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Research paper

The role of the wolf in endemic sylvatic *Trichinella britovi* infection in the Abruzzi region of Central Italy

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ABSTRACT

During the period 2004–2014 in the Abruzzi region (Central Italy), muscle samples gathered from hunted wild boars (n = 16,323) and retrieved from carcasses of other susceptible wild mammals (n = 838) and birds (n = 438) were tested for *Trichinella* larvae according to European Union regulations. Although no positive samples were found from wild birds, 91 wild mammals tested positive. Six species were found to harbor *Trichinella* spp. infections, namely wolf (*Canis lupus*, 59 positive samples out of 218), red fox (*Vulpes vulpes*, 24/480), wild boar (*Sus scrofa*, 3/16,323), stone marten (*Martes foina*, 2/27), pine marten (*Martes martes*, 2/6) and wildcat (*Felis silvestris*, 1/8). All isolates tested for species attribution belonged to *Trichinella britovi*. The overall prevalence was 0.52% (IC 95%: 0.4–0.6). The higher frequency of positive samples in wolf, compared to red fox, was statistically significant ($p = 0.001$). In spite of the limited geographical area of investigation and the random nature of sampling, this study provides new data on the circulation of *T. britovi* in Italy. In particular, the highest prevalence being found among wolves allows us to consider this species as a sentinel for *T. britovi* infection in the investigated area, and probably also in other apennine regions, which is different from the alpine regions where the red fox was reputed as the primary reservoir of *Trichinella* spp. infection.

1. Introduction

Trichinella is a nematode parasite that occurs as a larval stage in the striated muscles of a wide range of warm blooded animals, and is transmitted by the consumption of raw or undercooked infected meat. Wildlife represent the most important reservoir for parasites belonging to the genus *Trichinella* with the sylvatic cycle of infection being mainly based on predator–prey relationships. The consumption of uncooked or undercooked meat from hunted wild animals, or from pigs reared in farming systems that allow contact between pigs, rodents and wildlife are considered the primary modes of parasite transmission to humans (EC, 2001; Pozio, 2007; Pozio et al., 2009).

Trichinellosis is still cause for concern in Italy, where during the period 2009–2013 the average incidence rate was 0.01 per 100,000 hospitalizations (Graziani et al., 2016). With the exception of some recent reports on *T. pseudospiralis* in wild boar (*Sus scrofa*) and red fox (*Vulpes vulpes*), *T. britovi* has been the only species reported in the peninsular territory of Italy. In the alpine regions *Trichinella* spp. was mainly found in red fox. In the apennine regions, it has been found in red fox, wolf (*Canis lupus*) and sporadically in wild boar populations (Remonti et al., 2005; ITRC, 2015). After World War II, when it was close to extinction, the Italian wolf population began to expand and re-colonize part of its historical territory along the apennine ridge and the western Alps, even in low-hill, plain and coastal areas. It has proven to be a carnivore with a highly adaptable and flexible food habits including as an opportunistic scavenger (Pezzo et al., 2003; Galaverni et al., 2015).

The aim of the present study is to provide new data on the circulation of *T. britovi* infections in susceptible sylvatic hosts and to discuss the potential role of the wolf in maintaining the endemic status of infection in the Abruzzi region of Central Italy.

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Table 1
Number of wild birds and mammals tested for *Trichinella* spp from 2004 to 2014 (no. of positive samples in brackets) in Abruzzi region.

Species	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Wild birds[a]	6	31	92	79	12	5	17	26	64	77	29
Wild mammals	387 (5)	229 (14)	426 (3)	1301 (5)	2648 (3)	1507 (4)	1334 (9)	1312 (9)	1515 (7)	2057 (23)	4445 (9)
Wild boar	311	173	391	1261	2629 (1)	1479 (1)	1237	1212	1419	1953	4258 (1)
Stone marten	4	2				3	6	2	5 (1)	2 (1)	3
Wildcat	3 (1)	3					1				1
Wolf	15 (3)	22 (9)	14 (2)	25 (5)	8 (2)	10 (2)	22 (7)	15 (3)	20 (5)	43 (15)	24 (6)
Pine marten		2 (2)					2	2			
Red fox	51 (1)	16 (3)	20 (1)	15	7	13 (1)	56 (2)	65 (6)	57 (1)	47 (7)	133 (2)
Other species[b]	3	11	1	0	4	2	10	16	14	12	26
Total	393 (5)	260 (14)	518 (3)	1380 (5)	2660 (3)	1512 (4)	1351 (9)	1338 (9)	1579 (7)	2134 (23)	4474 (9)

[a] Including Accipitriformes (n = 200), Falconiformes (89), Strigiformes (149).
[b] Including the crested porcupine (9), European badger (75), European polecat (7), Marsican brown bear (8).

2. Material studied, area descriptions, methods, techniques

From 2004 to 2014, muscle samples collected during official inspection of hunted wild boars and during necropsy of carcasses of other susceptible wild mammals and birds (Table 1) were systematically tested for *Trichinella* larvae.

The investigated area was Abruzzi, an apennine region of central Italy with a surface area of 10,763 square km. Protected areas, namely three national parks, a regional park, and 38 protected natural reserves, cover more than one third of its territory.

Detection of *Trichinella* larvae was performed using either the magnetic stirrer method or the Trichomatic™ method according to official methods described by the EU regulation (EC, 2005: Annex I, chapter I or chapter II, meth. C). Briefly, 5 g of diaphragm muscle from wild boars or 10 g of striated muscle from specific predilection sites of other wild mammals and birds (EC, 2001; EC, 2005: Annex III) were analyzed by enzymatic digestion of individual or pooled samples. *Trichinella* larvae were detected and counted either by microscopic examination of the membrane filter (Trichomatic™ method) or the sediment of the digestion fluid (magnetic stirrer method). Beginning in 2008, samples testing positive for *Trichinella* spp. were submitted to the European Union Reference Laboratory for Parasites (EURLP) for species attribution. A multiplex PCR targeted to most of the epidemiologically relevant taxa (*T. spiralis, T. nativa, T. britovi, T. pseudospiralis, T. murrelli, T. nelsoni, T. papuae, T. zimbabwensis and Trichinella-T6*) was performed according to the internal procedures of the reference laboratory (EURLP, 2006).

Positive animals were georeferenced using the World Geodetic System (WGS84), associated with the Universal Transverse Mercator (UTM33) cartographical representation. If available, the geographical coordinates were normalized in WGS84 or they were obtained by pointing the location where the animal had been found, using Google Maps™. When no information on animal location was available, coordinates reflecting the centroid of the appropriate municipality were utilized. All the Abruzzi municipalities were classified according to the altitudinal zonation scheme provided by the National Institute for Statistics. According to this scheme, the natural layering of ecosystems is classified into five categories (internal mountain area, coastal mountain, internal hill, coastal hill, flat land).

A chi-square test was used to evaluate the probability that differences observed among prevalences of infection was simply due to chance. A p-value < 0.05 was considered to be significant. 95% confidence intervals were calculated using the Bayesian approach of beta distribution.

3. Results

In the period under study, 17,599 muscle samples were tested from 265 of 305 municipalities. Table 1 provides data on animals tested by species and by year. No positive samples were detected in wild birds (n = 438). In contrast, 91 positive samples were detected in six wild mammal species, namely wolf (59/218 samples), red fox (24/480 samples), wild boar (3/16,323 samples), stone marten (2/27 samples), pine marten (2/6 samples) and wildcat (1/8 samples). All larvae tested for species attribution (55/91) were identified as *T. britovi*.

Table 2 provides information on prevalence, 95% confidential interval (IC 95%) and larval burden in host species which tested positive for *Trichinella* spp. (prior to 2008) or *T. britovi*. The overall prevalence was 0.52% (IC 95%: 0.4–0.6%). Prevalence during the period under study ranged from 0.1% and 1.3%, except during 2005 (5.4%) (Fig. 1). Among the species that tested positive, due to the small number analyzed (stone marten, pine marten and wildcat), no inference was possible about the level of circulation of *Trichinella* in these populations. Wild boar was the most investigated species (92.75% of tests). In this species, the frequency of positive samples was sporadic (three positives in three different years) but the average of larval burden in these animals was higher than in other species. Eighty-three (91.2%) positive samples were collected from wolves and foxes and the higher frequency observed in the wolf was determined to be statistically significant (chi square test = 69.6, $p < 0.001$).

Forty-eight municipalities had at least one positive animal, 38 of which were situated within parks. All municipalities, except three, with at least one positive case, were classified as internal mountain areas, with an altitude ranging from 250 ma.s.l to 1,322 ma.s.l (average 740 ma.s.l).

4. Discussion

Our data describes the status of *Trichinella* spp. infection in the Abruzzi region during the period of 2004–2014, specifically the circulation of *T. britovi* in wild mammals species living in territories classified as 'internal mountain area'. Data from *Trichinella* tests carried out during official *post mortem* inspection of pigs covering the same period of time, demonstrated the absence of domestic pig infections in Abruzzi (Badagliacca et al., 2015).

Despite the low number of positive animals, the wild boar should be considered the main source of human trichinellosis in Abruzzi, both due to the large consumption of wild boar meat and the high larval burdens found in positive animals. Infection in wild boar was recently detected in 2014 in the Lazio region near the Abruzzi border (two cases) and in one animal in 2015 again in the investigated area (ITRC, 2015).

Infected wolves and foxes have been detected in all their territorial ranges. The larval burden in these wild carnivores showed a large variability. The median value detected in both wolf and red fox (6–6.4 l/g) is consistent with the larval burden of *T. britovi* reported by recent surveys targeted to other canid populations,

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