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Short Communication

Susceptibility of chickens (*Gallus gallus domesticus*) to *Trichinella patagoniensis*



M. Pasqualetti^{a,*}, F. Fariña^{a,c}, E. Falzoni^d, N. Cardillo^{a,c}, T. Aronowicz^a,
S. Krivokapich^b, A. Rosa^a, M. Ribicich^a

^a Cátedra de Parasitología y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Av. Chorroarín 280, C1427CWO Buenos Aires, Argentina

^b Departamento de Parasitología, INEI, ANLIS, Dr. Malbrán, Buenos Aires, Argentina

^c Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

^d Cátedra de Enfermedades Infecciosas, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Av. Chorroarín 280, C1427CWO Buenos Aires, Argentina

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ABSTRACT

Trichinella spp. is a genus of parasites that is widespread all over the world. In Argentina, *T. spiralis* was for years the only species involved in human and animal outbreaks. During the last decade, *T. patagoniensis*, a new *Trichinella* species, was discovered in Argentina. Up to now, this species has only been found in cougars (*Puma concolor*). Experimental infections in pigs, cats, mice and rats with this new genotype showed that cats and mice were the most susceptible hosts. The aim of the present work was to evaluate the susceptibility of chickens to infection with *T. patagoniensis*. In order to study the intestinal and muscular phase, and the histopathological changes, 27 Leghorn chickens were inoculated *per os* with 1000 muscle larvae of *T. patagoniensis* and were euthanized on days 4, 5, 6, 7, 11, 14, 21, 28 and 35. Adult worms of *T. patagoniensis* were recovered from the small intestine of chickens up to day 14 p.i. Gross examination of small intestine showed a moderate congestive appearance. Microscopically, an inflammatory response with lymphocytes and eosinophils in lamina propria, slight hyperemia, oedema and some haemorrhagic areas were detected. Lesions observed in chickens were similar to those described in different animal species during the intestinal phase. No muscular larvae were recovered from the muscle samples. These results suggest that *T. patagoniensis* is not capable to complete its entire life cycle in chickens.

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1. Introduction

Trichinellosis is a food-borne zoonotic disease transmitted in Argentina primarily by the consumption of undercooked or raw pork meat (Ribicich et al., 2005). The average number of people infected per year from 2008 to 2010 was 417. In 2013 the number of human cases

notified by the Argentinian Ministry of Health was 1159 (Anonymous, 2013a).

Although molecular analysis is not always performed, the main species of *Trichinella* frequently involved in human and animal outbreaks is *Trichinella spiralis*. Nevertheless, in 2004 near the locality of Trapalcó, Río Negro, Argentina, Krivokapich et al. (2008) isolated a new genotype of the genus *Trichinella* in a cougar: *Trichinella* T12. Moreover, in 2008 near the locality of El Calafate (Santa Cruz province) and in 2009 in the district of La Paz (Catamarca province), *Trichinella* T12 muscle larvae were

* Corresponding author. Tel.: +54 1145802820.
E-mail address: mpasqualetti@fvvet.uba.ar (M. Pasqualetti).

identified from cougars hunted in those places. This new genotype is now a new species of the *Trichinella* genus: *Trichinella patagoniensis* (Krivokapich et al., 2012).

Cougars inhabit many parts of Argentina. They occupy a territory that extends from north to south of the country. The cougar is an opportunistic predator which bases its diet on ungulates, but can feed on any available prey (Iriarte et al., 1990) such as reptiles, rodents or birds.

Researches in rats, pigs, and cats with *T. patagoniensis* showed that cats are the most suitable hosts for this parasite (Krivokapich et al., 2012; Ribicich et al., 2013).

Considering that *T. patagoniensis* has been found only in cougars, specially from the southern part of the country, and that birds are not only possible preys for cougars, but they also live in the same geographic area, and that no data concerning Trichinellosis in birds is available in Argentina, the purpose of the present study is to evaluate the infectivity of *T. patagoniensis* in chickens (*Gallus gallus domesticus*).

2. Materials and methods

Thirty-three five-week-old male Leghorn chickens were obtained from a commercial farm. Twenty-seven animals were inoculated *per os* with 1000 muscle larvae of *T. patagoniensis*, and six animals were used as uninfected control group. The *Trichinella* isolate (code ISS2311) was originally obtained from a naturally infected cougar from Patagonia (Argentina) and was maintained by periodic passages in CF1 mice.

T. patagoniensis larvae were identified by molecular methods (Krivokapich et al., 2012). Muscle larvae used for the inoculation of chickens were obtained by artificial digestion (Gamble et al., 2000) of the carcasses of four CF-1 mice that had been infected for 35 days.

Animals were housed under standard conditions. Food and water were given *ad libitum*. Twelve hours before necropsy, animals were deprived of food to reduce the amount of intestinal content.

The experimental study was approved under permit number 2012/2020 by the Institutional Committee for Use and Care of Laboratory animals of the Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina.

Animals were sedated by intra muscular injection of a mixture of ketamin (20 mg/kg body weight) and midazolam (2 mg/kg body weight) and then were euthanized with an intraperitoneal injection of 100 mg/kg body weight of pentobarbital sodium, according to the AVMA Guidelines for the Euthanasia of Animals (Anonymous, 2013b).

In order to recover adult worms, three chickens per day were euthanized on days 4, 5, 6, 7, 11, 14 and 21-post infection (p.i.). The entire small intestine of each animal was removed immediately after euthanasia. Intestines were opened longitudinally and then, gross gut contents were removed by dipping intestine gently into a beaker of saline twice. Thereafter, guts were cut into pieces of 5–10 cm in length and then were hung over a hook in a conical glass with 200 ml 0.9% saline at 37 °C for 4 h. Finally the rest of intestines were removed and samples were left to sediment for 1 h (Blair, 1983; Webster et al., 2004). Adult worms were recovered from the bottom of the conical glass,

Table 1

Total number of adult worms *T. patagoniensis* recovered from the gut of three chickens per day inoculated with 1000 muscle larvae.

Day post-infection	No of chickens	No worms recovered
4	3	2
5	3	4
6	3	0
7	3	1
11	3	1
14	3	2
21	3	0

identified according to Krivokapich et al. (2012) and counted using a stereoscopic microscope. Differentiation of males and females was based on size and the absence/presence of copulatory appendages.

Chickens were euthanized at days 14, 21, 28 and 35 p.i. skinned, and eviscerated. The whole pectoral, limb, abdominal, intercostal, tongue, and cervical muscles of each animal were subjected to artificial digestion. The reproductive capacity index (RCI) was calculated as the number of larvae collected after artificial digestion/number of larvae given *per os*. The presence of L1 in striated muscle was determined by the artificial digestion method described by Gamble et al. (2000). Animals euthanized on days 14 and 21 p.i. were used for both purposes, adult worms and muscle larvae recovery.

Six animals of the control group were euthanized on days 5 and 35 p.i. in groups of 3 chickens per day.

During the necropsy of each animal, samples of heart, lungs, liver, small intestine, gizzard, cecal tonsils, tongue, limbs and pectoral muscles were obtained for histopathological studies. Samples were fixed in 10% formaldehyde, embedded in paraffin, cut in 5 µm thick sections and finally stained with haematoxylin–eosin (H&E). They were examined by light microscopy.

3. Results

Intestinal worms were recovered from infected chickens on days 4, 5, 7, 11 and 14 p.i. (Table 1). Four male and six female adult worms were identified by microscopic observation (Fig. 1). The average length for male adults was 698 and 1164 for female. The presence of embryos inside female's uterus was not observed.

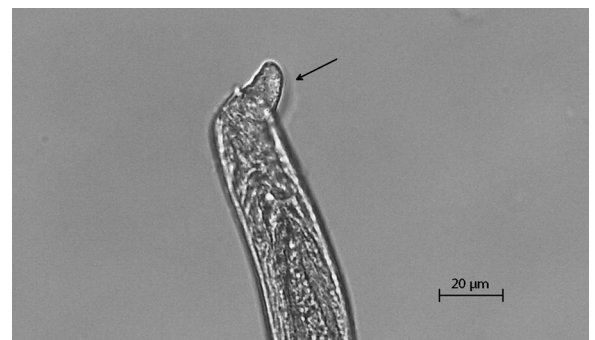


Fig. 1. Male of *T. patagoniensis* recovered from intestine of infected chicken. The copulatory appendages (arrow) are shown.

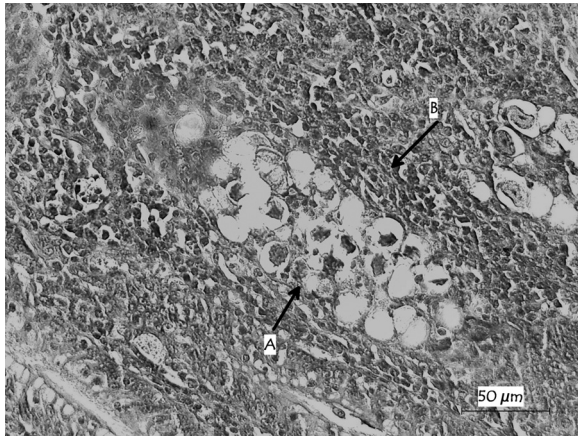


Fig. 2. Presence of *T. patagoniensis* worms (A) surrounded by slight interstitial inflammatory reaction (B) in small intestine of infected chicken.

Macroscopical and microscopical changes were observed in the small intestine. Gross examination of intestine showed a moderate congestive appearance. Microscopically, an inflammatory response with lymphocytes and eosinophils in lamina propria, slight hyperemia, oedema and some haemorrhagic areas were detected. Moreover, the presence of worms with moderate interstitial inflammatory reaction was observed (Fig. 2).

No gross or histopathological lesions were found in the rest of the samples analyzed.

Muscle larvae were not recovered from the carcasses neither from the infected nor from the uninfected group.

4. Discussion

Since the discovery of the new *Trichinella* species *T. patagoniensis*, some studies have been carried out leading to know this species better. Experimental assays in animals showed that *T. patagoniensis* had low infectivity in pigs and rats (Gonzalez Prous et al., 2011; Krivokapich et al., 2012). Moreover, the number of new borne larvae (NBL) produced by *T. patagoniensis* female *in vitro* over a 72 h period is lower than for *T. spiralis* and more similar to numbers reported previously for other sylvatic taxa (Krivokapich et al., 2012).

As it has been pointed out before, carnivores are at the top of the food chain representing the best hosts for *Trichinella* (Pozio, 2005). *T. patagoniensis* would not be the exception, Ribicich et al. (2013) showed that cats were susceptible and supportive hosts for this parasite reinforcing the theory that carnivores may act as reservoirs for encapsulated species (Pozio, 2005; Kapel, 2000). Krivokapich et al. (2012) suggested that this new parasite could be included inside the group of sylvatic encapsulated species.

Several authors have studied the effect of *Trichinella* in birds. Experimental infections with *T. spiralis*, *T. zimbabwensis* and *T. papuae* demonstrated that these parasites were not able to develop at the muscular phase in chicken (Gómez Barrio et al., 1989; Pozio et al., 1999, 2002) showing that these animals are not suitable hosts for these *Trichinella* species. Studies with *T. pseudospiralis* have proven that this parasite is able to develop the entire life

cycle in chicken (Lindsay et al., 1995; Pozio et al., 1999, 2002; Pozio, 2005), being the only species known up to now that can infect not only mammals, but also birds. Our results suggest that *T. patagoniensis* would not be able to develop the entire life cycle in chickens, hosts which have 42 °C of body temperature. According to Pozio (2005), the encapsulated species of the genus *Trichinella* only complete their life cycle in mammals in that they require a host body temperature ranging from 37 to 40 °C. Contrary to *T. patagoniensis*, the non-encapsulated species *T. pseudospiralis*, can complete its life cycle at host body temperatures ranging from 37 to 42 °C.

Once *Trichinella* L1 reaches a susceptible host, the small intestine is the primary site for the establishment, development and maturation of infective larvae (Weatherly, 1983). In our study, inflammatory response, slight hyperemia, oedema, and haemorrhagic areas were detected in the gut. Lesions observed in chickens were similar to those described by other authors in different animal species during the intestinal phase (Weatherly, 1983; Reina et al., 2000; Gamito Santos et al., 2009; Kocięcka, 2000). Intestinal worms were recovered from the small intestine until day 14 p.i., in agreement with previously published data about chickens experimentally infected with *T. spiralis* (Gómez Barrio et al., 1989). Nevertheless, the number of recovered worms in our study was lower than the values documented by other studies (Gómez Barrio et al., 1989; Ooi et al., 1984). An explanation to this could be related to the connection between chicken's age and resistance to parasite infection, as some authors have pointed out (Ackert et al., 1935; Idi et al., 2004). Ikeme (1973) reported that worm burdens were inversely proportional to host age. Besides, the low number of recovered adults in our study might have been a consequence of the number of infected larvae administered, which in comparison with other studies (Gómez Barrio et al., 1989; Ooi et al., 1984) was a low dose.

As there were no embryos in female worms or any larvae recovered from striated muscle and no lesions produced by larval migration, we can conclude that *T. patagoniensis* was not able to complete its life cycle. What is more, chicken would not act as a link in the epidemiologic chain of this parasite. Despite that the RCI values in experimentally infected chicken with *T. pseudospiralis* are apparently low (Lindsay et al., 1995; La Rosa et al., 2001), this fact does not exclude the possibility that carrion-eating avian species may function as better hosts, resulting in a higher RCI (La Rosa et al., 2001). Although *T. pseudospiralis* does not behave the same way as *T. patagoniensis* in terms of infecting chickens, we suggest that further studies should be conducted in carrion-eating avian species, in order to elucidate if these animals could act as susceptible hosts of *T. patagoniensis* due to its eating habits which are quite different from that of chicken.

Conflict of interest statement

No financial or personal relationships are maintained with other individuals or organizations that could inappropriately influence or bias this paper.

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