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Research paper

Reprint of: Comparison between *Trichinella patagoniensis* and *Trichinella spiralis* infection in BALB/c mice☆

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ABSTRACT

In Argentina, trichinellosis is an endemic disease acquired mainly through consumption of raw pork infected with nematodes larvae from the *Trichinella* genus. For years, the only species involved in outbreaks in humans and pig foci in Argentina was *Trichinella spiralis*. In 2008 the presence of a new *Trichinella* taxon from a cougar (*Puma concolor*) was detected and recorded in the province of Rio Negro, Argentina, and the finding was established as a new species in 2012: *Trichinella patagoniensis*. To the best of our knowledge, there is no information available on the intestinal phase and antibody response in a susceptible host during *T. patagoniensis* infection. Therefore, our research has been designed to study experimental infection with *T. patagoniensis* compared to infection with *T. spiralis* in BALB/c mice. One hundred and twenty eight BALB/c mice were divided into two groups and individuals in each group were infected per os with 500 larvae of *T. patagoniensis* or 500 larvae of *T. spiralis*, respectively. After that, they were euthanized on different days. Adult worm recovery from small intestines and artificial digestion of each carcass was performed. Histopathology of small intestines was performed using hematoxylin-eosin staining. Systemic cytokines and antibody kinetics were evaluated. Intestinal adult worm recovery of *T. patagoniensis* and *T. spiralis* took place until day 17 and 25, respectively. Systemic IFN-γ, IL-10, and TNF showed significant variations in *T. patagoniensis* infected mice. Seroconversion was detected in animals as from 15 days post-infection (pi) for both *T. patagoniensis* and *T. spiralis,* reaching the highest OD value at 42 days pi. Similar microscopic lesions were observed in the small intestine from mice infected with the same dose of *T. spiralis* and *T. patagoniensis*. Our findings contribute new information regarding the intestinal phase and the antibody kinetics of *T. patagoniensis* in BALB/c mice.

1. Introduction

Trichinellosis is a widespread zoonosis transmitted through the consumption of raw or undercooked meat and meat sub-products from animals infected with *Trichinella* spp. (Pozio, 2015). In Argentina, it is an endemic disease transmitted mainly by pigs. In 2018, the National Service for Health Surveillance notified 1242 cases in humans. The provinces that recorded the highest number of human cases were Cordoba (44.52 %), Buenos Aires (19.48 %), Mendoza (15.37 %), San Luis (8.05 %), and Santa Fe (7.57 %) (Anonymous, 2019).

For years, the only species involved in outbreaks in humans and pig foci in Argentina was *Trichinella spiralis* (Pasqualetti et al., 2014). However, in 2008 the presence of a new *Trichinella* taxon from a cougar (*Puma concolor*) was detected and recorded in the province of Rio Negro, Argentina, and the finding was established as a new species in 2012: *T. patagoniensis* (Krivokapich et al., 2012, [Krivokapich et al., 2008]

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2008). It was demonstrated that domestic cats and mice are more susceptible to the *T. patagoniensis* infection than rats and pigs (Krivokapich et al., 2012; Ribicich et al., 2013). *T. patagoniensis* can reach the adult stage but is not able to complete the entire life cycle in chickens (*Gallus gallus domesticus*) (Pasqualetti et al., 2014). Moreover, it can infect guinea pigs (*Cavia porcellus*), reaching high parasitic loads, hence it was considered as a potential host of this parasite species (Fariña et al., 2017).

The intestine constitutes the initial *Trichinella* point of contact with its host. The length of the enteral phase and the number of newborn larvae (NBL) produced by females in the host guts determine the length of the disease (Ding et al., 2016). The presence of adult worms in the intestinal niche, as well as the release of NBL by gravid females, stimulates a local inflammatory reaction that culminates with the expulsion of the intestinal worms (Wakelin, 1978). Villous atrophy, crypt hyperplasia, mucosal mast cell hyperplasia, hyperplasia of goblet cells, and an increase in the mucous permeability are produced during *T. spiralis* intestinal infection in mice. *Trichinella* infection distinctive immunological characteristics such as eosinophilia, mastocytosis, and IgE hypergammaglobulinemia are induced by cytokines of the Th2 subset (Finkelman et al., 1997).

To the best of our knowledge, there is no information on the intestinal phase and antibody response in *T. patagoniensis* infection in a susceptible host. Thus, the present research aimed to study the experimental infection with *T. patagoniensis* compared to that of *T. spiralis* in BALB/c mice.

2. Materials and methods

2.1. Parasites

T. patagoniensis (ISS2311) and *T. spiralis* (ISS3558) isolates were maintained in BALB/c mice by serial passages. Muscle larvae (ML) were recovered from infected mice by means of artificial digestion of the carcasses (Nöckler et al., 1995).

2.2. Animals

Seven-week-old female BALB/c mice from the vivarium at the Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina were used for the present study. Animals were maintained in a conventional animal cage exposed to 12 h light cycle (lights on between 06:00 to 18:00). Animals were fed with commercial rodent pellets and water, *ad libitum*.

The experimental protocol was approved by the Institutional Committee for Use and Care of Laboratory Animals of the Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina (CICUAL), under permit number 2014/01.

2.3. Intestinal phase

One hundred and twenty eight female BALB/c mice were divided into two groups of 64, and of the individuals in each group were infected per os with 500 *T. patagoniensis* larvae or 500 *T. spiralis* larvae, respectively. Four animals were euthanized on the first 10 days and then day 12, 15, 17, 18, 20, and 25 post-infection (pi). The entire length of the small intestine was removed, opened longitudinally, cut into 5 cm-long pieces, and then placed in 50 mL falcon tubes with saline solution and incubated for 4 h at 37 °C. The solution was poured into a Petri dish, and a worm count was performed using a LEICA™ DMi1 inverted microscope (Leica, Wetzlar, Germany). Four animals were used as negative control.

2.4. Small intestine histopathology

Samples from the duodenum, jejunum, and ileum were studied in an attempt to demonstrate qualitative microscopic changes. Samples were fixed in 10 % phosphate-buffered formalin, embedded in low-fusion paraffin, cut in 5 μm-thick sections, and finally stained with hematoxylin-eosin. Slide-mounted sections were examined using a LEICA™ DM500 microscope (Leica, Wetzlar, Germany). Three additional animals were used as uninfected controls.

2.5. Cytokine assay

Blood samples from sedated individuals were taken by means of intracardiac puncture and stored at −80 °C. A systemic cytokine profile was evaluated during the intestinal phase of *T. patagoniensis* infection from sera of the animals euthanized on days 0 (negative control), 2, 4, 6 and 9 pi, using a mouse Th1/Th2/Th17 cytometric bead array kit (BD Biosciences, San Jose, CA) (Morgan et al., 2004). The concentrations of IL-2, IL-4, IL-10, IL-17A, IFN-γ, TNF, and IL-6 were measured on a dual-laser FACSCalibur™ flow cytometer (BD Biosciences). The kit protocol was used and measurements were taken with the FCAP Array™ Software v1.0.1 (BD Biosciences).

2.6. Anti-Trichinella spp. antibody kinetics

The larval excretory-secretory (E/S) products used as antigens for the serological tests were prepared from *T. spiralis* ISS643, which was maintained in CF1 mice, according to Larrieu et al. (2004).

Serum antibody levels were evaluated in five BALB/c mice experimentally infected with 500 L1 of *T. patagoniensis* and 500 L1 of *T. spiralis,* respectively, using an in-house ELISA with E/S *T. spiralis* antigen on days: -5, 0, 5, 10, 15, 20, 30, 42 and 70 pi. Blood samples were collected by means of tail artery puncture at different times pi, and then samples were centrifuged at 200 *g* for 10 min and stored at −20 °C.

Flat-bottomed, polyvinyl, microtitre plates (MaxiSorp™; Nunc, Roskilde, Denmark) were coated with the antigen (5 μg/mL and 100 μL/well) in a carbonate-bicarbonate buffer, pH 9.6, overnight at 4 °C. Each well was then washed three times with wash buffer (phosphate-buffered saline, pH 7.2, containing 0.05 % Tween 20). The wells were then blocked with blocking buffer (3% albumin in wash buffer) for 30 min at 37 °C. After three more rinses with wash buffer, 100 μL of a diluted test serum, (1:250 in blocking buffer) were added to each well, and the plates were incubated for 30 min at 37 °C. Then a horseradish-peroxidase rabbit anti-mouse-IgG conjugate (whole molecule; Sigma A-5670), diluted at 1:3000 in blocking buffer, was left in the wells for 30 min at 37 °C. After the final three washes, 0.04 % orthophenylenediamine and 0.04 % H_2O_2 in a citrate–phosphate buffer (pH 5) were added as substrate. After incubation for 5 min in a dark box, the optical densities (OD) of the well contents were read at 450 nm in an automated microplate reader (BioTek ELx800).

2.7. Statistical analysis

All analyses have been conducted using R (R Core Team, 2018). In order to establish whether there were significant differences in the recovery of adult worms, a non-parametric Kruskal-Wallis analysis of variance was used, taking into account two factors: parasite species (*T. spiralis* and *T. patagoniensis*), and days pi. Dunn's post-test was used for comparisons. Differences were considered significant when $P < 0.050$.

Cytokines were analyzed by a factorial ANOVA using IL-2, IL-4, IL-10, IL-17A, IFN-γ, TNF, and IL-6 values as dependent variables and again the parasite species and days pi as factors. The Bonferroni test was used when significant differences were found in the levels of the factors, or in the term of interaction. Differences were considered significant when $P < 0.050$.

Antibody kinetics were analyzed with a Repeated Measures ANOVA, with the OD value as the dependent variable; samples from the same mouse were classified as repeated measures, with parasite species and

days pi as factors. A mixed linear model with a level of significance set a 5% was applied.

3. Results

3.1. Intestinal phase

Intestinal adult worm recovery of *T. patagoniensis* took place until day 17, in 3 out of 4 infected animals (Fig. 1). No adult worms were recovered after day 18. Intestinal *T. spiralis* adult worm recovery took place until day 25.

The interaction between parasite species and days pi was significant ($P = 0.042$). The recovery of adult worms showed statistical differences in the following comparisons: day 9 on against day 0 for *T. spiralis* ($P = 0.035$); and day 12 on against day 0 for *T. patagoniensis* ($P = 0.039$).

3.2. Cytokine assay

Systemic cytokine levels are shown in Table 1. IFN-γ values progressively increased after infection with *Trichinella*, until they reached a peak at 9 days pi in animals infected with either *T. spiralis* or *T. patagoniensis*. The concentration of IL-10 reached a peak at 4 days pi in animals in both experimental groups. The level of TNF showed a slight increased on day 3 pi.

The interaction between days pi and parasite species was significant for IFN-γ ($F = 3.45$, $P = 0.019$), IL-10 ($F = 8.86$, $P < 0.001$), and TNF ($F = 4.55$, $P = 0.002$). The concentration of IFN-γ showed significant differences from day 2 pi onwards compared to day 0 ($F = 120.24$, $P < 0.001$) for both *T. patagoniensis* and *T. spiralis*. Significant differences were seen in IL-10 values between day 0 and days 2, 4, and 6 ($F = 382.47$, $P < 0.001$). There were also significant differences in TNF between day 0 and day 4 ($F = 155.10$, $P < 0.002$) for both *T. patagoniensis* and *T. spiralis*.

Significant differences were also observed in the concentrations of IFN-γ ($F = 120.24$, $P < 0.001$), IL-10 ($F = 19.40$, $P < 0.001$) and TNF ($F = 10.20$, $P = 0.004$) along the days pi in *T. spiralis* and *T. patagoniensis* infected animals. IL-2, IL-4, IL-6, and IL-17 showed no significant variation during the study period ($P > 0.050$).

3.3. Muscle larvae recovery

Muscle larvae were recovered as from days 18 and 20 pi onwards from mice infected with *T. spiralis* or *T. patagoniensis*, respectively.

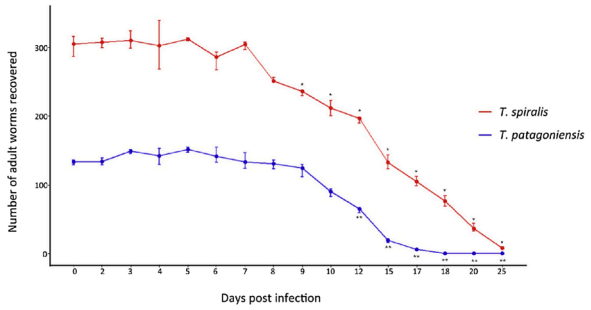

Fig. 1. Intestinal parasitic burden in BALB/c mice infected with 500 L1 of *Trichinella patagoniensis* or *Trichinella spiralis*. Intestinal parasitic burden is the number of worms recovered from the intestine. Each time point represents the median value and range of 4 mice for each parasite species. Differences among dates within parasite species were analyzed with the Kruskal-Wallis non-parametric ANOVA. Differences between parasite species at specific dates were determined with Dunn´s post-test. Asterisks (* and **) indicate statistically significant differences in the following comparisons: day 9 on against day 0 for *T. spiralis* ($P = 0.035$); and day 12 on against day 0 for *T. patagoniensis* ($P = 0.039$).gr1.

3.4. Small intestine histopathology

Hyperemia and edema were observed in the mucosae with scarce lymphocytic infiltrate as from day 1 pi in both *T. patagoniensis* and *T. spiralis* infected animals. As from day 2 pi, goblet cells and some eosinophils were observed infiltrating the mucosae. On days 4, 5, 6, and 7 pi, active goblet cells with abundant mucin secretion were observed in the intestinal lumen. The presence of edema and an infiltrate of inflammatory cells, such as plasmatic cells, macrophages, eosinophils, neutrophils, and lymphocytes, were found. From day 15 pi on, lymphocytic and plasmatic cells started to prevail in the infiltrate. Hyperplasia of Peyer's patches was observed as from day 20 pi (Fig. 2). Lesions were more evident in the jejunum and ileum than in the duodenum. From day 1–17 pi and from day 1–25 pi, adult and pre-adult worms were found invading intestinal epithelial cells in animals infected with *T. patagoniensis* and *T. spiralis*, respectively (Fig. 3).

3.5. Serum anti-Trichinella spp. antibodies

Seroconversion for *T. patagoniensis* and *T. spiralis* was detected in animals as from day 15 pi, reaching the highest OD value at 42 days pi. OD values remained positive throughout the experiment (Fig. 4). Animals in the control group did not present positive IgG antibodies for *Trichinella* spp. The interaction between parasite species and days pi proved significant ($P = 0.020$). The Newman-Keuls post-test was used to determine the significance of differences in mean OD between parasite species at specific days pi; differences were significant on day 20, 42, and 70 pi ($P < 0.050$).

4. Discussion

Our study showed that adult worms were recovered until day 17 and 25 pi from BALB/c mice infected with 500 L1 of *T. patagoniensis* and *T. spiralis*, respectively. Results obtained from mice infected with *T. spiralis* differed from those found by Dvorožňáková et al. (2016) and Ding et al. (2016), in which they recovered adult worms from BALB/c mice infected with the same infection dose and species until day 18 and 17 pi, respectively. In another study using mice infected with 100 and 400 *T. spiralis* or *T. pseudospiralis* L1, Wakelin et al. (1994) recovered a small number of worms after 15 days pi from the animals infected with 400 L1. Variability in the length of *T. spiralis* expulsion phase, particularly in mice, seems to be dependent upon different factors, such as the experiment methodology, the host, the parasite strain, and inoculum dosage (Kozar and Kozar, 1965; Ding et al., 2016; Wakelin et al., 1994).

Under the present conditions, the *T. spiralis* expulsion phase was longer compared with *T. patagoniensis*. Krivokapich et al., 2012; Krivokapich et al. (2012) found that *T. patagoniensis* infectivity in mice was approximately four times lower than that of *T. spiralis*. Therefore, it could be hypothesized that the duration of the expulsion phase could be related to infectivity levels in both species.

Bruschi and Chiumiento (2012) stated that at least three concepts support the host's intestinal expulsion of *Trichinella* spp.: 1) the parasite is eliminated by immunological mechanisms, 2) the immune response is directed against stage-specific antigens, and 3) the inflammatory response regulated by T-helper lymphocytes also plays a fundamental role in the expulsion of the parasite.

Both *T. patagoniensis* and *T. spiralis* infection induced an intense inflammatory reaction at the intestinal level during the first days of infection. Results were in line with what was described for *Trichinella* spp. by Despommier (1983) and Weatherly (1983). *T. patagoniensis* and *T. spiralis* microscopic lesions found were consistent with findings obtained by other authors in goats (Reina et al., 2000), mice (Weatherly, 1983) and humans (Kocięcka, 2000), and were produced as a result of the parasite's intestinal phase.

In this study, no differences were found between small intestine microscopic lesions in mice infected with the same dose of *T. spiralis* and

Table 1
Effect of time post-infection on serum cytokine levels (pg/mL) from mice experimentally infected with 500 L1 larvae of *Trichinella patagoniensis* or *Trichinella spiralis*.

		Days post infection				
cytokine (pg/mL)	species	0	2	4	6	9
IFN g	***T. spiralis***	382 ± 095	817 ± 044	155 ± 2,1	2064 ± 404	373 ± 686
	T. patagoniensis	342 ± 063	694 ± 063	1201 ± 192	1677 ± 228	2726 ± 207
IL-2	***T. spiralis***	189 ± 061	195 ± 040	203 ± 038	195 ± 037	208 ± 059
	T. patagoniensis	190 ± 069	228 ± 055	117 ± 065	195 ± 037	190 ± 054
IL-4	***T. spiralis***	237 ± 051	245 ± 061	224 ± 071	232 ± 054	227 ± 082
	T. patagoniensis	228 ± 081	240 ± 054	201 ± 059	2,3 ± 076	218 ± 067
IL-6	***T. spiralis***	563 ± 025	514 ± 058	582 ± 043	575 ± 064	581 ± 088
	T. patagoniensis	576 ± 011	447 ± 051	478 ± 034	511 ± 068	607 ± 091
IL-10	***T. spiralis***	144 ± 041	428 ± 027	1767 ± 196	913 ± 070	169 ± 037
	T. patagoniensis	155 ± 056	419 ± 043	1529 ± 112	938 ± 205	184 ± 015
IL-17A	***T. spiralis***	216 ± 028	218 ± 051	216 ± 045	292 ± 057	262 ± 034
	T. patagoniensis	217 ± 048	236 ± 041	226 ± 034	301 ± 075	321 ± 081
TNF	***T. spiralis***	502 ± 061	547 ± 024	1012 ± 110	579 ± 057	485 ± 017
	T. patagoniensis	497 ± 044	492 ± 056	910 ± 1,2	581 ± 1,3	530 ± 085

Data are expressed as mean ± SD, n = 4 per cytokine, parasite species, and days post infection.

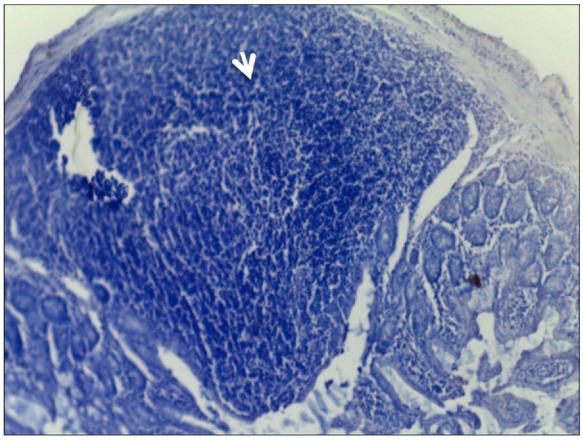

Fig. 2. Representative micrograph illustrating the small intestine histology of BALB/c mice infected with 500 *Trichinella patagoniensis* or *Trichinella spiralis* L1 larvae, on day 20 post-infection. The image shows marked hyperplasia in Peyer's patches (white arrow). No differences attributable to the parasite species were observed in the intestinal histology. H&E staining. Magnification 100 × .gr2.

T. patagoniensis. Similar results were obtained by Airas et al. (2012) in rats infected with *T. nativa* and *T. spiralis*. Moreover, Sukhdeo and Meerovitch (1980) were unable to find any differences in the intestinal inflammation elicited by temperate, Arctic or African isolates, using 500 L1 in mice, even though adult worm survival differed considerably.

Systemic cytokine levels were evaluated during the intestinal phase (days 2, 4 and 6) and the beginning of the systemic phase of infection (day 9). IFN-γ showed a progressive increase, reaching a peak at 9 days pi both in the animals infected with *T. patagoniensis* and *T. spiralis*. Yu et al. (2003) obtained similar results in C57BL/10 mice, observing that *T. spiralis* evoked mixed systemic Th1/Th2 response and inhibited Th17, with a predominance of the Th1 response during intestinal phase (Yu et al., 2013). In turn, Frydas et al., 2001 observed a slight increase in IFN-γ levels on day 1 pi, which remained relatively constant until day 14 pi.

The concentration of IL-10 started to grow as from day 2 pi, reaching a maximum value on day 4 pi; then it began to decrease until it reached basal values on day 9 pi. These results are in line with those obtained by Yu et al. (2013). In contrast, Frydas et al. (2001) found rising IL-10 serum values throughout the intestinal phase of *T. spiralis*. According to Mosman (1994), IFN-γ had an antagonistic effect in connection with IL-10, because it inhibited IL-10 secretion. In a study with non-infected mice treated with neutralizing anti-IL-10 receptor antibodies, as well as in knockout mice for the IL-10 coding gene, Helmby and Grencis (2003a) observed that the mice were highly susceptible to primary *T. spiralis* infection, demonstrating a marked delay in the expulsion of adult worms and a significant increase in muscle parasite load. The balance between IL-10 and IFN-γ seems to determine the development of immunity against the different parasite life stages (Helmby and Grencis, 2003b).

The relevance of TNF-α lies in the fact that it is required for the induction of a Th2 response against gastrointestinal helminths (Bruschi and Chiumiento, 2012). TNF values evidenced a slight increase on day 4

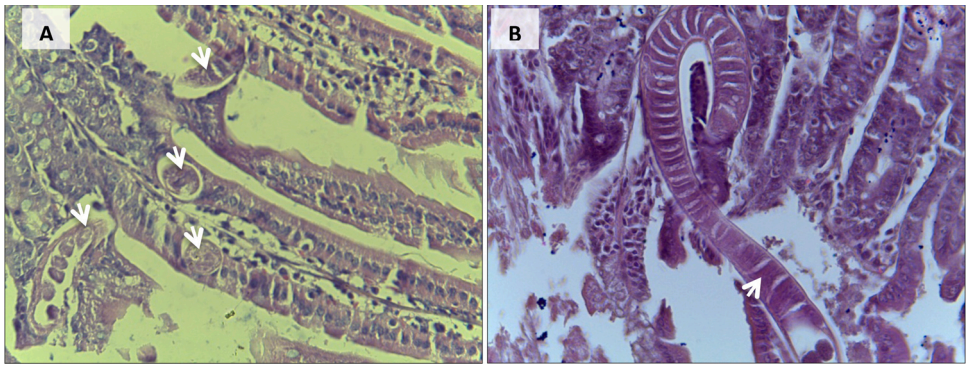

Fig. 3. Light micrograph of small intestinal section stained with H&E from BALB/c mice infected with *Trichinella patagoniensis* or *Trichinella spiralis* L1 larvae, on days 2 (A) and 14 (B) post-infection. The white arrows indicate the adult worms inside the epithelial cells of the small intestine. No differences attributable to the parasite species were observed in the intestinal histology. Magnification 400 × .gr3.

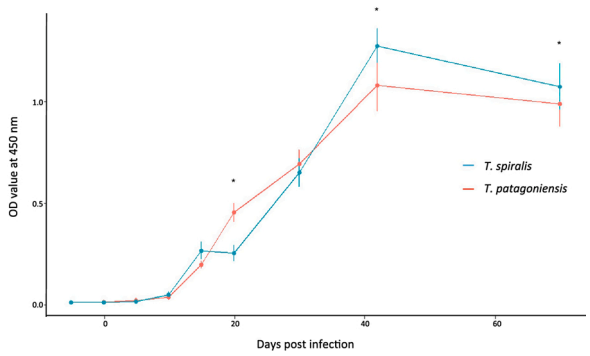

Fig. 4. Anti-*Trichinella* spp. optical density (OD) serum values from mice experimentally infected with 500 *Trichinella patagoniensis* or *Trichinella spiralis* L1 larvae. Each time point represents the mean value ± SD of 5 mice for each parasite species. Differences among dates within parasite species were analyzed with a repeated measure ANOVA, with OD value as the dependent variable, samples from the same mouse were classified as repeated measures, with parasite species and days pi as factors. A mixed linear model with a level of significance set a 5% was applied. Asterisks (*) indicate statistically significant differences in mean OD between *T. patagoniensis* and *T. spiralis* at days 20, 42, and 70 pi ($P < 0.050$).gr4.

pi, and then they decreased to basal levels. Similar results were obtained by Yu et al. (2013). On the contrary, Frydas et al. (2001) observed a marked increase in TNF-α values that remained relatively constant throughout the intestinal phase.

The evaluation of the cytokine profiles in this experiment was performed as a general approach to the host response against *T. patagoniensis* invasion.

Each *Trichinella* stage can evoke a protective immune response against cuticular or E/S antigens (Bruschi and Chiumiento, 2012). Host immune response, reflected in a specific species/isolate variation, can lead to a rapid expulsion of adult intestinal worms or to systemic NBL destruction (Bolas-Fernández, 2003; Dvorožňáková et al., 2016).

Several researchers have demonstrated that there exists a correlation between *Trichinella* spp. infective dose and the moment when seroconversion appears in rats (Airas et al., 2012), mice (Dvorožňáková et al., 2010; Reiterová et al., 2009;), and pigs (Kapel and Gamble, 2000; Kapel et al., 1998; Kapel, 2001; Nöckler et al., 1995; Oltean et al., 2012). Reiterová et al. (2009) observed IgG seroconversion at 40 and 50 days pi in mice experimentally infected with 50 and 5 *T. spiralis* L1 larvae, respectively.

In our study, seroconversion occurred in all infected animals. The antibody response showed similar dynamics in *T. patagoniensis* and *T. spiralis* infected animals. An increase in the mean value of OD in mice infected with *T. patagoniensis* and *T. spiralis* was observed from day 15 pi until its peak on day 42 pi. Then, OD values slightly decreased at day 70 pi. Wang et al. (2012) observed seroconversion from week three onwards, reaching its peak on day 42 pi in mice infected with 500 *T. spiralis* L1. Frydas et al. (2001) showed seroconversion from day 10 pi on, in BALB/c mice infected with 220 *T. spiralis* L1. Also, Hu et al. (2020) showed seroconversion at 18 days pi in BALB/c mice infected with 200 *T. spiralis* L1 larvae, which peaked at 24 days pi.

OD values in animals infected with 500 *T. patagoniensis* and *T. spiralis* L1 larvae show statistically significant differences on days 20, 42, and 70 pi. These differences could be attributed to *T. spiralis* higher immunogenicity, as shown by Pozio et al. (2002), who found higher anti-*Trichinella* IgG levels in horses experimentally infected with *T. spiralis* compared to those infected with *T. murrelli*. In contrast, Airas et al. (2012) did not find statistically significant differences between OD values in rats infected with 2000 L1 larvae of *T. nativa versus T. spiralis*.

Under the study conditions, animals infected with *T. patagoniensis* showed a more rapid expulsion phase than *T. spiralis*. Both *T. patagoniensis* and *T. spiralis* induced a similar inflammation pattern at the intestinal level. Although antibody kinetics and IL-10, TNF and IFN-γ values showed differences between the two species, both *T. patagoniensis* and *T. spiralis* presented similar dynamics.

Our findings contribute new information regarding the intestinal phase and the antibody kinetics of *T. patagoniensis* in BALB/c mice. It would be interesting to characterize the cells involved in the immune response against *T. patagoniensis* and the cytokines produced by them in a further study.

CRediT authorship contribution statement

Fernando A. Fariña: Conceptualization, Methodology, Writing - original draft. **Mariana I. Pasqualetti:** Data curation, Writing - original draft, Resources. **Clara Bessi:** Investigation, Resources. **Mariano E. Ercole:** Visualization, Investigation. **Claudia Vargas:** Investigation, Validation. **Patricia Arbusti:** Resources, Investigation. **Graciana Ayesa:** Resources, Investigation. **M. Mabel Ribicich:** Writing - review & editing, Data curation.

Declaration of Competing Interest

The authors report no declarations of interest.

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