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Trichinella Infection in Culled Wild Boar (*Sus scrofa*) from El Palmar National Park, Argentina, and Exposure Risk in Humans and Dogs Consuming Wild Boar Meat

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ABSTRACT: Trichinellosis is a foodborne disease caused by ingestion of raw or undercooked meat containing *Trichinella* spp. larvae. Consumption of wild boar (*Sus scrofa*) meat represents an important source of human trichinellosis worldwide. In El Palmar National Park (EPNP), Argentina, invasive alien wild boars are controlled and meat from culled animals is released for public consumption following on-site artificial digestion (AD) testing. Meat trimmings and offal from the control program are often used as food for dogs (*Canis familiaris*). We evaluated infection and exposure to *Trichinella* spp. in wild boars from EPNP, as well as exposure to *Trichinella* spp. and associated risk factors in dogs and human consumers of wild boar meat. *Trichinella* spp. larvae were detected in muscle samples from 5/49 wild boars by AD (10.2%; 95% confidence interval [CI], 3.8%–23%), with a mean burden of 0.24 larvae per gram (lpg; range, 0.06–0.95 lpg). Anti-*Trichinella* antibodies were not detected in wild boar serum samples (n=42). In dogs, 12/34 were seropositive to *Trichinella* spp. (35.29%; 95%, CI, 20.3%–53.5%). Immunoglobulin (Ig) G antibodies were not detected in human serum samples (n=63). Our results reveal the presence, albeit at low prevalence, of *Trichinella* spp. in wild boars and exposure in dogs fed game offal. These findings suggest that the low prevalence and parasitic load in wild boars, together with the best practices applied by EPNP culling program personnel, contribute to keeping the risk of infection in people low. The dog results highlight that the parasite is circulating in the area, and therefore the risk of infection is not negligible. We recommend the implementation of an animal surveillance strategy in order to monitor the evolution of this zoonosis in the study area.

Key words: Alien species control, domestic dogs, food safety, game meat, public health, trichinellosis.

INTRODUCTION

Trichinellosis is a foodborne disease caused by the ingestion of raw or undercooked meat containing muscular larvae (ML) of *Trichinella* spp. nematodes (Food and Agriculture Organization 2014). Carnivores are the main hosts of *Trichinella* spp., with the exception of *Trichinella spiralis,* which is also well adapted to suidae and consequently represents the main causative agent of human trichinellosis (Dupouy-Camet and Bruschi 2007).

After consumption of *Trichinella* spp. –infected muscle by a host, ML are released by digestion and burrow into the villi of the small intestine, where they rapidly develop into adults. Adults breed and 1 wk later

release larvae that migrate to striated muscle, where they develop into infective ML and become encapsulated (World Organisation for Animal Health [WOAH] 2023). The life cycle repeats when meat containing these ML is consumed by another individual (Centers for Disease Control and Prevention [CDC] 2019).

Trichinella spp. transmission presents a domestic cycle in which pigs are the main hosts, with transmission between domestic and synanthropic animals such as rodents, and a sylvatic cycle, involving mainly carnivores and wild boars (*Sus scrofa*; Pozio and Murrell 2006). Human infection is strongly related to consumption of raw or undercooked game meat (Pozio 2015; Yera et al. 2022). Wild boar is one of the most frequently consumed game species and a source of human trichinellosis worldwide (Faber et al. 2015; Sevillano Morales et al. 2018).

Hunting practices can play an important role in the epidemiology of trichinellosis, increasing the availability of game carcasses and offal infected with *Trichinella* larvae, thus promoting transmission to wild and domestic scavengers (Pozio and Murrell 2006; Dupouy-Camet and Bruschi 2007). Game meat trimmings and offal are usually used as food for dogs owned by hunters; therefore, sera from these animals may be tested to monitor the circulation of *Trichinella* spp. among wildlife (Gómez-Morales et al. 2016; Miterpáková et al. 2017).

Trichinellosis is an endemic zoonosis in Argentina and is an important public health problem because of its high morbidity rates (Pasqualetti et al. 2014; Ribicich et al. 2020). Although infection is commonly associated with domestic pigs, between 2013 and 2018, 84 *Trichinella* spp. foci infections in animals were reported in Argentina, with wild boar being the main species involved (Ministerio de Salud 2021). The recommended technique for detection of *Trichinella* spp. in meat intended for consumption is artificial digestion (AD; Servicio Nacional de Sanidad y Calidad Agroalimentaria 2006; European Union [EU] 2015; WOAH 2023). This method can detect <1 larva per gram (lpg) of tissue, but at these low levels of infection, the amount of digested muscle and the distribution of larvae within tissues are limiting factors for accurate diagnosis (WOAH 2023). Thus, the International Commission on Trichinellosis and the European Commission recommend using 10 g in wild animal testing (EU 2015; Gajadhar et al. 2019; Noeckler et al. 2019). Serological methods, such as ELISA using excretory-secretory (E-S) antigens released from *Trichinella* spp. ML in vitro, have high sensitivity (detect antibodies with parasite burdens under 0.01 lpg) and provide critical information for surveillance and monitoring studies (Gamble et al. 2004).

Since 2006, El Palmar National Park (EPNP) in Entre Ríos, Argentina, has implemented a multistakeholder control program targeting invasive alien mammals, including wild boars (Gürtler et al. 2017). The meat of hunted animals is inspected by AD in a local laboratory, and meat testing negative is distributed for consumption by hunters and EPNP personnel, with a hindquarter of each hunted animal being donated to residents from neighboring towns. Meat trimmings and offal from the control program, as well as those from private hunts, are often used as food for dogs.

Using samples collected in 2007, Cohen et al. (2010) reported ML in 11.4% (13/114) of wild boars by AD in EPNP. Since then, no positive findings have been recorded by on-site testing (C. Sosa pers. comm). Therefore, the aim of our study was to update epidemiological surveillance of *Trichinella* spp. in EPNP by evaluating 1) variation in infection and exposure to *Trichinella* spp. in wild boars since the last prevalence study by Cohen et al. (2010) and 2) exposure to *Trichinella* spp. and associated risk factors in dog and human consumers of wild boar meat.

MATERIALS AND METHODS

Study area

The EPNP (31°51′54″ S, 58°15′34″ W) is a protected area covering approximately 8500

ha (Batista et al. 2014), divided in two areas by the Palmar stream: the northern area, with trails and a recreational zone, and the southern area, where access is restricted to authorized personnel. The alien species culling program is carried out collaboratively by park rangers and authorized local hunters, through controlled still shooting from watchtowers that are widely distributed within the park and with no bag limit for hunting. During the program's first 10 yr, 1,999 wild boars were hunted (Gürtler et al. 2017), and in 2019, 276 wild boars were removed by 146 hunters that participated in the program (C. Sosa pers. comm).

Wild boar sample collection and analysis

Between March and May 2018 and August and November 2019, wild boar muscle and blood samples were collected from culled individuals by convenience sampling. Samples were identified with the number of the watchtower where each wild boar was killed. Individuals were classified into four age categories according to body length and sex (Gürtler et al. 2017). Blood samples from the jugular vein were taken by hunters within 5 min of death, kept at room temperature, and centrifuged within 4 h postcollection. Serum samples were stored at −20 C. To establish the distribution of wild boar *Trichinella* spp. infection, the watchtowers where the animals were killed were identified, georeferenced, and classified according to their location in the northern or southern area of the park. The spatial distribution of the watchtowers and the results of the wild boar ML analysis by AD were described using QGis 3.10.3 (QGIS Association 2022).

Muscle samples were obtained from diaphragm pillars, tongue, and masseter. In the absence of a cold chain, the salting method with 2% sodium chloride was applied in the range of percentages mentioned in other studies to inhibit decomposition (Childers et al. 1982; Pal and Devrani 2018; Johne et al. 2020). Salted muscle samples were kept at room temperature for no more than 2 wk until processing. Briefly, samples were stripped and rehydrated with distilled water (100 mL/20 g) for 4 h (Ministerio de Salud 2021). Pooled samples from each animal weighing at least 4.2 g were then ground and individually processed by AD (Gamble et al. 2000). A mean of 16.1 g (range, 4.2–25.9 g) from each individual was analyzed and results were expressed as lpg.

We detected anti-*Trichinella* antibodies using an indirect ELISA. This ELISA had been developed and validated for the detection of specific antibodies in domestic swine serum samples, but a similar ELISA has been used to detect anti-*Trichinella* antibodies in sera from wild boars with slight modifications (Riva et al. 2021). Briefly, plates were coated with E-S antigens (5 µg/mL) developed in house using E-S antigens of ML of *T. spiralis* produced at Laboratorio de Trichinellosis (Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires [FCV-UNCPBA]) according to the OIE protocol (Dupouy-Camet and Bruschi 2007) and incubated with serum samples diluted at 1:100 in buffer. The optimal dilution of sera had been determined by previous checkerboard titration assay using twofold serial dilutions from 1:25 to 1:200 of positive and negative control sera from wild boars in which *Trichinella* spp. larvae had been detected or not, respectively, by AD.

Bound antibodies were detected with rabbit antipig IgG conjugated to horseradish peroxidase (HRP; diluted at 1:2,500 in buffer; Sigma, St. Louis, Missouri, USA), previously tested to corroborate reactivity to wild boar sera. The reaction was developed by adding ortho-phenylene-diamine in a citric acid buffer 0.07% hydrogen peroxide and read at 450 nm. The cutoff point was calculated as the mean of optical density for five negative sera plus three standard deviations (Riva et al. 2021). Positive and negative controls were tested, as described below.

Dog questionnaires, sample collection, and analysis

We gave dog owners a questionnaire to collect demographic and feeding data for each dog

sampled (Supplementary Material Appendix 3). Dogs were classified by sex and age (young ≤18 mo, old >8 mo). Frequency of feeding with game was classified as high (10–14 times/wk), medium (5–9 times/wk), low (1–4 times/wk), or very low (once every 15 d or less). Questions included whether the feeding was based on raw or cooked meat and whether the diet was supplemented with other nutrient sources. The time since the last feeding of dogs with game prior to sampling was classified as very recent (1 d prior), recent (approximately 1 wk prior), or not recent (approximately 2 wk prior).

Dogs fed game meat and offal from culled wild boars from EPNP or from private lands neighboring the park were enrolled. Within the same period as for wild boar sampling, dog owners voluntarily participated in the study, providing their written informed consent (see Supplementary Material). Ethical approval was granted by the Comité de Bienestar Animal (FCV-UNCPBA).

Blood samples (3 mL) were drawn by venipuncture of the cephalic vein, kept at room temperature, and centrifuged within 2 h of collection. Serum samples were stored at −20 C. Detection of anti-*Trichinella* antibodies was carried out by ELISA as described earlier with some modifications: E-S antigens were used at 2.5 μg/mL, samples were diluted 1:100, and bound IgG antibodies were detected by adding antidog IgG conjugated to HRP (Jackson, West Grove, Pennsylvania, USA) diluted 1:25,000 in buffer. Serum samples from three *Trichinella*-free dogs were used as negative controls. To evaluate possible cross-reaction with related helminth infection, we tested sera from 10 *Trichinella*-free dogs infected with *Eucoleus* spp. Later, we included one of these sera as negative control. Positive serum samples were tested by WB as described (Ilić et al. 2014) with some modifications (Gómez-Morales et al. 2016). Briefly, E-S antigens (developed as described earlier) were separated in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis, then transferred onto nitrocellulose membranes. Membranes were cut into strips and incubated with serum samples from dogs (diluted 1:50 in buffer). The detection was done with rabbit antidog IgG conjugated to HRP (Jackson) diluted 1:5,000 in buffer. Reactive protein bands were visualized by adding 0.05% 3,3′diaminobenzidine and 0.01% hydrogen peroxide in 50 mM Tris-hydrochloride buffer (pH 7.2). Positive and negative control sera were tested in each assay. The specific band patterns at 45, 49, and 53 kDa were compared with the positive control and with the molecular weight marker (Sigma). Wild boar and dog samples were processed in the same laboratory.

Human questionnaires, sample collection, and analysis

People who consumed wild boar hunted in EPNP and were at least 18 yr of age were included in this study. Written informed consent was obtained from each volunteer participant, endorsed by the Comité Central de Bioética en la Práctica y en la Investigación Biomédica, Entre Ríos (Supplementary Material Appendix 2). Demographic data and role in the alien species control program (hunter, park ranger, or other) were recorded for each participant. Blood samples (5 mL) were drawn by venipuncture by the staff of Hospital Público San Benjamín (Entre Ríos) on 26–30 August and 11–15 November 2019. Sera were stored at −20 C until processing at the reference laboratory (Departamento de Zoonosis Rurales).

Detection of anti-*Trichinella* antibodies in human serum samples was carried out by indirect fluorescent antibody test (IFAT; Dupouy-Camet and Bruschi 2007). Bound antibodies were detected by the addition of goat antihuman antibodies (mixed IgG and IgM) conjugated to fluorescein isothiocyanate (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The 1:32 dilution was set as the cutoff limit, considering this titer as a probable case and values greater than or equal to 1:64 as a confirmed case (Zumaquero Ríos et al. 2018).

We used a structured questionnaire with enrolled participants to obtain information about their game meat consumption habits and identify exposure risk factors (see

TABLE 1. Sample mass and recovered larvae per gram (lpg) from muscle (diaphragm pillars, tongue, and masseter) of individual wild boar (*Sus scrofa*) positive for muscular larvae of *Trichinella* spp. by artificial digestion at El Palmar National Park, Entre Ríos, Argentina, 2018–19.

Year of sampling	Sex	Age category	Muscle mass analyzed (g)	*Trichinella* spp. larvae recovered (lpg)
2018	Male	Juvenile	4.2	0.95
2018	Female	Older adult	14	0.07
2019	Female	Young adult	17.6	0.06
2019	Male	Young adult	18.1	0.06
2019	Female	Young adult	16.4	0.06

Supplementary Material). The questionnaire asked whether they made raw sausages (yes or no) and from what meat (mix of deer [*Axis axis*] and wild boar meat, mix of deer and domestic pig, or only deer meat). It also asked about the three most frequent meals prepared with game meat; this was used to represent the frequency of occurrence of cured (rather than cooked) game meat consumption. Participants were also asked if they had been previously diagnosed with trichinellosis.

Statistical analysis

The positive rate of *Trichinella* spp. was estimated by each host species with its corresponding 95% confidence interval (CI). Descriptive statistics were performed for the categorical and continuous variables under study. To evaluate association between categorical variables, an independence test (χ^2 test and Fisher exact test, as appropriate) was performed, and analysis of variables was used for continuous variables. The odds ratio (OR) with its corresponding CI was calculated. All data were calculated using R Studio software (R Core Team 2020) and were considered statistically significant if the P value was <0.05.

RESULTS

Wild boars

We tested 49 wild boars. Epidemiological data and AD results are summarized in Supplementary Material Table S1. *Trichinella* spp. ML were detected in muscle samples from 5/49 individuals (10.2%; 95% CI, 3.8%–23%), with a mean burden of 0.24 lpg (range, 0.06–0.95 lpg; Table 1). In these five individuals, analyzed muscle sample mass median was 16.4 g (range, 4.2–18.1 g). The lowest-mass sample (4.2 g) from a juvenile individual (4–8 mo) was the one with the highest larva burden (0.95 lpg). No significant statistical associations were found between categorical or continuous variables and the presence of *Trichinella* spp. larvae. Anti-*Trichinella* antibodies were not detected in any of the 42 wild boar sera tested.

Watchtower locations where wild boars were hunted and the proportions of *Trichinella* spp.–positive animals are shown in Figure 1. No significant statistical associations were found between the prevalence of wild boars killed in the northern and southern areas (Fisher exact test, $P=1$).

Dogs

Owners reported that the diet consisted of meat and offal from wild boars and deer collected after hunting trips. A total of 48/63 (76%) of EPNP culling program participants reported feeding their own dogs, those of neighbors, or strays with game offal. Most owners (35/48, 73%) indicated feeding dogs uncooked game remains. This diet was supplemented with other sources of nutrients at the discretion of each owner, which in most cases included rice, cornmeal, and occasionally chicken and beef bones. Overall, 33/34 (97.1%) dog sera tested positive by ELISA and 12/34 (35.29%; 95% CI, 20.3%–53.5%)

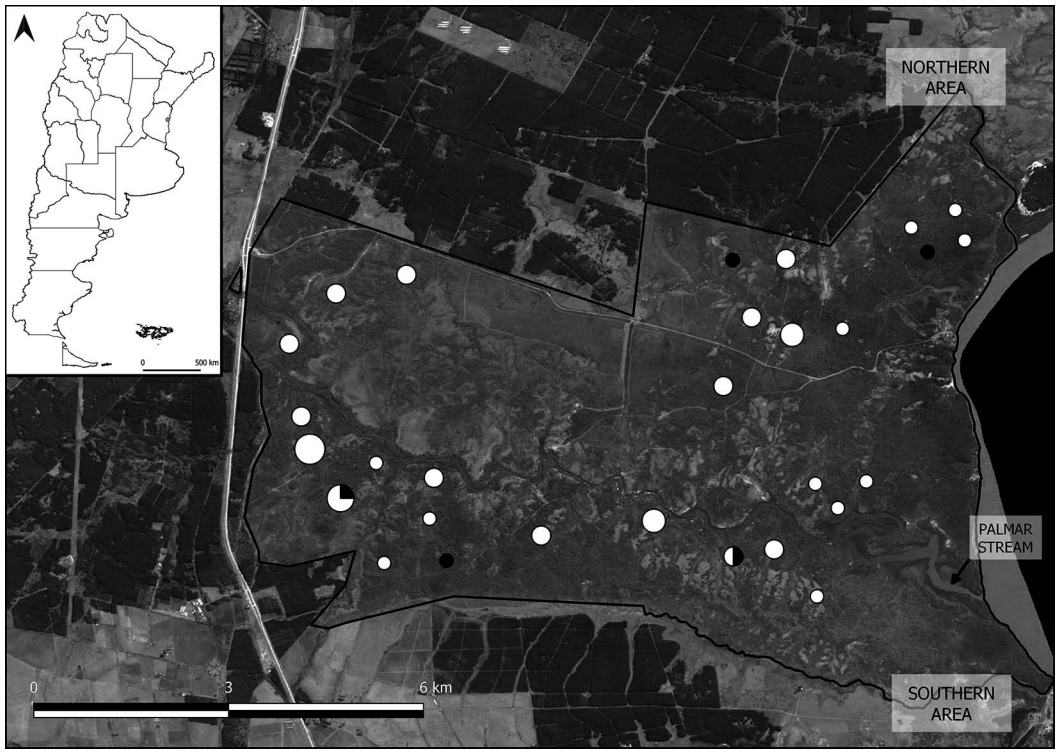

FIGURE 1. Map of hunting sites for wild boar (*Sus scrofa*) sampled in El Palmar National Park, Entre Ríos, Argentina, 2018–19. The location of the watchtowers is represented by circles of different sizes according to the number of wild boars killed in each area. The proportion of animals in which *Trichinella* spp. larvae were detected is indicated in black. Inset: Map of Argentina showing the location of El Palmar National Park.

were confirmed by WB. Sera from controls (*Trichinella* free and *Eucoleus* spp. positive) were negative, suggesting that no serological cross-reaction occurred (Fig. 2). There was no significant association between serologically positive dogs and their categorical and continuous variables under study (Supplementary Material Table S2).

Humans

Questionnaire answers and blood samples were obtained from 63 participants: 52 males and 11 females. Enrolled participants included 33 hunters, 15 park rangers, and 15 people with "other" duties. The "other" duty category comprised firefighters (n=10), support staff (n=3), and researchers (n=2). Housewives were grouped into the same duty categories as their husbands, because they shared similar game meat consumption habits. The questionnaire results and demographic information are summarized in Table 2. No previous diagnosis of trichinellosis was reported by participants. Antibodies to *Trichinella* infection were not detected in human serum samples by IFAT. Consumption of cured game meat was associated with the specific duty of the individuals (P=0.03): hunters were six times more likely to eat cured foods than people in the "other" category (OR, 6.15; 95% CI, 1.45–26.1). An association between duty and making sausages was found (P=0.00004), with hunters 5.5 times more likely to make game meat sausages than park rangers (OR, 5.5; 95% CI, 1.42–21.3).

The participants who reported cured meat as a frequent mode of game consumption reported making raw sausages more frequently (P<0.0001; OR, 62; 95% CI, 11.47–334). In addition, significant statistical differences

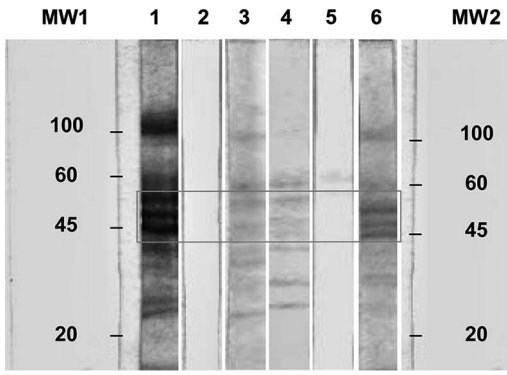

FIGURE 2. Representative Western blot analysis of serum samples from dogs (*Canis familiaris*) fed game meat and offal from culled wild boar (*Sus scrofa*) from El Palmar National Park, Entre Ríos, Argentina, or from private lands neighboring the park (2018–19). Line 1: positive control serum from an experimentally infected domestic pig (*Sus scrofa*) with *Trichinella spiralis*. Line 2: negative control serum from a *Trichinella*-free dog. Lines 3, 4, and 6: representative Western blot of serum from positive dogs (appearance of specific *Trichinella* band triad of antigens at 45, 49, and 53 kDa). Line 5: representative Western blot of sera from a negative dog where the specific *Trichinella* band triad was not observed. MW1=molecular weight marker for strips 1, 2, 3, 4 and 5. MW2=weight marker for strip 6.

between study groups were found related to how they prepared raw sausages (P=0.02): The majority of hunters (18/22, 82%) reported using a mixture of deer and wild boar meat, whereas park rangers used deer meat (2/4, 50%), a mixture of deer and pork (1/4, 25%), or deer and wild boar meat (1/4, 25%).

DISCUSSION

We found the presence of *Trichinella* spp. in wild boars and antibodies reflecting exposure in dogs that consumed game meat from EPNP-culled boars, but no evidence of exposure to *Trichinella* spp. was found in humans. These results suggest that the low prevalence and parasitic load in wild boars, together with the practices applied by EPNP culling program personnel, contribute to keeping the risk of infection in people low. The results in dogs highlight the fact that the parasite is circulating in the area and therefore the risk of infection is not null or negligible.

Our study, using a sample mass mean >10 g, yielded positive results with a prevalence of infection similar to that of the previous study, 10.2%, compared with 11.4% by Cohen et al. (2010), and low parasitic loads (0.06–0.95 lpg vs 0.2–0.3 lpg in the previous study in the wild boar analyzed by AD). These findings suggest that the *Trichinella* spp. scenario has not changed in the last decade, despite the reduction in wild boar abundance and carcass removals achieved by the ongoing control efforts in the park (Gürtler et al. 2017). The prevalence that we found also was within the range reported in most studies conducted in wild boars in Argentina, which varies from zero (0/423, Winter et al. 2019; Ribicich et al. 2020), and low (3.45, 28/828, Lauge et al. 2015); to 25% (3/12, Ribicich et al. 2010) and is higher than the global seroprevalence (6%) in wild boars (Rostami et al. 2018).

Conversely, the lack of detection of *Trichinella*-specific antibodies in wild boars by ELISA may be attributed to low parasite loads and early infections (particularly in juveniles), as reported by Gamito-Santos et al. (2011) in wild boars, with low infection loads at 40 d postinfection. It is also possible that negative ELISA results in our study resulted from the high dilution selected for serum samples (1:100 vs. 1:50 used in other studies, e.g., Gómez-Morales et al. 2014). Future studies involving a much larger sample size would be needed to further optimize this technique, because titration was based on available samples confirmed positive or negative by AD.

We did not find any relationship between infected wild boars and their spatial distribution within the park, whereas Cohen et al. (2010) reported a higher prevalence (85%, 11/13) near the recreational area. Given similar muscle mass of tested samples in both areas in this study, we presume that larger numbers and broader geographic representation would be needed to identify potential relationships between disease prevalence and distribution in the park.

TABLE 2. Summary of the interview results with demographic details and information related to game meat (deer, *Axis axis*, and wild boar, *Sus scrofa*) and domestic pig (*Sus scrofa*) meat consumption in humans according to their duties within the alien species control program at El Palmar National Park, Entre Ríos, Argentina, 2019.

	Hunter	Park ranger	Other	Total
Sample size	33	15	15	63
Gender				
Male	30	11	11	52
Female	3	4	4	11
Age (yr)				
Mean ± SD	42.4±10.9	43.1±7.5	35.9±11.5	41±10.6
Minimum–maximum	24–67	34–58	27–61	24–67
Frequency of consumption[a]				
Yes (%FO)[b]	20 (61%)	7 (47%)	3 (20%)	30 (48%)
No (%FO)	13 (39%)	8 (53%)	12 (80%)	33 (52%)
Preparation of raw game meat sausages				
Yes	22 (67%)	4 (27%)	*0 (0%)*	26 (41%)
Deer and wild boar	18 (82%)	1 (25%)	0 (0%)	19 (73%)
Deer and domestic pig	3 (14%)	1 (25%)	0 (0%)	4 (15%)
Only deer	1 (4%)	2 (50%)	0 (0%)	3 (12%)
No	11 (33%)	11 (73%)	15 (100%)	37 (59%)
Feeding dogs game meat				
Yes	25 (76%)	12 (80%)	11 (73%)	48 (76%)
Raw meat	21 (84%)	8 (67%)	6 (55%)	35 (73%)
Cooked meat	4 (16%)	4 (33%)	5 (45%)	13 (27%)
No	8 (24%)	3 (20%)	4 (27%)	15 (24%)

[a] Frequency of consumption = frequency of consumption of cured meat within the three most frequent game meat meal types.
[b] FO = Frequency of occurrence. The FO of cured meat consumption was determined in relation to the three most frequent meals prepared with game meat reported by the participants.

The sensitivity of AD is influenced by the amount of tissue examined and the body site from which the sample is obtained (WOAH 2023). For epidemiological surveillance purposes of *Trichinella* spp. in wildlife, greater diagnostic sensitivity is recommended to provide more accurate data and overcome field sampling limitations (EU 2015). We performed AD using, in general, a relatively high weight of *Trichinella* spp.–preferred muscles, with the aim of increasing diagnostic sensitivity. This, plus heterogeneous distribution of larvae (Kapel et al. 2005), may explain the contrast between our findings and the negative results in the local laboratory for the same individuals (C. Sosa pers. comm). Although we found a parasite load in wild boars lower than 1.0 lpg, which should prevent clinical trichinellosis in humans, this does not rule out the possibility of asymptomatic infections acquired by eating meat containing very low numbers of larvae (WOAH 2023). Additionally, at 0.95 lpg, our finding is only just below the WOAH limit.

Wild boars are generalists that may consume animal matter in the form of carrion, including carcasses of domestic pigs, deer, and even hunter-killed boar remains (Carrasco-Garcia et al. 2018). The availability of wild boar carcasses may be a transmission route of *Trichinella* spp. for scavenging wild boars (Pozio and Murrell 2006; Carrasco-Garcia et al. 2018). Moreover, infective larvae can survive in decaying carcasses and may remain viable for at least 6 wk in winter (Riva et al. 2012). For example, during 2015, 9/197 wild boars (4.6%) shot by EPNP hunters escaped injured (Gürtler et al. 2017). Assuming those animals

were infected and died of their wounds, they may have contributed to the maintenance of *Trichinella* spp. circulation.

In many countries where hunting is practiced, hunting dogs are commonly infected with *Trichinella* spp. because they have easy access to muscle offal such as diaphragm during slaughter (Gómez-Morales et al. 2016; Rostami et al. 2017). As the infection in animals is generally asymptomatic (Ministerio de Salud 2021), serological confirmation by WB has been considered a useful tool to inform on the circulation of these zoonotic nematodes (Oivanen et al. 2005). In our study, all sampled dogs regularly consumed raw wild boar meat and 36% were seropositive to *Trichinella* spp., higher than previous prevalence reports in hunting dogs from central Italy (56/384, 14.58%) and Slovakia (56/439, 12.76%; Gómez-Morales et al. 2016; Miterpáková et al. 2017). This finding suggests that the risk of infection is not negligible in the study area. Our finding that some owners fed strays and neighbors' dogs with game offal indicates that the risk of infection from wild boars might extend beyond the dogs sampled. Dogs may also be exposed to *Trichinella* spp. by consuming other species such as rodents (CDC 2019). We found no evidence either that dogs are part of the *Trichinella* spp. cycle in the study area or that dogs are dead-end hosts.

In general, hunters and their families and friends are at high risk of acquiring trichinellosis after consumption of raw or improperly cooked wild boar meat (Pozio 2015). The severity of clinical disease is directly correlated with the number of infective larvae ingested by the person; thus, infection may result in a large spectrum of clinical forms (Gottstein et al. 2009). In the convalescent stage, most people are asymptomatic, but ML persist in the muscles for many years (Ministerio de Salud 2021). The early stage of *Trichinella* spp. infection is characterized by a flawed diagnostic window of 2–3 wk with serological false-negative results (Wang et al. 2017; Sun et al. 2018). Seroconversion usually occurs between the second and fifth weeks; the time required for seroconversion is inversely correlated with the infective dose and depends on several factors, such as the *Trichinella* spp. involved and the individual's immune response (Gamble et al. 2004; Gottstein et al. 2009). We found neither positive serological results in people nor any self-reports of previous trichinellosis diagnosis. Although Cohen et al. (2010) detected anti-*Trichinella* spp. antibodies in 2/44 human serum samples tested by ELISA, IFAT, and WB, no clinical signs of trichinellosis were reported by participants of that study. The absence of detected specific antibodies in our study may reflect either a lack of exposure to *Trichinella* spp. or that antibodies had declined below detectable levels. It is also possible that these were false-negative results at the beginning of infection (Gómez-Morales et al. 2008; Zumaquero Ríos et al. 2018). A limitation of our study was that only the IFAT was used, which may have resulted in false negatives (Costantino et al. 2001).

Domestic preparation and consumption of raw sausages made with untested wild boar meat is a frequent practice in Argentina (Ribicich et al. 2010). One of the most commonly used meat processing methods is curing, which is not considered a safe method because the ML of *Trichinella* spp. can survive in salted meat (Gamble et al. 2000). Hunters in our study reported preparing homemade sausages and consuming cured meat as one of the most frequent modes of wild boar consumption; they were thus identified as the group with the highest risk of exposure to *Trichinella* spp. Likewise, the majority of participants indicated consuming salted sausages made with meat from a mix of deer, wild boar, and/or domestic pig. Sausages made by combining wild boar and deer meat may reduce the risk of infection compared with the use of only wild boar meat, as herbivores are atypical hosts of *Trichinella* spp. (Wilson et al. 2015).

Currently in the EPNP, AD diagnosis is routinely performed on wild boar, and meat

cooking and other best practices are encouraged for hunters and others who consume the meat handed out by the control program. Importantly, the meat is not formally released for consumption until the AD result is available. Although using culled animals is of ethical relevance, following preventive actions is essential to keep the risk of infection low. In the future, to increase the sensitivity of AD, we recommend the implementation of a surveillance strategy in wild boars that involves an annual study with higher sample mass. This would enable assessment of the evolution of this zoonosis in the study area over time and reinforcement of prevention should the larvae load in wild boar increase. In addition, correct disposal of animal remains is essential, avoiding leaving meat trimmings and offal available for scavengers. In our research area, as in similar contexts, a combination of actions for the prevention of trichinellosis and other foodborne zoonoses continues to be necessary to ensure food safety in consumers of wild boar meat.

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SUPPLEMENTARY MATERIAL

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