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# Assessment of *Trichinella* Infection in Animals from Argentina

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**Abstract:** Parasites of the genus *Trichinella* are widely distributed and infect a wide range of domestic and wild animals. Their presence spans across various continents, impacting agricultural and wildlife ecosystems alike. In Argentina, *Trichinella spiralis*, *Trichinella patagoniensis*, and *Trichinella pseudospiralis* have been detected infecting both domestic and wild animals. The aim of the present study is to detect *Trichinella* spp. in samples from domestic pigs and wildlife across Argentina between 2019 and 2024 and to identify these parasites at the species level. A total of 216 samples from domestic and wild animals underwent artificial digestion. In cases where a positive result was obtained, muscle larvae were further processed using PCR targeting the ESV region. Samples originating from 16 animals were analyzed. Among the animals tested, 52 were positive for *Trichinella* spp., including two pumas infected with *T. patagoniensis*; 43 domestic pigs, with 34 identified as *T. spiralis* at the species level; and seven wild boars, with five identified as *T. spiralis* at the species level. The present study enhances our understanding of the distribution and transmission dynamics of *Trichinella* among domestic and wild animals in Argentina.

**Keywords:** *Trichinella*; Argentina; domestic pigs; wild boars; wild fauna

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## 1. Introduction

The genus *Trichinella* comprises 13 recognized taxa that are phylogenetically divided into two distinct clades: encapsulated species, which induce the formation of a collagen capsule around the larvae in host muscle tissues, and non-encapsulated species, which lack this adaptive trait [1,2]. These parasites infect a broad range of mammals, birds, and reptiles, with humans serving as accidental hosts through the consumption of raw or undercooked meat containing infective larvae. In humans, infection by *Trichinella* spp. results in trichinellosis, a disease characterized by clinical manifestations ranging from gastrointestinal distress to severe systemic complications, including myositis and myocarditis [3]. The global distribution of these parasites, coupled with their ability to exploit both domestic and sylvatic cycles, underscores their persistent threat to public health and food security.

The trophic transmission of *Trichinella* spp. depends on predation and scavenging, with wild carnivores and omnivores serving as ideal reservoirs. Due to their greater biomass in wildlife compared to domestic animals, the eradication of these parasites is virtually impossible [2]. Historically, *Trichinella* nematodes caused widespread zoonotic disease, and they continue to impose costly inspection regimes and trade barriers for animal products. Before molecular methods enabled species discrimination, all occurrences were erroneously attributed to a single species, *Trichinella spiralis* [4].

In Argentina, *Trichinella spiralis*, *Trichinella patagoniensis*, and *Trichinella pseudospiralis* have been detected infecting domestic and wild animals. *Trichinella britovi*, one of the most widespread species in Europe, was detected for the first time in Argentina in homemade sausages [5]. To date, there are no reports of this species in animals.

*Trichinella spiralis* has been detected in pigs, wild boars (*Sus scrofa*), dogs, cats, armadillos (*Chaetophractus villosus*), cougars (*Puma concolor*), opossums (*Didelphis albiventris*), rats (*Rattus norvegicus*), and in South American sea lions (*Otaria flavescens*) from South America, Argentina. *T. patagoniensis* has been found only in cougars from provinces in the western region [6,7]. During the period of 2013–2023, there were 642 outbreaks of trichinellosis in domestic and wild animals. The most affected provinces were Buenos Aires and Neuquén, with 370 outbreaks. The majority of these outbreaks occurred in domestic pigs (77%), followed by wild boars (22%) and pumas (1%). Regarding the months of the year, the highest number of reported outbreaks occurred between May and September, which coincides with the winter season and homemade slaughter for the production of cured meats in rural areas without the mandatory veterinary checks carried out in slaughterhouses [8,9].

Argentina is an endemic zone of trichinellosis, with several human outbreaks throughout the year. During the period from 2019 to 2024, a total of 5006 notified human cases of trichinellosis were reported [8,10]. The implementation of artificial digestion in slaughterhouses and laboratories helps prevent disease outbreaks. Meanwhile, the application of molecular biology techniques for identifying *Trichinella* spp. has enabled more detailed epidemiological studies, leading to the prevention of outbreaks in previously unexplored areas.

The aim of the present study is to detect *Trichinella* spp. in samples from domestic pigs and wildlife across Argentina between 2019 and 2024 and to identify these parasites at the species level.

## 2. Materials and Methods

### *2.1. Sampling*

This study utilized a convenience sample consisting of muscle samples that were submitted to the Laboratory of Parasitology and Parasitic Diseases of the University of Buenos Aires, the Laboratory of Parasitology, and the Laboratory of Sede Atlantica, University of Rio Negro from 2019 to 2024. Samples were obtained from various regions of Argentina, including the provinces of Buenos Aires, Córdoba, Entre Ríos, La Pampa, Neuquén, Río Negro, San Luis, Santa Cruz, and Antarctica. While most samples were traceable to the provincial level, geolocation information for some of them was missing.

The analyzed animal samples included domestic pigs, wild boars, and a range of other wildlife species. Wild boar samples were collected by hunters. Pig samples were submitted by the National Service of Agri-Food Health and Quality (SENASA) or private veterinarians. The remaining wild animal samples originated from animals found deceased (authorization of scientific collection Nº 085206-SAYDC-2015_218/222). No animals were sacrificed for this study. No data related to hunters were collected or analyzed. The authorities of the Secretaría de Ambiente y Cambio Climático and Dirección de Fauna of Río Negro Province provided the permits for sampling the sea lion rookeries located on the coasts of Rio Negro province (RES. 1203/2019, 173/2021, and 2022-5-E-GDERNE-SAYCC#SGG).

Muscle samples were obtained from different tissues, including the diaphragm, tongue, masseters, intercostal muscles, forelimbs, hind limbs, and axial and pectoral muscles. Then, they were refrigerated at 4 °C until they were processed. Upon arrival at the laboratory, tissue samples were subjected to the artificial digestion technique as described in [6]. The resulting sediment was examined under an inverted LEICA DMi1™ microscope (Wetzlar, Germany) at 10× magnification for the presence of *Trichinella* larvae. Isolated larvae were preserved in 70% ethanol for further analysis.

### 2.2. DNA Extraction

For each *Trichinella* isolate, DNA was extracted from five individual MLs using the DNeasy Blood & Tissue Kit (Qiagen™, Venlo, The Netherlands) according to the manufacturer's instructions.

### 2.3. PCR Analysis

The expansion segment V (ESV) of the ML DNA was amplified using PCR for species identification following a previous protocol [6]. PCR reactions were performed in a 25 µL volume containing 0.5 µM of each primer (forward: 5′-GTTCCATGTGAACAGCAGT-3′; reverse: 5′-CGAAAACATACGACAACTGC-3′), 12.5 µL of Platinum™ II Hot-Start PCR Master Mix (Thermo Fisher Scientific, Vilnius, Lithuania), 10 ng of DNA from each *Trichinella* larva, and deionized water. PCR cycling was carried out on a TC1000-G thermocycler (DLAB Scientific), with initial denaturation at 95 °C for 1 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min. A final extension step at 72 °C was included for 2 min.

The DNAs from *T. spiralis* (ISS), *T. patagoniensis* (ISS), and *T. pseudospiralis* (ISS) were used as positive controls, while deionized water served as a negative control. PCR products were separated on a 2% agarose gel stained with SyBR SAFE™ and visualized under UV transillumination. DNA band sizes were determined by comparison to a 50 base pair (bp) ladder.

Data analysis and figure generation were performed using the R software (version 4.2.3) [11] along with the appropriate R packages [12–18].

## 3. Results

A total of 216 samples were analyzed (Table 1), including 208 from the class Mammalia and 8 from the class Aves. Of these samples, 70.37% belonged to the Artiodactyla order, 22.22% to the Carnivora order, 3.24% to the Sphenisciformes order, 2.32% to the Cingulata order, 1.39% to the Didelphimorphia order, and 0.46% to the Charadriiformes order (Figure 1).

**Table 1.** Number of muscle samples collected from various animal species across different provinces of Argentina between 2019 and 2024. Samples were analyzed using artificial digestion and PCR. For animals that tested positive in artificial digestion but did not show amplification in PCR, the results are reported as "unidentifiable species".

| Animal Species | Number of Animals | Location | Muscle Samples | Results |
|---|---|---|---|---|
| *Sus scrofa* | 2 | Buenos Aires | diaphragm, masseters, and intercostal muscles | *T. spiralis* |
| *Sus scrofa domestica* | 32 | Buenos Aires | diaphragm, masseters, and intercostal muscles | *T. spiralis* |
| *Sus scrofa domestica* | 14 | Buenos Aires | diaphragm, masseters, and intercostal muscles | negative |
| *Sus scrofa domestica* | 4 | Buenos Aires | diaphragm, masseters, and intercostal muscles | unidentifiable species |
| *Otaria flavescens* | 2 | Buenos Aires | diaphragm, masseters, and tongue | negative |
| *Sus scrofa domestica* | 1 | Córdoba | diaphragm, masseters, and intercostal muscles | *T. spiralis* |
| *Sus scrofa domestica* | 13 | Córdoba | diaphragm, masseters, and intercostal muscles | negative |
| *Sus scrofa domestica* | 2 | Córdoba | diaphragm, masseters, and intercostal muscles | unidentifiable species |
| *Sus scrofa domestica* | 1 | Entre Ríos | diaphragm, masseters, and intercostal muscles | negative |

**Table 1.** *Cont.*

| Animal Species | Number of Animals | Location | Muscle Samples | Results |
|---|---|---|---|---|
| *Sus scrofa* | 3 | La Pampa | diaphragm, masseters, and intercostal muscles | *T. spiralis* |
| *Sus scrofa domestica* | 1 | La Pampa | diaphragm, masseters, and intercostal muscles | *T. spiralis* |
| *Sus scrofa* | 66 | La Pampa | diaphragm, masseters, and intercostal muscles | negative |
| *Sus scrofa* | 1 | La Pampa | diaphragm, masseters, and intercostal muscles | unidentifiable species |
| *Puma concolor* * | 1 | Neuquén | diaphragm and masseters | *T. patagoniensis* |
| *Chaetophractus villosus* | 5 | Río Negro | diaphragm and hind limbs | negative |
| *Conepatus chinga* | 1 | Río Negro | diaphragm and hind limbs | negative |
| *Delphinus delphis* | 1 | Río Negro | diaphragm and hind limbs | negative |
| *Didelphis albiventris* | 3 | Río Negro | diaphragm and hind limbs | negative |
| *Galictis cuja* | 7 | Río Negro | diaphragm and hind limbs | negative |
| *Larus dominicanus* | 1 | Río Negro | pectoral muscles | negative |
| *Leopardus geoffroyi* | 3 | Río Negro | diaphragm and hind limbs | negative |
| *Lycalopex gymnocercus* | 15 | Río Negro | diaphragm and hind limbs | negative |
| *Lycalopex gymnocercus* | 1 | Río Negro | diaphragm and hind limbs | negative |
| *Otaria flavescens* | 16 | Río Negro | diaphragm, masseters, and tongue | negative |
| *Pontoporia blainvillei* | 5 | Río Negro | axial muscles | negative |
| *Puma concolor* | 1 | Río Negro | diaphragm and masseters | negative |
| *Spheniscus magellanicus* | 7 | Río Negro | pectoral muscles | negative |
| *unidentified Delphinidae* | 1 | Río Negro | axial muscles | negative |
| *Sus scrofa* | 1 | San Luis | diaphragm, masseters, and intercostal muscles | negative |
| *Sus scrofa* | 1 | San Luis | diaphragm, masseters, and intercostal muscles | unidentifiable species |
| *Sus scrofa domestica* | 3 | San Luis | diaphragm, masseters, and intercostal muscles | unidentifiable species |
| *Puma concolor* * | 1 | Santa Cruz | diaphragm and masseters | *T. patagoniensis* |

* Previously reported in Fariña et al., 2024 [6]. New records of Trichinella patagoniensis from Argentina.

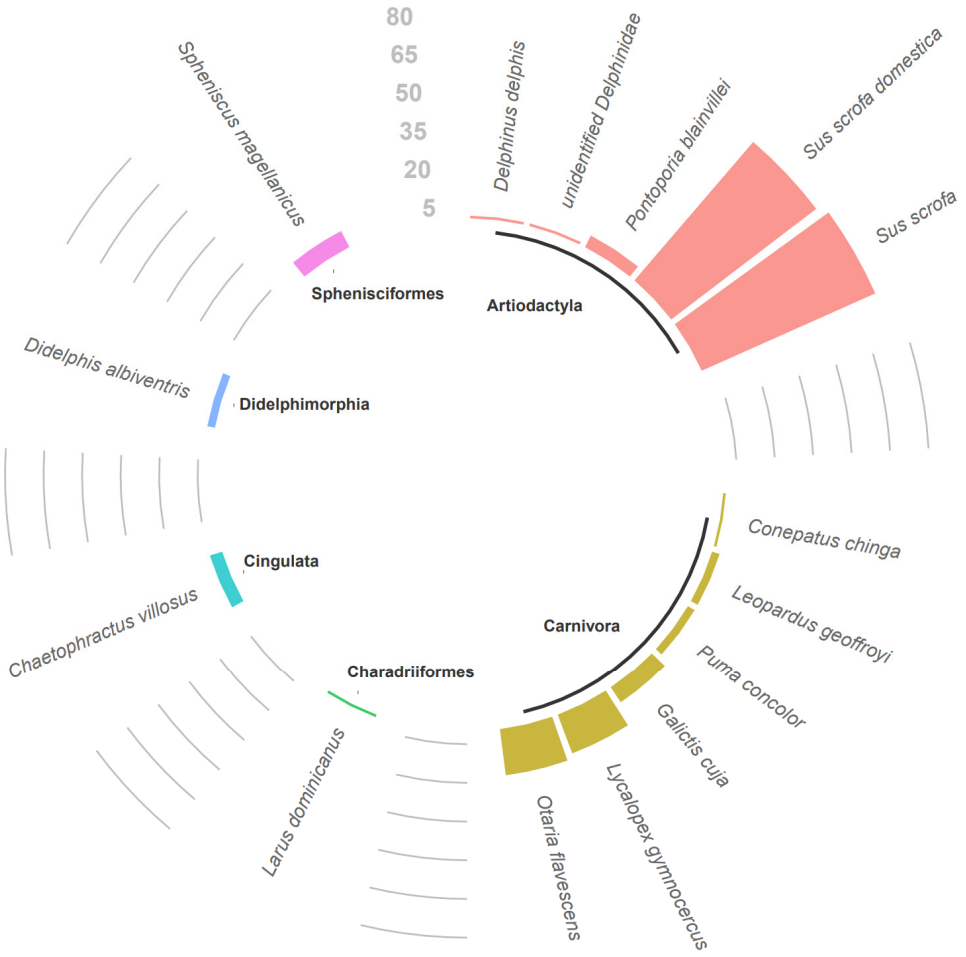



**Figure 1.** Circular bar plot illustrating the distribution of taxonomic orders and the corresponding animal samples analyzed.

Regarding geographical origin, 32.9% of the samples came from La Pampa, 31% from Rio Negro, 25% from Buenos Aires, 7.41% from Córdoba, and 2.31% from San Luis province. Additionally, 0.463% of the samples were each from Entre Ríos, Neuquén, and Santa Cruz (Table 1).

*Trichinella* larvae were isolated from 52 animals. *T. spiralis* L1 larvae were detected in two wild boars from Buenos Aires province, four wild boars from La Pampa Province, 32 pigs from Buenos Aires Province, 1 pig from Córdoba Province, and 1 pig from La Pampa Province. T. patagoniensis isolates from Puma concolor from Neuquén and Santa Cruz Provinces have been previously reported [6]. DNAs from Trichinella detected in one wild boar from San Luis Province, four pigs from Buenos Aires Province, two pigs from Córdoba Province, and three pigs from San Luis Province could not be amplified and therefore were not identified at the species level (Figure 2).

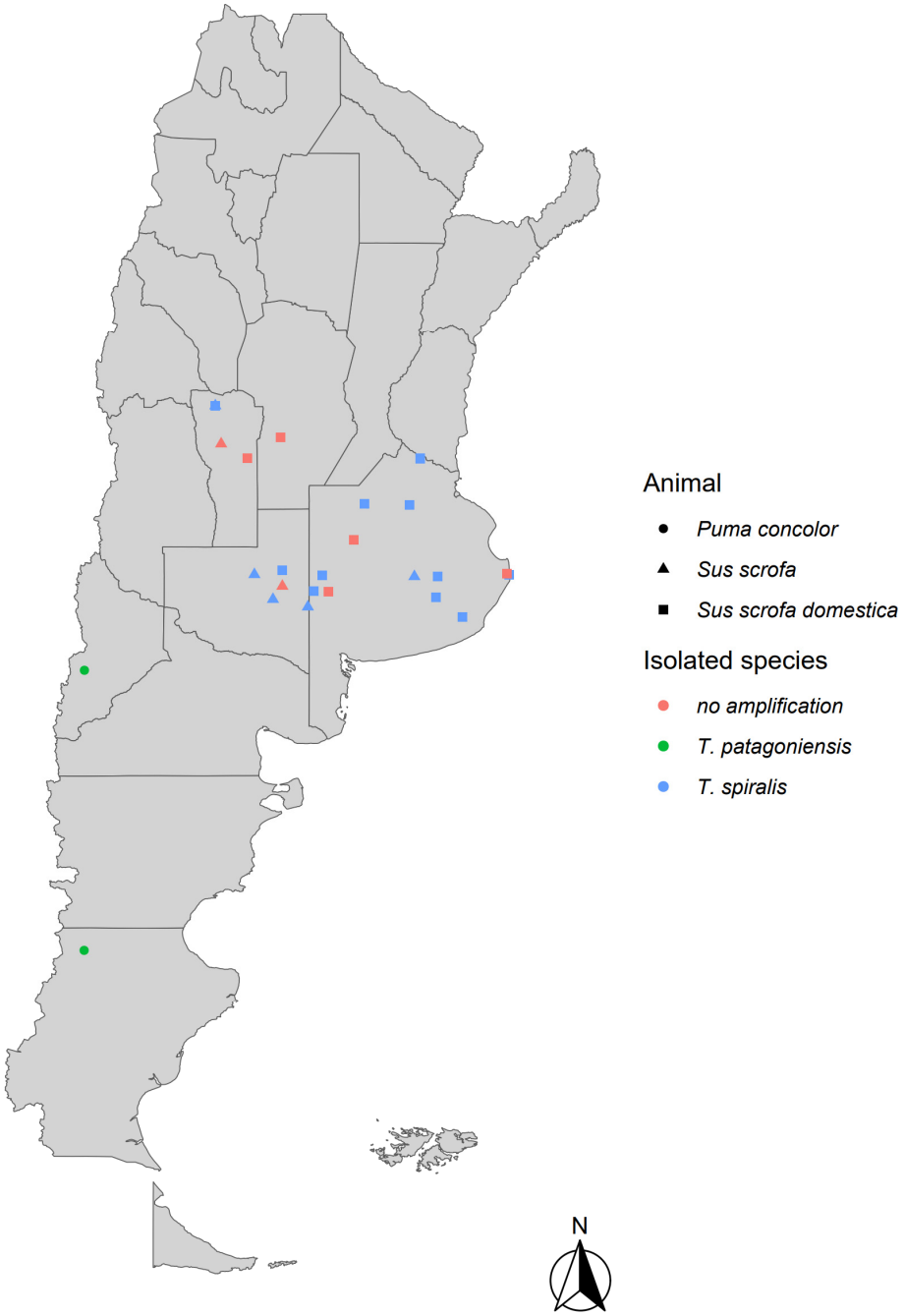



**Figure 2.** Spatial distribution of Trichinella-positive animals in Argentina for the time period of 2019–2024.

## 4. Discussion

This research enhances the understanding of *Trichinella* species distribution in Argentina. In our study, 82.7% of the infected animals were pigs, 13.47% were wild boars, and 3.85% were pumas. Geographically, 73.09% of the positive samples came from Buenos Aires Province, 9.61% from La Pampa, 7.70% from San Luis, 5.77% from Córdoba, and 1.92% each from Neuquén and Santa Cruz. However, the fact that the majority of pigs that tested positive for *Trichinella* originated from animal outbreaks may limit the generalizability of our findings to the broader pig population in Argentina.

Pig farming in Argentina is distributed across various regions, with the highest concentration in the central part of the country [19]. Family and subsistence pig farming, where slaughtering is performed at home, accounts for the majority of pig infection sources (68% of cases). To a lesser extent, infections occur in commercial farms (18%) or in establishments that gather or fatten pigs from various sources (7%) [20]. Between 2013 and 2022, 88% of the detected swine outbreaks were not associated with human outbreaks, with only 12% being a source of human infection [9]. These results highlight the importance of detection in slaughterhouses to prevent the occurrence of human cases.

In recent years, Argentina has seen an increase in the detection of *Trichinella* spp. in domestic and wild animals, including wild boars and cougars. In 2023, SENASA reported a total of 45 outbreaks in pigs and 19 in wild animals such as wild boars and cougars [9]. Seasonal patterns indicate that human outbreaks are more common between April and September, largely due to the consumption of homemade cured meats that lack proper veterinary oversight [19]. This seasonal trend underscores the need for improved regulation and the monitoring of meat processing practices to mitigate the risk of *Trichinella* transmission.

In South America, trichinellosis is a concern not only in Argentina but also in Chile. The highest number of cases in Chile typically occurs during national holidays and Mapuche New Year celebrations, especially between July and September, as well as October and December [7]. Landaeta-Aqueveque et al. (2021) studied the annual series of reported trichinellosis cases in Chile, demonstrating a downward trend that has become more evident since the 1980s [21]. The results suggest that trichinellosis is not a (re)emerging disease in Chile. However, several factors such as the high poverty rate among the Mapuche indigenous people in Chile and the large number of backyard pigs raised in uncontrolled housing conditions appear to be associated with the elevated risk of trichinosis in the Araucanía region [21].

Although *T. spiralis* primarily infects swine, it also affects a wide variety of mammals. This includes domestic animals such as horses, dogs, and cats, as well as synanthropic species like rats, mice, armadillos, and opossums. Additionally, it can infect various wild mammals, including rodents and carnivores [22]. The transmission of *Trichinella* spp. depends on trophic interactions such as predation and scavenging, which are essential for maintaining the parasite's life cycle. Consequently, wild carnivores and omnivores serve as significant reservoirs. *Trichinella* spp. often have a higher biomass in wildlife compared to domestic animals, making eradication efforts particularly challenging [23]. In subsistence farming systems where proper management is lacking, pigs are at a heightened risk of exposure to *T. spiralis* from wild boars or other wild carnivores [24]. Additionally, there is a risk of spillback, where *T. spiralis* from swine infects wildlife [2].

In the present study, most wild animal samples were collected from the southern part of Buenos Aires and the northeastern region of Río Negro Province. Our findings are consistent with a previous report from these areas, which also found no *Trichinella* larvae in *D. albiventris*, *L. geoffroyi*, *G. cuja*, *C. chinchilla*, or *L. gymnocercus* [25]. Despite previous documentation of *T. spiralis* infecting *O. flavescens* from northeastern regions [26], we did not find *Trichinella* larvae in any of the 18 *O. flavescens* specimens analyzed in

our study. Although we did not find *Trichinella* larvae in our tested samples, continued surveillance in various wild animal species is essential for a comprehensive understanding of the parasite's distribution. This study also reports the first examination of samples from *D. delphis*, *L. dominicanus*, *P. blainvillei*, and *S. magellanicus* in Argentina.

In Argentina, the wild boar has spread across half of the country and is present in a wide range of ecosystems. As an omnivorous species, it can serve as a reservoir for several pathogens [27]. Products derived from its meat are the second most common source of trichinellosis outbreaks [20]. Necrophagy, combined with high humidity and low temperatures, facilitates the persistence of *Trichinella* in an ecosystem. Hunting activities contribute to the abundance of carcasses and promote necrophagy when wild boar carcasses (or parts thereof) are not removed from the hunting site. This may explain the disparity in recorded cases of trichinellosis in wild boars between western and eastern Argentine Patagonia (and Chile) [10,12,18]. From west to east, the average temperature increases and humidity decreases, affecting the persistence and infectivity of the larvae [28].

*T. spiralis* is the most commonly detected *Trichinella* species in pigs in Argentina. While *T. pseudospiralis* was identified in a single pig from the province of Santa Cruz [29], no further detections of this species in pigs or other animals have been reported since. In addition to pigs, *T. spiralis* has been observed in various domestic and wild animals throughout Argentina. It has been detected in a dog and a cat from Neuquén, as well as in wild species such as armadillos (*C. villosus*) from Buenos Aires; *Rattus* spp. from Buenos Aires, Neuquén, and Río Negro; *Rattus norvegicus* from Neuquen; wild boars from Entre Ríos and Neuquén; pumas (*P. concolor*) from Catamarca; *O. flavescens* from Río Negro; and both *D. albiventris* and *L. crassicaudata* from Buenos Aires [30–35]. Furthermore, *T. patagoniensis* has been detected exclusively in Argentina, specifically in pumas from the provinces of Catamarca, Córdoba, Neuquén, Río Negro, and Santa Cruz [6]. Although there are additional reports of *Trichinella* larvae in wild and domestic animals, species-level identification has often been challenging. These findings underscore the widespread circulation of *T. spiralis* across various provinces in Argentina.

In Chile, *T. spiralis* is the only species that has been isolated in both domestic and wild animals. Specific detections include domestic pigs [30], wild boars from La Araucanía and Los Ríos [36], *R. norvegicus* from the Metropolitan Region [30], cougars (*P. concolor*) from Bío Bío and Ñuble [21,37], American minks (*Neovison vison*) from Los Ríos [38], and kodkods (*Leopardus guigna*) from Ñuble [39]. In countries neighboring Argentina, there is no information on *Trichinella* at the species level. In Brazil, the seroprevalence of *Trichinella* in wild boars has been documented in São Paulo, Mato Grosso, Santa Catarina, and Rio Grande do Sul states [40,41]. In Bolivia, antibodies against *Trichinella* spp. have been detected in humans [42] and pigs [43], with earlier reports indicating positive results via digestion and ELISA but not PCR in 1992 [44]. Despite Uruguay's proximity to Argentina and similar dietary practices, including the consumption of raw pork products, there are no current documented reports of *Trichinella* in animals [45]. Finally, there is no information on the presence or absence of *Trichinella* spp. in Paraguay [7].

It is important to note that the samples from wild animals analyzed in the present study were obtained from two sources: hunted animals and those found deceased along roadways. Consequently, while the presence of *Trichinella* in these samples can provide valuable insights into the circulation of the parasite in a specific locality, its absence should be interpreted with caution. Given that these samples represent a convenience sample, it is not possible to definitively conclude the absence of *Trichinella* species in the environment. Therefore, while our findings are informative, they do not necessarily rule out the presence of *Trichinella* species in the studied area, and further, more systematic sampling would be required to draw more definitive conclusions about the parasite's distribution.

This study highlights the complexity of *Trichinella* transmission in Argentina, where anthropogenic, ecological, and climatic factors converge to sustain endemicity. Addressing these challenges requires interdisciplinary collaboration to mitigate risks to both human and animal health.

**Author Contributions:** Conceptualization, F.A.F., M.M.R. and M.I.P.; methodology, F.A.F., M.M.R. and M.I.P.; software, F.A.F. and M.I.P.; validation, F.A.F., T.A., M.W. and S.A.; formal analysis, M.I.P. and M.M.R.; investigation, F.A.F., M.I.P. and M.W.; resources, F.A.F., M.M.R. and M.I.P.; data curation, M.I.P., C.L. and F.A.D.; writing—original draft preparation, F.A.F., M.I.P., M.M.R. and T.A.; writing—review and editing, M.I.P., M.W., S.A., G.D., A.H., C.C., C.L., F.A.D. and M.M.R.; visualization, F.A.D., G.D., A.H., and C.C.; supervision, M.I.P. and M.M.R.; project administration, M.M.R.; funding acquisition, F.A.F. and M.M.R. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** No animal was killed for the purpose of this study, and no representative sampling was conducted. All performed analyses were based on samples submitted by SENASA or private veterinarians.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author due to privacy.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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