Veterinary Parasitology 212 (2015) 262–266

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Trichinella britovi from domestic to wild animals of Sardinia, Italy

Bandino E.[a,*], Goddi L.[a], Mulas M.[a], Murgia M.C.[a], Soddu M.[a], Marucci G.[b], Pezzotti P.[b], Cabras P.A.[a], Pozio E.[b]

[a] *Istituto Zooprofilattico Sperimentale della Sardegna, via F.lli Kennedy 2, 08100 Nuoro, Italy*
[b] *Department of Infectious, Parasitic and Immunomediated Disease, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy*

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 15 July 2015
Accepted 17 July 2015

Keywords:
Trichinella britovi
Sardinia
Pig
Wildlife
Epidemiology
Rearing practices
African swine fever

ABSTRACT

The zoonotic nematode *Trichinella britovi* has been documented in animals and/or humans of the Mediterranean islands of Corsica and Sardinia since 2004. From 2005 to 2007 in the Sardinia island, several surveys had shown that *T. britovi* was circulating among backyard and free-ranging pigs reared in the Orgosolo municipality but all attempts had failed to detect this parasite in wild susceptible animals. The aim of the present work was to investigate the circulation of *T. britovi* in pigs and wildlife of the Orgosolo municipality, and of surrounding municipalities and provinces in the 2010–2014 slaughtering/hunting seasons. The results show that the *T. britovi* circulation was still restricted to the Orgosolo municipality with a prevalence of 2.6% in free-ranging pigs and 0.2% in backyard pigs but, for the first time, this parasite was detected also in 0.4% of wild boar, and 27.6% of red foxes. No infection was detected in backyard pigs, wild boar, and red foxes of the other municipalities and provinces. Since 1978, African swine fever is endemic in Sardinia and foci of this virus are still active in the investigated areas favoring cannibalism and, consequently, the *T. britovi* transmission, due to the high mortality rate caused by this virus. This is the first documented report on the transmission of *T. britovi* between the domestic and the sylvatic cycle. The health authority of the island must provide a service to dispose animal carcasses and offal, stamping out illegal free-ranging pigs, and train hunters and pig owners to manage waste and by-products according to the EU regulations.

1. Introduction

Trichinellosis is a zoonotic disease caused by nematode worms of the genus *Trichinella* circulating in domestic and wild animals worldwide (Gottstein et al., 2009). The main sources of *Trichinella* spp. infection for humans are meat and meat derived products from domestic and wild swine (Murrell and Pozio, 2011). These zoonotic nematodes are parasites of both carnivore and omnivore wild animals and, sometimes, they reach the domestic habitat infecting pigs and horses. The importance of wildlife as reservoir hosts for all species of *Trichinella* is underscored by the parasite's biomass, which is greater in wild than in domestic animals, unlike other nematode infections involving both sylvatic and domestic animals. When humans fail in the proper management of domestic animals and wildlife, *Trichinella* (especially *Trichinella spiralis*) infection is transmitted from the sylvatic environment to the domestic one, sometimes through synanthropic (intermediary between domestic and sylvatic) animals (Pozio and Murrell, 2006).

Today, of the 12 recognized taxa in the genus *Trichinella*, four, namely *T. spiralis*, *T. nativa*, *T. britovi*, and *T. pseudospiralis*, circulate in Europe (Pozio and Zarlenga, 2013). Since the most important *Trichinella* hosts in Europe are terrestrial mammals, these nematodes do not colonize most of the European islands, excluding those of the Northern regions which are linked to mainland by ice during the winter (Pozio et al., 1998). However, *T. spiralis* has been passively introduced in Ireland and Sicily in historical ages (Pozio and La Rosa, 1998; Rafter et al., 2005; Zimmer et al., 2009).

With the exception of Sicily where *T. spiralis* was documented in pigs and humans from 1933 to 1961 (Pozio and La Rosa, 1998), *Trichinella* spp. were never documented in the islands of the Mediterranean Basin up to 2004 when *T. britovi* was detected in free-ranging domestic pigs and in a red fox of a remote area of the Corsica island (Boireau and Vallée, 2004; Richomme et al., 2010). One year later, a human outbreak of trichinellosis occurred in the Sardinia island following the consumption of pork from a free-ranging pig reared in a remote area of this island (Pozio et al., 2006, 2009a).

* Corresponding author.
E-mail address: ennio.bandino@izs-sardegna.it (E. Bandino).

http://dx.doi.org/10.1016/j.vetpar.2015.07.020
0304-4017/

The aim of the present work was to investigate the prevalence of *T. britovi* infection in domestic and wild animals of the Sardinia focus from October 2010 to March 2014, to monitor the transmission of this pathogen between domestic and wild animals circulating in the same areas.

2. Material and methods

Study area

Sardinia is the second largest island of the Mediterranean basin with a surface of 24,100 km^2 and 1662,045 inhabitants (69 inhabitants/km^2). The investigated area of 5788 km^2, approximately a fourth of the total surface of the island, is predominantly a mountainous area covered by forests in the north-eastern part of the island, and includes: the Orgosolo municipality (223 km^2 with 20 inhabitants/km^2), where a *T. britovi* focus in domestic pigs was active from 2005 to 2009, area 1; the municipalities surrounding Orgosolo (Oliena, Dorgali, Urzulei, Talana, Villagrande Strisaili, Fonni, Mamoiada, and Nuoro) (total surface about 1202 km^2 with an average of 54 inhabitants/ km^2), area 2; and the Nuoro and Ogliastra provinces surrounding the municipalities above reported (total surface about 4362 km^2 with an average of 35 inhabitants/ km^2), area 3 (Fig. 1).

The core of the investigated area, named Supramonte, is a mountain area northeast of the Gennargentu massif, spanning eastwards until it reaches the Tyrrenian Sea. The populated areas border the Supramonte, which, for the most part, is a largely uninhabited continuum of sharp limestone cliffs and deep, lush canyons with an average altitude of 900 m asl.

In the three investigated areas, wild mammalian species susceptible to *Trichinella* spp. infections were: wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*), marten (*Martes martes*), and weasel (*Mustela nivalis buccamela*). Among livestock, backyard pigs (about 10,500 heads reared in 1784 farms of 1–5 heads and in 647 farms of 6–10 heads) (National Database 2014, https://www.vetinfo.sanita.it) were present as well as illegally reared free-ranging pigs, which were roughly estimated to be about 15,000 heads. These pigs are not registered, are free-ranging for their whole life span, and occasionally fed by their owners during the winter and summer seasons, when there is a shortage of natural feed resources in the environment. In the slaughtering season 2010/2011, just after the last human outbreak of trichinellosis, the mayor of the Orgosolo municipality in agreement with the Regional Health Department, ordered the veterinary control for *Trichinella* spp. of all pigs slaughtered at home regardless of whether they were registered or not.

2.2. Animals, muscle sampling and diagnostic tests

From October 2010 to March 2014, muscle samples were collected from backyard pigs slaughtered for private consumption and from wild animals hunted in the course of the 2010/11, 2011/12, 2012/13, and 2013/14 hunting seasons in the three investigated areas. Furthermore, 351 illegal free-ranging pigs were tested in the winter season 2010/11 (Table 1), when pigs are slaughtered for family consumption. Domestic pigs slaughtered in approved slaughterhouses of the investigated areas, all of which tested negative for *Trichinella* spp. larvae, were not included in this study.

Muscle specimens were chosen according to the predilection sites: diaphragm pillar from domestic pigs and wild boar, and the anterior tibial muscle from red foxes and martens. If the amount of available muscle was lower than 10 g, muscle samples from the masseter and/or tongue was collected to reach at least 10 g. To detect *Trichinella* spp. larvae, pools of 10 g muscle/animal from 10 animals were digested according to the commission regulation 2075/2005 (European Commission, 2005) at the Nuoro section of the Istituto Zooprofilattico Sperimentale of Sardinia. When a pool tested positive, muscle samples from each individual animal were tested separately to identify the positive animal.

Trichinella spp. larvae were collected, stored in 90% ethyl alcohol and sent to the European Union Reference Laboratory for Parasites, Rome, Italy, for the taxon identification by multiplex PCR (Pozio and La Rosa, 2010).

2.3. Statistical analysis

The association between investigated areas and the detection of *Trichinella* spp. infection was evaluated by calculating frequency and percentages of positive animals, stratified by place of sampling, and host species. chi-square (or exact Fisher test when the expected frequency in each cell was <5) was calculated to evaluate the probability that the observed distribution was simply due to chance. A P-value < 0.05 was considered to be significant. Stata software (Stata Cooperation, College Station, TX, USA) was used for the statistical analysis.

3. Results

In the period under study (2010–2014), *Trichinella* spp. larvae were detected in the area 1 (Orgosolo municipality) with a prevalence of 2.6% in free-ranging pigs, 0.2% in backyard pigs, 0.4% in wild boar, and 27.6% in red foxes. No infection was detected in backyard pigs, wild boar, red foxes and martens of the other two investigated areas (Table 1). Specifically in the period October–March 2010–11, *Trichinella* spp. larvae were detected in 9 (2.6%) free-ranging pigs of 1–10 years of age (average 5.3 years). In the following autumn–winter period (2011/12), *Trichinella* spp. larvae were detected for the first time in red foxes (10/28, 35.7%). In the two following seasons (2012/13 and 2013/14), *Trichinella* spp. larvae were detected in both backyard pigs and wild animals (wild boar and red foxes) (Table 1). The average larval per gram (LPG) was 127 (range 0.4–543) in pigs and 79 (range 3.4–565) in foxes. In wild boar, LPG was: 50, 20.6 and 41.8 in the diaphragm, rib muscles and ham muscles, respectively, of one animal; 0.1 and 0.8 in the diaphragm and rib muscles, respectively, of a second animal; and 0.2 in the diaphragm of a third animal. All the larvae isolated from domestic and wild animals were identified as *T. britovi*. No one out of 18 martens tested for *Trichinella* spp. was positive; however, only one specimen originated from area 1, the only investigated area in which *T. britovi* was detected in both domestic and wild animals.

The difference in the *T. britovi* prevalence between domestic pigs and foxes and between wild boar and foxes, was highly significant ($P < 0.01$); whereas, the difference in the *T. britovi* prevalence between domestic pigs and wild boar was not statistically significant ($P = 0.32$). In addition, the difference in prevalence between area 1 and 2 and between area 1 and 3 was highly significant ($P < 0.01$). The temporal trend of *T. britovi* prevalence in domestic pigs was significantly increasing in the period under study; whereas, the temporal trend of prevalence was not significant in wild boar ($P = 0.82$) and in foxes ($P = 0.58$).

4. Discussion

The similar *T. britovi* prevalence between pigs and wild boar ($P = 0.32$) and the highly different prevalence ($P < 0.01$) between swine and foxes, confirm the different infectivity of *T. britovi* to swine and carnivores already documented in other surveys (Pozio et al., 2009b). Both domestic and sylvatic swine are not a good reser-