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Short communication

Trichinella spiralis a new alien parasite in Italy and the increased risk of infection for domestic and wild swine

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ABSTRACT

In Europe, *Trichinella spiralis*, the most dangerous species for humans of the genus *Trichinella*, has a patchy distribution with important foci in Eastern countries and Spain. This zoonotic pathogen was apparently not circulating among wild and domestic animals of Italy. In 2016, muscle larvae belonging to this nematode species were detected in a red fox (*Vulpes vulpes*) shot in the Piacenza province (Northern Italy). This parasite may have been introduced into northern Italy from eastern Europe by hunters, by a hunting dog, or by immigrants, who illegally carried infected meat in their personal baggage. In the same year, *T. spiralis* infected sausages illegally introduced by personal baggage into Italy from Romania, were inadequately disposed of in the garbage of a central Italian town. Even though these two episodes may not be connected in time and space, they represent an increased risk of infection for domestic and wild swine, which are highly susceptible to this pathogen. In these animals, *T. spiralis* shows a higher larval burden and a longer survival time than other *Trichinella* species. Since most of the Italian pig production plants are in northern Italy, the circulation of *T. spiralis* should be strictly monitored in wildlife living in these areas.

1. Introduction

Most species and genotypes of the zoonotic nematodes of the genus *Trichinella* are circulating in well-defined distribution areas with two exceptions, *T. spiralis* and *T. pseudospiralis*, which show a cosmopolitan distribution (Rosenthal et al., 2008; Pozio and Zarlenga, 2013). Originally, *T. spiralis* distribution area was probably circumscribed to eastern and south-eastern Asia, however, due to its high infectivity to swine, it was imported in the historical age to Europe and then it spread to several continents through the European colonization (Pozio and Zarlenga, 2013). The cosmopolitan distribution of *T. pseudospiralis* is probably related to its wide host spectrum encompassing both mammals and birds, which spread this pathogen through the world (Pozio, 2016).

In Europe, *T. spiralis* has a patchy distribution with important foci in Eastern countries (e.g., Bulgaria, Croatia, Lithuania, Poland, Romania and Serbia), in Germany and Spain, and with restricted foci in Austria, Czech Republic, Estonia, Finland, Hungary, Ireland, Latvia and Slovak Republic (Pozio et al., 2009). Foci of *T. spiralis* had been reported in France, Sweden, and The Netherlands in the past, but this parasite has not been documented in wild and domestic animals in the last 20 years (EFSA, 2016).

During the last decades, there has been a strong increase in immigration from countries known to be highly endemic for *T. spiralis* such as Bulgaria, Poland, Romania and Serbia, to Italy and to other western European countries. At the same time, there has been an increase in the number of Italian individuals who went to these countries for business purposes, to visit friends and relatives, for hunting activities and for tourism, where they acquired *trichinellosis* (Pozio, 2015).

The aims of the present work were to report the detection of a *T. spiralis*-infected fox in the Piacenza Province, Emilia-Romagna region, northern Italy, and the illegal importation into Italy of *T. spiralis*-infected meat in personal baggage, followed by incorrect disposal, which is a way by which *T. spiralis* can easily reach wild animals and free-ranging and backyard pigs.

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2. Materials and methods

2.1. Data collection and parasite identification

In January 2016, *Trichinella* sp. vital larvae were detected in the muscles of a 2-year-old male fox (*Vulpes vulpes*) shot in the Travo municipality (333 m asl), Trebbia valley, Piacenza province, northern Italy, by the magnetic stirrer method for pooled sample digestion (Commission Implementing Regulation, 2015). Larvae were identified at the species level by multiplex PCR (Pozio and La Rosa, 2010).

An Italian citizen (N.M.) consumed raw sausages at a friend's home in Cluj Napoca (Romania) on December 12, 2015. The sausages had been made with pork from a backyard pig, which had been slaughtered at home without any veterinary control. On his return home in Central Italy by car, he carried in his baggage several kilograms of raw sausages, received as a gift from Romanian friends. At the beginning of January 2016, he developed a serious symptomatology with fever and myalgia. He was hospitalized with a diagnosis of *trichinellosis* confirmed by serology. He was successfully treated with an anthelminthic, and discharged from the hospital 12 days later. A piece of sausage was provided to the physician, who sent it to the Istituto Superiore di Sanità, Rome, Italy. The sausage (85 g) was digested and larvae identified at the species level as above reported. On his return home, the patient disposed of the sausages by throwing them in the garbage.

2.2. Microsatellite study

To acquire information on the possible origin of the *T. spiralis* isolate from the fox shot in the Piacenza province, 30 single larvae collected from the fox muscles and 30 single larvae collected from the sausage, were investigated at five microsatellite loci according to previous published protocols (La Rosa et al., 2012).

3. Results and discussion

The fox hunted in the Piacenza province harboured more than 100 larvae/g in the diaphragm pillars. Larvae were identified as *T. spiralis* (isolate code ISS6185). Since 2006 according to European Union regulation (Commission Implementing Regulation, 2015), a *Trichinella* monitoring program in wildlife has been carried out in the Emilia-Romagna region. *Trichinella* spp. were widely investigated in hunted wild boar and in other indicator animals such as carnivore and omnivore mammals and birds (Table 1). During this period, besides *T. spiralis* in a fox from the Piacenza province, *T. britovi* was detected in five foxes (one from the Piacenza province) and three wolves (one from the Piacenza province), *T. pseudospiralis* in a wild boar. The microsatellite analysis of the 30 *T. spiralis* larvae from the isolate ISS6185 did not reveal any polymorphism and the allelic pattern falls within the genetic variability detected in *T. spiralis* isolates from Europe so far studied, which meant that the possible geographical origin of the parasite could not be traced (data not shown).

The wild boar population of the Emilia Romagna region has been estimated to be of 60,875 in 2016 (http://agricoltura.regione.emilia-romagna.it/caccia/doc/osservatorio-faunistico-venatorio/abbattimenti/cinghiali-abbattuti-in-emilia-romagna/at_download/file/cinghiali%20abbattuti%20in%20Emilia-Romagna%2020_09_2016.pdfhttp://agricoltura.regione.emilia-romagna.it/caccia/doc/osservatorio-faunistico-venatorio/abbattimenti/cinghiali-abbattuti-in-emilia-romagna/at_download/file/cinghiali%20abbattuti%20in%20Emilia-Romagna%2020_09_2016.pdf; Merli et al., 2017); of them about 32% (19,764 heads) were tested for *Trichinella* in the year (Table 1).

At the national level, the estimated wild boar population ranges from 625,000 and 937,000 (Massei et al., 2015; Merli et al., 2017), 617,524 heads were tested for *Trichinella* in the last ten years (roughly 10% of the population per year) and 68 (0.01%) of them were positive

Table 1
Wild animals tested for *Trichinella* in the Emilia-Romagna region from 2006 to 2016.[a]

Year	No. of positive/tested animals (%)		
	Wild boar (*Sus scrofa*)	Fox (*Vulpes vulpes*)	Wolf (*Canis lupus*)
2006	0/3514	0/24	0
2007	0/6624	0/208	0
2008	0/8864	1[c]/204 (0.49)	0/1
2009	0/9033	0/350	0/2
2010	1[b]/11,267 (0.009)	1[c]/312 (0.32)	0/4
2011	0/15,499	0/989	0/7
2012	0/16,147	2[c]/719 (0.28)	0/13
2013	0/15,434	0/593	0/6
2014	0/15,614	0/595	1/2
2015	0/17,523	1[c]/477 (0.21)	1[c]/11 (9.09)
2016	0/19,764	1[d]/509 (0.19)	1[c]/10 (10.00)
Total	1/139,283 (0.0007)[e]	6/4980 (0.12)[f]	2/56 (3.57)[g]

[a] In the same years, 36 mustelids (badgers, *Meles meles*; stone martens, *Martes foina*), 12,968 corvids and 156 raptorial birds were investigated for *Trichinella*; no one tested positive.
[b] *Trichinella pseudospiralis*.
[c] *Trichinella britovi*.
[d] *Trichinella spiralis*.
[e] 0/13,267 wild boars were from the Piacenza province.
[f] 2/285 (*T. britovi* and *T. spiralis*) foxes were from the Piacenza province.
[g] 1/1 wolf (*T. britovi*) was from the Piacenza province.

Table 2
Trichinella species detected in wild and domestic animals of Italy from 1968 to 2016.

Host	*T. britovi*	*T. pseudospiralis*	*T. spiralis*	Total
Badger (*Meles meles*)	2			2
Black rat (*Rattus rattus*)	1			1
Brown bear (*Ursus arctos*)	1			1
Brown rat (*Rattus norvegicus*)	5			5
Cat (*Felis silvestris*)	5			5
Domestic pig	18			18
Red fox (*Vulpes vulpes*)	166 (98.2%)	1 (0.6%)	2 (1.2%)	169
Little owl (*Athene noctua*)		1		1
Stone marten (*Martes foina*)	8			8
Stray dog	5			5
Tawny owl (*Strix aluco*)		1		1
Wild boar (*Sus scrofa*)	35 (87.5%)	5 (12.5%)		40
Wolf (*Canis lupus*)	114			114
Total	365 (97.3%)	8 (2.1%)	2 (0.5%)	375

(EFSA, 2007, 2011, 2012, 2013, 2014, 2015a, 2015b, 2016). Most of positive animals were infected by *T. britovi* and very few by *T. pseudospiralis* (Table 2). No data are available on the estimated fox population of Italy. However according to EFSA (2007, 2011, 2012, 2013, 2014, 2015a, 2015b, 2016), 20,237 foxes were tested for *Trichinella* in Italy from 2006 to 2015 and 89 (0.44%) heads were positive. Positive animals were prevalently infected by *T. britovi* (87, 97.7%), only one by *T. pseudospiralis* (1.1%) and only one by *T. spiralis* (1.1%).

Before the discovery of *T. spiralis* in a fox of the Piacenza province, the only *T. spiralis* isolate from Italy originated from a fox, which had been shot in 1991 at Jaffereau (Bardonecchia, Turin) about 2 km from the border between Italy and France, and about 300 km from the Travo municipality. From 1968 to 2016, the identification at the species level of *Trichinella* spp. larvae collected from muscles of 375 wild and domestic animals from Italy, showed that 365 (97.3%) were *T. britovi*, 8 (2.1%) *T. pseudospiralis*, and 2 (0.5%) *T. spiralis* (Table 2, Fig. 1).

In the sausage imported from Romania, 42.8 dead larvae/g was detected and the larvae were identified as *T. spiralis* (isolate code ISS6333). Since the larvae collected after digestion were dead, the microsatellite analysis did not provide any result. The illegally disposal of infected sausages represents a serious risk of introduction of this zoonotic pathogen among free-ranging and backyard pigs and wildlife population including the wild boar, which can be the source of human

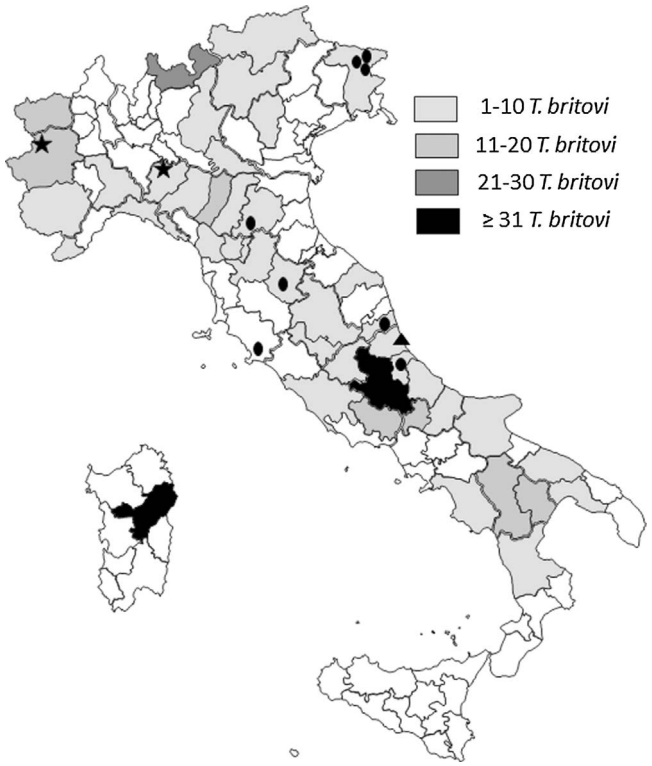

Fig. 1. Map of autochthonous *Trichinella*-positive animals detected in Italy from 1968 to 2016 by Italian provinces. Increasing grey tones show increasing numbers of *Trichinella britovi* isolates detected per province; star, *Trichinella spiralis* isolate; circle, *Trichinella pseudospiralis* isolate; triangle, *T. spiralis* infected sausages incorrectly disposed.

trichinellosis. *T. spiralis* shows a higher larval burden and a longer survival time in domestic and wild swine than that of the other *Trichinella* species (Kapel and Gamble, 2000; Kapel, 2001; Nöckler et al., 2005). *T. spiralis* may have been introduced into the Emilia Romagna region from Eastern Europe by hunters, by the carcass of a hunting dog, wrongly disposed, or by travellers and immigrants, who illegally carried infected meat in their personal baggage. However, other introduction avenues cannot be excluded.

In September 2015, a flooding caused by Trebbia and Nure rivers, involved the respective valleys of the Piacenza province. The flooding killed many wild and domestic animals and overflowed many houses. We can speculate that an infected animal (e.g., a hunting dog, which had hunted in Eastern Europe) drowned and wild animals fed on its carcass. Alternatively, game meat, pork or derived raw products, illegally imported, were removed by the water from houses and scattered throughout the area.

Between 1933 and 1946, *trichinellosis* outbreaks caused by the consumption of *T. spiralis* infected pork occurred in Sicily. However, the last Sicilian infected pig was detected in 1961 and this infection focus has ceased to exist (Pozio and La Rosa, 1998). According to literature, *T. spiralis* infected animals or meat reached the Italian territory over the years. Three *T. spiralis* infected horses imported from eastern Europe and slaughtered in Italy where the source of three outbreaks of *trichinellosis* in Apulia region (Southern Italy) in 1990 and in 2000, and in Piacenza (Emilia Romagna region, Northern Italy) in 1998. From 1996 to 2008, *T. spiralis* was detected during routine inspection at the slaughterhouse in five horses imported from eastern Europe (Pozio, 2015). In the period 2000–2003, pig meat illegally imported in personal baggage into Italy from Serbia and Romania, was the source of infection for humans (Pozio and Marucci, 2003).

In 2008, it was estimated that about five tons of bush meat per week were illegally imported in personal baggage from Africa to Europe through the Charles de Gaulle airport in Paris, France (Chaber et al., 2010). From 2008 to 2011, 5.5 ton of meat was confiscated at Swiss international airports per year (Falk et al., 2013). Meat and meat-derived products of pig origin illegally introduced by personal baggage from Asia to the European Union have been discovered in two German international airports from 2012 to 2013 (Beutlich et al., 2015). These three examples show the high risk for the introduction of *Trichinella* sp. infected meat, which can be the source of human outbreaks thwarting the efforts made in the EU to control these zoonotic parasites in the food chain.

Border authorities should implement control systems to avoid the importation of infected food in personal baggage and educate travellers, tourists, hunters, immigrants and consumers on the health risk of consuming uncontrolled meat and on the risk of introducing into a country new pathogens when infected meat is improperly disposed. In the EU, there is an increase of *Trichinella* susceptible wild animals (e.g., foxes and wild boar), which colonize the urban areas and survive feeding on garbage (Cahill et al., 2012). In the Piacenza province, both wild boar and red fox populations increased in recent years. The fox population nearly doubled from 1999 to 2016 (E. Merli, unpublished data), and the number of hunted wild boar heads increased from about 1300 in the 2004/05 hunting season to approximately 3300 in the 2015/16 hunting season (http://agricoltura.regione.emilia-romagna.it/caccia/doc/osservatorio-faunistico-venatorio/abbattimenti/cinghiali-abbattuti-in-emilia-romagna/at_download/file/cinghiali%20abbattuti%20in%20Emilia-Romagna%2020_09_2016.pdf).

Peri-urban and urban areas can offer resources such as food, water and refuge to wild animals when conditions change in surrounding areas (winter, drought, hunting season), making these areas even more attractive when population densities are high because they firstly feed on vegetable material found in gardens (wild boar), and secondly, on domestic garbage (both foxes and wild boar). Natural disasters, such as flooding or earthquakes, can help this process. The juxtaposition of urban, rural and forest areas facilitates foxes and wild boar presence in urban settings, as does the existence in cities of corridors such as rivers (Széll et al., 2013). If these animals acquire *Trichinella* infection by feeding on garbage, they can play the role of a Trojan horse introducing *Trichinella* in the wild cycle when they die due to car accidents, after being wounded by a hunter or for natural causes. In peculiar cases, synanthropic rats (*Rattus norvegicus*) can amplify the *T. spiralis* biomass and act as a vector of the parasite to backyard pigs (Pozio, 2014).

Since most of the Italian pig production plants are located in the northern Italy, the circulation of *T. spiralis* should be strictly monitored in wildlife. The question is to know if the detection of this zoonotic pathogen in a wild animal of Italy was an isolated event or the tip of the iceberg of a *T. spiralis* biomass not detectable by monitoring the infection in wild animals, because the number of infected animals is still low and with a low worm burden.

Conflict of interest

No financial or personal relationships are maintained with other people or organizations that could inappropriately influence or bias this paper.

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