications in the levels of certain cytokines and to the massive exit of eosinophils from the vascular system, leading to huge tissue infiltrates.

Muscle enzymes

The levels of all muscle enzymes increase in serum during the course of trichinellosis: CPK, LDH, aldolase and, occasionally, aspartate aminotransferase (AspAT). Increased muscle enzyme levels are found in 75% to 90% of infected persons. The increase, which is several-fold, occurs between the second and the fifth week of infection (Capo & Despommier, 1996). No correlation has been found between increased CPK and the severity of infection, although a correlation has been found with the intensity of muscular pain (Ferraccioli *et al.*, 1988).

Total IgE

During trichinellosis, patients undergo immunoglobulin level increases, particularly in total IgE, as occurs in many other helminthic infections. However, an increase in total IgE levels is not a consistent phenomenon, and it is not possible to exclude trichinellosis on the basis of its absence. A low correlation between total and specific IgE has been observed for both *T. spiralis* and *T. britovi* infections, suggesting that higher production of IgE is due to a polyclonal activation, rather than to an effective host defence process (Watanabe *et al.*, 2005). Clinical observations suggest that *Trichinella*-specific IgE are responsible for allergic manifestations typical of the clinical picture of trichinellosis, such as cutaneous rash or oedemas (Watanabe *et al.*, 2005).

Differential diagnosis

Isolated cases

Isolated cases can be mistaken for auto-immune disease or other infectious diseases. For example, persons with high fever and myalgia are often misdiagnosed with *flu*, particularly in winter. Protracted diarrhoea is often attributed to *salmonellosis*, *shigellosis*, or other infections of the alimentary tract. Eosinophilia combined with myalgia and an inflammatory response should be differentiated from eosinophilia-myalgia syndromes, such as *toxic oil syndrome*, *tryptophan intake* and *eosinophilic fasciitis*. Eosinophilia combined with fever should be differentiated from tissular parasitosis such as *fascioliasis*, *toxocarosis* or invasive *schistosomiasis*. Periorbital or facial oedema with fever should be differentiated from *glomerulonephritis*, *serum sickness*, *allergic reactions* to drugs or allergens, *polymyositis*, *dermatomyositis* and *periarteritis nodosa*. Intense headaches and stiff neck with confusion, drowsiness, irritability, and neurological symptoms should be differentiated from infectious *meningitis* and *encephalitis*. Haemorrhages of the conjunctiva or haemorrhagic skin petechiae associated with fever should be differentiated from *leptospirosis*, *bacterial endocarditis* and *typhus exanthematicus*. Persons without periorbital oedema but with high fever and neurological symptoms may be misdiagnosed with *typhoid fever*. Table II gives an algorithm which can help for diagnosis.

Table II - Algorithm for diagnosing the probability of being infected with acute *Trichinella* in humans

Group A	Group B	Group C	Group D
Fever	Diarrhoea	Eosinophilia (> 1.G/l) and/or increased total IgE levels	Positive serology (with a highly specific test)
Eyelid and/or facial oedema	Neurological signs	Increased levels of muscular enzymes	Seroconversion
Myalgia	Cardiological signs Conjunctivitis Subungual haemorrhages Cutaneous rash		Positive muscular biopsy

The diagnosis is:

Very unlikely: one A or one B or one C

Suspected: one A or two B and one C

Probable: three A and one C

Highly probable: three A and two C

Confirmed: three A, two C, and one D; any of groups A or B and one C and one D

Grouped cases

Grouped cases are also highly suggestive of the disease but can even be seen for other infectious diseases. Although a definitive diagnosis of trichinellosis can only be made with highly specific immunodiagnostic tests or by detecting larvae in a muscle biopsy, the infection can be suspected on clinical and epidemiological grounds. If two or more persons in the same household or a number of persons in the same community have high fever, periorbital or facial oedema, and myalgia, trichinellosis can be suspected. When cases are sporadic or the clinical course is atypical, it is more difficult to suspect infection or less likely that the infection will be suspected. Once infection is suspected, information should be collected on the consumption of raw or undercooked meat or meat products, including the place and time of purchase and consumption. The European Center for Disease control has issued case definitions to be used when suspecting a human outbreak (Annex I).

Detection of main complications

Complications usually develop within the first two weeks. They are observed mainly in severe cases, but they have also been reported in moderate cases, in persons who were improperly treated (including those for whom treatment was begun too late) and, particularly, in the elderly. A positive correlation has been reported between age and the frequency and severity of complications (Dupouy-Camet *et al.*, 1985). Encephalitis and myocarditis, which are both life-threatening, are often simultaneously present (Fourestié *et al.*, 1993).

Cardiovascular

Cardiovascular disturbances can occur in moderate or severe cases of trichinellosis, usually later in the infection (i.e. between the third and fourth week p.i.) (Bessoudo *et al.*, 1981; Compton *et al.*, 1993, Lazarevic *et al.*, 1989; Puljz *et al.*, 2005).

Myocarditis

Myocarditis develops in 5% to 20% of all infected persons. The symptoms include pain in the heart region, tachycardia, and electrocardiogram (ECG) abnormalities. The ECG disorder most frequently observed are non-specific ventricular repolarisation disturbances (with ST-T wave changes), followed by bundle-branch conduction disturbances, and sinus tachycardia. The other ECG disorders recorded, during various phases of the infection, are sinus bradycardia, right bundle-branch block, supraventricular and ventricular extrasystoles, low-voltage QRS complexes in standard limb leads, first-degree atrio-ventricular block, and atrial fibrillation. Although ECG abnormalities appear to be a common feature of trichinellosis, especially during the invasive phase of the disease, they are rarely associated with a poor prognosis. A transient, nonspecific, ventricular-repolarisation disturbance is the abnormality most commonly observed. High levels of troponin have been observed in patients with myocarditis (personal observation). The persistence of the ECG abnormalities, even if other signs and symptoms of trichinellosis have already subsided, usually reflects hypokalaemia. Compensation of the potassium deficit in such persons promotes normalisation of the ECG. Echography can identify myocardium functional anomalies (segmentary hypokinesis or ventricular dilation).

Others

Another cardiovascular complication is thromboembolic disease, specifically, deep thrombophlebitis, intraventricular thrombi, and/or pulmonary embolism, all of which can lead to death. Cardiovascular complications may be accompanied by oedema of the lower limbs due to hypoalbuminaemia. Sudden death may result from embolism of the pulmonary artery or from paroxysmal tachycardia. Echography can identify pericardial effusion or a transitory intracavitary thrombus (Ancelle *et al.*, 1988).

Neurological complications

Neurological complications include a variety of signs and symptoms (Ellrodt *et al.*, 1987; Ryczak *et al.*, 1987; Fourestié *et al.*, 1993; Mawhorter & Kazura J.W. 1993). Neurological complications could be less frequent if the infected person is treated early.

Encephalopathy

Persons with severe disease can show consciousness disorders or excessive excitement, and frequently somnolence and apathy; some of the persons with these symptoms show signs of meningitis or encephalopathy. Dizziness, nausea, and tinnitus are transient. Anisocoria, facial nerve paresis, and Babinsky's reflexes have also been observed in severe cases. Brain damage, which is usually observed within a few days after the onset of fever, can result in diffuse encephalopathy or focal signs such as disorientation, memory disturbances, frontal syndrome, behavioural disturbances, transient hemiparesia or hemiplegia, oculomotor dysfunction, aphasia, and cerebellar syndrome. The electroencephalogram shows a total deceleration of the cortical electric activity without critical aspect. Small hypodensities are seen with the CT scan or magnetic resonance (MRI) (Feydi *et al.*, 1996; De Graef *et al.*, 2000; Gelal *et al.*, 2005). CT scan can find nodular multifocal hypodensities which can be bilateral and of cortical or under-cortical topography or within the hemispherical white substance (Figure 3). After injection of contrast medium, cortical lesions and, much more rarely, those of the white substance are enhanced. This enhancement translates the ischaemic nature of the first, while the seconds are rather regarded as being of granulomatous origin. The imagery by MRI confirms these aspects. The lesions are found in iso- or hyposignal T1, but especially in hypersignal T2 and protonic density, and can be enhanced after gadolinium injection; in particular for the cortical and under-cortical lesions (Figure 3). These images are not very specific. There is no narrow correlation of radiological signs and clinical signs and symptoms. Most

CT scan or MRI brain abnormalities disappear in four to eight weeks p.i. as well as the clinical signs and symptoms.

Neuromuscular disturbances

Decreased muscular strength and tendon reflexes, dysphagia, and trismus usually occur at the beginning of the disease and may persist for a long period of time. Bioelectric disorders in *Trichinella*-infected muscles are revealed by electromyography (EMG). The disturbances reveal muscle lesions and are characterised by a decreased amplitude of muscle contraction and incomplete interference. However, they are not pathognomonic for trichinellosis. The disturbances regress simultaneously with clinical improvement and with the subsiding of histological lesions in muscle tissue. In most persons, bioelectric disturbances at the acute stage of trichinellosis correspond to the severity of the clinical course and to the intensity of the disease (Kociecka, 2000).

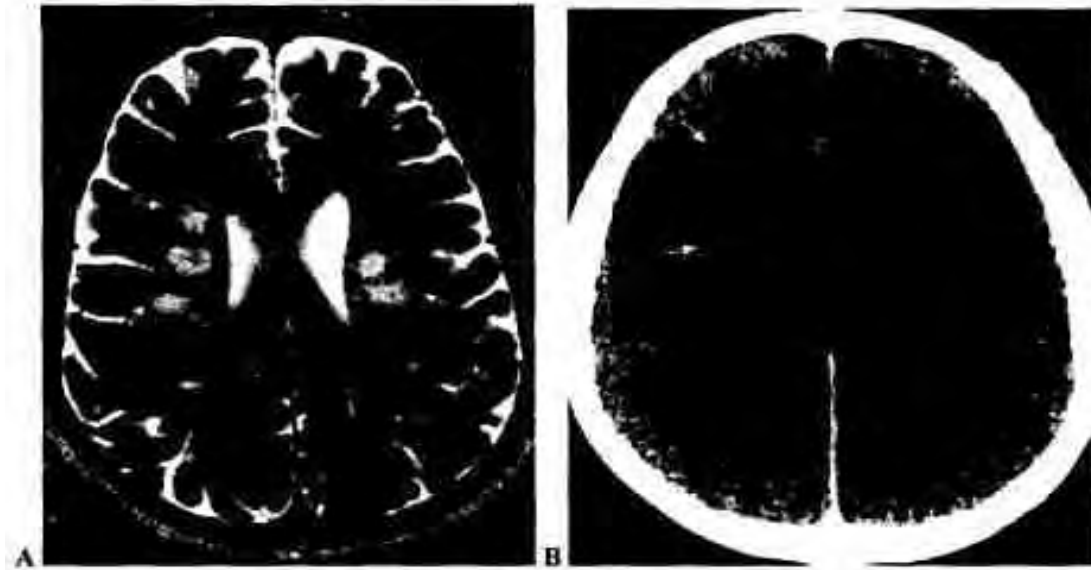


Figure 3 - Magnetic resonance imaging

A: axial T2 weighted spin echo magnetic resonance images showing bilateral signal areas (arrows) without mass effect

B: contrast enhanced CTscan revealing low densities areas (arrows) (A. Bonnafé)

Ocular

Ocular lesions appear during the acute stage of the disease and result from disturbances in microcirculation. The typical traits are oedema and vascular lesions within the conjunctiva, the uvea, the retina, and, in some cases, the optic nerve. Rarely, retinal lesions may be induced by migrating *Trichinella* larvae which penetrate ciliary arterioles and the central artery of the retina, leading to irreversible damage to eye sight. An intense invasion of muscles of the ocular bulb provokes pain when moving the eyeballs, muscle paralysis, diplopia, or a disturbed accommodation.

Respiratory

Dyspnea is relatively common and is caused primarily by parasite invasion and subsequent inflammation of respiratory muscles such as the diaphragm. Respiratory complications are uncommon. They can occur during both the early and late stages of trichinellosis. They consist of pneumonia, obstructive bronchitis or Löffler-type infiltrates or ventilature failures (Compton *et al.*, 1993). Following glucocorticosteroid treatment,

the respiratory disturbances regress within a few days. In the late stages of the disease, pneumonia and pleuritis of bacterial aetiology may appear, as well as lung infarction (Pawlowski, 1983).

Digestive

Digestive complications occur during the acute stage of infection, and they consist of:

- 1) massive protein exudation leading to hypoalbuminemia and localised oedemas
- 2) acute intestinal necrosis
- 3) prolonged diarrhoea.

In some outbreaks (Ancelle *et al.*, 1988; Ancelle *et al.*, 1998), oedema of limbs was reported in 6% to 8% of infected persons. A particular syndrome has been described in persons who regularly eat infected meat (i.e. Inuit populations). In these persons, trichinellosis manifests itself as a chronic diarrhoeal syndrome which is due to the strong intestinal immune reaction; this reaction consists of a rapid expulsion of adult worms from the intestine, thus preventing the muscular phase (Viallet *et al.*, 1986; MacLean *et al.*, 1989).

Evaluation of the severity of the disease

The severity of trichinellosis depends on a number of variables which are often interrelated, including the infecting dose (i.e. the number of larvae ingested); the frequency of consumption of infected meat; how the meat was cooked or treated (e.g. whether it was raw or rare or whether it had been smoked or salted); the amount of alcohol consumed at the time of meat consumption, given that alcohol could increase the resistance to the infection (Pawlowski, 1986); the *Trichinella* species involved (the number of NBL shed by females differs by species); and individual susceptibility which depends on ethnic factors as well as sex, age and the immune status of the host.

Infective doses

There are no precise data defining the minimal infective dose able to exert clinical trichinellosis in an individual person. Murrell and Bruschi (1994), quoting Piekarsky (1954) reported that 70 live larvae were sufficient to provoke clinical disease. It is also assumed that meat containing at least one larva per gram is necessary to induce a clinical infection in man (Zimmermann, 1983), which could correspond to an infective dose of approximately 150 larvae for the usual consumer (assuming a meat consumption of 150 g). On the other hand, an infection is clinically patent in humans when the number of larvae per gram (lpg) of muscle biopsy is around ten and severe when the number of lpg of muscle biopsy is above 100 (Pawlowski, 1983). From these data and from the theoretical number of NBL shed by *T. spiralis* females (around 1,000/female), the minimum infective dose could be estimated around 100 and 300 larvae. An intake of more 1,000 to 3,000 larvae could lead to a severe disease.

Infections with different species

Although clinical differences have been observed among persons infected with different species of *Trichinella* (Bruschi & Murrell, 2002), it has not been possible to attribute these differences to the species of the pathogen because the number of infecting larvae ingested by each person was generally unknown. However, *T. spiralis* infections could be more severe than those caused by *T. britovi* and this could be due to the fact that *T. britovi* females are less prolific (Pozio *et al.*, 1993). *Trichinella murrelli* seems to be more likely to provoke skin reactions and less likely to cause facial oedema (Dupouy-Camet *et al.*, 2001). *Trichinella pseudospiralis*, which is non-encapsulated, seems to provoke signs and symptoms that last longer (Jongwutiwee *et al.*, 1998; Ranque *et al.*, 2000).

Immunocompromised persons

To our knowledge, only three cases of trichinellosis have been reported in immunocompromised persons. In a renal graft recipient, the infection was asymptomatic, even in the presence of 1,400 larvae/g in the deltoid muscle (Doby *et al.*, 1984), and in an HIV-positive person the clinical symptoms were not particularly severe (Louthrenoo *et al.*, 1993). A very severe case was described in a person with chronic myeloid leukemia (Jacobson & Jacobson, 1977). Hypereosinophilia could be absent from these patients (Kociecka pers. comm.)

Incubation period

The length of the incubation period depends upon the same variables as disease severity. Furthermore, it has been observed that for the more severe forms of trichinellosis, the incubation period is generally shorter, specifically: the incubation period lasts approximately one week for the severe form, two weeks for the moderately severe form, and at least three to four weeks for the benign and abortive forms.

Clinical forms

Based on disease severity, the five following clinical forms of trichinellosis can be recognised:

Severe form

All of the signs and symptoms are very pronounced (see paragraph 'Acute trichinellosis'), and there are metabolic disturbances and circulatory and/or neurological complications. The fever can last for more than two weeks and high numbers of larvae can be recovered from the muscular biopsies (usually more than 100 lpg)

Moderately severe form

All of the signs and symptoms are pronounced, yet complications are rare and, if present, they are benign and transient.

Benign or mildform

The signs and symptoms are mild, and there are no complications; consequently, this form is rarely suspected, unless the infected person is involved in an investigated outbreak.

Abortive form

The clinical signs and symptoms frequently appear individually and not as a syndrome; they are mild and last only for a few days.

Asymptomatic form

It can only be diagnosed by means of serology or muscle biopsy performed for other reasons.

HOW TO PROVE TRICHINELLA INFECTION?

Immunodiagnosis

The three main objectives in the immunodiagnosis of human trichinellosis are:

- a) recognising the acute infection to allow early anthelmintic treatment
- b) making a retrospective diagnosis
- c) adding information to the epidemiology of the infection (Ljungström, 1983).

Many techniques have been used for detecting antibodies against *Trichinella* antigen. At present, enzyme-linked immunosorbent assay (ELISA) is the most highly recommended technique and is best used in combination with immunoblotting (western-blot) to confirm ELISA-positive samples or to exclude false-positive ELISA results. The use of two techniques can also allow diagnosis to be made earlier (Costantino *et al.*, 2001).

Antigens

Four different antigens can be used for serological diagnosis:

- 1) cryo-sections of infected muscles or isolated larvae (muscle-larva cuticle antigen), which are generally used for indirect immunofluorescence (IIF),
- 2) a crude antigen prepared from muscle larvae,
- 3) an excretory/secretory antigen (E/S antigen) produced in vitro after 18 h of cultivation of the muscle larvae,
- 4) a 3,6-dideoxyhexose sugar (tyvelose), one of the major highly specific immunodominant epitopes of *Trichinella*.

Tyvelose is highly specific, yet it is less sensitive than crude and E/S antigens. Crude (ELISA, IIF) and, less often, E/S antigens can cause cross-reactions with non-specific *Trichinella* antibodies (Morakote, 1991). The antigenic pattern is quite similar among all *Trichinella* species and genotypes; thus, the antigen prepared with one species, genotype or strain can be used to detect specific antibodies in persons infected with any species.

Antibody response

The humoral response consists of the production of anti- *Trichinella* antibodies, and the detection of specific antibodies has great diagnostic value. However, antibodies are usually not detectable at the onset of clinical signs, and they appear with a distinct time sequence according to the specific class of antibodies (Van Knapen *et al.*, 1982; Lungström, 1983, Bruschi *et al.*, 1990). IgE class antibodies are thought to appear first and are typical of the acute stage of the disease. However, they are seldomly detected because their half-life in serum is relatively short, although an amplified ELISA or the use of tyvelose antigen can greatly increase the probability of detection (Bruschi *et al.*, 2001). The levels of antibodies increase during the following two to three weeks, particularly in patients with severe infection. IgG antibodies may persist for many years after infection, even if the disease has developed as benign or asymptomatic (Harms *et al.*, 1993). Most of the time, the serology is negative during the first days of the febrile phase; in such case a second test performed a few days later is advisable. If serological positivity can help in diagnosis, antibody

levels do not help too much in estimation of prognosis, in fact they do not correlate with the severity or the clinical course of the disease in humans (Murrell & Bruschi, 1994). Seroconversion usually occurs between the second and fifth week of infection and the time required for seroconversion is inversely correlated with the infective dose. Serum may remain positive up to one year or more (19 years has been reported) after the end of the acute phase of infection (Pozio *et al.*, 1993). In *T. britovi* human infections, seroconversion has been documented up to two months post-infection. Serological testing performed in a large outbreak of human trichinellosis due to *T. nativa* revealed a positivity rate of 45% and 87% at three to four and ten to eleven weeks post-infection among confirmed cases. However, seroconversion from acute to convalescent samples was 55% (Schellenberg *et al.*, 2003).

An evaluation of the antibody kinetics in infected persons should be conducted every three months to follow up the effects of chemotherapy. In persons infected with *T. britovi*, circulating antibodies disappear (in about one half of patients) within six months and within three years all persons are seronegative (Pozio *et al.*, 1993).

In *T. spiralis* infected persons, who received a delayed or ineffective therapy, specific antibodies can be detected for years (Pozio *et al.*, 2001), whereas in those who received effective treatment in the first two weeks after infection specific antibodies can disappear in a shorter period of time.

Indirect immunofluorescence

Indirect immunofluorescence can be carried out alternatively with frozen sections of infected tissue (Ljungström, 1974) or formalin fixed whole larvae (Brzosko *et al.*, 1965; Pozio *et al.*, 1988), as antigens, the former being more sensitive. High sensitivity (up to 100%) was obtained with IIF (Pozio *et al.*, 1988, Dupouy-Camet *et al.*, 1988). With this test, all relevant immunoglobulins can be detected. Cross-reactions with *Trichinella* antigens were observed in persons with autoimmune diseases (Robert *et al.*, 1996). Persons reading the fluorescent sections should be aware that only sections with a uniform fluorescence should be considered as positive, whereas those, which show a non-uniform fluorescence along the cuticle, should be considered as false-positive.

Enzyme-linked immunosorbent assay

Although many kits for ELISA are commercially available, only those few that do not produce false-positive results due to cross-reaction with other parasitic antigens (e.g. visceral *larva migrans* and *Loa loa*) should be used. Absolute sensitivity (100%) has been obtained in humans infected with *T. spiralis* by ELISA (measuring IgG) using both a crude larval extract or ES antigens (van Knapen *et al.*, 1982, Bruschi *et al.*, 2001). This high rate of sensitivity, observed 50 days after infection, declined to about 80% after two years. However, parasite-specific IgM was detected for up to 11 years after infection. Serological tests measuring other classes of antibodies (IgA, IgE) resulted on lower sensitivity rates (Murrell & Bruschi, 1994). The study of the humoral response against stage-specific antigens has not resulted in an improvement in diagnosis; however, the search for NBL specific IgA has shown promising for use in detection of the early phase of infection (more than 80% of positivity after three weeks of infection) (Mendez-Loredo *et al.*, 2001). The sensitivity of ELISA is reported to be higher with E/S antigen than with tyvelose for detecting circulating antibodies in the early infection (E. Pozio, unpublished data). However, in another report, asymptomatic infections are detected using the tyvelose antigen (Owen *et al.*, 2001). Antibody directed to tyvelose were found three to eight years after *T. spiralis* infection (Bruschi *et al.*, 2005) and sporadically even after 15 years after a *T. britovi* outbreak (Piergili-Fioretta *et al.*, 2005). Specificity depends primarily on the type of antigen used and the cut-off value established. Using the ELISA (Engvall & Ljungström, 1975), E/S antigens are preferable to crude extracts of *T. spiralis* muscle larvae, since the former gave a higher specificity. This is particularly important in regions where other human helminth infections (e.g. *Ascaris*, *Trichuris*, filariae such as *Onchocerca*, *Dracunculus*) are common and cross-reactions with these parasites

could give false positive results (Au *et al.*, 1983; Mahannop *et al.*, 1995, Escalante *et al.*, 2004). In industrialised countries, the risk of cross-reactions using E/S antigen is low, but cross-reactions do occur with human larva migrans syndromes of unknown species. The use of the synthetic tyvelose antigen in the ELISA has resulted in even greater specificity (Bruschi *et al.*, 2001; Owen *et al.*, 2001), although some exceptions have been noted (Dea-Ayuela *et al.*, 2001). Further study on this synthetic antigen is required.

A capture ELISA (cELISA) using TSL-1 antigens (tyvelose bearing epitopes) immobilised with a specific monoclonal antibody (MAb) on the plates was a reliable method for serodiagnosis of human trichinellosis compared to other antigens (crude antigen, deglycosylated crude antigen and affinity-purified TSL-1 antigens); in fact, this was the only method giving 100% specificity and 100% sensitivity at the patent stage of the infection, and it was also the most sensitive for sera obtained prior positivity in IIF. These results suggest that cELISA with TSL-1 antigens was more sensitive than IIF or ELISA allowing an earlier detection of infections. It was also shown by ELISA inhibition with the above MAb that human IgG1 antibody responses against TSL-1 antigens were directed almost exclusively against tyvelose-containing epitopes and that antibodies specific for tyvelose-containing epitopes accounted for a large proportion of anti- *T. spiralis* reactivity in most patients (Escalante *et al.*, 2004).

Western blot

Western blot (WB) is able to discriminate efficiently patients with trichinellosis from patients with other helminth infection, although possible cross-reactions may occur with cases of anisakiasis (Yera *et al.*, 2003) and schistosomiasis (Dupouy-Camet, unpublished data). Western blot can be used as a primary or confirmatory test and when E/S antigens are used it is quite specific and useful for follow-up studies (Andrews *et al.*, 1995). Antibodies were detected earlier in the course of the disease by western-blot than by ELISA or IIF (Yera *et al.*, 2003). The presence of antibodies specific for the TSL-1 antigen family (40 kDa to 70 kDa in the reduced form) should be considered of diagnostic value. Western blot has been used also with the purified 45 kDa. glycoprotein to evaluate IgG subclasses (IgG4) (Pinelli *et al.*, 2001).

Other techniques

Counterimmunoelectrophoresis or latex agglutination are recommended when a rapid confirmation of infection is required (the result is obtained in less than 1 h), yet these tests are not commonly used for the diagnosis of trichinellosis because they are less sensitive and specific than ELISA. Competitive inhibition assay, which detects specific antibodies, is a valuable test, but it is used less frequently (Ivanoska *et al.*, 1989).

Kit evaluation

For humans, it is imperative to consider the cut-off value which derives from the mean \pm two or three standard deviations of optical densities obtained with a panel of at least 100 to 200 sera which are representative of the human population for which the test will be used. Different factors such as the human genotype, food habits and environmental characteristics can influence the background of a serological test. This preliminary evaluation should be done for both commercial kits or *in house* developed tests. It is also important to confirm the cut-off value, whenever the antigen, reagents or materials (e.g. ELISA plate) are modified or changed.

Muscle biopsy

For parasitological diagnosis, a muscle biopsy must be collected, preferably from the deltoid muscle, although any skeletal muscle could be used. The surgeon should carefully collect 0.2 g to 0.5 g of muscle tissue (less than a pea size) without fat or skin. One part of the muscle biopsy should be weighed and

stored without any fixative, avoiding dehydration; the other part be processed for histological examination. The sensitivity of the parasitological diagnosis depends on the amount of muscle sample tested and the number of lpg.

Trichinelloscopy

This method is widely used in diagnosis because it detects *Trichinella* larvae, defines the intensity of infection (i.e. the number of lpg of examined tissue), and allows the collection of individual larva, which can then be used to identify the parasite at the level of species or genotype. The number of lpg is correlated with the severity of infection: if at least approximately 1,000 lpg are present, the infection is very severe (Pawlowski, 1983). This technique, like all techniques for parasitological diagnosis, is also useful for diagnosing sporadic cases of the infection, doubtful cases (e.g. atypical clinical course, the absence of circulating antibodies, as occurs in immunosuppressed persons, and retrospective analysis of persons), and, frequently, for purposes of compensation claims. To perform trichinelloscopy, small muscle samples (no larger than a grain) are compressed between two thick slides held together with two screws and examined under a trichinelloscope or a dissection microscope at a magnification of 30 to 40 X, or between two microscopy slides, and examined under a light microscope at a magnification of 50 to 100 X. The larvae are easier to detect when the muscle biopsy is performed in the late stage of infection, which is characterised by a fully developed capsule (Figure 2, chapter 1). However, trichinelloscopy may fail when the larval density is low or for not yet encapsulated larvae or larvae from non encapsulated species, resulting in false-negative results.

Digestion

Digestion of muscle samples using pepsin and HCl digestion fluid (see later) is very useful for accurately determining the number of lpg of muscle tissue and for isolating larvae for molecular identification (Figure 2, chapter 1). However, if the muscle biopsy is taken too early after infection, the larvae can be destroyed by digestion: only muscle larvae that are at least 10 to 12 days of age are not destroyed by artificial digestion (i.e. from muscle biopsies collected two to three weeks p.i.). The muscular biopsy is cut in small pieces and incubated at 41°C for 30 min in a small beaker containing 2 ml of digestion fluid for 100 mg of muscle (see Chapter 3, Annex I). Non-encapsulated species would necessitate shorter digestion times such as 15 min to 20 min. The microscopic analysis is carried out directly in the beaker by using a binocular magnifier microscope. The sensitivity of this method depends on the amount of muscle sample tested. This method permits the isolation of larvae by feeding mice.

Histology

The histological analysis of muscle tissue reveals fragments of larvae at various stages of development, the presence of the collagen capsule (for encapsulated species) or that which remains of a destroyed capsule, the presence of muscle-cell basophilic transformation, and the type and composition of cellular infiltrates. The basophilic transformation of muscle cells represents a valuable diagnostic criterion of *Trichinella* invasion even when no larvae have been detected. This method is more sensitive than trichinelloscopy in the early stage of muscle invasion, when larvae are very small and cannot be easily differentiated from the muscle fibres (Wranicz *et al.*, 1998)

Molecular analysis

Molecular biology has also been extensively used to type *Trichinella* isolates. Techniques based on DNA amplification of various targets are capable of typing samples containing as few as a single larva, by means of polymerase chain reaction (PCR). Two typing methods are commonly used: multiplex PCR (Zarlenga *et al.*, 1999) and sequencing of the conserved 5S rRNA gene (Rombout *et al.*, 2001, De Bruynes *et al.*,

2005). This identification is possible at the International *Trichinella* Reference Centre (Istituto Superiore di Sanita, viale Regina Elena 299, 00161 Rome, Italy) where it is possible to send infected samples or infected mice.

TREATMENT

There are 'areas of uncertainty in the management of human trichinellosis' because there have been very few prospective, controlled clinical trials of treatment for this infection (Watt & Silachamroon, 2004). However, on an empiric basis, most experts recommend the association of diffusible anthelmintics and corticosteroids (Dupouy-Camet *et al.*, 2002).

Anthelmintics

Only three comparative studies have been made to evaluate the efficacy of the different anthelmintics for trichinellosis (see Annex II). The principal anthelmintics used for trichinellosis are mebendazole (Vermox^R, Janssen) and albendazole (Zentel^R, GlaxoSmithKline). Thiabendazole is no longer used because of its side effects. Pyrantel (Combantrin^R, Pfizer) has been proposed for children and pregnant women (Kociecka, 1988), and flubendazole has been used in some countries. However, the efficacy of these two products is doubtful. To eliminate adult worms from the intestinal lumen, thus preventing production of NBLs and muscle invasion, and the development of trichinellosis, anthelmintics must be used during the period of intestinal invasion (i.e. less than one week after infection). However, this is rarely possible, and treatment is usually started at the beginning of larval development in muscle cells. Since it has not been clearly established how long the adult females survive and produce NBL in the human intestine, it is recommended that anthelmintics should be administered to all persons with trichinellosis during the four to six weeks following infection. Mebendazole was shown to prevent the occurrence of clinical disease when given to persons 48 h after consumption of meat highly infected with *Trichinella* (Kociecka *et al.*, 1996). The later the treatment is prescribed, the higher the probability that the infected person will harbour viable larvae in their muscles for years (Ozereztkovskaya *et al.*, 1980), with possible persistent myalgia.

Mebendazole

Several studies have reported that mebendazole is effective against trichinellosis (Klein *et al.*, 1980; Ozereztkovskaya *et al.*, 1980; Kociecka *et al.*, 1996). Mebendazole, an anthelmintic benzimidazole, is poorly absorbed in the intestinal lumen. The plasma concentrations differ considerably from individual to individual, ranging from 17.5 ng/ml to 500 ng/ml (Braithwaite *et al.*, 1982), although concentrations have been shown to increase when cimetidine is administered concomitantly (Bekhti & Pirotte, 1987). Mebendazole is available in tablets (100 mg) or as a suspension (30 ml bottle at a concentration of 100 mg/5 ml) and should be administered at a daily dose of 5 mg per kg body weight (administered in two doses) (e.g. in adults, two tablets twice daily) for 10 to 15 days. The whole treatment cycle may be repeated after five days. In some countries (e.g. Germany, Italy and Lithuania), higher doses are recommended (20 mg/kg/day to 25 mg/kg/day administered in three doses for 10 to 14 days), and in this case 500 mg tablets are used. However, compared to lower doses, this dose has been more frequently associated with adverse effects, such as allergic reactions, increased liver enzymes values, alopecia and bone marrow depression. Persons receiving high doses should be strictly supervised, monitoring blood counts and liver function (Martindale, 1996). Persons with liver failure should receive reduced doses (Braithwaite *et al.*, 1982). The efficacy of mebendazole against larvae in muscle tissues depends on the time between infection and treatment and could be dose-dependent. For example, when using a cumulative dose of 7.5 g to 15 g of mebendazole for 10 to 13 days started one month after infection, the treatment failed to kill muscle larvae (Pozio *et al.*, 2001). A larvicidal effect was obtained only when using a cumulative dose of 77 g of mebendazole for 56 days, started four months after infection (Levin, 1983).

Albendazole

Albendazole, an anthelmintic benzimidazole carbamate, is absorbed in the intestinal lumen relatively quickly. After oral administration of a single dose of 400mg, peak plasma concentrations of the sulphoxide metabolite (i.e. between 0.04 and 0.55 microg/ml) were obtained after 1 h to 4 h (Marriner *et al.*, 1986). When the drug was administered with a fatty meal, a two to four-fold increase in plasma concentration was observed, although large intra-and inter-individual variability in the plasma concentration has been reported (Lange *et al.*, 1988). Concentrations of 0.45 mg/l to 2.96 mg/l were obtained in persons treated with 15mg/kg, and the half life of the active sulphoxide metabolite was between 10 h and 15 h (Jung *et al.*, 1992). It is not clear if these higher concentrations of albendazole, compared to those obtained with mebendazole, are correlated with a higher antiparasitic activity. Albendazole is well tolerated in persons with trichinellosis (Kociecka *et al.*, 1989; Cabie *et al.*, 1996; Watt *et al.*, 2000). In addition, when high doses were used to treat persons with echinococcosis, for repeated sequences of 800 mg/day for four weeks, gastrointestinal side effects were observed in 4% of these persons, dizziness and headaches in 2.4%, urticaria and itching in 1.2%, leukopaenia in 2.4% and elevated serum transaminases in 16.6% (Horton, 1989). Alopecia has also been reported (Martindale, 1996). Albendazole is available in tablets (200 mg) or as a suspension (20 ml bottle at a concentration of 100 mg/5 ml). In adults, it should be used at a daily dose of 800 mg/day (15 mg/kg/day) administered in two doses, for 10 to 15 days; in children over two years of age, the drug is given at 10 mg per kg body weight. For severe infection, the treatment may be repeated after five days. Blood cell counts and liver function should be regularly monitored.

New formulations

The efficacy of benzimidazoles is limited by their poor water solubility, and the consequent poor bioavailability in intestinal fluids and poor absorption through the intestinal lumen. To increase the rate of absorption and the concentration of these drugs in plasma, certain formulations have been developed and only used in animal models. Specifically, mebendazole and albendazole have been supplemented with polyvinyl-pyrrolidone to create solid dispersions, and albendazole in liquid solutions has been supplemented with absorption promoters, mixed with crystalline complexes of cyclodextrin or with arachis oil-polysorbate 80 as an excipient (Lopez *et al.*, 1997; Castillo *et al.*, 1999). Further studies are needed but it could be a promising approach to treat humans.

Glucocorticosteroids

Though no valid controlled studies have been performed, glucocorticosteroids are used by most physicians to treat the signs and symptoms of type I hypersensitivity. They must always be used in combination with anthelmintics and never alone, since they could increase the larval burden by delaying the intestinal worm expulsion. Klein *et al.* (1980) showed that steroids used in combination with mebendazole would significantly shorten the duration of fever. They could also provoke a prolonged eosinophilia resulting from a delayed encapsulation process of the muscular larvae (Kociecka *et al.*, 1989). Glucocorticosteroids could also be used to treat acute vasculitis and myositis, in this case they could also help to prevent complications by inhibiting eosinophil activation, degranulation and consequent cytotoxicity for endothelium (Fourestié *et al.*, 1993) Moreover, dexamethasone administered with albendazole has been reported to increase the serum levels of albendazole sulphoxide by about 50% (Jung *et al.*, 1990). The most commonly used glucocorticosteroid is prednisolone, which is available in tablets of 1 mg or 5 mg and is administered at a dosage of 30 mg per day to 60 mg per day, in multiple doses, for 10 to 14 days.

Practical recommendations

For severe and moderately severe diseases:

- 1) hospitalisation is compulsory for severe forms and debatable for moderately severe forms
- 2) administration of anthelmintics (albendazole or mebendazole)
- 3) monitoring of the pharmacokinetics of anthelmintics (if possible)
- 4) administration of glucocorticosteroids (e.g. prednisolone)
- 5) compensation of fluid and electrolyte deficits
- 6) administration of pain killers.

For benign, abortive and asymptomatic diseases:

- 1) administration of anthelmintics (albendazole or mebendazole)
- 2) administration of non-steroidal anti-inflammatory drugs if necessary
- 3) reporting and surveillance forms are proposed in Annex III and IV.

EVOLUTION

The evolution of the disease is usually simple but depends on the severity of the disease (see paragraph 'Evaluation of the severity of the disease'). A severe disease will have a complicated evolution. Complicated evolutions are seen after an important infective dose, in elderly patients and in patients with associated debilitating factors.

Death

Death is rare. For example, of the more than 6,500 infections reported in the EU in the past 25 years, only five deaths have been observed, all of which were due to thromboembolic disease, in persons over 65 years of age; deaths has been reported in two outbreaks involving more than one thousand cases (Ancelle *et al.*, 1988). Twenty fatalities out of 10,030 cases were reported in a worldwide survey performed by the ICT between January 1995 and June 1997 (Dupouy-Camet, 2000). During a horsemeat outbreak in France, caused by the newly described *T. murrelli*, a 0.46% mortality rate was observed. (Ancelle *et al.*, 1988). No deaths were reported in outbreaks caused by *T. britovi*. (Bruschi & Murrell, 2002). In 2005, three deaths were recorded in Serbia amongst 339 cases (Sofronic-Milosavljevic & Djordjevic, personal communication, 2005) and two deaths in Romania amongst 574 cases (Cretu, personal communication, 2005).

Convalescent stage

The convalescent stage of trichinellosis begins when the adult females cease to release migrating larvae and the already established larvae have completed their development in the muscle cells. The transition to this stage is characterised by the progressive disappearance of the signs and symptoms of the disease and by the return of laboratory parameters to normal values (see paragraph 'Non-specific biological signs'). This stage usually begins between the sixth and the eighth week p.i., and infected persons could still have a severe asthenia for several weeks and chronic muscular pain for up to six months. Most persons will then become asymptomatic, though live larvae will persist in their muscles for years.

Chronic trichinellosis and sequelae

Definition

Whether or not a chronic form of trichinellosis actually exists is still under debate, and chronic trichinellosis could be difficult to distinguish from sequelae of the acute phase. However, its existence is supported by reports of persons who complain of chronic pain and a feeling of general discomfort and who show signs of paranoia and a syndrome of persecution, months or even years after the acute stage. Persistent formication, numbness, and excessive sweating have been observed more frequently in persons who have had severe trichinellosis (Pielok, 2001). Impaired muscle strength, conjunctivitis, impaired coordination and IgG antibodies have been reported in some persons up to ten years post infection (p.i.) (Harms *et al.*, 1993), whereas live larvae in muscles were detected without clinical signs and symptoms up to 39 years p.i. (Fröscher *et al.*, 1988). Electromyographic disturbances can be observed for several years after the acute stage (i.e. in persons considered to be chronically infected), usually in persons who had not been adequately treated in the early period of invasion. In these cases, the disturbances are characterised by a mixed type of electrical alterations, revealing a disturbed function of motor neurons and of impulse transmission at the neuromuscular junction (Kociecka, 2001). Five (two of which were treated at onset of infection) out of thirteen patients re-evaluated 15 years after a *T. britovi* infection still presented EMG changes (Piergili-Fioretti *et al.*, 2005). The existence of a chronic form is supported by the presence of IgG antibodies in the serum, of bioelectric muscle disturbances, and of inflammatory cells in the muscles, all due to the chronic presence of live larvae. Moreover, this syndrome can also result from unnoticed brain localisations during the acute phase of the disease. An algorithm is given in Table III to help to the diagnosis of chronic trichinellosis and sequelae.

Table III - Algorithm for diagnosing chronic trichinellosis and sequelae

Group A	Group B	Group C	Group D
Chronic myalgia	Neuropsychiatric disorders	Positive serology (with a highly specific test)	Electromyographic alterations
Cutaneous rash or formication	Cardiological signs	Positive muscular biopsy	Myositis at histology
Conjunctivitis			

The diagnosis of chronic trichinellosis is.-

Probable: any C and/or one A or B and one D; confirmed: any C and two A or any D

Sequelae are: Probable: any B acquired during acute infection and one C; confirmed: any B acquired during acute infection

Treatment of sequelae and of chronic trichinellosis

At this stage, anthelmintics are useless; on the contrary, glucocorticosteroids or non-steroidal antiinflammatory drugs prescribed for short periods can lead to some transient improvement of myalgia. Physiotherapy and psychotherapy could certainly alleviate muscular and neurological sequelae.

TRICHINELLOSIS IN PREGNANT WOMEN AND CHILDREN

Pregnancy

In pregnant women, trichinellosis can cause abortion or premature delivery (Ancelle *et al.*, 1988). Although the underlying mechanisms have not been clarified, these complications could be due to modified

production of choriogonadotropin, progesterone or cytokines (Kociecka, 1988). The existence of congenital trichinellosis has not been clearly established; however, most women infected during their pregnancy have delivered healthy babies (Kociecka, 2000). Since mebendazole is teratogenic in rats, it is contraindicated in pregnant women and in children less than two years of age. However, a recent study showed that mebendazole therapy during pregnancy (but at 200mg/day for three days) was not associated with a significant risk for major congenital defects when administered during the second and third trimesters, but not during the first trimester (De Silva *et al.*, 1999). Thus during pregnancy, especially in the first trimester, mebendazole should be used only when the infection is severe, and treatment must begin no later than one to three weeks from infection, because at the recommended dose for pregnant women, it is not *effective after this period*. *Albendazole is contraindicated in pregnant women although offspring of pregnant women accidentally receiving albendazole at high dosages did not show any damage at birth* (Horton, 1993; Auer *et al.*, 1994; Bradley & Horton, 2001). Therefore, during pregnancy hospitalisation is compulsory for symptomatic forms. Only anthelmintics that are poorly absorbed by the intestinal lumen should be used (i.e. pyrantel at 10 mg/kg body weight for one to three days), although the efficacy of these drugs has not been evaluated in humans or is doubtful. However, for severe infection, mebendazole could be administered under the physician's control and responsibility. In the acute stage of trichinellosis, with severe disease, prednisolone may be administered at a dose of 20 mg/day to 30 mg/day for 10 to 12 days, gradually reducing the dose (particularly for women in the third trimester of pregnancy). Salicylates are not administered because of their negative effects on the foetus.

Children

In children, the signs and symptoms of trichinellosis are the same as those found in adults, although myalgia and diarrhoea are less frequent, the clinical signs and symptoms are less pronounced and regress more quickly, and the frequency of complications is lower. The clinical picture is milder possibly because of lower infecting doses and a less intense allergic reaction to the larvae invasion. Children should be treated by administration of anthelmintics (albendazole or mebendazole) if older than two years of age; the use of these drugs in younger children is, in principle, contraindicated but trichinellosis is not frequent at that age. Glucocorticosteroids (e.g. prednisolone) will be prescribed if necessary. Regarding treatment in children, the use of mebendazole has been given before the age of two years in situations where it was deemed necessary (Gendrel *et al.*, 2000).

CONCLUSIONS

Any physician who observes a case of trichinellosis should alert public-health and veterinary authorities so that other cases and the source of infection can be identified and so that treatment can be started as soon as possible. Additional information on all aspects of human trichinellosis or assistance with specific problems can be obtained by contacting the ICT at the following web site: www.med.unipi.it/ict/welcome.htm.

Although it has not been clearly proven by case-control studies, early treatment with anthelmintics and glucocorticosteroids must be used to alleviate the general syndrome of the disease, to prevent complications and to reduce persistent muscular pain. Anthelmintics are effective in the intestinal stages of the parasite and should be prescribed in all occurrences, although efficacy against muscle larvae decreases as the time between infection and treatment increases. But there are still several important unresolved questions about the treatment of trichinellosis: whether or not to treat a pregnant woman with a severe disease with benzimidazoles? What is the anthelmintic drug of choice for severe, life threatening disease? Would higher doses of benzimidazoles have a greater effect on encapsulated larvae? Could new formulations or absorption promoters increase the efficiency of benzimidazoles? Information from prospective, controlled trials is urgently needed.

ANNEX I
EUROPEAN CENTER FOR DISEASE CONTROL CASE DEFINITION
TRICHINELLOSIS (TRICHINELLA SPP.)

Clinical criteria

At least three of the following six:

- fever
- muscle soreness and pain
- gastrointestinal symptoms (diarrhoea)
- facial oedema
- eosinophilia
- subconjunctival, subungual and retinal haemorrhages.

Laboratory criteria

At least one of the following two laboratory tests:

- demonstration of *Trichinella* larvae in tissue obtained by muscle biopsy
- demonstration of *Trichinella* specific antibody response by IIF, by ELISA or western-blot (i.e. seroconversion).

Epidemiological criteria

At least one of the following three:

- consumption of laboratory confirmed parasitised meat
- consumption of potentially parasitised products from a laboratory confirmed animal
- an epidemiological link to a laboratory confirmed human case by exposure to the same common source.

Case classification

- Possible case: not applicable
- Probable case: any person meeting the clinical criteria and with an epidemiological link
- Confirmed case: any person meeting the laboratory criteria and with clinical criteria within the past two months
- To be reported to EU level: confirmed cases should be reported to the EU level.

ANNEX II

COMPARATIVE STUDIES IN HUMANS

Albendazole vs thiabendazole and flubendazole

In a prospective study, Fourestié *et al.* (1993) compared two regimens (i.e. 1) albendazole and 2) thiabendazole followed by flubendazole) in 117 persons infected in a single outbreak of *T. spiralis* infection related to horsemeat consumption. Disease activity was evaluated at 1, 7, 15, and 45 d.p.i. No difference was found between the two groups with regards to the evolution of myalgia, fever, fatigue, new clinical manifestations, or laboratory and serological data. Both treatment regimens were well tolerated. Sixteen months p.i., 30 of the persons treated with albendazole and 29 of those treated with thiabendazole and flubendazole were re-evaluated. Serology was negative for 70% of the albendazole group and for 34.5% of the thiabendazole-flubendazole group. Moreover, of the four muscle biopsies performed on persons treated with albendazole, one was positive, and it revealed a low number of larvae, whereas three of the five biopsies performed on persons receiving thiabendazole plus flubendazole were still positive and they revealed a high number of larvae. Although no difference was observed between the two regimens in the early response to therapy, albendazole could be more effective against residual larval presence.

Albendazole vs thiabendazole

Cabie *et al.* (1996) compared the immediate and midterm efficacy and tolerability of thiabendazole and albendazole as therapy for 46 persons also infected in a horsemeat related outbreak due to *T. spiralis*. A total of 26 persons received thiabendazole and 18 received albendazole. All infected persons were also treated with prednisone. Eight relapses occurred (seven in the thiabendazole group and one in the albendazole group). Side effects were reported by seven persons, all of them were treated with thiabendazole. Six months after treatment, 16 of the 31 persons who responded to a questionnaire still had symptoms, the most frequent of which were myalgia (81%) and fatigue (69%), with no significant differences between the two treatment groups. The authors concluded that the immediate efficacy of thiabendazole and albendazole as therapy for trichinellosis was comparable but that albendazole was better tolerated.

Mebendazole vs thiabendazole vs fluconazole vs placebo

In a recent study, Watt *et al.* (2000) conducted a double-blind, placebo-controlled comparison of three antiparasitic drugs during an outbreak of trichinellosis in northern Thailand. Forty-six adults were randomised to receive ten days of oral treatment with either mebendazole (200 mg twice a day), thiabendazole (25 mg/kg twice a day), fluconazole (400 mg initially, then 200 mg daily), or a placebo. The persons receiving fluconazole or placebo were also treated with pyrantel for five days. All infected persons were seropositive; 19 persons (41%) had a positive biopsy. Improvement was observed among a significantly higher proportion of persons treated with mebendazole (12/12) and thiabendazole (7/7) compared to those receiving the placebo (6/12; $p < 0.05$) or fluconazole (6/12). Muscle tenderness resolved in a greater number of patients treated with thiabendazole and mebendazole than in those treated with placebo ($p < 0.05$). Although the results of this study may have been greatly affected by bias, they show that 77% of the persons treated with pyrantel still had myalgia ten days after beginning treatment, compared to 16% of the persons treated with mebendazole or thiabendazole. Moreover, as already reported by Cabié *et al.* (1996), the persons treated with thiabendazole showed side effects: intolerable dizziness, urticarial rash, generalised maculopapular rash, hand rash (Walsh, 2001), tinnitus, and gastrointestinal disturbances.

Conclusion

Thiabendazole is no longer recommended as a drug of choice because of severe side effects. In light of the results of these three studies, mebendazole and albendazole should be considered as the first-line drugs for treating the acute phase of trichinellosis.

ANNEX III
INDIVIDUAL REPORT FORM

INDIVIDUAL REPORT FORM		Reporting physician						
Date (dd/mm/yyyy)		Address						
		Phone, fax, mail						
Patient identification								
3 first letter of the name, first letter of the surname								
Birth's date				Sex (M, F)				
Town of residence		Country						
Date of diagnosis (dd/mm/yy)								
Muscular biopsy	done	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>			
				Result: Positive	<input type="checkbox"/>			
					Negative			
					<input type="checkbox"/>			
Serology	done	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>			
				Result: Positive	<input type="checkbox"/>			
					Negative			
					<input type="checkbox"/>			
Serology	Type	Threshold value		Result				
Technique 1								
Technique 2								
Signs and symptoms	Yes	No	?	Complications	Yes	No	?	
Fever > 39°C	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Neurological	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Myalgias	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Cardiovascular	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Facial oedema	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Death	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Increased muscular enzymes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Eosinophilia	c/mm3			
Treatment	Name (ICD)	Doses		How many days?				
Anthelmintics								
Corticosteroids								
Other								
Other cases (family, relatives...)								
Probable date of contamination								
Probable place of contamination								
Involved meat:	Wild boar	<input type="checkbox"/>	Horse	<input type="checkbox"/>	Pork	<input type="checkbox"/>	Other	<input type="checkbox"/>
Coming from:	Shop	<input type="checkbox"/>	Supermarket	<input type="checkbox"/>	Hunting	<input type="checkbox"/>	Restaurant	<input type="checkbox"/>
Other	<input type="text"/>							

ANNEX IV

SURVEILLANCE FORM

DO is the day when surveillance begins

SURVEILLANCE FORM	DO	D7	D14	D28	D60	D360
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Fever

Maximum:

Cutaneous rash

Diarrhoea

Myalgia

Localisation:

Facial oedema

Other oedema

Localisation:

Conjunctivitis

Asthenia

Other

Specify:

Neurological complication

Specify:

Cardiovascular complication

Specify:

Other complications

Specify:

BIOLOGY	DO	D7	D14	D28	D60	D360
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Blood cells counts

Haematocrit %

Haemoglobin (g/l)

WBC per mm

Neutrophils

Eosinophils

Lymphocytes

Monocytes

Platelets per mm-

ESR (first hour)

Enzymes

Specify int. Units

ASAT/ALAT

CPK

Serology

Specify threshold

IIF

ELISA

WB

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CHAPTER 3

DETECTION AND SURVEILLANCE FOR *TRICHINELLA*: MEAT INSPECTION AND HYGIENE, AND LEGISLATION

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Summary

The present chapter outlines the diagnostic tools available for *Trichinella* inspection of meat for consumption, and indirect tools for detection of infection in single animals, herds of animals or wildlife.

Keywords

Artificial digestion - Enzyme-linked immunosorbent assay - Legislation - Meat inspection - *Trichinella* detection - Trichinoscopy.

METHODS FOR DETECTION OF *TRICHINELLA*

According to the *Manual for Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE, 2004a), the diagnosis of *Trichinella* infection in animal falls into two categories:

- *direct methods*.; identification and visualisation of the first-stage muscle larvae encysted or free in striated muscle tissue
- *indirect methods*.; detection of infection by demonstration of specific circulating antibodies.

Direct methods: detection of *Trichinella* muscle larvae

The identification of *Trichinella* larvae in muscle samples is limited to *post-mortem* inspection of carcasses. In many countries, in order to prevent human trichinellosis, the examination of muscle samples from pigs and other animal species used for consumption (e.g. horses, wild boars, bears etc.), potential sources of this food-borne infection, should be a part of routine slaughter inspection (Gamble *et al.*, 2000). Direct detection is also widely applied in wildlife epidemiology, where indicator animals (e.g. foxes or raccoon dogs) are examined to assess the existence of infection among wildlife reservoirs and the risk of introduction into production animals. Indicator animals provide an estimation of the prevalence of *Trichinella* in the environment (Nöckler *et al.*, 2000).

Factors important in larval detection

Direct methods for the detection of *Trichinella* larvae in muscle samples are designed for optimal sensitivity for a particular sample size, but sensitivity is greatly influenced by the muscle selected for sampling and the specific method used (Nöckler *et al.*, 2000).

Sample location

Selection of muscles for sampling in meat inspection requires identification of predilection sites in a particular animal species. Experimental studies using doses that mimic natural infection have been carried

out in different species to determine typical predilection sites for *Trichinella* larvae. In domestic pigs, the three main predilection sites for *T. spiralis* are the diaphragm crus, the tongue and the masseter muscle (Gamble, 1996; Forbes and Gajadhar, 1999). Comparable results were also observed in experimental *T. britovi* and *T. pseudospiralis* infection in pigs (Nöckler *et al.*, 2005). The neck muscle appears to be another predilection site in addition to the tongue, the diaphragm and the masseter in pigs experimentally infected with *T. spiralis* and *T. britovi* (Kapel *et al.*, 1998). In horses experimentally infected with *T. spiralis* and *T. britovi*, the tongue and masseter were found to be typical predilection sites (Soulé *et al.*, 1989, 1993; Gamble *et al.*, 1996) whereas diaphragm, masseter and tongue yielded the highest larval burden after *T. pseudospiralis* inoculation (Kapel, 2005). In naturally infected horses, most larvae of *T. spiralis* were detected in different muscles of the head. Of these, the tongue and the masseter proved to be the fourth and the fifth most important predilection sites (Pozio *et al.*, 1998; Murrell *et al.*, 2004). Results of experimental infections with *T. pseudospiralis* in poultry (cock-broilers) demonstrated that the muscles of the head (e.g. masseter and the neck) were typical predilection sites (Petrov *et al.*, 1999).

In wild boars (*Sus scrofa*) experimentally infected with *T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. murrelli*, *Trichinella* T6 and *T. nelsoni* most larvae were found in the diaphragm and the tongue (Kapel, 2001). In silver foxes (*Vulpes vulpes fulva*) experimentally infected with *T. spiralis*, the diaphragm crus and the forearm musculature proved to be predilection sites most suitable for examination (Nöckler and Voigt, 1998a). There are comparable results for arctic foxes (*Alopex lagopus*) experimentally infected with *T. nativa* (Kapel *et al.*, 1995), red foxes (*Vulpes vulpes*) naturally infected with trichinae (Cristea, 1996; Malczewska *et al.*, 1997) and for raccoon dogs (*Nyctereutes procyonoides*) naturally infected with *T. spiralis* (Thiess, 2001).

Some of the sampling site recommendations (Gamble *et al.*, 2000) of the ICT are summarised in Table I.

Table I - Predilection sites for *Trichinella* larvae in different animal species (Gamble *et al.*, 2000, Kapel, 2000, Pozio *et al.*, 2004, Kapel *et al.*, 2005)

Animal species	Predilection sites	Aim of examination
Domestic pig	Diaphragm, tongue, masseter	Meat inspection
Horse	Tongue, masseter	(domestic meat)
Wild boar	Forearm, diaphragm	
Bear	Diaphragm masseter, tongue	Meat inspection (game
Walrus	Tongue	meat)
Seal	Tongue, diaphragm flippers, masseter	
Fox	Diaphragm, forearm muscles	
Raccoon dog	Diaphragm, forearm muscles	Epidemiological studies
Crocodile	Intercostal, tongue, forearm, tail	(reservoir animals)

Sample size

The amount of sample used for the detection of *Trichinella* larvae must be chosen to provide an adequate level of sensitivity and an acceptable cost-benefit-relationship. For routine meat inspection, it is necessary to ensure a sensitivity of approximately 1 lpg to 3 lpg as this is the level above which infection constitutes a food safety issue (see Chapter 2, Patient Management). Theoretically, a 1 g sample would be enough for the detection of at least 1 lpg of tissue, on condition that there is a homogeneous distribution of larvae in the tissue investigated. In practice, this is applicable for high larval densities, but in cases of low infection

rates, larvae are not distributed homogeneously. Using 1 g muscle samples from pigs and horses experimentally infected with *T. spiralis*, the sensitivity of the pooled sample method of artificial digestion was between 3 lpg and 5 lpg, whereas a 5g sample increased the sensitivity of this method to approximately 1 lpg (Gamble, 1996, 1998; Gamble *et al.*, 1996; Forbes and Gajadhar, 1999).

For routine slaughter inspection of pig carcasses, using the pooled sample digestion method, a minimum of a 1 g sample of tissue from a predilection site is examined. Using trichinoscopy, the examination of 28 oat kernel-size pieces of diaphragm muscle is recommended which corresponds to a 0.5 g sample (Gamble *et al.*, 2000). Thus, the sensitivity for trichinoscopy is lower than for the digestion method since only half of the sample weight is available for examination. In endemic areas especially, a 5 g sample should be used for digestion in order to adequately increase the sensitivity of the detection method. Because no significant difference in detection sensitivity was found using either 3 g and 5 g samples for detection of infection in pig tissue containing 1.0 lpg to 1.9 lpg, 3 g samples may be considered the minimum sample size under these conditions (Forbes and Gajadhar-1999). To ensure a high sensitivity in horse meat, 5g samples should be examined in the pooled sample digestion method. In France, the requirements for sample size has been changed to 10 g for horsemeat which originates in countries with high *Trichinella* prevalence (Boireau *et al.*, 2000). If the muscles from predilection sites are not available for inspection, carcasses should be tested using larger amounts of available muscle (up to 100 g samples) in order to achieve adequate sensitivity. For epidemiological studies in reservoir animals (wildlife), the sample size should also be adjusted upward to achieve a sensitivity of less than 1 lpg. Because the mean intensity of larvae in muscles of wild carnivores is typically less than 5 lpg, the samples to be tested should have a weight of at least 5 g or more (Gamble *et al.*, 2000). As discussed below, predilection muscles in carnivores may require a prolonged digestion time (up to 2 h) (Kapel *et al.*, 2005).

Choice of method

The correct choice of a suitable diagnostic method is necessary in order to obtain reliable results for all *Trichinella* genotypes. *Trichinella spiralis*, *T. nativa*, *T. britovi*, *T. nelsoni* and *T. murrelli* induce the formation of a nurse cell in the striated muscles of the host, whereas the non-encapsulating species *T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis* are characterised by the lack of a capsule around the muscle larva (Murrell *et al.*, 2000; Pozio & Zarlenga, 2005; Pozio & Murrell, 2006). Larvae of non-encapsulating species are more difficult to detect by trichinoscopy. Because of this limitation, and the lower sensitivity of trichinoscopy compared with artificial digestion methods, trichinoscopy and similar compression methods are not recommended for the routine examination of food animals and game intended for meat consumption (OIE, 2004a).

Since 1970, various methods for artificial digestion have been introduced which allow the examination of a pool of muscle samples from up to 100 carcasses. Although the digestion method requires more technical equipment, it meets the requirements for efficiency, reliability and cost effectiveness far better than trichinoscopy; consequently it has become the method of choice for routine slaughter inspection in most industrialised countries (Webster *et al.*, 2005). Trichinoscopy, however, is still practised in many places in Central and East Europe, and in small slaughterhouses in Western Europe (Kapel, 2005).

Compressorium method/trichinoscopy

As early as the 1860s, microscopy, and later trichinoscopy, were introduced in slaughterhouses for systematic inspection of pigs for *Trichinella* larvae (Nöckler *et al.*, 2000). Later, special trichinoscopes with a large shaded magnification screen on which the technician more easily could detect larvae were developed. When carried out properly by skilled personnel, trichinoscopy has proven to be reliable for detecting a larval burden of approximately 3 lpg, which is considered the threshold for clinical trichinellosis in humans. Trichinoscopy is a fairly simple method and can be employed in any place where ordinary light

microscopy is available. However, it is a laborious method for the inspection of individual carcasses, and it is time-consuming. After slaughter, muscle tissue of the size of a walnut is collected from each of prescribed predilection sites (e.g. diaphragm crus in pigs). A total of 28 small sub samples of muscle of about 2 mm x 10 mm (size of an oat grain), with a total weight of about 0.5 g, should be taken. The muscle pieces are compressed between two glass plates until they become translucent, examined individually for *Trichinella* larvae, using a trichinoscope or a conventional stereo-microscope with 15 to 40 x magnification (OIE, 2004a).

Artificial digestion methods

The main principle of this method is that muscle larvae are released after digestion of the muscle tissue by means of artificial digestion fluid composed of pepsin and hydrochloric acid. Such digestion methods are used for individual or pooled muscle samples, followed by selective screening, filtration, or sedimentation procedures and a final microscopic examination for the presence of larvae (OIE, 2004a).

The efficacy of digestion tests may vary because digestibility depends on muscle types and animal species. For routine *Trichinella* meat inspection in swine, the time for digestion of diaphragm takes not more than 30 min at 44°C to 46°C. In horses, muscle samples from diaphragm, tenderloin, filet, and rump are readily digested within 30 min, whereas masseter, tongue and leg muscles may require digestion times two to three times longer (Kapel *et al.*, 2005). The maximal enzymatic efficacy is reached in the range of 42°C to 46°C, but digestion may take 3 h at 35°C to 37°C compared to only 30 min at 44°C to 46°C. The digestion time can be prolonged to 60 min at 44°C to 46°C, for example, but should not exceed a point at which the structure of larvae becomes degraded and the enzymatic activity of pepsin begins to decrease.

There are various published procedures for the pooled digestion technique for the detection of *Trichinella* in meat (Gamble, 1996; Gamble *et al.*, 2000). According to current legislation of the EU, the following four artificial digestion methods are acceptable (see also paragraph 'Meat hygiene and EU legislation for *Trichinella* inspection'):

- the magnetic stirrer method
- the stomacher sedimentation method
- the stomacher filtration technique
- the Trichomatic 35' automated digestion method.

The magnetic stirrer method (Figure 1) is considered the gold standard because it is a method specifically designed for pooled samples and it has been subjected to validation studies (Kapel *et al.*, 2005). This widely used magnetic stirrer method for pooled samples can be employed in a variety of circumstances with a minimum of equipment (OIE, 2004a). Therefore, this method will be explained in more detail, and for the other three methods cited above see details in European Community (2005).

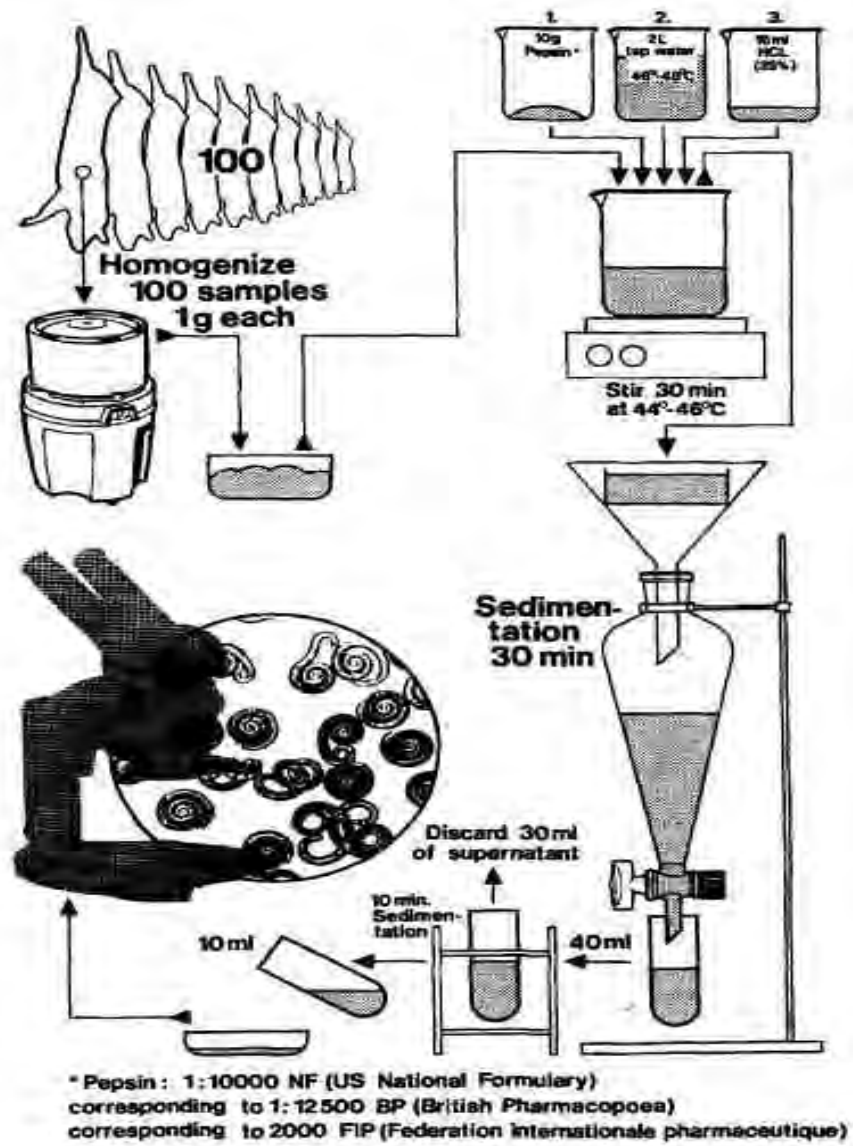


Figure 1 - Outline of the artificial digestion technique (magnetic stirrer method)

For the magnetic stirrer method (Figure 1) the essentials are: a maximum of 100 g of pooled samples of muscle tissue from prescribed predilection sites of the animals under inspection. The sample pool is digested using 2 l of artificial digestive fluid consisting of 0.5% pepsin (1:10,000 USA National Formulary) and 25% HCl to achieve a final normality of 0.06N. The digest is stirred for 30 min at 44°C to 46°C in a 3 l glass beaker using a heated plate magnetic stirrer. During this process, the *Trichinella* larvae are released from the muscle. The digestion fluid is then poured through a sieve (mesh size 180 µm), which retains any undigested tissues, but allows the passage of *Trichinella* larvae, into a 2 l separatory funnel. Following sedimentation for 30 min, a 40 ml sample of the sediment is quickly released into a 50 ml tube. After a further 10 min of sedimentation to clarify the suspension, 30 ml of supernatant is withdrawn and the remaining 10 ml of sediment is poured into a gridded Petri dish. The 50 ml tube is rinsed with 10 ml of tap-water, shaken, and this is added to the gridded Petri dish. The sample in the petri dish is then examined by either trichinoscope or stereo-microscope (15 to 40 x magnification) for the presence of *Trichinella* larvae (Nöckler *et al.*, 2000). A detailed protocol for this magnetic stirrer method for detection of *Trichinella* muscle larvae in pork is described in Regulation (EC) No 2075/2005 and is reproduced in Annex I. Molecular diagnosis to detect *Trichinella*-DNA in meat samples by means of PCR has shown a low sensitivity due to the huge amount of muscle cells DNA diluting the larva target sequence.

Validation of direct detection methods

The ICT recommends that all slaughter testing methods for *Trichinella* detection in pigs, other livestock and game should be validated by standard procedures and any new methods be subjected for evaluation by at least three reference laboratories (Gamble *et al.*, 2000). The ability to perform validation studies depends on proficiency samples of consistently high quality (Forbes *et al.*, 1998). Such a proficiency panel consists of a minimum of 40 samples including ten negative samples, ten samples containing 3 lpg to 5 lpg, ten samples containing 10 lpg to 20 lpg and ten samples containing 20 lpg to 50 lpg. Acceptable results of testing among the three reference laboratories are detection of at least one larva, at a 95% confidence level, in those samples containing 3 lpg to 5 lpg, and recovery of a minimum of 75% of total larvae from samples containing 10 lpg to 20 lpg and 20 lpg to 50 lpg.

In a validation study for the magnetic stirrer method, a larval burden of more than 3 lpg was consistently detected with a currently accepted sample size of 1 g from the individual pig carcass, whereas larval burdens of 1.0 lpg to 1.9 lpg required a sample size of 3 g to 5 g (Forbes and Gajadhar, 1999). Improved larval recovery can be obtained by changing the filter size from 177 µm (180 µm) to 355 µm (Gamble, 1999). In another validation study, the sensitivity of the magnetic stirrer method was 3.2 times better than the most sensitive Compressorium technique for identification of samples with low infection level (less than 2 lpg). Thus, trichinoscopy was less sensitive than the digestion method regardless of the larval muscle density evaluated (Forbes *et al.*, 2003). A comparative study pointed out that both sensitivity and reproducibility of the stomacher method are considerably lower in comparison to the magnetic stirrer method (Webster *et al.*, 2006). *Trichinella* larvae were lost at various steps of the stomacher procedure and these steps need to be identified and corrected through optimisation to improve detection methods.

Quality assurance system for artificial digestion methods

The ICT recommends that all laboratories which are involved in *Trichinella* meat inspection should establish a suitable quality assurance system. Such a quality assurance system is required to document that properly trained analysts perform the method under controlled conditions in order to produce reliable and consistent results during *Trichinella* meat inspection according to a predetermined level of sensitivity and specificity (Gamble *et al.*, 2000).

Consumers demands for safe food, and the world wide trade standards for adequate food safety have led to the introduction of tests that are subject to, in order to qualify meat for export, validation and quality assurance systems (Forbes *et al.*, 1999). An internationally recognised quality assurance system was developed for the artificial digestion method by means of the magnetic stirrer procedure according to ISO/IEC Standard 17025 (Gajadhar and Forbes, 2002). The main components for the laboratory accreditation are briefly described in recent papers (Gamble *et al.*, 2000; Forbes *et al.*, 2005):

The quality assurance manual provides information on the programme's organisation and describes staff qualifications, training requirements, mechanisms for monitoring adherence to written protocols, criteria for certification of analysts, equipment maintenance, reporting, record keeping, handling deviations, corrective actions, handling of complaints, documentation and audits.

A standardised protocol must be developed to ensure that accurate and repeatable results can be achieved by any laboratory performing the detection method. A protocol for the validation method must be clearly written, and include a detailed description of all necessary equipment, reagents and procedures. The protocol has to be performed exactly as written, and must include critical control points (CCP's) which are defined as those procedures, equipment or reagents which could adversely affect the results of the detection method, if not used exactly as stated in the protocol (see Annex I).

The technical certification demonstrates the knowledge and ability of analysts to perform the method accurately. A regular training programme for meat inspectors by qualified persons should be provided and evaluations for competency by testing of proficiency samples must be instituted.

During regular proficiency sample testing programme analysts must test a set of unknown samples prepared by a reference or another competent laboratory. A standardised protocol should be used to prepare and distribute samples. Such proficiency sample programme can be adapted for implementation and/or validation of a new method, by ring testing among a group of qualified laboratories.

A reporting and trace back system must be in place. This includes a complete documentation on operability of technical equipment and quality of consumables as well as sample entrance, inspection scheme and laboratory report. Each sample must be linked to the associated carcass to ensure trace back success in case of a positive or doubtful finding.

The audit system should monitor the maintenance of the quality assurance system. It must identify deficiencies and it controls the progress of corrective actions. Internal audits that are performed by the designated laboratory employee (quality manager) take place annually whereas external audits are conducted by the official regulatory body (e.g. state accreditation office) every two years.

An appropriate laboratory facility provides a controlled environment for sample testing and the health and safety of persons working in the laboratory is ensured. At least one door should be used to separate common areas from the laboratory which must have adequate, illuminated bench space, hot and cold running water, a sink suitable for the glassware used in the procedure, surfaces impervious to common disinfectants, a fume hood, adequate ventilation, heating and cooling system capable of maintaining a comfortable working temperature, appropriate signage, a pest control programme if necessary, and immediate access to an emergency shower and a first aid kit, staff washrooms, and appropriate laboratory wear such as gloves, safety glasses and lab coats (Gamble *et al.*, 2000).

Indirect methods for surveillance: detection of *Trichinella* antibodies

Serological methods are used either for ante-mortem or post-mortem examination of serum samples for *Trichinella-specific* antibodies (OIE, 2004a).

According to the ICT, indirect (serological) methods are not recommended as a substitute for meat inspection of individual carcasses (Gamble *et al.*, 2000). However, serological methods for detection of *Trichinella* infection are considered to be suitable for surveillance and epidemiological investigations in animal populations, where the prevalence of infection is high (Gamble *et al.*, 2004).

The immunofluorescence antibody test, Western blot analysis, complement fixation test and haemagglutination test are examples of conventional, serodiagnostic methods that are labour intensive. These methods are often employed in human clinical medicine for the examination of individual samples or as confirmatory tests, although they are relatively expensive (Nöckler *et al.*, 2000).

The ELISA method, is the most commonly used method for the detection of *Trichinella* infection because it is economical, reliable, readily standardised and provides an acceptable balance of sensitivity and specificity (OIE, 2004a). Infection levels as low as one larva per 100 g of muscle tissue have been detected by ELISA (Gamble *et al.*, 1983). In many experimental and/or field studies the successful use of indirect ELISA for the detection of specific *Trichinella* antibodies in pig serum and meat juice samples has been demonstrated (Murrell *et al.*, 1986; Smith, 1987; Smith and Snowdon, 1989; Van der Leek *et al.*, 1992; Jakob *et al.*, 1994; Nöckler *et al.*, 1995; Gamble, 1996, Gamble and Patrascu, 1996; Nöckler *et al.*, 2004).

Host immunity and antibody response

Host invasion by *Trichinella* larvae induces a complex cellular and humoral immune response during the intestinal stage and the migratory and muscle invasion stage of infection. Specific antibody isotypes such as IgA, IgM and IgG can usually be detected two to three weeks after ingestion. Among the different classes of immunoglobulins, IgG has the highest concentration in the serum as well as in other body- and secretory fluids. Of importance is the fact that IgG, with its long half-life of 23 days is normally detectable for a long time. However, species-specific factors, variations in the individual's immune response, the presence of maternal antibodies and immunodeficiency syndromes may compromise the interpretation of test results (Nöckler *et al.*, 2000).

The early stage of *Trichinella* infection is characterised by a 'diagnostic window' in which larvae have become encysted in muscle tissue as early as 17 d.p.i., but specific antibodies can not yet be detected in the host animal. In this case, false-negative results may occur when compared with direct tests (OIE, 2004a). Under normal conditions, serum antibodies decline slowly after an initial peak. Except in experimental infections, it is usually impossible to determine at what point after infection the serum sample was taken, and in general, it can be assumed that the blood sample was taken at a stage of infection after the peak of antibody titer (Nöckler *et al.*, 2000).

Enzyme-linked immunosorbent assay

Since the 1980s, considerable research has led to the widespread adaptation of the indirect ELISA for detecting antibodies in infected hosts. The main aspects of this test are briefly explained in Figure 2 and are as follows: microtiter plates with *Trichinella* antigen are coated with control reagents and the 'unknown' serum or meat juice samples added. During incubation at room temperature or at 37°C, specific antibodies present will bind to the antigen. Unbound serum components are removed by washing the microtiter plate and in the next step, the antigen-antibody complex is exposed to an anti-host IgG (typically) antibody linked to an enzyme (the conjugate). After adding a substrate (chromogen) together with hydrogen peroxidase, the enzyme component of the conjugate induces the production of free oxygen radicals which result in a redox reaction of the substrate. The colour reaction is measured as optical density using a photometer and the measured intensity correlates with the antibody level or amount of immunoglobulin that had bound to the antigen fixed in the microtiter plate. Details for performing an ELISA based on using E/S antigens are

presented in Annex II. The sensitivity and specificity of ELISA depends on specific test components which are discussed below:

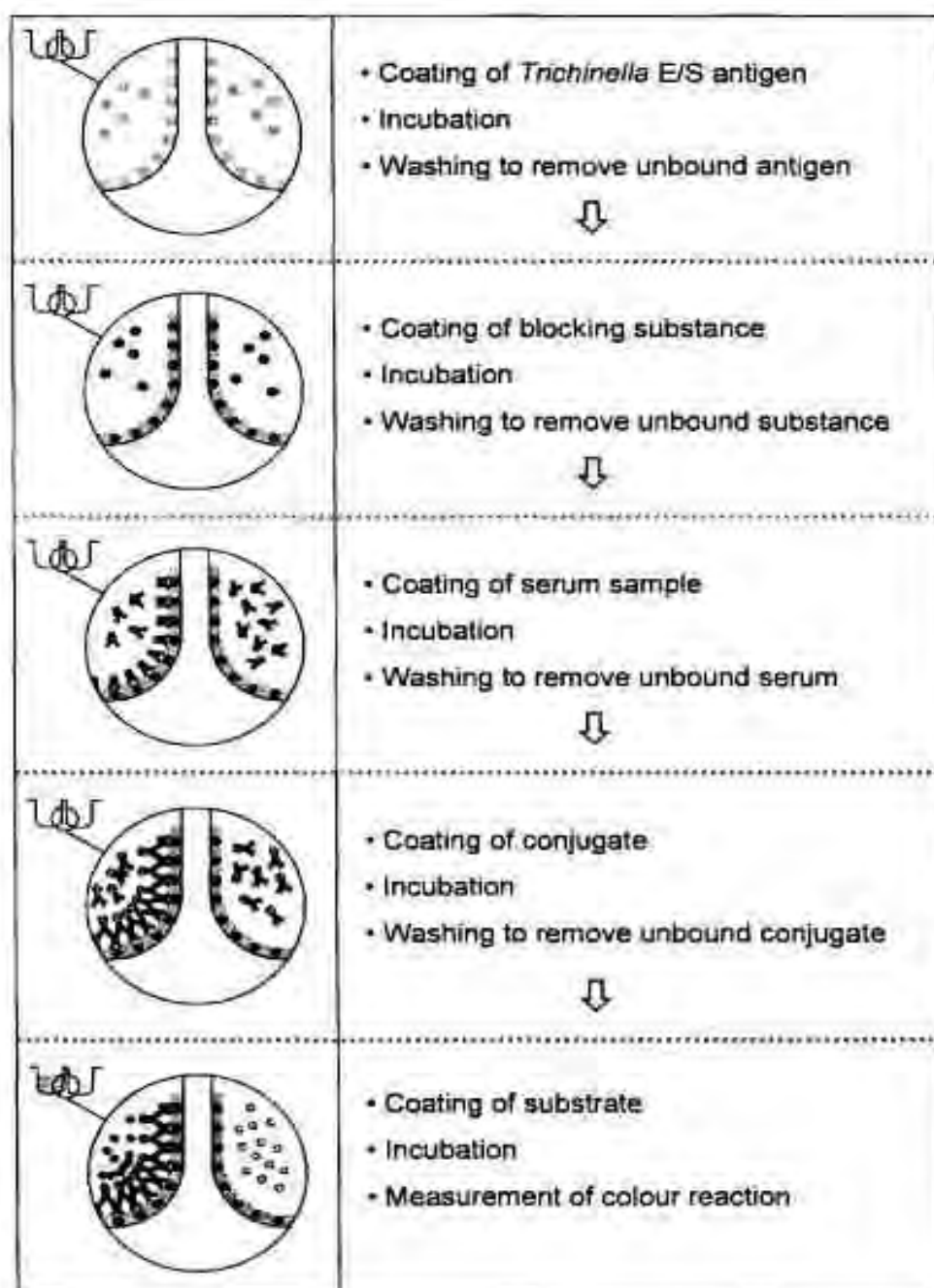


Figure 2 - Scheme for indirect *Trichinella*-enzyme-linked immunosorbent assay based on excretory/secretory antigen

Antigen

Initially, somatic antigens prepared from whole body extracts of muscle larvae were used in the ELISA for detection of *Trichinella* antibodies in pigs (Ruitenber *et al.*, 1974). However, this crude worm extract produced from first stage muscle larvae shows poor specificity, due to cross-reactions (Ruitenber *et al.*, 1976; Ruitenber and Van Knapen, 1977; Clinard, 1978; Taylor *et al.*, 1980).

During the 1980s, the specificity of the ELISA was greatly improved by utilising E/S antigens released from *Trichinella* muscle larvae incubated *in vitro*. These antigens, consist of a group of structurally related glycoproteins (Gamble *et al.*, 1983; Gamble and Graham, 1984). The predominant *Trichinella* antigen epitope recognised by animals and humans belongs to the so-called TSL-1 group. TSL-1 antigens are found in the stichocyte cells and on the surface of the parasite's cuticle and they are actively secreted by first-stage larvae in the muscle (Appleton *et al.*, 1991; Ortega-Pierres *et al.*, 1996). The TSL-1 antigen epitopes are highly conserved and are recognised by antibodies in all *Trichinella* infected animals and humans. (Appleton *et al.*, 1991). Therefore, species-specific *Trichinella* antigens for serology are not normally necessary (Kapel and Gamble, 2000; Gamble *et al.*, 2004; Nöckler *et al.*, 2005), because E/S antigens obtained from one species such as *T. spiralis* muscle larvae will detect specific antibody induced by both *T. spiralis* and the other *Trichinella* species.

In order to produce the TSL-1 antigens, optimal culture conditions are required, as well as meticulous isolation of the *Trichinella* metabolites. Critically, the incubation period should not exceed 18 h to prevent non-specific antigens being released from dead or dying *Trichinella* larvae to contaminate the E/S antigen mixture (Gamble *et al.*, 1988). Such somatic constituents have certain epitopes which may also occur in other helminths, and can cause reduced antigen specificity and cross-reactions in the ELISA (Gamble and Graham, 1984).

A synthetic glycan antigen (tyvelose) which possesses the common carbohydrate epitope of the TSL-1 antigens has been developed for use in ELISA (Wisnewski *et al.*, 1993; Reason *et al.*, 1994). The synthetic carbohydrate antigen offers the advantages of stability and standardisation. In several studies, this glycan antigen has been compared with E/S antigens for its suitability for testing in humans, pigs and other animals (Gamble *et al.*, 1997; Bruschi *et al.*, 2001; Owen *et al.*, 2001; Møller *et al.*, 2005). Although, producing a higher specificity in many species, a low rate of false-positive results has been obtained in some studies (Dea-Ayuela *et al.*, 2001). The tyvelose antigen may also sacrifice sensitivity in some instances (Gamble *et al.*, 2004).

Sample matrix

Serum is the preferred sample for conducting serological tests for *Trichinella*. After collection, blood samples should be clotted and the sera frozen at -20°C as soon as possible. Repeated freezing and thawing of samples should be avoided since serum titers will decline. Therefore, samples that will be used frequently should be divided into aliquots and frozen at -80°C or lyophilised. If freezing is not possible, 1% merthiolate, or another suitable preservative, should be added to each serum sample at a dilution of 1:10,000 (Gamble *et al.*, 2004). Serum samples of poor quality due to extensive hemolysis or microbial contamination, especially in samples from game or wild carnivores, may significantly decrease the specificity of the test (OIE, 2004a).

Other host fluids that can be used for testing include plasma, whole blood, and tissue fluids (Gamble and Patrascu, 1996). Results from experimental studies indicate that tissue fluids such as meat juice from slaughtered pigs or from other dead animals (e.g. wild boars and foxes) may be suitable for serologic examinations using ELISA (Gamble and Patrascu, 1996; Kapel *et al.*, 1998; Møller *et al.*, 2005). However, antibody levels in meat juice are usually lower than in serum, therefore meat juice samples should be used at a lower dilution in serological assays than that normally employed with serum. In pigs experimentally infected with 200, 1,000 and 20,000 larvae of *T. spiralis*, *T. britovi*, *T. pseudospiralis* and *T. nativa*, there was a good correlation for antibody between serum and diaphragm meat juice samples by E/S ELISA when the meat juice was tested at a lower dilution (1:10) than that of blood serum (1:100). In this study, meat juice samples were obtained from 10 g of the diaphragm muscle and cut into small pieces before frozen overnight at -20°C in a plastic bag. After thawing at room temperature, the meat juice was collected with a pipette into an Eppendorf tube (Nöckler *et al.*, 2005). Comparable correlations and dilution ratios between

blood sera and muscle fluids was observed in a comparative study on pigs, wild boars and foxes employing both E/S and tyvelose antigen (Møller *et al.*, 2005).

Conjugate and substrate

The conjugate, called the 'detection antibody', in the ELISA consists of the anti-species-antibody (usually anti-IgG) and an enzyme which is bound to it, for example horse-radish peroxidase or alkaline phosphatase. The conjugate then detects specific host serum antibodies that are bound to the antigen. Conjugates are available commercially only for certain animal species. Anti-dog-IgG conjugate is suitable for detection of specific antibodies in foxes (Gamble *et al.*, 2004). Protein G which is a non-specific ligand for IgG can effectively detect specific antibodies in both pigs and dogs.

Several substrates are commercially available and some are known to be potentially mutagenic and/or carcinogenic (e.g. orthophenyldiamine). Therefore, adequate handling and precautionary measures are necessary to avoid health hazards. Recently, substrates such as 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) and tetramethylbenzidine provided as ready to use chromogens together with hydrogen peroxidase are preferred for routine ELISA testing.

Validation of enzyme-linked immunosorbent assay

Generally, the capacity of a positive or negative serological test result to predict accurately the infection status of the animal or population of animals is the most important consideration of assay validation. This capacity is not only dependent on a highly precise and accurate assay and carefully derived estimates of diagnostic sensitivity and specificity, but is heavily influenced by the prevalence of the infection in the targeted population or the likelihood that an animal is infected based on clinical criteria (OIE, 2004b). In this connection, the positive predictive value for an expected *Trichinella* prevalence in the targeted population is usually very low.

The user of any test should always conduct an independent evaluation of test performance using panels of defined *Trichinella* positive and negative sera. The negative control group should be representative of the local population and positives should reflect various stages and levels of infection. The Office International des Epizooties (OIE, 2004b) recommends a minimum of 300 known positive and 1,000 known negative samples to establish the diagnostic sensitivity and specificity.

The cut-off can be established by visual inspection of the frequency distributions of test results from uninfected and infected reference animals (Jacobson, 1999), by receiver-operator characteristics (ROC) analysis (Greiner *et al.*, 2000), or by selection that favours either diagnostic sensitivity or specificity, depending on the intended use for a given assay (Smith, 1991).

In pigs, the sensitivity of the ELISA using an E/S antigen ranged from 93.1% to 99.2% whereas the specificity varied from 90.6% to 99.4% (Murrell *et al.*, 1986; Oliver *et al.*, 1989; Van der Leek *et al.*, 1992). According to results of a field study with a total of 1627 German and Croatian pigs, the meat juice ELISA achieved a sensitivity and specificity of 81.8% and 99.3%, respectively if results of the digestion method were considered as the 'gold standard'. In comparison, the serum ELISA had a sensitivity of 72.7% and a specificity of 99.6%. However, false-negative results were observed in serum and meat juice samples from those pigs where the larval density in the muscle was very low with 0.17 lpg to 0.38 lpg (Nöckler *et al.*, 2004). In another study, two ELISA systems based on E/S and glycan antigen were evaluated for detection of *Trichinella* infection in pig. Sensitivity was estimated by examination of 113 samples collected between three and 21 weeks p.i. from 15 experimentally infected pigs, and specificity was estimated using 397 samples from a *Trichinella*-free pig population. At 49 d.p.i. the sensitivity and specificity of the ROC-optimised glycan-ELISA were 94.3% and 96.7%, respectively, as compared with the E/S-ELISA at 84.9%

and 96.0%, respectively. This study indicates that the glycan-ELISA is as good or better than the E/S-ELISA for the detection of *Trichinella* antibodies in pig (Forbes *et al.*, 2004). With special regard to specificity, results of western blot examination showed that no cross-reactions were found between *Trichinella* antigen and serum antibodies to various other parasitic nematodes of pigs (*Ascaris suum*, *Trichuris suis*, *Strongyloides ransomi*, *Hyostrongylus rubidus*, *Oesophagostomum dentatum*) (Nöckler *et al.*, 1995).

Results from field studies revealed a high serological prevalence for *Trichinella* in wild animals with an absence of larvae in muscles when obtained from regions where *Trichinella* infection was never observed, or the prevalence rate detected by artificial digestion was 100 times lower than that recorded using serological methods (Nöckler and Voigt, 1998b; Wacker *et al.*, 1999). Although this observation is likely due to a certain percentage of false positive reactions, the higher sensitivity of ELISA may still be of importance for wildlife populations where worm burdens may be very low. Therefore, the ICT recommends that serological methods used for determination of *Trichinella* prevalence in wildlife must be preceded by a thorough evaluation of test sensitivity and specificity in the species and population being tested (Gamble *et al.*, 2004). Western blots have shown promise for discriminating cross-reacting antibodies; however, such tests are time consuming and expensive.

Quality assurance system for enzyme-linked immunosorbent assay

Valid laboratory results have to be achieved by the use of good management practices, valid test and calibration methods, proper technique, quality control, and quality assurance, all working together within a quality system (OIE, 2004c). Such a quality assurance system should be established for serological testing as described for direct larval detection by the artificial digestion method. There are main components for laboratory accreditation according to ISO/IEC Standard 17025 or another internationally recognised quality assurance system such as: quality assurance manual, standardised protocol for serological test, technical certification of analysts, proficiency sample programmes for laboratory, reporting and trace back system for doubtful or positive samples and audit system for monitoring the maintenance of the quality assurance system (see paragraph 'Quality assurance system for artificial digestion methods').

Commercially available serological tests such as the ELISA have to meet specific standards of sensitivity and specificity established by an individual country's licensing authority. Users of commercial tests should verify that the test has been adequately evaluated using international reference standards and has received the approval of any relevant regulatory authorities (Gamble *et al.*, 2004).

All components of the test that are critical for maintaining suitable performance (CCP's) should be identified and appropriately controlled, and the test should be conducted within a laboratory quality system (Gajadhar and Forbes, 2002). In particular, the suitability of each batch or lot of antigen for ELISA should be demonstrated by checkerboard titration against a standardised positive control serum. Furthermore, each batch of antigen coated microtiter plates should be tested against a standardised panel consisting of negative, positive and doubtful serum samples.

By special arrangements, standard antigens, reference sera and scientific consultation can be obtained from ICT members' laboratories (www.med.unipi.it/ict/welcome.htm) and the OIE Reference Laboratories (www.oie.int/eng/oie/organisation/en_listeLR.htm).

Antibody responses in different host species

Pigs

The time of seroconversion is related to infection dose and the larval burden in the muscle as has been demonstrated by comprehensive experimental studies with *T. spiralis* in pigs (Table II). With 100, 500 and 2,500 *T. spiralis* larvae per animal, seroconversion in pigs was observed by ELISA at five to seven, four to five or four weeks p.i, respectively (Gamble, 1996, 1998). With higher infection doses of 8,000 and 64,000 larvae per pig and an increasing larval density in the muscle sample, seroconversion occurred earlier, at 2.5 to 3 weeks p.i. (Smith and Snowdon, 1989). Analogous results were reported for Yorkshire pigs (kept under specific pathogen free conditions) and Iberian pigs experimentally infected with 100, 1000 and 20,000 *T. spiralis* larvae per animal (Nöckler *et al.*, 2005). If Yorkshire and Iberian pigs were infected with the same larval doses of *T. pseudospiralis* and *T. britovi*, larval densities were lower compared to *T. spiralis* and consequently, time of seroconversion was prolonged up to eight to nine weeks p.i. In the same study, experimental infection with 1,000 and 20,000 larvae of *T. nativa* per pig induced seroconversion in pigs with either no or only a few muscle larvae. Investigators propose that this phenomenon is due to stimulation of the host immune system during the early enteral (intestinal) and/or migration stage of infection (Nöckler *et al.*, 2005). In another study, in pigs experimentally inoculated with *T. nativa*, *T. murrelli* and *Trichinella* T6, decreasing antibody levels were related to the disappearance of muscle larvae (pigs are a poor host for these species); but sustained high antibody levels were observed in animals infected with *T. spiralis*, *T. britovi* and *T. nelsoni* (all of which support infection to varying degrees), presumably because of sustained antigen stimulation from muscle larvae (Kapel and Gamble, 2000). *Trichinella* antibodies may persist in pigs for a long time (Nöckler *et al.*, 1995). It can be assumed that in slaughter pigs, which have a live weight of 90 kg to 100 kg at an age of 25 to 30 weeks, it is unlikely that a false-negative result will be obtained because of a declining antibody titer (Nöckler *et al.*, 2000).

Horses

Horses experimentally infected with 1,000, 4,000, 10,000 and 40,000 larvae of *T. spiralis* per animal seroconverted by two to seven weeks p.i. (Table II) (Gamble *et al.*, 1996). In another study in which horses were experimentally inoculated with 5,000 *T. spiralis* larvae per animal, seroconversion was detected 2 to 4.5 weeks p.i. but in contrast to hosts such as pigs, specific IgG persisted for only a relatively short time. The earliest decline of antibody titer below the cut-off occurred in week 15, at which time infective larvae were still present in the muscle tissue (Voigt *et al.*, 1998). Infection in ponies with 50,000 larvae of, respectively, *T. spiralis*, *T. britovi* and *T. pseudospiralis* resulted in a rapid seroconversion two to three weeks p.i. peaking at eight weeks p.i., but rapidly decreasing thereafter, although average larval burdens of 112 lpg, 5 lpg and 66 lpg, respectively, were detected ten weeks p.i. for the three *Trichinella* species (Kapel, unpublished data). These observations confirm the results of other studies which showed that specific antibody titers fell below cut-off levels of the ELISA as soon as 14 weeks p.i. (Soulé *et al.*, 1989, 1993), and that in naturally infected horses, specific circulating antibodies were not detected in spite of the presence of a high worm burden in muscles (Pozio *et al.*, 1997, 1999). Similar observations for horse infection serology by ELISA were reported by others, although antibody could be detected up to 32 and 40 weeks p.i. by the indirect fluorescence test (IFT) (Soulé *et al.*, 1989; Pozio *et al.*, 2002; Murrell *et al.*, 2004). Considering the present state of knowledge, the ICT cannot recommend serological methods for use in horses either for detection of single infections or for reliable surveillance until serological tests are more thoroughly validated (Gamble *et al.*, 2004).

Table II - Correlation between time of seroconversion and infection dose (*T. spiralis*) in pig (Smith and Snowdon, 1987, Gamble, 1996, Nöckler *et al*, 2005), horse (Gamble *et al*, 1996, Voigt *et al*, 1998), wild boar (*Sus scrofa*) (Kapel, 2001), red fox (*Vulpes vulpes*) (Möller *et al*, 2005) and silver fox (*Vulpes vulpes fulva*) (Nöckler and Voigt 1998)

Animal species	Infection.dose (number of larvae per animal)	Larvae per gram	Time of seroconversion, post-infection (weeks)
Pig	100	1.62-6.50 ^(a)	5-7
	500	18.4-48.6 ^(a)	4-5
	1,000	26.3-90.6 ^(d)	4-6
	2,500	87.6-99.5 ^(a)	4
	8,000	12.1-81.4 ^(b)	3
	20,000	699.2-1103.5 ^(d)	3-4
	64 000	221.4-466.6 ^(b)	2.5-3
Horse	1000	0.10-0.26 ^(a)	3-4
	4 000	0.39-7.8 ^(a)	37
	5,000	0.02-8.9 ^(d)	2-4 5
	10,000	6.6-60.0 ^(a)	3-4
	40,000	484-1060 ^(c)	2-3
Wild boar	10,000	43-100 ^(d)	3-4
Silver fox	500	7.4-14.7 ^(d)	4-6
	2,000	4.7-66.9 ^(d)	2
Red fox	10,000	7.7-202.7 ^(d)	3

a) of tongue

b) mean of tongue, masseter, diaphragm, intercostal, psoas and rectus abdominis

c) of masseter

d) of diaphragm

Wild boars

In a large, comprehensive experimental study, 36 wild boars were infected with nine different sylvatic and domestic *Trichinella* species, and the resulting muscle larvae distribution was compared with antibody responses (Kapel, 2001). An antibody response was detected in all wild boars by three to four weeks p.i. (Table II). For *T. spiralis* and *T. nelsoni*, a high antibody level persisted throughout the observation period of 70 d.p.i., however, seroconversion was delayed in infections with non-encapsulated *T. pseudospiralis*. The antibody level remained stable in wild boars infected with *T. spiralis*, *T. britovi* and *T. nelsoni*, but the decline of antibodies directed against *T. nativa*, *T. murrelli* and *Trichinella* T6 was associated with the rapid disappearance of larvae in the muscle sample (Kapel, 2001).

Other wild animals

There are only a few studies on antibody detection after infection with *Trichinella* larvae in wild carnivorous animals. Silver foxes (*Vulpes vulpes fulva*) that were experimentally infected with 500 and 2,000 larvae of *T. spiralis* sero-converted at four to six and two weeks p.i., respectively (Table II). Long-term follow up studies revealed that specific IgG could be detected up to the end of the study at 76th week p.i. This period corresponds to the mean life expectancy of the fox in its natural habitat (Nöckler and Voigt, 1998a). Similar

results were obtained in another study, in which two farm foxes (*Vulpes vulpes*) were infected by oral inoculation of 150 *T. spiralis* muscle larvae per kg body weight and one fox with a total of 5,000 larvae. High IgG levels were recorded three to seven weeks p.i. and the immune response persisted at high levels in all infected animals throughout the observation period of 700 d.p.i. (Wacker *et al.*, 1999). In red foxes (*Vulpes vulpes*) experimentally infected with nine species of *Trichinella* (10,000 larvae per animal), all foxes seroconverted three weeks p.i., and maintained a high titer until the end of experiment (40 weeks p.i.) (Møller *et al.*, 2005).

MEAT HYGIENE AND EUROPEAN UNION LEGISLATION FOR TRICHINELLA INSPECTION

Trichinella infections have a worldwide socio-economic significance, and are of medical and veterinary concern (Webster *et al.*, 2006). The costs for inspection of pork in the EU is estimated to 570 million euros annually (Pozio, 1998). In a survey performed in Europe in 2004, more than 1,100 trichinellosis cases were identified with 984 cases being reported from Serbia, Croatia, Bulgaria and Rumania. Therefore, suitable and sensitive methods to detect parasitised animals are of crucial importance (Dupouy-Camet, 2006). The ICT recommends that all domestic and sylvatic animals that may harbour *Trichinella* and intended for food should be tested for *Trichinella* infection using an accepted methodology. In this connection, slaughter inspection methods are designed to prevent clinical trichinellosis in humans and are not designed to prevent infection entirely (Gamble *et al.*, 2000).

As early as 1860, the German pathologist Friedrich Albert Zenker discovered the biology and pathogenic role of *T. spiralis* in humans (Giese, 1996). As a result it became possible to detect the sources of epidemics of trichinellosis and to devise specific means to control food-borne infections. Rudolf Virchow followed up on these discoveries and was instrumental in introducing inspection for *Trichinella* infection in the domestic pig in Germany. From his many studies, Virchow concluded that trichinellosis was an infection of individual animals, and that a single infected pig could be the cause of infection in hundreds of people. He justifiably demanded that every pig slaughtered for food be inspected individually for trichinae; to use his own words: 'If one has slaughterhouses, then nothing is simpler than setting up microscopes there, and preventing the sale of any pork unless there is an official permit certifying the purity of the said animal' (Virchow, 1864).

In the European Community, until 2005 *Trichinella* meat inspection in pigs, horses and game was separately regulated by Directive 64/433/EEC on health problems affecting intra-Community trade in fresh meat (European Community, 1964), Directive 77/96/EEC on the examination for trichinae (*Trichinella spiralis*) upon importation of fresh meat derived from domestic swine from third countries (European Community, 1977) and Directive 92/45/EEC on public health and animal health problems relating to the killing of wild game and the placing on the market of wild-game meat, respectively (European Community, 1992). From 1 January 2006, the legislation for *Trichinella* meat inspection in the EU has been bundled by Regulation (EC) No 2075/2005 laying down specific rules on official controls for *Trichinella* in meat (European Community, 2005). This new Regulation (EC) No 2075/2005 on *Trichinella* meat inspection mainly refers to Regulation (EC) No 853/2004 (European Community, 2004a) that stipulates specific hygiene rules for food of animal origin. Furthermore, Regulations (EC) No 854/2004 (European Community 2004b) which describe specific rules for the organisation of official controls on products of animal origin intended for human consumption and (EC) No 882/2004 (European Community, 2004c) on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules have to be considered.

Slaughter testing in pigs, horses and game in the European Union

According to Regulation (EC) No 2075/2005 (European Community, 2005), carcasses of domestic pig, horse, wild boar and other farmed and wild animal species susceptible to *Trichinella* infestation shall be systematically sampled in slaughterhouses or game-handling establishments as part of the post-mortem examination.

A muscle sample shall be collected from each carcass with consideration to predilection site of larvae in the respective animal species (Table I). In the absence of predilection muscle or for cuts of meat, a higher sample weight has to be taken for analysis (Table III). The sample shall be examined for *Trichinella* in a laboratory that is designated by the competent authority. As discussed above, four methods are accepted for pooled sample digestion technique:

- 1) magnetic stirrer method
- 2) stomacher sedimentation method
- 3) stomacher filtration technique
- 4) 'Trichomatic 35' automated digestion method.

Table III - Sampling for *Trichinella* meat inspection in pig, horse, wild boar and bear by artificial digestion method according to Regulation (EC) No 2075/2005 (European Community 2005)

Animal species	Sample location	Sample weight	Sample weight (for alternative muscle)	Weight for examination
Pig	Diaphragm	1 g (fattening pig)	2g	Whole sample
		2 g (boar sow)	4 g (boar, sow)	
			5 g (muscle pieces)	
Horse	Tongue or masseter	10 g	Larger sized specimen of diaphragm	5g
Wild boar	Foreleg, tongue or diaphragm	10 g	-	5g
Bear	Diaphragm, masseter or tongue	10 g	-	10 g

The magnetic stirrer method is considered as the reference method of detection and a detailed protocol for this method is given in Annex I. The number of samples that can be examined in one run depends on the total weight of the complete pool (100 g for the first three methods and 35 g for the Trichomatic 35 method) and the weight of each single sample to be examined (1 g, 2 g, 4 g, 5 g, 10 g).

The trichoscopic examination is less sensitive than artificial digestion method and fails to detect non-encapsulated *Trichinella* larvae infecting domestic and sylvatic animals. Therefore, this method is no longer permitted as a detection method for standard use. The trichoscopic method should only be used under exceptional circumstances for the examination of a small number of animals slaughtered per week, provided that measures are taken by the food business operator to process the meat in such a way that it is completely safe for consumption. However, the method should be replaced by a more reliable detection method within a transitional period, thus the trichoscopic method is accepted for examination of domestic pig and wild boar carcasses in exceptional cases until 31 December 2009. In these cases, single carcasses need to be examined individually in an establishment that does not slaughter more than 15 domestic swine per day or 75 domestic swine per week or prepare for placing on the market more than ten wild boar per day and artificial digestion methods are not available. Where the trichoscopic method is used, the competent authority shall ensure that the meat is marked with a health mark that is clearly different from

the health mark provided for meat subjected for examination by one of the artificial digestion methods. Furthermore, the meat has to be supplied directly to the final consumer or to retail establishments directly supplying the final consumer. It is not permitted to use the meat for the production of products if processing does not kill *Trichinella*. For domestic pig meat, the inspector must cut 28 oat-kernel-size pieces from each diaphragm pillar from a whole carcass, making a total of 56 pieces that corresponds to a sample weight of about 1 g. If only one diaphragm pillar is present, 56 oat-kernel pieces are cut at different places of the sample, if possible from the transition to the sinewy part. In wild boars, additional samples i.e. seven oat-kernel-size pieces each from the jaw, the muscles of the lower leg, the intercostal muscles and the tongue muscles should be examined together with the 56 pieces from diaphragm pillar (total 84 pieces).

Carcasses or other parts of an animal intended for human or animal consumption which contain striated muscle tissue may not leave the premises, until the result of the *Trichinella* examination is found to be negative.

Derogation from testing pigs by freezing treatment of meat

Freezing meat under specified conditions can kill any parasites present but certain *Trichinella* species occurring in game and horses are resistant when freezing is carried out using the recommended temperature and time combinations. Thus, freezing is not a measure for inactivation of *Trichinella* in meat from these host species. The Scientific Panel on Biological Hazards of the European Food Safety Authority has addressed this subject and adopted an opinion on the suitability and details of freezing methods to allow human consumption of meat infected with *Trichinella* or *Cysticercus* (European Food Safety Authority, 2004).

According to Regulation (EC) No 2075/2005 (European Community, 2005), meat of domestic pigs that has undergone a freezing treatment under the supervision of the competent authority shall be exempt from *Trichinella* examination. Meat of domestic pigs may be imported without having undergone examination, if a freezing treatment has been carried out under the supervision of the competent authority in the third country.

During freezing treatment, the temperature must be measured using calibrated thermo-electric instruments and recorded continuously, and the instruments must be kept under lock and key. The temperature charts must include the relevant data from the meat inspection register on import and the date and time of commencement and completion of freezing, and must be retained for one year after compilation. There are two main principles that are applicable to the freezing treatment of meat undergoing *Trichinella* examination:

Meat obtained already frozen in the refrigeration room is to be kept in this condition

The technical equipment of the refrigeration room must ensure that the required temperature is reached very rapidly and maintained in all parts of the room and of the meat. Packaging must be removed before freezing, except meat must be so packaged that it will not prevent the required temperature to be reached within the specified time. Consignments in the refrigeration room must be kept separately and under lock and key. Date and time of freezing must be recorded. The temperature in the refrigeration room must be at least -25°C and the freezing time is calculated from the point when the prescribed temperature in the freezing room has been reached. The temperature may not be measured directly in the cold air. Meat of a diameter or thickness of up to 25 cm and from 25 cm to 50 cm must be frozen for at least 10 and 20 consecutive days, respectively. Freezing treatment of meat may also be carried out if the following time-temperature combinations are applied: Meat of a diameter or thickness of up to 15 cm must be frozen

either 20 days at -15°C, ten days at -23°C or six days at -29°C; meat of a diameter or thickness of between 15 cm and 50 cm must be frozen either 30 days at -15°C, 20 days at -25°C or 12 days at -29°C.

Freezing of meat with temperature monitored at the centre of each cut

The thermometer probe is inserted into the centre of a cut of meat no smaller in size than the thickest piece of meat to be frozen. This cut must be placed at the least favourable position in the refrigeration room, not close to the cooling equipment or directly in the cold air flow. The freezing treatment has to be carried out for the following time-temperature combinations: either 106 h at -18°C, 82 h at -21°C, 63 h at -23.5°C, 48 h at -26°C, 35 h at -29°C, 22 h at -32°C, 8 h at -35°C, or 30 min h at -37°C. However, freezing of meat cannot be recommended in areas where *T. britovi* is found (Pozio *et al.*, 2006).

Derogation from testing in pigs from a ' *Trichinella*-free' farm or region

According to Regulation (EC) No 2075/2005 (European Community, 2005), carcasses and meat of domestic pigs kept solely for fattening and slaughter shall be exempt from *Trichinella* examination where the animals come from a holding or category of holdings that has been officially recognised by the competent authority as free from *Trichinella*. Furthermore, an exemption from *Trichinella* examination is possible if the fattening pigs come from a region where the risk of *Trichinella* in domestic pig is officially recognised as negligible (see Chapter 4 for more details).

ANNEX I

PROTOCOL FOR ARTIFICIAL DIGESTION TECHNIQUE (MAGNETIC STIRRER METHOD) ACCORDING TO REGULATION (EC) NO2075/2005

Apparatus and reagents

- a) Knife or scissors and tweezers for cutting specimens,
- b) trays marked off into 50 squares, each of them can hold samples of approximately 2 g of meat, or other tools to ensure the traceability of the samples,
- c) a blender with a sharp chopping blade. If the samples are larger than 3 g, a meat mincer with openings of 2 mm to 4 mm or scissors must be used. In the case of frozen meat or tongue (after removal of the superficial layer, which cannot be digested), a meat mincer is necessary and the sample size should be increased considerably,
- d) magnetic stirrers with thermostatically controlled heating plate and teflon-coated stirring rods approximately 5 cm long,
- e) conical glass separation funnels, capacity of at least 2 l, preferably fitted with teflon safety plugs,
- f) stands, rings and clamps,
- g) sieves, mesh size 180 µm, external diameter 11 cm, with stainless steel mesh,
- h) funnels, internal diameter not less than 12 cm, to support the sieves,
- i) glass beakers, capacity 3 l,
- j) glass measuring cylinders, capacity 50 ml to 100 ml, or centrifuge tubes,
- k) a trichinoscope with a horizontal table or a stereo-microscope, with a substage transmitted light source of adjustable intensity,
- l) a number of 9 cm diameter petri dishes (for use with a stereo-microscope), marked on their undersides into 10 mm x 10 mm square examination areas using a pointed instrument,
- m) a larval counting basin (for use with a trichinoscope), made of 3 mm thick acrylic plates as follows:
 - the bottom of the basin should have 180 mm x 40 mm, marked off into squares
 - the sides 230 mm x 20 mm
 - the end 40 mm x 20 mm. The bottom and the ends must be inserted between the sides to form two small handles at the ends. The upper side of the bottom must be raised 7 mm to 9 mm from the base of the frame formed by the sides and the ends. The components must be stuck together with glue suitable for the material,
- n) aluminium foil,
- o) 25% hydrochloric acid,
- p) pepsin, strength: 1: 10,000 NF (USA National Formulary) corresponding to 1: 12,500 BP (British Pharmacopoea) and to 2,000 FIP (Federation internationale de pharmacie),
- q) tap water heated to 46°C to 48°C,
- r) a balance accurate to at least 0.1 g,
- s) metal trays, capacity 101 to 151, to collect the remaining digestive juice,

- t) pipettes of different sizes (1 ml, 10 ml and 25 ml) and pipette holders,
- u) a thermometer accurate to 0.5°C within the range 1°C to 100°C,
- v) siphon for tap water.

Sampling

- In the case of whole carcasses of domestic swine, a specimen weighing at least 1 g has to be taken from a pillar of the diaphragm at the transition to the sinewy part. Special trichinae forceps can be used if an accuracy of 1 g to 1.15 g can be guaranteed
- In the case of breeding sows and boars, a larger sample weighing at least 2 g has to be taken from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimen of twice the size 2 g (or 4 g in the case of breeding sows and boars) has to be taken from the rib part or the breastbone part of the diaphragm, or from the jaw muscle, tongue or abdominal muscles.
- For cuts of meat, a sample weighing at least 5 g of striated muscle, containing little fat has to be taken, if possible from close to bones or tendons. A sample of the same size has to be collected from meat that is not intended to be cooked thoroughly or other types of post-slaughter processing.
- For frozen samples, a sample weighing at least 5 g of striated muscle tissue has to be taken for analysis. The weight of meat specimens relates to a sample of meat that is free of all fat and fascia. Special attention must be paid when collecting muscle samples from the tongue in order to avoid contamination with the superficial layer of the tongue, which is indigestible and can prevent reading of the sediment.

Procedure

Complete pools (100 g of samples at a time)

- a) 16 ml \pm 0,5 ml of hydrochloric acid is added to a 3 l beaker containing 2 l of tap water, preheated to 46°C to 48°C; a stirring rod is placed in the beaker, the beaker is placed on the preheated plate and the stirring is started.
- b) 10 g \pm 0,2 g of pepsin is added.
- c) 100 g of samples collected in accordance with point 2 is chopped in the blender.
- d) The chopped meat is transferred to the 3 l beaker containing the water, pepsin and hydrochloric acid.
- e) The mincing insert of the blender is immersed repeatedly in the digestion fluid in the beaker and the blender bowl is rinsed with a small quantity of digestion fluid to remove any meat still adhering.
- f) The beaker is covered with aluminium foil.
- g) The magnetic stirrer must be adjusted so that it maintains a constant temperature of 44°C to 46°C throughout the operation. During stirring, the digestion fluid must rotate at a sufficiently high speed to create a deep whirl without splashing.
- h) The digestion fluid is stirred until the meat particles disappear (approx. 30 min). The stirrer is then switched off and the digestion fluid is poured through the sieve into the sedimentation funnel. Longer digestion times may be necessary (not exceeding 60 min) in the processing of certain types of meat (tongue, game meat, etc.).
- i) The digestion process is considered satisfactory if not more than 5% of the starting sample weight remains on the sieve.
- j) The digestion fluid is allowed to stand in the funnel for 30 min.

- k) After 30 min, a 40 ml sample of digestion fluid is quickly run off into the measuring cylinder or centrifuge tube.
- l) The digestion fluids and other liquid waste are kept in a tray until reading of the results is completed.
- m) The 40 ml sample is allowed to stand for 10 min 30 ml of supernatant is then carefully withdrawn by suction to remove the upper layers and leave a volume of not more than 10 ml.
- n) The remaining 10 ml sample of sediment is poured into a larval counting basin or petri dish.
- o) The cylinder or centrifuge tube is rinsed with not more than 10 ml of tap water, which has to be added to the sample in the larval counting basin or petri dish. Subsequently, the sample is examined by trichinoscope or stereo-microscope at a 15 to 20 times magnification. Visualisation using other techniques is allowed, provided examination of positive control samples has been shown to give an equal or better result than traditional visualisation methods. In all cases of suspect areas or parasite-like shapes, higher magnifications of 60 to 100 times must be used.
- p) Digests have to be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

If the digests are not examined within 30 min of preparation, they must be clarified as follows. The final sample of about 40 ml is poured into a measuring cylinder and allowed to stand for 10 min. 30 ml of the supernatant fluid is then removed, leaving a volume of 10 ml. This volume is made up to 40 ml with tap water. After a further settling period of 10 min, 30 ml of the supernatant fluid is withdrawn by suction, leaving a volume of no more than 10 ml for examination in a petri dish or larval counting basin. The measuring cylinder is washed with no more than 10 ml of tap water and these washings are added to the sample in the petri dish or the larval counting basin for examination.

If the sediment is found to be unclear on examination, the sample is poured into a measuring cylinder and made up to 40 ml with tap water and then the above procedure is followed. The procedure can be repeated two to four times until the fluid is clear enough for a reliable reading.

Pools of less than 100 g

- If necessary, up to 15 g can be added to a total pool of 100 g and examined together with these samples,
- more than 15 g must be examined as a complete pool. For pools of up to 50 g, the digestion fluid and the ingredients may be reduced to 11 of water, 8 ml of hydrochloric acid and 5 g of pepsin.

Positive or doubtful results

- If examination of a collective sample produces a positive or uncertain result, a further 20 g sample must be taken from each pig. The 20 g samples from five pigs are pooled and examined using the method described above. In this way samples from 20 groups of five pigs will be examined.
- If *Trichinella* is detected in a pooled sample from five pigs, further 20 g samples must be collected from the individual pigs in the group and each is examined separately using the method described above.

Critical control points (Gamble *et al.*, 2000)

- 1) A verifiable system of sample collection and identification must be maintained. The process must assure that samples of 1 g or greater size originate from the appropriate number of pigs and that samples are clearly identified back to the pig.

- 2) Digestion fluid must be consistent in quality and prepared in a manner that does not affect the activity of the pepsin. The most critical step in preparation of digestion fluid is the addition of the hydrochloric acid to the water prior to the introduction of pepsin. This step will protect the pepsin from degradation by direct contact with concentrated hydrochloric acid. Other factors in the preparation and use of digestion fluid (the source and quality of pepsin, the amounts of pepsin and hydrochloric acid used, and the ratios of tissue to digestion fluid) should conform to published guidelines.
- 3) The temperature maintained during the digestion process should not exceed 46°C. Higher temperatures will result in the inactivation of pepsin, incomplete digestion and poor recovery rates.
- 4) Following digestion, no undigested muscle tissue should remain (as evidenced by material retained on the sieve). Digestion must be complete to assure the integrity of the test. Remedies for incomplete digestion include increasing digestion times, and, if this is not effective, verifying the quality of the pepsin.
- 5) Sedimentation procedures and times should be conducted to maximise recovery of larvae. Existing methods employing sedimentation times of 30 min are sufficient. Shortening the recommended times will result in reduced recovery rates. Sedimentation could be improved by periodically vibrating or tapping funnels during settling. Recovery of sediment from separatory funnels must include complete opening of the stopcock to avoid larval retention.
- 6) Digest samples must be clarified sufficiently to allow visualisation of larvae. The classical measure of clarity is the ability to read newsprint through the bottom of the petri dish. Digests which are not clarified properly may result in a false-negative result.
- 7) Microscope optics must be sufficient to provide clear magnification at 20 to 40 x. In addition, regular microscope maintenance is required.
- 8) Digests should be examined prior to the removal or release of carcasses. This system is necessary to assure that positive carcasses are not distributed for human consumption.
- 9) Records are kept which assure the accurate identification of samples and carcasses.

ANNEX II PROTOCOL FOR INDIRECT TRICHINELLA-ELISA BASED ON EXCRETORY/SECRETORY ANTIGEN ACCORDING TO OIE MANUAL FOR DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS (2004)

Antigen production

- a) *T. spiralis* muscle larvae (larval stage 1) are recovered from the muscle by artificial digestion technique by means of magnetic stirrer method (as described above).
- b) Then, larvae are washed (three times for 20 min each) in Dulbecco's modified Eagle's medium (DMEM) with penicillin (500 units/ml) and streptomycin (500 units/ml), and then placed (at a density of 5,000 larvae per ml) into DMEM supplemented with HEPES (N-2-hydroxyethylpiperazine, N-2-ethanesulphonic acid) (10 mM), glutamine (2 mM), pyruvate (1 mM), and penicillin (250 units per ml)/streptomycin (250 µg per ml) (complete DMEM) at 37°C in 10% CO₂ in air.
- c) After 18 h to 20 h culture medium is recovered and worms are removed by filtration. The fluid is concentrated under pressure with a 5,000 Da molecular weight retention membrane.
- d) Recovered E/S antigens may be stored frozen for short periods at -20°C or for longer at -70°C.
- e) E/S-antigen should have a 280:260 nm absorbance ratio of > 1.0. The final dilution should be calibrated by checkerboard titration and the antigen batch should be tested against a panel of known negative and positive sera.

Procedure

- a) Coat 96-well microtiter plates 100 µl/well of *T. spiralis* ES antigens diluted to 5 µg/ml in coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6). Coating is performed for 60 min at 37°C or overnight at 4°C.
- b) Wash antigen-coated wells three times with wash buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 5% non-fat milk powder and 1% Triton X-100. Following each washing, plates are blotted dry.
- c) Dilute pig sera 1/10 or 1/100 in wash buffer. Alternative sources of antibodies that may be used in place of sera include whole blood or tissue fluids. Add 100 µl of diluted sera to antigen-coated wells. A known positive and known negative serum sample should be used on each plate at the same dilution as the test sera. Incubate at room temperature for 30 min.
- d) Wash wells three times as in step (b).
- e) Add 100 µl per well of an affinity-purified rabbit anti-swine IgG-peroxidase conjugate at an appropriate dilution in wash buffer and incubate the plates for 30 min at room temperature.
- f) Wash wells three times as in step (b), and rinse once with distilled water.
- g) Add 100 µl per well of a suitable peroxidase substrate (e.g. 5'-aminosalicylic acid [0.8 mg/ml] with 0.005% hydrogen peroxide pH 5.6 to pH 6.0).

After 5 min to 15 min, read plates for colour density at 450 nm on an automated microplate reader. Values obtained in the ELISA four times that of normal serum pool controls are considered to be positive. Values three times higher than normal are classified as suspect.

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CHAPTER 4

PREVENTION OF *TRICHINELLA* INFECTION IN THE DOMESTIC PIG

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Summary

*There is limited risk associated with exposure of conventionally-raised domestic pigs to *Trichinella* spp. and the risk factors for exposure are well documented. Pigs raised in confinement housing with effective rodent control systems and good general farm hygiene have a very low risk of exposure to this parasite; such pigs may potentially be certified *Trichinella*-free and exempted from meat inspection. Confirming good management practices through individual farm audits and conducting regular monitoring of pigs produced is required to document the effectiveness of certification systems. Due to the ubiquitous nature of *Trichinella* in wildlife, *Trichinella* -free areas cannot be assured and therefore geographic freedom from infection should not be considered as an effective means of assuring public health.*

Keywords

*Certification - Pig production - Risk assessment - *Trichinella*.*

INTRODUCTION

Worldwide efforts to control *Trichinella* infection in pigs, or rather to control the risk of human exposure to *Trichinella* from infected pigs, have relied on three strategies:

- 1) education of consumers as to the risk of acquiring *Trichinella* infection from eating raw or undercooked pork or pork products
- 2) processing by cooking, freezing or curing of pork products intended for human consumption
- 3) inspection of individual pig carcasses for the presence of *Trichinella* larvae. These topics are covered in large part in Chapter 3 (Control) and will not be discussed in detail here.

In contrast to control measures, prevention of pig infection with *Trichinella* has received substantially less attention, and most gains in reducing infection in domestic pigs have been the by-product of other disease prevention initiatives. For example, the introduction of garbage cooking laws in the USA were intended to control vesicular exanthema (1953-1954) and hog cholera (1962) (Zimmerman and Zinter, 1971; Zimmerman *et al.*, 1973). Likewise, improvements in swine husbandry, including the introduction of confinement housing systems, generally occurred without any intention to prevent exposure of pigs to *Trichinella*. Despite an overall reduction in the prevalence of *Trichinella* infection in domestic pigs in some countries, resulting from a transition to confined management systems and improved veterinary public health efforts, the increase in prevalence rates in other countries where organised farming systems have broken down underscores the ongoing risk of infection with this parasite in domestic pigs. Although most pigs are produced in confinement, the production of free-ranging pigs has increased in many countries, and obviously such pigs have a higher risk of exposure.

ROUTES OF TRANSMISSION OF TRICHINELLA TO THE DOMESTIC PIG

Trichinella infection in pigs can only result from the ingestion of first-stage larvae in the musculature of an infected animal. For this reason, there are limited, and well documented, risk factors for exposure of pigs to *Trichinella* infection on the farm. These have been extensively reported (Hanbury *et al.*, 1986; Schad *et al.*, 1987; Murrell *et al.*, 1987; Leiby *et al.*, 1988; Gamble *et al.*, 1999; Malakauskas *et al.*, 2006) and include: feeding of raw or undercooked waste products which contain meat, exposure of pigs to infected rodents, exposure to infected wildlife, and exposure to infected pig carcasses.

Because several *Trichinella* species occur in nature (Pozio, 2005; European Food Safety Authority, 2005), it must be assumed that pigs raised under conditions which expose them to contact with wild animals or wild animal carcasses are at risk. Transmission between the domestic (synanthropic) and wildlife (sylvatic) cycles has been well documented (Murrell *et al.*, 1987; Leiby *et al.*, 1988; Malakauskas *et al.*, 2006). Similarly, pigs exposed to infected rodents rapidly acquire infection (Leiby *et al.*, 1990; Schad *et al.*, 1987). However, populations of rodents living in and around pig farms often serve as bystanders rather than primary reservoirs, and only become infected after introduction of the infection in pigs.

The occurrence of *Trichinella* infection in pigs can be linked to specific conditions or events which increase the risk of exposure. Feeding of raw garbage in the early 1900s led to a high rate of infection in pigs in the USA. As mentioned, the advent of garbage cooking laws resulted in a rapid decline in the infection rate in such countries. Recently, in some eastern European countries, the breakdown of organised farming systems and a decline in the availability and quality of veterinary services (including such problems as improper disposal of carcasses of infected pigs and ineffective inspection of pork meat for *Trichinella* infection) have resulted in higher prevalence rates in pigs and outbreaks of trichinellosis in humans (Djordjevic *et al.*, 2003; Cuperlovic *et al.*, 2005; Blaga *et al.*, 2007).

The risk of *Trichinella* infection for pigs raised in outdoor farming systems is clear in all parts of the world (Gamble *et al.*, 1999; Nöckler *et al.*, 2004; Liu and Boireau, 2002; Malakauskas *et al.*, 2006). Nevertheless, the degree of risk to pigs raised outdoors depends in great part on the infection levels in local wildlife, and this degree of risk is of substantial importance for 'organic' or 'green' pig producers, who provide products to consumers seeking meat from animals raised under natural conditions. An example of different risk levels for outdoor pigs was demonstrated by Nöckler *et al.* (2004) who tested pigs raised outdoors in Germany and Croatia. While all pigs raised outdoors in Germany tested negative, all outdoor farms from Croatia had pigs that tested positive for *Trichinella*. This observation points out the importance of understanding infection pressure from the wildlife population, in situations where pigs are otherwise at risk for exposure. Further, infection pressure is not static, but changes over time with new foci of infection emerging. For example, there have been reports of *Trichinella* infection in unexpected areas such as Mediterranean islands (European Food Safety Authority, 2005). Despite the fact that these islands were considered to be *Trichinella*-free geographic areas, *Trichinella* infection was detected in outdoor pigs and cases of human trichinellosis were reported (Pozio *et al.*, 2006).

In areas where *Trichinella* infection is known to be endemic in wildlife, it is relatively easy to identify the conditions under which pigs are at greatest risk (Malakauskas *et al.*, 2006). These conditions include pigs raised on small holdings, with minimal confinement. These so-called 'backyard pigs' are often fed food scraps or other forms of meat-containing waste and have ready access to rodents and wildlife. To compound the problems, pigs raised in this manner are generally not sold through retail marketing channels, and therefore are not subjected to reliable methods of veterinary inspection. While this scenario might be more typical of developing countries, the situation exists to some extent in most countries of the world.

CERTIFYING PIG PRODUCTION SYSTEMS

The knowledge of modes of transmission of *Trichinella* to domestic pigs, allows pig farmers/producers to design management systems which prevent or drastically reduce the risk of exposure. By following a series of good management practices, combined with documentation of these practices and regular official control to verify that these practices are effective, it is possible to certify the safety of pork without subsequent slaughter inspection or further processing.

Requirements for *Trichinella*-free pig production

The requirements for producing pigs under conditions which greatly reduce or preclude exposure to *Trichinella* infection are outlined by several published sources. The ICT provides guidance for *Trichinella*-free pig farming in a document entitled 'Recommendations on Methods for the Control of *Trichinella* in Domestic and Wild Animals Intended for Human Consumption' (Gamble *et al.*, 2000; <http://www.med.unipi.it/ict/Recomm.htm>). The essential elements of these recommendations relative to *Trichinella*-free pig farming are reiterated in the USA Trichinae Herd Certification Program Standards (www.aphis.usda.gov/vs/trichinae) and in Commission Regulation (EC) No 2075/2005 of the EL). According to Regulation (EC) No 2075/2005 (European Community, 2005a), carcasses and meat of domestic pig kept solely for fattening and slaughter are exempt from *Trichinella* examination at slaughter when the animals come from a holding (farm) or category of holdings that has been officially recognised, by a competent authority, as free from *Trichinella*.

The minimal requirements that need to be met for livestock to be considered *Trichinella*-free are summarised by the ICT in the following section. These recommendations are intended to describe the minimal conditions that must be established for a programme to certify pigs free from risk of exposure to *Trichinella*. Programmes that have already been established, such as those referred to above in the EU and the USA, have additional requirements (see Sections 'European Union legislation for certifying pigs free from *Trichinella* infection', and 'United States *Trichinella*-free certification programme').

International Commission on Trichinellosis recommendations for certification of *Trichinella*-free pig production (unless otherwise specified, all of the conditions listed below must be met)

Architectural and environmental barriers

- Pig buildings are constructed to prevent rodents from entering buildings (e.g. openings, such as those for air ventilation or water pipes are covered with wire or specific devices to limit entry of rodents)
- areas within 100 meters of pig buildings are free from debris and rodent harborage
- a 2 m perimeter consisting of gravel or vegetation mowed to a height of less than 10 cm is maintained around all pig buildings.

Feed and feed storage

- Feed is maintained in closed silos, which do not allow rodents to enter
- purchased feed is obtained from an approved facility, which produces feed by good production practices
- waste food, containing meat products is cooked in accordance with waste food laws intended to inactivate trichinae.

Rodent control

- A documented rodent control programme is maintained by a recognised pest control provider
- no evidence indicating the presence of rodents (burrows, tracks, faeces) is observed by a recognised pest control provider.

Farm hygiene

- Dead animals are disposed of within 24 h and by sanitary means
- no garbage dumps are present within a 2 km radius of the farm.

New animals

- New animals originate from *Trichinella* free farms
- new animals are held in quarantine and are tested serologically after three weeks to assure the absence of antibodies to *Trichinella*.

Programmes, which allow certification of pigs as free from trichinae, based on good management practices that eliminate risk of exposure, should be administratively organised so as to allow proper documentation of certified herds. This administration should perform the following functions:

- develop a system of documentation of *Trichinella*-free production practices, which addresses all the points raised above
- issue certifications and maintain records of certified farms
- periodically, conduct spot audits of certified producers to assure the integrity of the system
- conduct periodic serology testing of pigs originating from certified farms to verify absence of infection.

European Union legislation for certifying pigs free from *Trichinella* infection

Recent legislation of the EU (Commission Regulation (EC) No 2075/2005) describes requirements for certifying pigs from individual holdings (farms) or categories of holdings (e.g. farms which raise pigs under certain conditions such as confinement housing) as free from risk of *Trichinella* infection. Pigs from holdings or categories of holdings are exempt from requirements for *Trichinella* inspection at slaughter. The EU legislation is based on two advisory documents. The Scientific Committee on Veterinary Measures relating to Public Health adopted an opinion on trichinellosis, epidemiology, methods of detection and *Trichinella*-free pig production (European Commission, 2001). Subsequently, the Scientific Panel on Biological Hazards adopted an opinion on risk assessment of a revised inspection of slaughter animals in areas with low prevalence of *Trichinella* (European Food Safety Authority, 2005). Based on these two opinions, Commission Regulation (EC) No 2075/2005 allows holdings or categories of holdings to be officially recognised as *Trichinella*-free, provided specific conditions are met. Among these conditions, is a programme for regular monitoring of domestic swine, wild boar (*Sus scrofa*), horses and foxes (*Vulpes* spp.) or other indicator animals.

European Union requirements for holdings officially recognised as free from *Trichinella*

The competent authority may officially recognise holdings of fattening pigs as free from *Trichinella* if the following requirements are complied with:

The competent authorities in Member States where *Trichinella* has been detected in domestic pig in the last ten years may recognise a holding as free from *Trichinella* if:

- a) at least two control visits are made in the 12 months preceding recognition of the holding to verify compliance with the requirements to food business operators (e.g. pest-control programme, heat treated feed supplies, disposal of dead animals within 24 h, risk assessment if a rubbish dump is located in the neighbourhood of the holding, piglets are born and bred under controlled housing conditions, trace back of all pigs to the holding, an outdoor access during the first few weeks of life before weaning shall be permitted under certain conditions);
- b) all pigs sent for slaughter during the 24 months preceding recognition or a longer time period if the competent authority decides that a sufficient number of animals from the holding have been tested using one of the accepted parasite detection methods;
- c) the results of the tests have been negative;
- d) a risk-based wildlife monitoring programme has been put in place in those areas where wildlife and holdings applying for *Trichinella*-free status coexist; the monitoring programme optimises parasite detection by applying the most suitable indicator animal and direct detection technique, by sampling a relevant number of animals and taking as large a meat sample as is feasible; parasites detected in wildlife are identified at species level in a Community or National Reference Laboratory; the Community Reference Laboratory can assist by preparing a standardised protocol for a wildlife monitoring programme. Historical data may be used for the fulfillment of the requirements listed in this part.

The competent authorities in Member States where *Trichinella* has not been detected in domestic swine in the last ten years may recognise a holding as free from *Trichinella* if a risk-based wildlife monitoring programme has been put in place (see above).

In the event that a domestic pig or other animal species susceptible to *Trichinella* infestation tests positive for *Trichinella*, the competent authority shall withdraw the recognition of a *Trichinella*-free holding without delay, and the following measures should be taken:

- all domestic pigs shall be examined for *Trichinella* at the time of slaughter and a serological test on all animals susceptible to *Trichinella* infection on the holding should be conducted once a suitable test has been validated by the Community reference laboratory
- all breeding animals that arrived on the holding and, as far as possible, all those that left the holding in at least the six months preceding the positive finding should be traced and tested
- as far as is feasible, the spread of parasite infestation due to the distribution of meat from domestic pig slaughtered in the period preceding the positive finding should be investigated
- the Commission and the other Member States must be informed
- an epidemiological investigation must be initiated to elucidate the cause of infestation
- the frequency of testing under and the scope of the monitoring programme in domestic and wild animals should be increased
- appropriate measures where any infested carcass cannot be identified at the slaughterhouse should be taken by increasing the size of each meat sample collected for testing of the suspect carcass or declaring the carcass unfit for human consumption
- measures for the disposal of suspect carcass or parts thereof and those tested positive should be taken.

European Union requirements for categories of holdings officially recognised as free from *Trichinella*

The competent authority may officially recognise categories of holdings of fattening pigs as free from *Trichinella* if the following requirements are complied with:

- a) all the requirements to food business operators are met, with the exception of those for an outdoor access during the first few weeks of life before weaning (see above);
- b) no autochthonous *Trichinella* infestations in domestic animals have been detected in the country in the last ten years, during which time continuous testing has been conducted on slaughtered swine population such as to provide at least 95% confidence that where the prevalence of *Trichinella* exceeds 0.0001%, any infestations will be detected;
- c) a clear description must be available of the category of holdings, the type of farming and the type of animals involved;
- d) a risk-based monitoring programme for wildlife has been established (see above).

Requirements for maintaining *Trichinella*-free status for categories of holdings are similar to those for individual holdings; further details can be found in Commission Regulation (EC) No 2075/2005.

Region where the risk of *Trichinella* in domestic pig is officially recognised as negligible

Commission Regulation (EC) No 2075/2005 provides requirements for an exemption from *Trichinella* examination if the fattening pigs come from a region where the risk of *Trichinella* in domestic pig is officially recognised as negligible. The details of these requirements can be found in the Regulation. The ICT does not recommend recognising geographic regions as free from *Trichinella* (see Section ' *Trichinella*-free regions').

United States of America *Trichinella*-free certification programme

A system for certifying pig farms free from risk of exposure to *Trichinella* was first described by Gamble *et al.* (2000). Currently in a pilot phase awaiting publication of a final regulation, this programme emphasises auditing of good production practices which document the absence of risk factors that would expose pigs to *Trichinella* in feed, rodents and wildlife (www.aphis.usda.gov/vs/trichinae). As part of the audit requirements, farms must maintain accurate records of animal movement, animal disposal, and rodent control logs. Further, it provides for education of veterinary practitioners who are responsible for conducting regular audits of farms seeking and maintaining *Trichinella*-free, certification. Finally, it provides guidance on verification testing of pigs produced by *Trichinella*-free certified farms. A flow chart detailing the progress in achieving and maintaining *Trichinella*-free, status under the USA system is shown in Table I.

A substantial difference between the USA and EU certification programmes is the requirement for testing wildlife (required by the EU, but not by the USA). For the EU, the stringency of requirements for bio-security are less where *Trichinella* is not endemic in wildlife. For the USA programme, it is assumed that infection pressure is equal in all locations where the programme is in place and therefore all farms must meet the same level of bio-security. Extensive discussions of risk based decisions on requirements to be applied to *Trichinella* free farms and holdings can be found in the previously cited expert opinions (European Commission 2001; European Food Safety Authority, 2005).

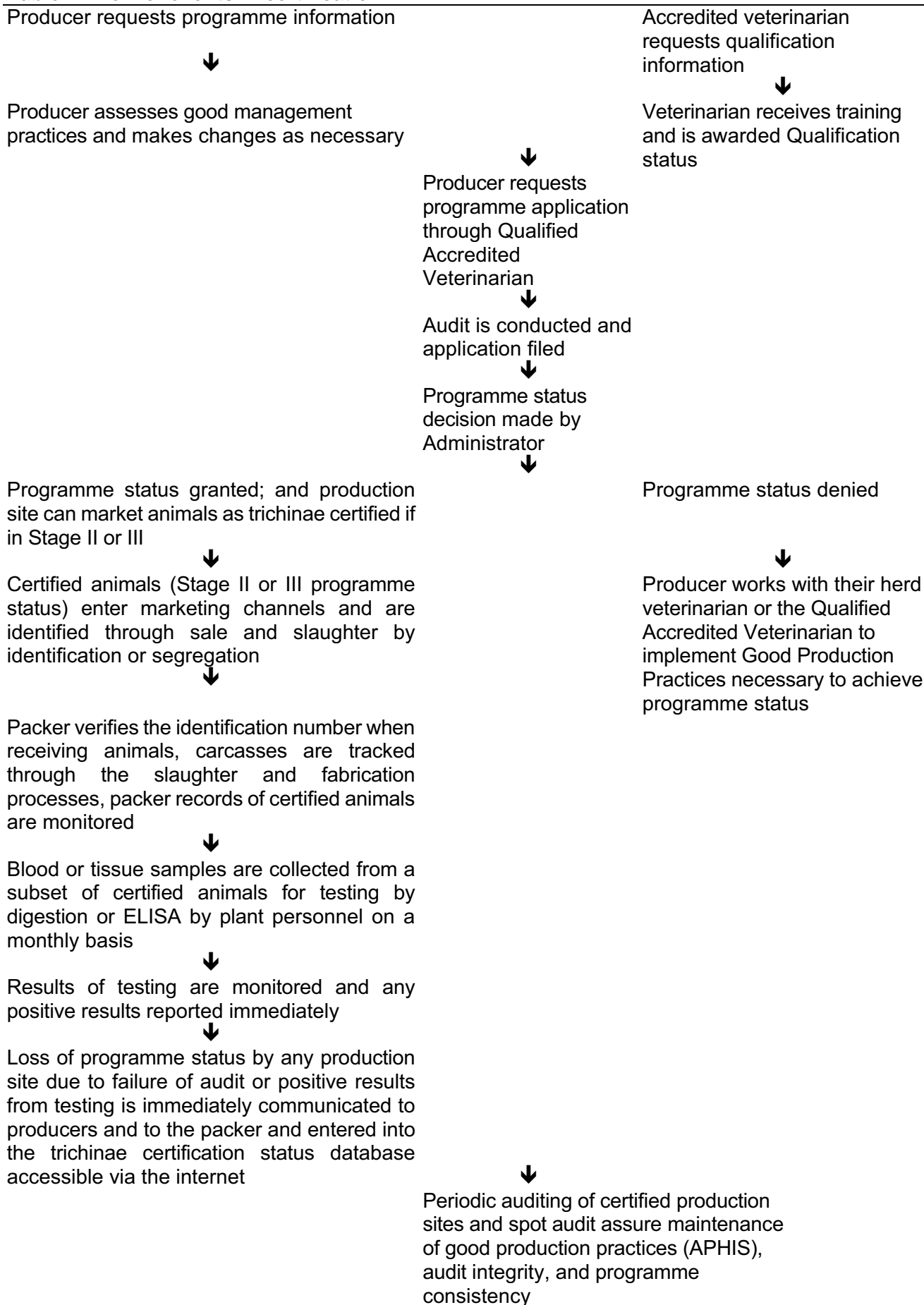
TRICHINELLA-FREE REGIONS

The ICT does not endorse any programme for assuring pigs to be free from *Trichinella* based on geographic boundaries such as a region, state or country (e.g. OIE *International Animal Health Code*, Article 2.2.9.3). This position is supported by a report of the EU Scientific Panel on Biological Hazards (EFSA-Q-2005-001, European Food Safety Authority, 2005). According to this report, a *Trichinella-free* area is a geographic area in which *Trichinella* has never been detected in wild (synanthropic) and domestic animals despite regular and systematic surveillance and domestic cases of trichinellosis has never been reported in humans. The opinion of the Committee, however, was that it is not possible to adequately address all the requirements of a *Trichinella-free* area. *Trichinella* occurs in a wide range of wildlife reservoirs both in terrestrial and marine mammals and birds that can serve as a reservoir for *Trichinella* spp. The absence of *Trichinella* infection cannot be reliably documented in these various species. Although the OIE *Terrestrial Animal Health Code*, Article 2.2.9.3, provides the requirements for a *Trichinella-free* zone or country, some experts do not agree with this concept (Gamble *et al.*, 2000; European Food Safety Authority, 2005) and therefore further review of this subject is required.

OUTDOOR HUSBANDRY

While confinement production systems can be managed in a manner to reduce or eliminate the risk of exposure of pigs to *Trichinella*, pigs raised outdoors, or under conditions that facilitate contact with wildlife will always be at risk for infection. The increasing interest in animals raised under organic or animal-friendly (free range) conditions increases the risk of exposure to this parasite, as well as other pathogens of concern to human health (e.g. *Toxoplasma gondii*). All pigs raised under conditions which dispose them to possible infection should be tested at slaughter by one of the detection methods described in Chapter 3 (Control) under a specific quality assurance programme.

Table I - Flow of events in certification



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