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ORIGINAL PAPER

Trichinella infection in wild animals from endemic regions of Argentina

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Abstract Natural infection with *Trichinella* has been described in more than 150 mammalian species. However, few reports of *Trichinella* infection in wild animals have come from Argentina. In this study, muscle tissue was obtained from wild animals in Argentina with the aim of evaluating the presence of *Trichinella*. A total of 169 muscle samples were collected to determine the presence of *Trichinella* larvae by artificial digestion. The 169 muscle samples originated from 12 species including 36 opossums (*Didelphis albiventris*), 19 armadillos (*Chaetophractus villosus*), 9 capybaras (*Hydrocaeris hydrocaeris*), 1 puma (*Puma concolor*), 3 grey fox (*Lycalopex gymnocercus*), 6 coypus (*Myocastor coypus*), 6 skunks (*Conepatus chinga*), 2 ferrets (*Galictis cuja*), 66 rats (*Rattus norvegicus*), 6 mice (*Mus musculus*), 12 wild boars (*Sus scrofa*), and 3 wild cats (*Felis geoffroyi*). *Trichinella* infection was detected in 1 puma [2 larvae per gram (LPG)], 3 wild boars (8–420 LPG), 3 armadillos (0.04–0.08 LPG), and 9 rats (0.1 to 150 LPG). Only 3 *Trichinella* isolates, of 1 rat and 2 wild boars from Neuquén, were identified as *Trichinella spiralis* by nested PCR. The presence of *Trichinella* infection among wild animal populations suggests a sylvatic cycle of transmission in Argentina, which can serve as a reservoir for humans and domestic animals. Further, evidence of high prevalence in rats emphasizes the need to improve pig management, mainly in small individual farms without adequate technology, to enhance the quality of feeds, and to improve veterinary services to avoid exposure of pigs to *Trichinella*.

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Introduction

Trichinella spp. infection has been documented in domestic animals (mainly pigs) and/or wildlife in at least 66 countries. *T. spiralis* is the species identified in 87% of all isolates from domestic pigs, in 67% from wild boars, in 88% from domestic horses, and in 79% from synanthropic rats. In countries of the Americas, Europe, and Asia, *T spiralis* is also a parasite of wildlife maintained in nature by a sylvatic cycle (Dupouy-Camet and Murrell, 2007) The separation of cycles into the domestic or synanthropic cycle, involving primarily pigs and rodents, and the wild animal or sylvatic cycle, involving a wide variety of carnivores and omnivores, does not preclude risk of exposure of pigs to species of *Trichinella* typically found in wild animals. Thus, knowledge of *Trichinella* prevalence in wildlife is useful from an epidemiological perspective, especially in areas where pigs are raised outdoors.

In Argentina, *Trichinella* infection has been detected in domestic and wild animals (in both the synanthropic and

sylvatic cycles), including pigs, dogs, cats, rodents, wild boars, armadillos, and pumas (Tesón et al. 1997; Huici et al. 1999; Larrieu et al. 2004; Krivokapich et al. 2006; Constantino et al. 2007). In the present study, muscle tissue was obtained from wild animals in some of these same regions with the aim of evaluating the presence of *Trichinella*.

Materials and methods

Study area and samples

Samples were collected in Azul, Ascasubi, 9 de Julio, Delta (Buenos Aires) and Córdoba, Chubut, Neuquén, Rio Negro, and Santa Cruz provinces, during the 2005–2008 period.

A total of 169 muscle samples (diaphragm, masseters, tongue, intercostals, and legs) were collected from wild animals, and these samples were used to determine the presence of *Trichinella* larvae by artificial digestion. The samples originated from Argentina.

The muscle samples were from 12 wild animal species including 36 opossums (*Didelphis albiventris*) from Buenos Aires; 19 armadillos (*Chaetophractus villosus*) from Ascasubi and 9 de Julio (Buenos Aires); 9 capybaras (*Hydrocaeris hydrocaeris*) from Delta (Buenos Aires); 1 puma (*Puma concolor*) from Santa Cruz; 3 grey fox (*Lycalopex gymnocercus*) from Ascasubi and Azul (Buenos Aires); 6 coypus (*Myocastor coypus*) from Ascasubi and 9 de Julio (Buenos Aires); 6 skunks (*Conepatus chinga*) from Ascasubi and 9 de Julio (Buenos Aires); 2 ferrets (*Galictis cuja*) from Ascasubi and 9 de Julio (Buenos Aires); 66 rats (*Rattus norvegicus*) from Ascasubi, Azul, and 9 de Julio (Buenos Aires), Río Negro, and Neuquén; 6 mice (*Mus musculus*) from Azul (Buenos Aires); 12 wild boars (*Sus scrofa*) from Córdoba, Río Negro, Chubut, Neuquén, and Buenos Aires; and 3 wild cats (*Felis geoffroyi*) from Azul and 9 de Julio (Buenos Aires) (Table 1).

Artificial digestion

Muscle samples were used to determine the presence of *Trichinella* larvae by artificial digestion of 10–100 g of tissue, according to Gamble et al. (2000).

PCR analysis

Muscle larvae of 3 *Trichinella* isolates (1 rat and 2 wild boars from Neuquén) were identified at the species level by nested PCR of the ESV region of the ribosomal DNA according to Zarlenga et al. (1999) with some modifications. For DNA preparation, a single larva was placed in 5 μl of lysis buffer (10 mM Tris, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween 20, 0.45% NP-40, and 0.01% gelatin) containing proteinase K (5 μg), overlaid with a drop of mineral oil, heated for 90 min at 65°C and then 15 min at 90°C, and frozen at −20°C. In the first and second round of nested PCR reactions, the primers Ne (5′-TCTTGGTGGTAGTAGC+5′-GCGATTGAGTTGAACGC) and I (5′-GTTCCATGTGAA CAGCAGT+5′-CGAAAACATACGACAACTGC) were used, respectively. Muscle larvae from *Trichinella* reference isolates *T. spiralis* (ISS599), *Trichinella nativa* (ISS532), *Trichinella britovi* (ISS447), *Trichinella pseudospiralis* (ISS13), *Trichinella murrelli* (ISS103), and *Trichinella* T6 (ISS34) were used as controls.

Results

Trichinella larvae were recovered from one species associated with the synanthropic cycle (*R. norvegicus*) (prevalence of 15.5%) and from three species associated with the sylvatic cycle (wild boar, armadillo, and puma) with a prevalence of 8.33% to 25% (Table 1). Worm burdens varied considerably. In rats, the number of LPG recovered ranged from 0.01 to 150. LPG for wild animals were 2.0 for the puma, 8.0–420 for the three wild boars, and 0.04–0.08 for the three armadillos (Table 2).

Samples from one infected rat and two infected wild boars, coming from Junín de los Andes, Neuquén, were analyzed by PCR of the ESV region. Results showed amplification of a 173-pb fragment, consistent with the pattern for *T. spiralis* (Fig. 1).

Discussion

Risk of infection of pigs with *Trichinella* can result from exposure to infected rodents or wild animals. While infection can only occur if muscle tissue from an infected animal carcass is ingested, it is well documented that pigs raised outdoors will eat animal carcasses and pigs have even been observed to catch and eat rats (Murrell et al. 1984). For that reason, knowledge of the prevalence of *Trichinella* infection in the synanthropic and sylvatic cycles is important in areas where pigs are raised outdoors, including certain areas of Argentina.

Several previous studies of *Trichinella* in rodents from Cordoba, Santa Fe, and Buenos Aires provinces of Argentina (*Mus domesticus*, *Calomys musculinus*, *Akodon dolores*, *Rattus rattus*, *Akodon azarae*, *R. norvegicus*, and *Calomys venustus*) produced negative results (Vazquez, 2005; Sequeira 2001; Gómez Villafañe et al., 2004). In contrast, our study found an infection rate of 15.5% in *R. norvegicus.* In another study in Río Negro Province, Argentina, 4 (15.4%) of 26 local rodents were found positive (Larrieu et al. 2004), similarly to the prevalence found in our study.

Table 1 Prevalence of *Trichinella* infection by animal species studied

Animal species	Number tested	Number positive	Prevalence (%)	Location
Puma	1	1	100	Santa Cruz
Opossums	36	–	0	Buenos Aires
Armadillo	19	3	15.7	Buenos Aires
Capybara	9	–	0	Buenos Aires
Grey fox	3	–	0	Buenos Aires
Coypus	6	–	0	Buenos Aires
Skunks	6	–	0	Buenos Aires
Ferrets	2	–	0	Buenos Aires
R. norvegicus	66	10	15.5	Buenos Aires, Río Negro, and Neuquén
M. musculus	6	–	0	Buenos Aires
Wild boars	12	3	25	Córdoba, Río Negro, Chubut, Neuquén, and Buenos Aires
Wild cats	3	–	0	Buenos Aires
Total	169	17	10.5	

Some studies have reported rats would be vectors for *Trichinella* infection in pigs, while other studies have found rats to serve as bystander populations, which are only infected once infection is established in a pig population. In research carried out in Croatia on 49 pig farms with poor sanitation, *Trichinella* was found in 31.8% of pigs and 2% of rats, while in farms with good sanitation, *Trichinella* was not found in either pigs or rats (Stojcevic et al. 2004). The role of the brown rat as vector of *T. spiralis* was studied by Smith et al. (1976) in swine herds from the Atlantic provinces of Canada. In these herds, a rat control program forced rats to migrate from *Trichinella*-positive herds to *Trichinella*-negative herds. A few months later, pigs of the *Trichinella*-negative herds became positive. A close association of rats with pigs is therefore a risk for exposure of pigs to *Trichinella* infection. The relatively high prevalence of *Trichinella* infection in rats in our study raises a concern regarding risk to pigs raised in these areas of Argentina.

Table 2 Worm burdens recovered from *Trichinella*-positive animals

Species	LPG of muscle
Puma	2
Wild boar	420
Wild boar	8
Wild boar	26
Armadillo	0.04
Armadillo	0.05
Armadillo	0.08
R. norvegicus	40
R. norvegicus	0.5
R. norvegicus	53
R. norvegicus	36.4
R. norvegicus	54.8
R. norvegicus	7.1
R. norvegicus	0.1
R. norvegicus	0.5
R. norvegicus	3
R. norvegicus	150

Wild animals are also a risk for introduction of infection into a domestic pig population. In research carried out in the USA on pigs from 91 farms, 58.3% of pigs had access to wild animals. On these farms, pigs were six times more likely to be infected with *Trichinella* than those with no access to wild animals (Gamble et al. 1999). In a similar study from Argentina, 52.4% of pigs from 11 establishments had access to wildlife and/or pig carcasses; this risk factor had a statistically significant association with pig infection ($p \leq 0.01$) (Ribicich et al. 2009).

Previous studies have reported *Trichinella* infection in armadillos (*C. villosus*) from Argentina (Tesón et al. 1997; Huici et al. 1999). In another study, *Trichinella* isolates were identified as *T. spiralis* by nested PCR (Krivokapich et al. 2006). In our study, the prevalence in armadillos was 15.7%. Because of its scavenging behavior, this animal could act as a reservoir of *Trichinella* (Pozio 2005).

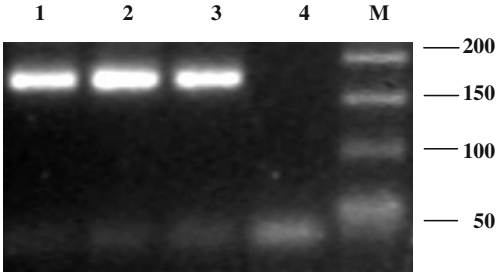

Fig. 1 Nested PCR result obtained from the three *Trichinella* isolates analyzed in Neuquén. *Lane 1*, rat isolate; *lanes 2 and 3*, wild boar isolates; *lane M*, 50 bp Step Ladder (Sigma). All *Trichinella* isolates generated a fragment of 173 bp, specific for *T. spiralis*

However, only very low worm burdens (0.04–0.08 LPG) were found in these animals, making them a poor source of infection for other animals.

A high prevalence rate (25%) was also found for wild boars tested in this study and work burdens in positive wild boars were quite high (8–420 LPG). Another study reported similar infection rates and recovery of large numbers of larvae (22–46 LPG) from wild boar in Argentina (Tesón et al. 1997). Based on this information, wild boar may serve as a significant reservoir of infection and may pose a risk for exposure of domestic pigs raised outdoors.

The occurrence of *Trichinella* infection in wild boar poses a substantial risk to hunters who may not be aware of the necessity to adequate cook boar meat to prevent infection. In our study, the wild boar that harbored 420 LPG would have been consumed by the hunter, but intervention of the health authorities based on the results of testing by artificial direction prevented an outbreak in humans. These results demonstrate the importance of testing wild animals for *Trichinella* infection at the local level because of the use of this meat in the preparation of homemade sausages and other recipes using wild boar meat in southern Argentina.

Larvae from the two wild boars collected in Junin de los Andes were analyzed by PCR of the ESV region and amplified a fragment of 173 bp, which was consistent with the pattern of *T. spiralis*. This result was not unexpected. There are currently 32 isolates of *Trichinella* from Argentina deposited in the International *Trichinella* Reference Centre (http://www.iss.it/site/trichinella/index.asp?lang=2). Of these, 29 are identified as *T. spiralis*, including two isolates from wild boars in Colón, Province of Entre Ríos. Further studies of wildlife should be conducted to determine if other species of *Trichinella* circulate in Argentina.

The presence of *Trichinella* infection among wild animal populations in Argentina poses a risk to humans and domestic animals. Evidence of a high prevalence in rats emphasizes the need of improving pig management practices on small farms that are currently lacking adequate technology, quality feeds, and veterinary services.

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