

Original Article

Trichinellosis surveillance in wildlife in northeastern Argentine Patagonia

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ARTICLE INFO

Keywords:
Trichinellosis
Trichinella
Zoonosis
Wildlife
Patagonia
Argentina

ABSTRACT

Trichinellosis is a food-borne parasitic disease produced by different nematodes of the genus *Trichinella*. In Argentina, it is an endemic zoonosis and an important public health problem. The infection has been detected in domestic and wild animals. *Trichinella* spp. muscle larvae have anaerobic metabolism, which allows their survival in decaying tissues. The aim of this study was to evaluate the presence of *Trichinella* spp. in carnivorous and/or scavenger wild vertebrates - birds, mammals and reptiles - in northeastern Argentine Patagonia. Skeletal muscle samples from 141 animals, which were found killed on northeastern Argentine Patagonia roads, were analyzed by the artificial digestion method. None of the 141 samples were positive for larvae of *Trichinella*. These results suggest that *Trichinella* does not use these species to complete its cycle in this region of the continent and the absence of a significant alteration in the study area makes it difficult to transmit parasitic diseases. However, due to the limited number of samples assessed for some species, this could not be confirmed. The relevance of this study resides in the fact that it is the first systematic study in South America that considers birds, reptiles and mammals as potential hosts for *Trichinella*.

1. Introduction

Trichinellosis is a food-borne parasitic disease produced by different nematodes of the genus *Trichinella*. It currently behaves as a re-emergent zoonosis with wide global distribution. This parasite has been reported in domestic and wild animals on all continents, except Antarctica (Dupouy-Camet and Murrell, 2007). Trichinellosis worldwide distribution is associated with the ability of *Trichinella* spp. to affect a large number of hosts that exceeds 100 species among mammals, birds and reptiles (Pasi et al., 2016). Until now, 12 species and genotypes of the genus *Trichinella* are recognized in the world grouped in two clades, the encapsulated clade, which included *T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, and *T. patagoniensis*; and three genotypes (*Trichinella* T6, T8 and T9), and the non-encapsulated clade, which included *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis* (Pozio and Murrell, 2006; Zarlenga et al., 2006; Krivokapich et al., 2012). Encapsulated species have been found naturally only in mammalian

muscle, whereas non-encapsulated species have been found naturally in mammals, birds, and reptiles (Pozio and Zarlenga, 2013), shown a high host range. In South America, trichinellosis is an endemic disease in countries such as Argentina and Chile (Ribicich et al., 2005). In Argentina, this parasitic disease is an important public health problem because of its high morbidity rates (Pasqualetti et al., 2014). Although almost human outbreaks are related to the consumption of domestic pig (Ribicich et al., 2005), in recent years, human cases of trichinellosis have been reported by consumption of cougar *Puma concolor* or wild boar *Sus scrofa* meat (Ribicich et al., 2010b; Pasqualetti et al., 2014). Several studies have detected *Trichinella* spp. in wild animals such as rodents species, wild boar *Sus scrofa*, big hairy armadillo *Chaetophractus villosus*, cougar *Puma concolor* (Tesón et al., 1997; García et al., 2005; Krivokapich et al., 2006; Ribicich et al., 2010a) white-eared opossum *Didelphis albiventris* and little water opossum *Lutreolina crassicaudata* (Castaño Zubietta et al., 2014). However, so far very little is known about the wild Cycle of *Trichinella* genus in Argentina.

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In addition, *Trichinella* spp. muscle larvae have an anaerobic metabolism in the tissue of their host (Despommier, 1990) that allow their survival in decaying tissues and serving as a source of infection in scavenger species. Encapsulated species show more persistence in decaying tissue (Stewart et al., 1990; Owen and Reid, 2007). *Trichinella patagoniensis*, which is the last encapsulated species described, is endemic to South America, and has been found only in cougars (Krivokapich et al., 2012). Also, it has been demonstrated *in vitro* that larvae of this new species are able to survive up to three weeks in decaying tissue and maintain their infective capacity for two weeks in decaying tissue (Fariña et al., 2016). The persistence of *Trichinella* spp. in decaying tissue also changes according to different environmental conditions: low temperature and high humidity favor survival of *Trichinella* larvae even when the muscle tissue is completely liquefied (Pozio, 2016). Recently, it has been determined that species with scavenger habits have a more important epidemiological role than predatory species in the *Trichinella* Wild Cycle (Pozio, 2016). Also, it is recognized that wild carnivores and omnivorous sustain the *Trichinella* spp. biomass (Pozio and Zarlunga, 2013), and some wild mammals constitute the best reservoir for this parasite (Campbell, 1988). Therefore, it is important to carry out surveillance studies to determinate the presence and circulation of *Trichinella* spp. in wildlife and know the spatial distribution of *Trichinella* spp. in the northeastern Patagonia. Also, it is important to investigate if *T. patagoniensis* may infect other sympatric hosts with *Puma concolor* in this region. The aim of this study was to evaluate the presence of *Trichinella* spp. in carnivorous and/or scavenger wild vertebrates - birds, mammals and reptiles - in the northeastern Argentine Patagonia.

2. Material and methods

2.1. Study area

The study was carried out in the northeastern Argentine Patagonia that includes the south of Buenos Aires province and the northeastern of Rio Negro province. This is a transitional area between Espinal and the Monte regions (Roig et al., 2009). The extensive ecotone is characterized by the transition from xerophilous forest to xerophila shrub (Fosberg, 1961). The vegetation is distributed as shrub-graminous islands dispersed in a soil matrix with little or no vegetation cover (Aguiar and Sala, 1998). The herbaceous stratum is formed by winter growth grassland. Most of the area are private fields, where the native vegetation alternates with semi-extensive livestock (bovine, ovine and porcine) and farming production. The weather is sub-temperate dry transition, with warm summers and moderate winters, and without any seasonal excess of water. The seasons with the highest precipitations are autumn and spring, although in winter it can rain occasionally. Windy, especially in spring and summer (Bran et al., 2000). Average annual temperatures range from 10° to 14 °C (Rey et al., 1988).

Patagonian wildlife shows a lower number of species than other regions of the country, and even than other ecologically similar regions of the world. However, there are very characteristic groups of great ecological significance and more abundance of native and endemic species than other parts of the world like *Lycalopex griseus*, *Dolichotis patagonum*, *Leopardus geoffroyi*, *Leopardus colocolo*, *Galictis cuja* and *Lyncodon patagonicus*. (Bonino, 2003). Wildlife animals coexist with livestock in peri-urban areas. The presence of cougars and wildboars are recognized and they are frequently hunting prey destined for consumption. Also, pig farms with risk factor associated with *Trichinella* infection are frequent in this area.

2.2. Muscle samples and artificial digestion

Samples of skeletal muscle from carnivorous and/or scavenger wild vertebrates - birds, mammals and reptiles - found as road-kill on national and provincial routes and rural roads of northeastern Argentine

Patagonia were taken between April of 2015 and February of 2017. Dead animals were found randomly by the authors. Muscle samples of mammals were collected from tongue, anterior and/or posterior limbs and intercostal muscles. For birds were collected from limbs, tongue and pectoral muscles, and in reptiles were collected from axial musculature. Samples were kept at 4 °C until the laboratory and were processed by artificial digestion method (Gamble et al., 2000). Digestion assays are the only reliable procedures for the direct detection of *Trichinella* larvae in meat (Gamble et al., 2000; OIE, 2008).

Identification of the mammals, birds and reptiles was performed according to Chebez et al., 2014; Ferguson-Lees and Christie, 2001; König and Weick, 2008; Hoyo et al., 1992, 1996, 1997 and Scrocchi et al., 2010.

The collection protocol was approved by Secretary of Environment and Sustainable Development of Rio Negro province (085026-SAYDS-019/2015).

3. Results

A total of 141 wild vertebrate samples were analyzed: 94 mammals, 37 birds and 9 reptiles that covered an area of 105 km² in northeastern Argentine Patagonia. All the species analyzed are endemics, and none of them is at risk of conservation. All the samples were *Trichinella* spp. negative. Table 1 shows the species, number of individuals by species and their eating habits. Fig. 1 shows the distribution of sampling points.

4. Discussion

In Argentina, the most important source of human trichinellosis infection is the domestic pig (Ribicich et al., 2005). In the study area, more than 50% of pig production is slaughtered and traded informally

Table 1
Species and number of each specie analyzed and description of their eating habits. C = carnivorous; O = omnivorous; H = hunter; S = scavenger.

Scientific name	Common name	n	Eating habits	
Mammals n total = 94			C/O	H/S
Didelphimorphia <i>Didelphis albiventris</i>	white-eared opossum	15	O	S
Cingulata <i>Chaetophractus vellerosus</i>	big hairy armadillo	19	O	S
Carnivora <i>Leopardus geoffroyi</i>	geoffroy's cat	10	C	H
<i>Galictis cuja</i>	lesser grison	13	C	H
<i>Conepatus chinga</i>	molina's Hog-nosed Skunk	1	O	S
<i>Lycalopex gymnocercus</i>	south american grey fox	35	O	S
Birds n total = 37			C/O	H/S
Procellariiformes <i>Macronectes giganteus</i>	southern giant petrel	1	C	H
Falconiformes <i>Cathartes aura</i>	turkey vulture	1	C	S
<i>Buteo polysoma</i>	red backed hawk	2	C	H
<i>Polyborus plancus</i>	southern crested caracara	1	C	S
<i>Milvago chimango</i>	chimango caracara	2	O	S
<i>Falco sparverius</i>	american kestrel	1	C	H
Charadriiformes <i>Larus dominicanus</i>	kelp gull	4	O	S
<i>Sterna hirudinacea</i>	south american tern	1	C	H
Cuculiformes <i>Guira guira</i>	guira cuckoo	2	O	S
Strigiformes <i>Tyto alba</i>	barn owl	9	C	H
<i>Athene cunicularia</i>	burrowing owl	9	C	H
<i>Asio flammeus</i>	short eared owl	2	C	H
<i>Bubo virginianus</i>	great horned owl	2	C	H
Reptiles n total = 9			C/O	H/S
Squamata <i>Philodryas patagoniensis</i>	parejera culebra	9	C	H

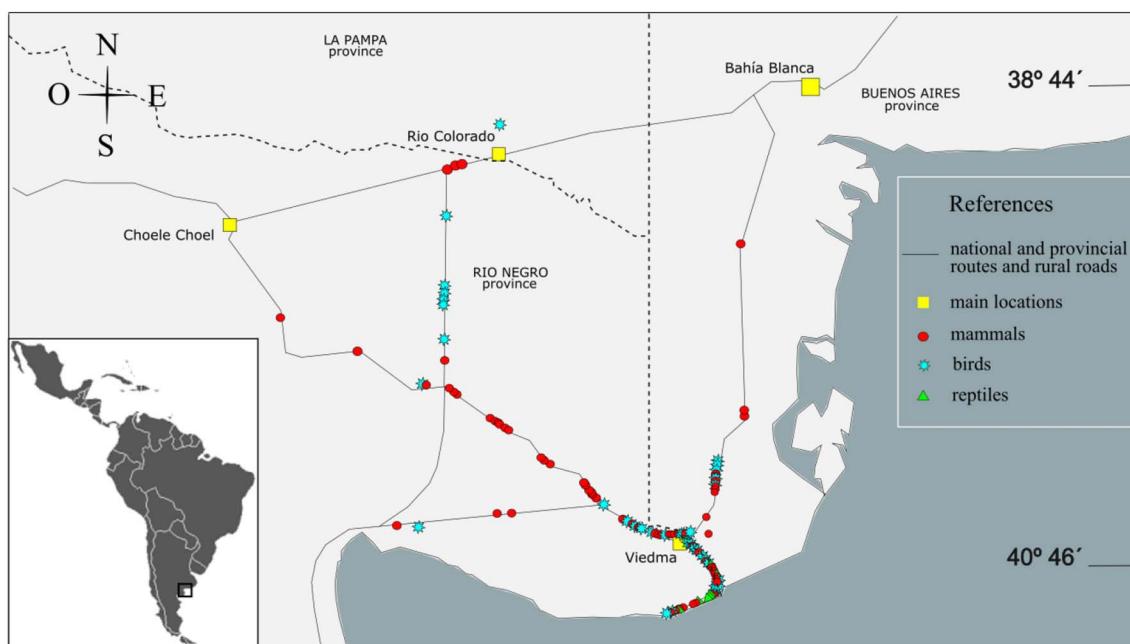


Fig. 1. Location of the study area and distribution of sampling points in northeastern Argentine Patagonia.

and most of the rural population of the region practice traditionally inherited pig production methods in the absence of adequate sanitary controls (Mohor, 2015). In this context, wild animals in search of food could participate in the life Cycle of *Trichinella* spp. Experimental studies in domestic cats have demonstrated that this species is susceptible and supportive hosts for *Trichinella patagoniensis* (Ribicich et al., 2013), this was evidenced by the high reproductive capacity of the organism compared with that observed in rats and pigs where *Trichinella patagoniensis* showed poor or null reproductive capacity (Gonzalez Prous et al., 2011). The negative results observed in the totality of the samples could indicate that the parasite does not use these species to complete its cycle in this region of Argentina. However, due to the limited number of samples assessed for some species this can not be confirmed. These results agree with the absence of evidence of circulation of *Trichinella* spp. in hunting wild species, as in the case of wild boar (Winter et al., 2015; Winter et al., 2016).

Numerous studies argue that the presence of pathogens in wildlife is associated with the degree of habitat fragmentation and degradation. Wildlife of degraded areas are at increased risk of infection. In addition, the sudden elimination of a habitat may cause the migration of animals from one area to another, along with its associated pathogens. So, host-pathogen dynamics between wild species, between wild species and domestic species, and between wild species and man, may be seriously affected as a result of the alteration, fragmentation or elimination of natural habitats (Weiss, 2001; Gillespie and Chapman, 2006; Langlois et al., 2001; Ostfeld and Keesing, 2000). Our results allow us to consider that the study area maintains as a conserved natural area and the absence of a significant alteration makes it difficult to transmit parasitic diseases between domestic and wild species.

The use of road-killed animals may allow to obtain information about the role of wild animals in the transmission Cycle of *Trichinella* and other infections and parasitic diseases and, hence, contribute to anticipate outbreaks in livestock and human population.

This is the first systematic study in South American to assess the presence of *Trichinella* spp. in wild birds, reptiles and mammals and more investigations are necessary in areas no explored of the continent to establish the role of animals species involved with new *Trichinella* species.

Funding

This study was supported by FONCyT ANPCyT PICT- 2013-0965 and PICT2015-2350.

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