FOUR-YEAR INVESTIGATION ON *TRICHINELLA* SPP. LARVAE IN WILD AND DOMESTIC ANIMALS IN NORTH-WESTERN ITALY *

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ABSTRACT

The results of analyses performed from 2011 to 2014 in Piedmont region for the detection of muscle larvae of *Trichinella* spp are reported. A total of 212.997 meat samples (61.177 in 2011, 53.709 in 2012, 61.493 in 2013 and 36.618 in 2014) were analyzed. All samples were analyzed according to the methods described in Regulation CE 2075/2005. Four fox (*Vulpes vulpes*) samples resulted positive for *Trichinella* spp. in 2011; one fox muscle in 2012 and two wild board (*Sus scrofa*) samples in 2013 tested positive. No samples of reared animals were found to be infected with *Trichinella* spp confirming that herd system is able to avoid contact between wild and domestic animals protecting herds from this type of infection.

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

RESUME

Cette étude rapporte les résultats des analyses effectuées de 2011 à 2014 pour la détection des larves de *Trichinella* spp. dans des échantillons de tissus musculaires prélevés dans la Région du Piémont, Italie. Un total de 212 997 échantillons (61 177 en 2011, 53 709 en 2012, 61 493 en 2013 et 36 618 en 2014) ont été analysés. Toutes les analyses ont été effectuées selon les méthodes décrites dans le Règlement CE 2075/2005. Quatre renards en 2011, un renard en 2012 et deux sangliers en 2013 ont fourni des résultats positifs pour *Trichinella* spp. Aucun des échantillons provenant des animaux d'élevage n'était contaminé par *Trichinella* spp., ce qui confirme que la biosécurité des élevages est capable d'éviter le contact entre les animaux sauvages et domestiques et protège les troupeaux de l'infection.

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

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I – INTRODUCTION

Trichinellosis is a zoonotic disease, caused by ingestion of raw or undercooked meat and meat derived products with live larvae of parasitic nematode belonging to the genus *Trichinella*. This parasite is characterized by an extremely wide host range and geographical distribution [Pozio, 2015].

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 that encapsulate in host muscle tissue and *T. papuae, T. pseudospiralis, T. zimbabwensis* that do not encapsulate following muscle cell invasion [Gajadhar *et al.*, 2009]. *T. spiralis* and *T. pseudospiralis* have a cosmopolitan distribution, while *T. britovi* circulates in temperate areas of Europe and Asia and in northern and western Africa. *T. nativa* is present in arctic and sub-arctic regions of North America, Europe and Asia; *T. murrelli* in temperate areas of North America; *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 [Pozio and Darwin Murrell, 2006; Krivokapich *et al.*, 2008]. All species are able to develop in mammals and some of them also in birds and reptiles. Biochemical or molecular analyses have to be performed to identify specie because no remarkable morphological differences exist between species [Pozio and La Rosa, 2003].

Larvae are ingested in raw or undercooked meat and develop into adults in the small intestine. Trichinella life cycle should be differentiated into domestic or sylvatic life cycle, depending on the host involved.

Domestic cycle most often involves pigs, rodents and occasionally horses. In the sylvatic cycle, a great range of different animals can acquire the infection, but animals most often associated as source of human infections are only represented by game. Wild carnivorous and omnivorous hosts are the main reservoirs of *Trichinella* spp. nematodes in nature [CDC, 2013]. 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].

Symptoms of trichinellosis are usually only reported in humans as infected animals do not generally show clinical signs. Clinical manifestations of the disease range from asymptomatic infection to fatal disease; the common signs and symptoms include eosinophilia, fever, periorbital edema, and myalgia. In 2013, 217 confirmed trichinellosis cases were reported in the EU. The EU notification rate decreased by 17.7% compared with 2012. The highest notification rates were reported in Romania, Latvia and Bulgaria. The temporal trend of trichinellosis in the EU in 2009-2013 was greatly influenced by a number of smaller and larger outbreaks with peaks often occurring in January [EFSA and ECDC, 2015].

Human fatalities are rare but a recent outbreak in Spain due to the consumption of wild boar meat involved one fatality and is a reminder of the importance of ongoing testing, especially of high risk products in endemic areas [Learmont *et al*., 2015]. In Italy, during past years there has been an increasing attention to the circulation of *Trichinella* spp. in wildlife due to an increasing human cases related to game meat consumption. *T. britovi* is the most common species involved in human and animal infections, that is maintained in nature by red fox (*Vulpes vulpes*), representing the main reservoir in Italy [Romano *et al*., 2011; Fichi *et al*., 2015].

Each Member State (MS) should 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.

This paper reports the results of the analyses for the detection of muscle larvae of *Trichinella* spp. performed from 2011 to 2014 in Piedmont Region (Northwest Italy) by Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta.

II – MATERIALS AND METHODS

According to Regulation CE 2075/2005 and local monitoring plans on wild animals, a total of 212.997 meat samples was collected by Regional Veterinary Service between January 2011 and December 2014. Swine (202.178), soliped (9.802), fox (897), mustelids (93), wild herbivore (16), birds of prey (10) and wolf (1) meat samples were analyzed (table 1).

Isolation of *Trichinella* larvae was obtained by magnetic stirrer method and automatic digestion method according to Reg. CE 2075/2005 Annex I Chapter I and II respectively. Different weight samples were 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.

Table 1

Number of meat samples analyzed to detect *Trichinella* spp.

Animal	Origin	Year			
		2011	2012	2013	2014
domestic swine	intensive breeding	52.248 (0)	42.821 (0)	42.855 (0)	18.281 (0)
	extensive breeding	3.504 (0)	3.388 (0)	3.563 (0)	3.524 (0)
wild boar	hunting	873 (0)	3.158 (0)	11.273 (2)	11.838 (0)
	reared	1.197 (0)	1.283 (0)	1.248 (0)	1.124 (0)
solipeds	reared	3.036 (0)	2.742 (0)	2.329 (0)	1.695 (0)
fox	carcass	280 (4)	300 (1)	186 (0)	131 (0)
mustelid	carcass	22 (0)	17 (0)	30 (0)	24 (0)
bird of prey	carcass	10 (0)	n.d.	n.d.	n.d.
fallow deer	reared	6 (0)	n.d.	6 (0)	n.d.
chamois	hunting	1 (0)	n.d.	1 (0)	n.d.
roe deer	hunting	n.d.	n.d.	1 (0)	n.d.
deer	reared	n.d.	n.d.	1 (0)	n.d.
wolf	carcass	n.d.	n.d.	n.d.	1 (0)

( ) positive samples

For magnetic stirrer method was processed up to 100g of tissue. A 2000 ml of water was heated up 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. Pooled meat sample was put in a blender and blended for 5-10 seconds, subsequently transferred to the beaker containing the digestion mix (water, pepsin and hydrochloric acid). The fluid was stirred at 47° C until the meat particles disappeared (approximately 30 minutes) and then poured into the sedimentation funnel through the 180 µm sieve. After 30 minutes 40 ml of digestion fluid was put into a 100 ml cylinder for sedimentation for 10 min. Subsequently 30 ml of supernatant was removed and the 10 ml remaining was poured in a Petri dish for analysis. The cylinder was rinsed with 10 ml of water and added to the Petri dish.

For the automatic digestion method the Trichomatic 35® was used. Muscle samples (up to 35 g) was put into the reactor chamber, water (approximately 400 mL), 30 ml hydrochloride acid (8.5%) and 7 g of pepsin were added. A membrane filter was placed in the appropriate filter holder. Trichomatic 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 the membrane filter was transferred into a gridded Petri dish. Samples obtained by both methods were observed using bright-field microscope [European Commission, 2005].

Isolated *Trichinella* spp. were identified at the species level by multiplex PCR according to Pozio and La Rosa (2003) with visualization by capillary electrophoresis, at the Istituto Superiore di Sanità, Rome, Italy, currently acting as the European Reference Laboratory for Parasites (EuRL).

III – RESULTS

Out of 212.997 meat samples, 7 tested positive for *Trichinella* spp. No larvae were demonstrated to be present in 179.986 samples of domestic animals (pigs, horses and donkeys). Five positive cases of infection with *Trichinella* spp. in a total of 897 red foxes examined were reported. Furthermore two of 31.994 wild boar samples resulted positive. No positive samples were reported in mustelids (*Meles meles, Martes foina, Martes martes* and *Mustela putorius furo*), wild herbivores (*Cervus laphus, Dama dama, Capreolus capreolus* and *Rupicapra rupicapra*), birds of prey and wolf (*Canis lupus*).

Larvae isolated from five samples (3 foxes and 2 wild boars) were identified as *Trichinella britovi*, while identification to species level was not possible for the other two positive samples. Positive foxes were found in South-Western Alps as wild boars in Eastern Piedmont (figure 1).

IV – DISCUSSION AND CONCLUSION

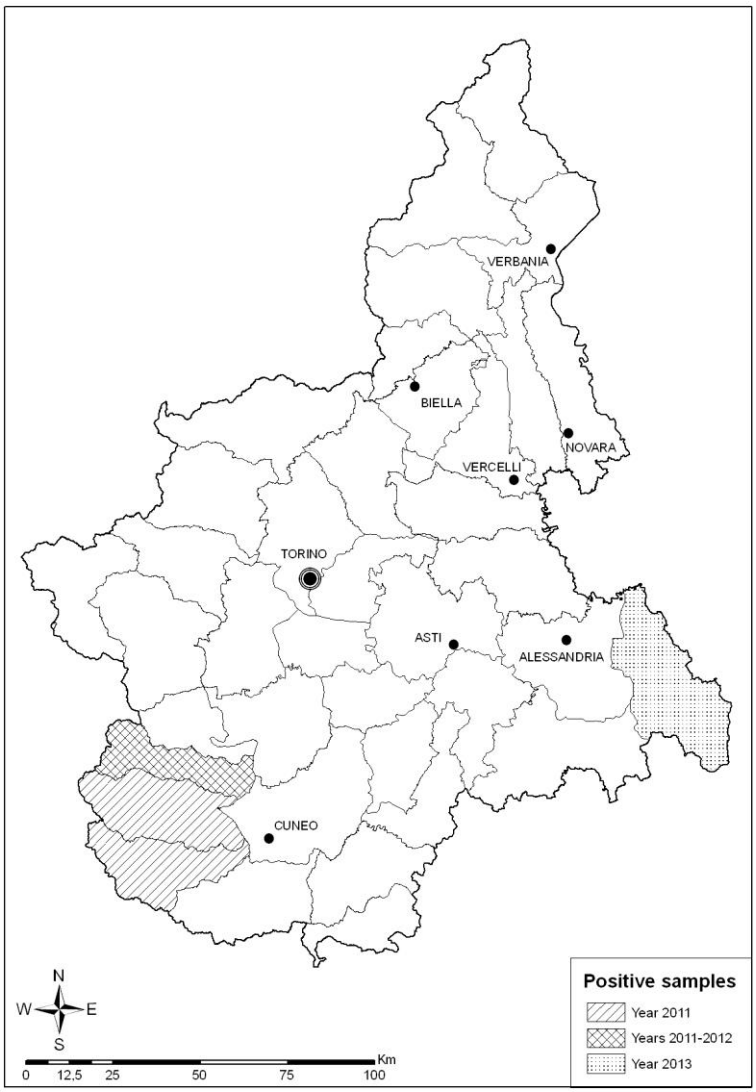
The prevalence of *T. britovi* in the wild boar population of Italy is low. From 1988 to 2014, *T. britovi* was detected in only 26 wild boars, hunted in the Regions of Abruzzi (n = 5), Piedmont (n = 4), Emilia Romagna (n = 2), Lazio (n = 2), Liguria (n = 2), Lombardy (n = 2), Marche (n = 2), Sardinia (n = 2), Umbria (n = 2), Valle d'Aosta (n = 2) and Basilicata (n = 1) (International Trichinella Reference Centre, www.iss.it/site/Trichinella/ index.asp). One of the main reasons for this low prevalence could be the low susceptibility of wild boars to *T. britovi* [Romano *et al*., 2011].

In Italy from 1982 to 2014, *T.britovi* was detected in 151 foxes. To investigate regional circulation of *Trichinella* spp. red foxes, that represent the most important indicator species for these parasites in the European wildlife, were sampled and examined for *Trichinella* spp. infection. Wild plan is of pivotal importance to assess the risk of introduction into the domestic cycle. Decreasing of pork samples during the last year was linked to an increase of holding that has been officially recognised by the competent authority as free from *Trichinella* in accordance to Regulation EC 2075/2005.

At the end of 2013, 555 pig farms had been declared Trichinella-free in Piedmont region. On the other hand, samples from wild boar increased during latter years due to an increase of monitoring plans on game meat in Piedmont.

Figure 1

Spatial distribution of positive samples



Our results suggest that the exposure to *Trichinella* for consumers is quite low, for none of the samples from breeding animals tested positive. Negative samples of reared animals show that herd system allow to keep down livestock infection from *Trichinella*; indeed contact between wild and domestic cycle seems to be avoided. Controlled housing conditions are often not applied to hand-reared wild boars. Despite this no positive samples were tested from this type of animals. There are strong epidemiological evidences supporting that pigs from controlled housing conditions in integrated production systems, most of pigs reared in Italy, are *Trichinella*-free. 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. Otherwise trichinellosis outbreak occurred in Piedmont Region in December 2008 showed that the definition of a region with a negligible risk for ***TRICHINELLA*** infection is not applicable to wild boar and stresses the need to test all ***TRICHINELLA*** - susceptible wild animals intended for human consumption and implement risk communication to hunters and consumers.

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