# *TRICHINELLA* SPP. IN WILD AND DOMESTIC ANIMALS IN THE TURIN PROVINCE, ITALY*

**Silvia Gallina**[1], **Daniela Manila Bianchi**[1], **Alberto Bellio**[1], **Ferdinando Pulitano**[1], **Fabio Zuccon**[1] and **Lucia Decastelli**[1]

**SUMMARY**

Pork, horse and game meat may be infected with muscle larvae of the zoonotic nematode *Trichinella*, which may cause a severe disease in humans.

This paper reports on analyses performed during the year 2011 by Laboratorio Controllo Alimenti (Food Control Laboratory) of Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta in Turin (Piedmont - Italy) for the detection of *Trichinella* spp. larvae in muscular tissues.

A total of 13.533 samples (12.050 samples from domestically slaughtered animals; 1.183 samples from game meat; 300 samples from wild reservoir animals) were analyzed according to the methods described in Commission Regulation (EC) No 2075/2005. Four samples from wild animals (foxes) were found to be positive for *Trichinella* spp. No samples from domestically slaughtered animals were found to be contaminated by *Trichinella* spp, confirming that the herd system effectively prevents contact between wild and domestic animals and thus protects herds from the infection.

**Keywords**: *Trichinella* spp., Meat, Food safety.

**RÉSUMÉ**

La viande de porc, de cheval et de gibier peut être infestée par des larves du nématode zoonotique *Trichinella*, qui peut causer des maladies graves chez le consommateur. Cet article rapporte les analyses effectuées pour la détection des larves de *Trichinella* spp., par le Laboratorio Controllo Alimenti (Laboratoire Côntrole des Aliments) de Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta de Turin (Piémont - Italie) pendant l'année 2011. Un total de 13 533 échantillons (12 050 échantillons d'animaux abattus, 1 183 échantillons d'animaux tués à la chasse, 300 échantillons d'animaux sauvages) ont été analysés conformément aux méthodes du règlement CE 2075/2005. Quatre échantillons d'animaux sauvages (renards) ont donné un résultat positif pour *Trichinella* spp. Aucun autre échantillon d'animaux pour la viande n'était contaminé par *Trichinella* spp. Les résultats de ce plan confirment que le système de gestion de *Trichinella* est capable d'éviter le contact entre les animaux sauvages et domestiques, assurant la protection de l'infection des troupeaux.

**Mots-clés** : *Trichinella* spp., viande, sécurité des aliments.

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[1] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, via Bologna 148, 10154, Torino, Italy

# I - INTRODUCTION

Nematode worms of the genus *Trichinella* are one of the most widespread zoonotic pathogen. This parasite is characterized by an extremely wide host range and geographical distribution. Up to date eight species and four genotypes are known and have been described worldwide: *T. britovi, T. murrelli, T. nativa, T. nelsoni, T. spiralis*, *Trichinella* T6, T8, T9, T12 which encapsulate in host muscle tissue and *T. papuae, T. pseudospiralis, T. zimbabwensis,* on the contrary do not encapsulate following muscle cell invasion [Gajadhar, 2009]. *T. britovi* circulates in temperate areas of Europe and Asia, also in northern and western Africa; *T. murrelli* in temperate areas of North America; *T. nativa* is present in arctic and sub-arctic regions of North America, Europe and Asia; *T. nelsoni* in eastern Africa; *Trichinella* T6 in arctic and sub-arctic regions of North America; *Trichinella* T8 in southern Africa; *Trichinella* T9 in Japan and *Trichinella* T12 in Argentina. *T. spiralis* and *T. pseudospiralis* have a cosmopolitan distribution [Pozio and Darwin Murrell, 2006; Krivokapich *et al.*, 2008].

All species can develop in mammals, and some of them also in birds (*T. pseudospiralis*) and reptiles (*T. papuae* and *T. zimbabwensis*). No clearcut morphological difference exists between species and biochemical or molecular analyses are necessary to distinguish them. The majority of the methods in use are based on Polymerase Chain Reaction (PCR) amplification of a single larva [Pozio and La Rosa, 2003].

Human infections in Europe are usually caused by *T. spiralis,T. britovi* and *T. nativa*, while a few cases caused by *T. pseudospiralis* and *T. murelli* have also been described [EFSA and ECDC, 2012]. In Italy, *T. britovi* is the most common specie involved in human and animal infections, which is maintained in nature by red fox (*Vulpes vulpes*), representing the main reservoir [Romano, 2011].

The life cycle of nematodes of the genus Trichinella is characterized by no exogenous stage and the development of two generation (larval and adult) in the same host. Humans acquire trichinellosis by ingesting raw or undercooked meat of swine or other animals containing the *Trichinella* spp. larvae. Gastric acid and pepsin are able to free larvae present in muscle tissue. Larvae invade the small intestine epithelial wall, where they become adult in about 30 hours [Bruschi, 2012]. After one week adult females release newborn larvae (NBL). Normally adult worms reside in the small intestine for few weeks, but they are able to persist much more in immune compromised hosts. NBL migrate through lymphatic and blood vessels reaching striated muscle cells. Larvae penetrate in muscle cells where they induce the formation of a collagen capsule (encapsulating species) and they gradually encyst. NBL develop to the L1 (muscle larva) in about 15 days. L1 is the infective larva stage and can persist for years waiting to be ingested by a new host [Pozio, 2007].

Trichinella life cycle should be differentiated into domestic or sylvatic life cycle, depending on the involved host. Pigs, rodents and occasionally horses, represent the classical host in domestic cycle. In the sylvatic cycle, a wide range of different animals can acquire the infection, but animals most often associated as source of human infections are only represented by game animals. Wild carnivorous and omnivorous hosts are the main reservoirs of *Trichinella* spp. nematodes in nature. Humans, pork and horses represent the most important host from a medical and veterinary standpoint [Romano, 2011].

Each European Member State (MS) must monitor zoonotic agents, including *Trichinella* spp. according to Directive 2003/99/EC and Regulation (EC) 2075/2005 [European Commission, 2003; 2005]. To allow the commercialization of safe meat, systematic official inspection of domestic slaughtered animals (pork, horse and donkey) and game meat is necessary. Furthermore, to study the presence of the parasite, monitoring plans on wild carnivorous have been developed.

In 2010, confirmed cases of human trichinellosis decreased sharply, by 70.2%, with 223 reported cases, versus 748 cases reported in 2009. Often human cases were associated with food-borne outbreaks due to consumption of meat from domestic home bred pigs. No deaths due to Trichinella infection were reported in 2010. The parasite was very rarely detected in pigs in 2010 and less than 0.0001% of pigs tested Trichinella-positive in the EU. The prevalence of the parasite in slaughter pigs was reduced compared to previous years. The parasite was isolated more frequently from farmed and hunted wild

boar. Trichinella was prevalent in wildlife species, with most member states reporting positive findings in wildlife [EFSA and ECDC, 2012].

In Italy six outbreaks of trichinellosis for the consumption of hunted wild boars (*Sus scrofa*) were documented from 1978 to 2008. In December 2008, in Piedmont Region a case of trichinellosis occurred for the consumption of meat from wild boar hunted in southwestern Alps in Italy [Romano, 2011].

This paper reports the results of the analyses for the detection of muscle larvae of *Trichinella* spp. performed during the year 2011 by Food Control Laboratory of IZSPLV in Turin (Piedmont - Italy).

## II - MATERIALS AND METHODS

During 2011 a total of 13.533 samples was collected by Regional Veterinary Service according to Regulation CE 2075/2005 and local monitoring plans on wild mustelids (e.g. badger and beech marten) and wild carnivorous; 12.050 samples of domestic slaughtered animals (9.694 pigs; 2.346 horses; 10 donkeys), 1.183 samples of game meat and 300 samples (278 foxes, 20 badgers and 2 beeches marten) of wild reservoir animals were tested. Magnetic stirrer method and automatic digestion method were used to isolate *Trichinella* larvae. Different weight samples was taken and pooled to be analyzed: from a pillar of the diaphragm at the transition to the sinewy part of whole carcasses of domestic swine (1 g), breeding sow (2 g) and boar (2 g). Specimens weighing 2 g (domestic swine) and 4 g (breeding sow and boar) were taken from the breastbone part or the rib part of the diaphragm, or from the jaw muscle, tongue or abdominal muscles. Samples weighing at least 5 g of striated muscle was taken from cuts of meat, frozen samples, meat of wild boar or other animals.

For magnetic stirrer method was processed up to 100g of tissue. 2 liter of water were heated to 46 to 48 °C in a 3 liter beaker and 16 ± 0.5 ml of hydrochloric acid (25%) was added. The beaker was placed on the preheated plate, a stirring rod placed in, and the stirring started. Then 10 ± 0.2 g of pepsin was added. Meat sample was put in a blender and subsequently transferred to the beaker containing water, pepsin and hydrochloric acid. The fluid was stirred until the meat particles disappear (approximately 30 minutes) and then transferred through the 180 µm sieve into the sedimentation funnel. After half an hour 40 ml of digestion fluid was put into a measuring cylinder and kept for sedimentation for 10 min. Subsequently 30 ml of supernatant was removed and the 10 ml remaining was poured in Petri dish. The measuring cylinder was rinsed with 10 ml of water and added to the Petri dish.

For automatic digestion method Trichomatic 35® blender with filtration insert was used. Muscle samples (up to 35 g) was put into the blender, water (approximately 400 mL) and 30 ml hydrochloride acid (8.5%) was added. A membrane filter was placed in the appropriate filter holder and then 7 g of pepsin was added. Blender was switched on and digestion program was chosen: 5 min for pigs at the normal slaughter age and 8 min for other samples. When the process was completed, membrane filter was transferred into a millimeter Petri dish.

Samples obtained by both methods were observed using bright-field microscope [European Commission, 2005].

Found larvae were sent to the European Union Reference Laboratory for Parasites (EURLP) to identify species by multiplex PCR protocol.

## III - RESULTS

Out of 13.533 meat samples, 4 tested positive for *Trichinella* spp. No larvae were demonstrated to be present in 12.050 samples of domestic slaughtered animals and 1.183 samples of meat game. Out of 300 samples of wild reservoir animals, 4 were found positive

for *Trichinella* spp. All the positive samples were collected from red foxes (*Vulpes vulpes*) which were examined according to monitoring plans on wild carnivorous. Positive animals were found in southwestern Alps: one sample from Valle Stura, two from Valle Varaita and one from Valle Gesso Vermagnana and Pesio Area. Three foxes were adult (2 female and 1 male) and no data were reported for one of them.

Positive samples were carried to Food Control Laboratory of IZSPLV of Turin in February and May. Two *Trichinella* spp. larvae isolated from foxes were identified as *Trichinella britovi*, while for the other two positive samples; identification to species level was not possible.

## IV - DISCUSSION AND CONCLUSION

Cases of confirmed human trichinellosis in EU decreased by 70.2% in 2010 (223) compared with 2009 (748). In 2010 *Trichinella* spp. was the second etiological agent (26.5%) of 34 strong evidence outbreaks caused by pig meat and products thereof in EU; although the main cases occur only in two MSs (Romania and Lithuania) [EFSA and ECDC, 2012].

Our results suggest that the exposure to *Trichinella* for consumers is low, as none of the samples from slaughterhouses tested positive. Negative samples of domestic slaughtered animals show that herd system allow to keep down livestock infection from *Trichinella*; indeed contact between wild and domestic cycle seem avoided. Therefore the pivotal role in the prevention from Trichinella infection in livestock is played by good farming practices as management, bio-security, feed and its storage, rodent control programs and general hygiene conditions.

These results are in accordance with the European situation of the last years where it is very rarely to detect *Trichinella* spp. in slaughtered pigs: all positive samples derived from pigs from free range farming.

Otherwise trichinellosis outbreak occurred in Piedmont Region in December 2008 shows that systematic meat inspection is necessary, especially for game meat.

Moreover, considering that *T.britovi* is the most common species detected in Italy and that this species is less pathogenic than others producing less number of larvae [Romano, 2011], human infection by *Trichinella* is likely to be underreported.

Positive samples of *Trichinella* spp. in red foxes (1.4%) are in accordance with Italian prevalence (1.5%) [EFSA and ECDC, 2012]. Results show the importance of the wildlife monitoring program covering foxes, mustelids and other carnivores including birds of prey. This plan is of pivotal importance to assess the prevalence of *Trichinella* spp. in wild reservoir animals and the risk of introduction into the domestic cycle.

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