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Toxoplasma gondii and *Trichinella* infections in wild boars (*Sus scrofa*) from Northeastern Patagonia, Argentina

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ABSTRACT

Wild boar (*Sus scrofa*) was introduced in many countries of the world and is recognized as carrier of many infectious diseases. Wild game meat consumption is recognized as a source of transmission of *Toxoplasma gondii* and *Trichinella* spp. The aim of the present study was to evaluate the prevalence of antibodies to *T. gondii* and *Trichinella* spp. in free-range wild boars in Northeastern Argentine Patagonia. Between 2014 and 2018, 144 blood samples and 423 muscle samples from 423 carcasses were collected. To detect *T. gondii* IgG, 144 sera were processed by an immunofluorescent antibody test, and to detect anti-*Trichinella* IgG, 125 sera and 304 muscle juice samples were processed by ELISA. Detection of first stage larvae in muscle was performed by artificial digestion. A total of 423 wild boars muscle samples were negative to *Trichinella* spp. by artificial digestion. Antibodies to *Trichinella* spp. were detected in 2.4% (3/125) of serum samples and in 1.64% (5/304) of meat juice samples. Antibodies to *T. gondii* infection were detected in 12.5% (18/144) of the serum samples. This is the first study to reveal the presence of antibodies to *T. gondii* in wild boars from Argentina. The present results suggest that consumption of raw or undercooked wild boar meat could represent a potential source risk for toxoplasmosis in humans and that *Trichinella* spp. is infrequent and/or that it circulates in low burdens among wild boars in Northeastern Patagonia.

1. Introduction

Wild boar (*Sus scrofa*) is an ungulate species that was introduced in many countries of the world. In Argentina, wild boar was introduced during Spanish colonization (Crosby, 1986). Also, around 1906, pure Eurasian wild boars were deliberately introduced for recreational hunting (Daciuk, 1978; Navas, 1987). Wild boar has a high adaptability to different environments and a wide tolerance to different climatic conditions. As it has happened in other countries, wild boars populations continue to increase and spread into new areas across Argentina (Merino and Carpinetti, 2003). Greater numbers of wild boars represent greater possibilities of interaction with livestock and humans (Massei et al., 2011; Castillo-Contreras et al., 2018). Hence, wild boar is recognized worldwide as a carrier of many pathogens, such as *Toxoplasma gondii* and *Trichinella* spp. (Ruiz-Fons et al., 2008; Meng et al., 2009; Wacheck et al., 2010), and recreational hunting and wild game meat

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consumption have been highlighted as an emerging risk factor of transmission of foodborne parasites (Kijlstra and Jongert, 2008; Kapel and Fredensborg, 2015).

Toxoplasmosis and trichinellosis are parasitic zoonotic diseases with worldwide distribution. *Toxoplasma gondii* and *Trichinella spiralis* are in the "top ten" foodborne parasites by the FAO-WHO (FAO, 2014). Toxoplasmosis is considered a food-borne, soil-borne and water-borne disease. (Dubey, 2010; Hill and Dubey, 2015). *T. gondii* is an intracellular protozoon parasite with a facultative heteroxenous life cycle that infects domestic animals, wildlife and humans. Wild and domestic felids are the definitive hosts, while many mammals and avian species are intermediate hosts. Among intermediate hosts, wild boars are frequently exposed to *T. gondii* through ingestion of sporulated oocysts from the environment or by ingesting tissue cysts in prey or through scavenging, and result commonly infected (Bengis and Kock, 2002; Rostami et al., 2017). Some studies showed that the detection of *T. gondii* antibodies in wild boars is positively correlated with the presence of bradyzoites in their muscles (Bartová et al., 2006; Richomme et al., 2010). Currently there is no data on prevalence of *T. gondii* in wild boars of Argentina.

Trichinella spp. larvae have been reported in domestic and wild animals in all continents, except Antarctica (Dupouy-Camet et al., 2007; Pozio and Zarlenga, 2013). Wild and domestic animals become infected by feeding on *Trichinella* infected muscles from infected prey or through scavenging (Dupouy-Camet et al., 2007; Gajadhar et al., 2009; Pozio, 2013; Fariña et al., 2017). Up to now, 12 species and genotypes of the genus *Trichinella* are recognized in the world, and are grouped in two clades. The encapsulated clade, include *T. spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. patagoniensis*; and three genotypes (*Trichinella* T6, T8 and T9), whereas the non-encapsulated clade, include *T. pseudospiralis, T. papuae* and *T. zimbabwensis* (Pozio and Murrell, 2006; Zarlenga et al., 2006; Krivokapich et al., 2012; Korhonen et al., 2016). *Trichinella* spp. muscle larvae have an anaerobic metabolism (Despommier, 1990) that allow their survival in decaying tissues and maintain their infective capacity (Fariña et al., 2016). Unlike other nematode infections that involve both wild and domestic animals, the biomass of *Trichinella* spp. is greater in wild than in domestic animals (Pozio, 2014). Wild boar is an important source of human trichinellosis worldwide (Rhee et al., 2011; Fichi et al., 2015; Holzbauer et al.,2014; Romano et al., 2011; Faber et al., 2015). In Argentina, trichinellosis is an endemic food-borne disease and an important Public Health problem (Pasqualetti et al., 2014). The aim of the present study was to evaluate the prevalence of antibodies to *T. gondii* and *Trichinella* spp. infection in free-range wild boars in Northeastern Argentine Patagonia.

2. Materials and methods

2.1. Study area

The study was carried out in Northeastern Argentine Patagonia, which includes the South of the province of Buenos Aires, and Northeast of the province of Rio Negro. This is a transitional area between *Espinal (thorn shrub)* and *Monte (arid forest)* 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 is private owned farmland, where the native vegetation alternates with semi-extensive livestock (cattle, sheep and swine) and farming.

The weather is sub-temperate dry transition, with warm summers and moderate winters, and no seasonal excess of water. The rainy seasons are autumn and spring, however it can occasionally rain in winter. It is windy, especially in spring and summer (Bran et al., 2000). Average annual temperatures range from 10° to 14 °C (Rey et al., 1988).

Historically, more than 50% of the swine production in the study area and in Northern Patagonia is informal and without bromatological control. However, production and consumption are increasing (Boletín del Instituto Nacional de Tecnología Agropecuaria, 2019).

In addition, four species of wild felids have been reported in extra-Andean Patagonia: *Puma concolor, Leopardus geoffroyi, Leopardus colocolo* and *Herpailurus yaguarondi* (Chebez et al., 2014), of which *P. concolor* has shown findings of *Trichinella* spp. (Ribicich et al., 2010a; Krivokapich et al., 2012).

As in other regions from Argentina and the world, wild boar has a high value as game animal species. They have been traditionally hunted in drive hunts by hunting teams with several dogs.

2.2. Sample collection

Between July 2014 and April 2018, 144 blood samples and 423 muscle samples were collected from 423 wild boars hunted during hunting seasons and in hunting tournaments (cross-sectional observational study with convenience sampling). Registered hunters are allowed to hunt between March and December in the province of Buenos Aires (Law 5786, decree 2578-1403/05, resolution 25) and between March and August in the province of Río Negro (Law 2056, decree 633/86, resolution 236). In most cases, hunters reported on the hunting location and the age of the animal. Wild boars were classified according to sex and to age groups as: piglets ($\leq$6 months of age), yearlings (6–12 months of age) and subadults or adults ($>$ 12 months of age), according to body size and estimated weight (Zelman et al., 2018).

2.2.1. T. gondii: antibody detection

Serum samples were kept at −20 °C until analyzes. Sera from 144 (34.0%) wild boars were analyzed by indirect immunofluorescent antibody test (IFAT). This test was already used with pig samples from Argentina and was available in our laboratories (Pardini et al., 2012). According to Basso et al. (2013), IFAT to detect IgG to *T. gondii* in swine samples has a sensitivity and specificity of 87.3 (80.9; 92.8) and 87.0 (81.4; 91.8), respectively. Slides with cell cultured *T. gondii* tachyzoites of the RH strain were used as antigen. Sera were tested at 1:50 and 1:200 in phosphate buffer solution (PBS) dilutions, antipig IgG fluorescein isothiocyanate was used as conjugate (Sigma, USA). A titre of 50 was considered positive (Pardini et al., 2012). Positive and negative naturally infected pig sera were used as controls (Pardini et al., 2012; Basso et al., 2013).

2.2.2. Trichinella spp.: parasite detection

Muscle samples were collected and kept at 4 °C until artificial digestion. Ten grams of diaphragm pillars free of fat and fascia were tested by artificial digestion (AD) according to Gamble et al. (2000). In some cases, tongue and masseter muscles were added to diaphragm muscle to reach 10 g.

2.2.3. Trichinella spp.: antibody detection

Serum and meat juice samples were kept at −20 °C until analyzes. To detect anti-*Trichinella* IgG, 125 serum samples were examined with an indirect multi-species commercial E/S IDScreen® IDVet ELISA (19 serum samples were discarded due to poor quality/quantity). Meat juice was extracted from 304 wild boars muscle samples. Meat juice was obtained by allowing the samples to thaw at 4 °C for 24 h and were tested by E/S PrioCHECK® ELISA. According to the manufacturers, both ELISA tests have high sensitivity and specificity, up to 100% (Buholzer et al., 2008). A total of 95 wild boars samples were analyzed by AD and both ELISA. Serum and meat juice samples were processed at 1:50 and 1:5 dilutions, respectively, proceeding according to manufacturer's instructions and using control sera supplied in the commercial kit.

2.3. Statistical analysis

The statistical analyzes were performed using the InfoStat®

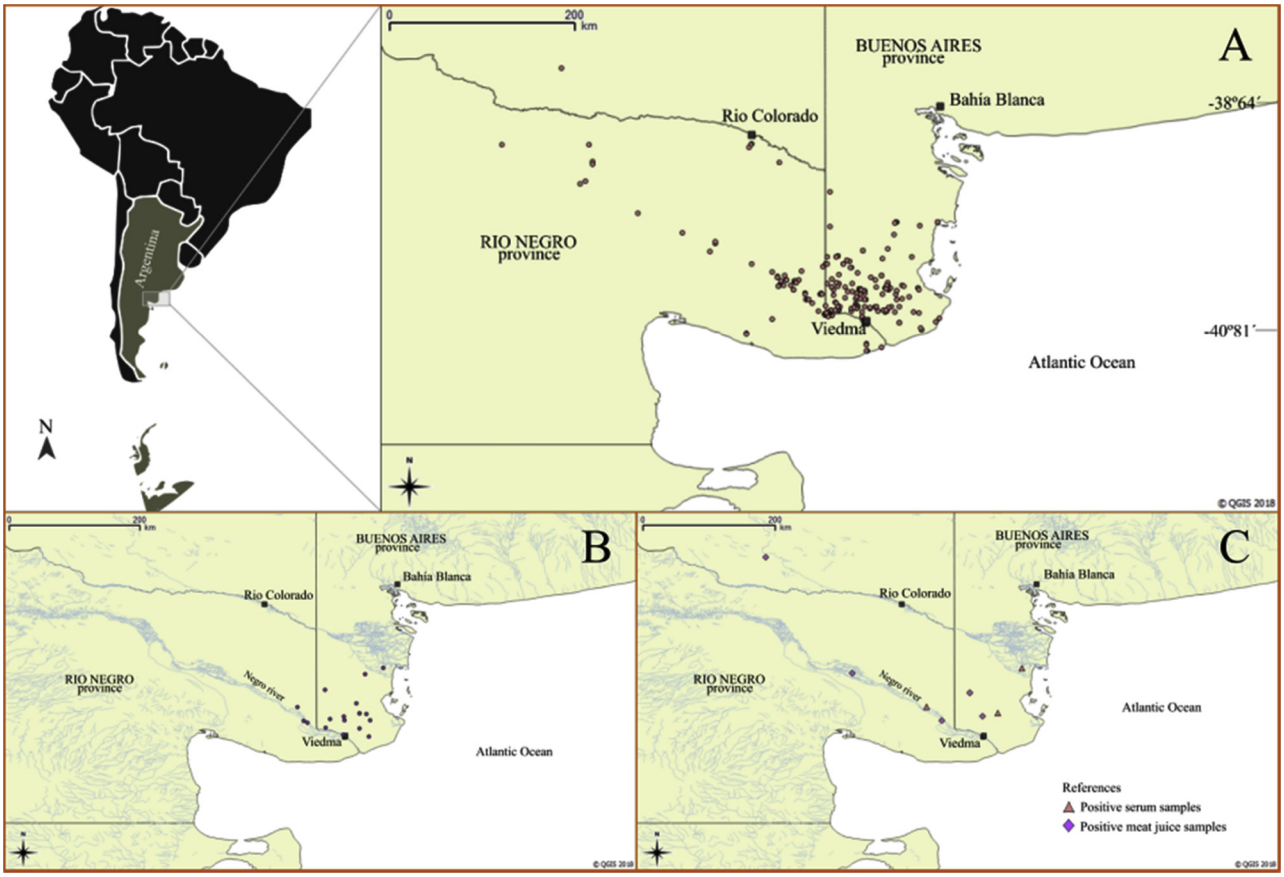

Fig. 1. Study area and distribution of total wild boars hunting locality from the northeastern Patagonia, Argentina (A); hunting locality of wild boars positive to antibodies to *T. gondii* (B); hunting locality of wild boars positive to antibodies to *Trichinella* spp. (C).

statistical software (Version 2018p). Significant differences between AD and ELISA results, sex and age group was determined by Fisher's test for proportions with a confidence of 95%.

3. Results

In five years, 423 wild boars carcasses were examined (2014 n = 26, 2015 n = 108, 2016 n = 141, 2017 n = 140, 2018 n = 8). From the total, 221 were males and 163 females (of 39, data of wild boars were not available), and 20 were classified as piglets, 57 yearlings and 225 subadults or adults (for 121 animals age was not determined). Fig. 1(A) shows the distribution of the hunting locations. Antibodies to *T. gondii* were detected in 18 (12.5%; 95% CI 6.56–18.44) serum samples (Fig. 1B). Positive results were obtained at serum dilutions of 1:50 in 11 (61.1%) and 1:200 in 7 (38.9%) wild boars samples. All muscle samples were *Trichinella* spp. negative by AD. Antibodies to *Trichinella* spp. were detected in 3 (2.4%; 95% CI 0.42–4.38) serum samples and in 5 (1.64%; 95% CI 0–3.24) meat juice samples (Fig. 1C). Distribution of positive samples to *T. gondii* and *Trichinella* spp. by sex is shown in Table 1. It was not possible to obtain muscle juice samples from the three *Trichinella* spp. positive wild boars sera. The availability of samples did not allow a correlation analysis of *Trichinella* spp. results between serological tests in serum and muscle juice results.

The statistical analysis showed that there are significant differences between AD and ELISA test ($p \leq 0.05\%$; ELISA sera vs. AD p value = 0.01; ELISA meat juice vs. AD p value = 0.01). The difference between males and females for each pathogen and test was not statistically significant ($p \leq 0.05$; ELISA sera p value = 0.16; ELISA meat juice p value = 0.24; IFAT p value = 0.1), but there was significant difference when considering the total positive males and females ($p \leq 0.05$; p value = 0.000029). Age had no significant effect on antibody seroprevalence to *Trichinella* and *T. gondii*. All the comparisons produced a p value = 1 ($p \leq 0.05$).

4. Discussion

Sus scrofa is one of the hundred worst invasive species (Lowe et al., 2000) and one of the most relevant wildlife species related to human and livestock health problems (Meng et al., 2009). Moreover it is the most important and widely distributed game animal species in the world (Massei et al., 2015).

The seroprevalence obtained to *T. gondii* antibodies in the present study is in agreement with the values reported by other authors in South America (Fornazari et al., 2009; Santos et al., 2016; Rostami et al., 2017). The difference among studies could be due to the use of different serological tests and cutoffs. The modified agglutination test (MAT) would have been the choice test but it was not commercially available in Argentina and the in-house method requires further development and validation. Nevertheless, the IFAT performed has shown a good sensitivity and specificity in swine samples (Basso et al., 2013). IFAT can be performed using a close related specie conjugate because antibodies cross-react to the IgG Fc fragment and it allows to detect, like in this case, specific *Toxoplasma* antibodies in wild boars using an anti-Pig IgG conjugate. Most *T. gondii* mammal infections are asymptomatic and chronic, where animals have tissue cysts and showed specific antibodies (Dubey et al., 2010). Therefore, seropositive animals could be considered potentially chronically infected. Because of their behavioral characteristics, wild boar is an important constituent of wildlife–livestock interface. It is unclear if *T. gondii* infection in wild boars primarily occurs by ingestion of oocysts from the environment or by ingestion of tissue cysts from prey meat or through scavenging. Nevertheless, free-living wild boars could be a good indicator for

Table 1
Distribution of the analyzed wild boar samples according to sex and positivity for *Trichinella* spp. and *Toxoplasma gondii*.

	Parasite detection			*Antibody detection*								
	AD *Trichinella* spp.			ELISA *Trichinella* spp.						IFAT *T. gondii*		
SAMPLE	MUSCLE			MUSCLE JUICE			SERUM			SERUM		
Sex	males	females	nd	males	females	nd	males	females	nd	males	females	nd
Nº tested	237	158	28	171	113	20	60	55	10	72	61	11
Nº positive	0	0	0	5	0	0	3	0	0	12	4	2
%	–	–	–	2.92[a]	–	–	5[b]	–		16.67[c]	6.56[d]	18.18[e]

*nd = no data. 95%.
[a] CI.0.94–4.9.
[b] CI.0–10.01.
[c] CI.9.99–23.3.
[d] CI.1.55–11.56.
[e] CI.0.47–35.88.

monitoring environmental contamination with *T. gondii* oocysts in a given region (Bartová et al., 2006; Dubey et al., 2010). The prevalence of *T. gondii* in livestock in Northeastern Patagonia is unknown. The results obtained in wild boars could be indicating that *T. gondii* is also circulating in domestic animals from the study area. Therefore, this information is of high relevance because meat from infected animals is considered the most important source of *T. gondii* human infections (Hill and Dubey, 2015). In the province of Rio Negro, the prevalence of seropositive pregnant women to *T. gondii* in the last five years was between 8.28% (2013) and 2.95% (2016) (*Ministerio de Salud de la Provincia de Río Negro, Departamento Provincial de Estadística*). Thus, these results suggest that wild boar meat or meat products consumed raw or undercooked are a potential risk of transmission of toxoplasmosis to humans in the study area. Additionally, the existence of four species of felids has been reported in the studied area, which could be related with the maintenance of *T. gondii* in the region, shedding oocyst which contaminate food or water (Dubey, 2010; Marcogliese, 2005).

In South America, trichinellosis is an endemic disease in Argentina and Chile (Ribicich et al., 2005). In Argentina, it is an important Public Health problem because of its high morbidity rates (Pasqualetti et al., 2014). In 2017, 91 cases of trichinellosis were confirmed (Boletín Integrado de Vigilancia, 2017), although it is recognized that there is a significant under-registration of the disease. Although most human outbreaks are related to the consumption of pork (Ribicich et al., 2005), in recent years the reported human cases of trichinellosis have been associated to the consumption of cougar (*Puma concolor*) or wild boar meat (Ribicich et al., 2010b; Pasqualetti et al., 2014). The global seroprevalence of host-*Trichinella* interaction in wild boars is 6% (Rostami et al., 2018). Lauge et al. (2015) reported a prevalence of 3.4% (28/828) by AD among wild boars from the Northwest of the Argentine Patagonia. Natural ecosystems play an important role in the maintenance of *Trichinella* spp. in the environment. The differences observed in the results between wild boars from Northeast Patagonia and Northwest Patagonia could be explained by the differences between the ecosystems and the presence of large cultivated areas in the study location. In this sense, despite their feeding habits of scavenging on animal carcasses they would have less chance to consume animals infected with *Trichinella* larvae. *Trichinella* spp. negative results by AD are in line with the negative results reported for other wild species in the study area, like white-eared opossum (*Didelphis albiventris*), big hairy armadillo (*Chaetophractus vellosus*), Geoffroy's cat (*Leopardus geoffroyi*), lesser grison (*Galictis cuja*), Molina's hog-nosed skunk (*Conepatus chinga*) and South American grey fox (*Lycalopex gymnocercus*) (Winter et al., 2018).

For human consumption, the analytical sensitivity (one larva per gram) in wild animals may depend on the total amount of muscle analyzed. The amount of tissue recommended by the International Commission on Trichinellosis is 10 g (Gamble et al., 2000). The use of serological tests, such as ELISA, are considered adequate for surveillance and monitoring studies, due to their high sensitivity (detect antibodies with parasite burdens under 0.01 larva per gram) (Gamble et al., 2004). A total of 95 wild boars samples could be analyzed by artificial digestion and both indirect methods. Unfortunately the availability of samples did not allow a comparison of results between serological tests in serum and muscle juice for *Trichinella* spp. However, the different results obtained between AD and ELISA (in serum and meat juice samples) could be due to false positive results (Bruschi et al., 2019) or as a consequence of the fact that *Trichinella* spp. infects wild boars of Northeast Patagonia with a parasite burden under one larva per gram. Additionally, those seropositive wild boars could be infected by *T. patagoniensis* which induces a very low infection parasite burden in swine hosts (Krivokapich et al., 2012; Ribicich et al., 2013).

Although most of the reviewed studies showed no dietary differences between male and female wild boars (Loggins et al., 2002; Adkins and Harveson, 2006; Skewes et al., 2007), significant differences were found between proportions of seropositive males and females to the two foodborne parasites. All *Trichinella* spp. seropositive and 75% of *T. gondii* seropositive wild boars were male, probably due to the fact that in the studied location the males distribution area was greater than that of females, as reported in other countries (Saunders and Mc Leod, 1999), allowing scavenging and predation in different environments and with a broader range of hosts (Jolley et al., 2010; Giménez-Anaya et al., 2008). Also, according to rural inhabitants, it is easy to observe male wild boars feeding on live or dead livestock. In order to observe differences between age groups, it may be necessary to increase the number of wild boars sampled. However, it would be expected that the greatest seroprevalence would be among the older animals, understanding that they have been longer exposed (Pannwitz, et al., 2010).

This kind of studies contributes to the knowledge of two endemic foodborne pathogens in relation to a growing source of food exposure. Further studies need to be addressed to fully understand, in depth, the role of wild boar in the transmission of *T. gondii* and *Trichinella* spp. in Argentine Patagonia.

5. Conclusion

The relevance of this study resides in the fact that it is the first study to reveal the presence of antibodies to *T. gondii* in free-living wild boars of Argentina. More studies are needed to know in depth wild life cycle of *T. gondii* and *Trichinella* spp. in Argentine Patagonia.

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Conflict of interest

I testify that none of the authors of our article "*Toxoplasma gondii* and *Trichinella* infections in wild boars (*Sus scrofa*) from the north-eastern Patagonia, Argentina" has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled

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