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Study of *Trichinella patagoniensis* in wild boars

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## Study of *Trichinella patagoniensis* in wild boars

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### **Highlights**

- *Trichinella patagoniensis* and *T. pseudospiralis* produced a rapid immunological response.
- *T. patagoniensis* had low infectivity in wild boars.
- OD values from muscle juices and sera samples had positive correlations.

### **Abstract**

Trichinellosis is a zoonotic disease, which represents a significant public health concern in some South American countries, such as Argentina and Chile. Its impact is essentially due to absence of adequate control measures on meat from game animals, as well as the presence of illegal slaughterhouses and the trade of meat products without being tested for this parasite. In Argentina, trichinellosis is an endemic disease. At present, *Trichinella spiralis*, *Trichinella patagoniensis*, *Trichinella pseudospiralis*, and *Trichinella britovi* have been detected in animals from Argentina. Until now, *T. patagoniensis* had only been found in mountain cougars (*Puma concolor*) in Argentina but there is limited information available. The present study intends to determine susceptibility, serological response and distribution of muscle larvae in wild boars infected with *T. patagoniensis*, *T. spiralis* and *T. pseudospiralis*. For each of the *Trichinella* species five wild boars were inoculated with 20,000 muscle larvae. Except for two specimens which died during the experiment, the animals were euthanized 19 weeks post infection (pi). Blood samples were collected throughout the study in order to determine the antibody kinetics. Also, nine muscle samples from each specimen were taken and analysed for determination of larval distribution. Additionally, four muscle samples were used to obtain muscle juices. Wild boars infected with *T. patagoniensis* showed little to no larvae in the muscle samples analysed while animals infected with *T. spiralis* and *T. pseudospiralis* had a significantly high larval load in all the samples analysed. Optical density (OD) values remained above the cut-off value throughout the

experiment. This is the first study to characterize the biological aspects of *T. patagoniensis* in wild boars.

Keyword: *Trichinella patagoniensis*; muscle larvae distribution; serological response; wild boars

## 1. Introduction

Trichinellosis is a zoonotic disease, which represents a significant public health concern in some South American countries, such as Argentina and Chile (Hidalgo et al., 2019; Pozio, 2007; Ribicich et al., 2005). Its impact is essentially due to absence of adequate control measures on meat from game animals, as well as the presence of illegal slaughterhouses and trade of meat products without being tested for this parasite (Dupouy-Camet, 2000; Pozio and Darwin Murrell, 2006; Pozio and Zarlenga, 2013; Ribicich et al., 2010). This parasite has a considerable impact on people's health, and affects the meat by-product markets, as well as its trade and economic repercussions (Pozio et al., 2009; Pozio and Zarlenga, 2005). The most prevalent animal species/ parasitic species association is that of pigs and *Trichinella spiralis* (Zarlenga et al., 2006), which is frequently involved in outbreaks in humans. *Trichinella* species found in Argentina are *T. spiralis*, *T. pseudospiralis*, *T. britovi*, and *T. patagoniensis*. This last one has only been found in mountain cougars (*Puma*

*concolor*) in Argentina but there is limited information available about that species (Krivokapich et al., 2012).

So far, *T. patagoniensis* is more frequently associated with carnivorous hosts rather than pigs (Krivokapich et al., 2012; Ribicich et al., 2013). Moreover, *T. patagoniensis* has been proven to be able to reach the adult stage in chickens (*Gallus gallus domesticus*). However no muscle larvae (ML) were recovered (Pasqualetti et al., 2014). In addition, *T. patagoniensis* ML are able to survive for long periods of time in decaying muscle tissue in guinea pigs (*Cavia porcellus*) (Fariña et al., 2017), and have some freezing tolerance in cat muscle (Krivokapich et al., 2012).

In some South American countries, (Bjorland et al., 1993; Fonseca-Salamanca et al., 2006; Ribicich et al., 2005) the incidence of outbreaks in humans entails a difficult problem, whose origin is connected to hog farming practices and meat consumption habits. In these countries, it is not infrequent to observe outdoor breeding where hogs are fed either with balanced pig feed or rubbish from domestic garbage disposal. Given these characteristics, there is a high risk of exposure for animals to get infected with different pathogens such as *Trichinella* spp. (Fariña et al., 2012), which represent a contributing factor for the appearance of trichinellosis outbreaks in humans.

Wild boars (*Sus scrofa*) are becoming more relevant in Argentina due to an increase in the consumption of their meat (Pisano et al., 2019). In the last decade, these

animals were involved in most of the sylvatic foci of *Trichinella* spp. (Poizio et al., 2009; SENASA, 2017).

The infective capacity of *T. patagoniensis* in wild boars is yet unknown. Hence, this study is intended to determine the role of wild boars in the cycle of *T. patagoniensis* by comparing their susceptibility, serological response, and ML distribution to those found for *T. spiralis* and *T. pseudospiralis*.

## **2. Materials and methods**

### **2.1.1 Parasites**

The isolates used in this study were *T. patagoniensis* (ISS2311, from a mountain cougar), *T. pseudospiralis* (Krivokapich et al., 2015 from a domestic pig), and *T. spiralis* (ISS1097, from a hybrid *Landrace* x *Yorkshire* pig). The parasites were maintained by means of serial passages using CF1 mice, and ML were recovered by artificial digestion (Gamble et al., 2000).

### **2.1.2 Animals**

Eighteen sixty-day-old castrated wild boars were purchased from an establishment in the province of Buenos Aires to be used in this study. Once the animals arrived,

their faeces were analysed using the Willis flotation technique, Dennis Stone Swanson, and Baermann's method (Ribicich et al., 2012). To evaluate their health condition, haematological parameters were measured. Blood samples were taken, to record the red and white blood cell counts (RBC and WBC, respectively), and anti-*Trichinella* antibodies were measured. The wild boars were clinically examined every two days. The physical examination consisted on checking respiratory rate, as well as posture, behaviour, and skin condition. The wild boars underwent a thirty-day adaptation period to the new environment in the experimental facility where the study was to be conducted.

### **2.1.3 Experimental design**

Fifteen wild boars were randomly divided into 3 groups of five animals. Each group was inoculated per os with one of the *Trichinella* spp. under study. Each wild boar received a dose of 20,000 ML. Three animals served as uninfected controls.

All animals were held at the experimental facilities of the Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina, in indoor stalls with a plastic slatted floor, and were fed off the shelf pig feed and water ad libitum.

The animals were slaughtered 19 weeks post infection (pi); euthanasia was performed with acepromazine maleate, ketamine and thiopental sodium (Leary et al., 2013).



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 2015/16.

## 2.2 Larval distribution

Muscle samples (20 gram each) from tongue, masseters, pork shoulder (*M. splenius*, *Mm. scalene*), oesophagus muscles, diaphragm, intercostal muscles, tenderloin (*M. psoas minor*), upper foreleg (*M. brachialis*, *M. tricipitis brachii*, *M. bíceps brachii*, *M. infraspinatus*, *M. supraspinatus*, *M. subclavius*, *M. cleidobrachialis*, *M. subcapularis*, *M. teres major*) and upper hind limb (*M. semitendinosus*, *M. semimembranosus*, *M. quadriceps femoris*, *M. gluteobiceps*, *M. gracilis*, *M. adductor*, *M. pectineus*) were taken from each wild boar and were analysed using artificial digestion (Gamble et al., 2000) to determine larval distribution. As no ML were recovered after the digestion of the 20 g samples from wild boars inoculated with *T. patagoniensis*, 100 g samples were subsequently analysed. All muscles were freed from fascia and tendons, and were individually processed using artificial digestion (Gamble et al., 2000). The recovered ML levels were expressed as larvae per gram (lpg).

## 2.3 Serology

Blood samples were collected by jugular venipuncture at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, and 19 weeks pi. The sera were separated by centrifugation and then stored at  $-80\text{ }^{\circ}\text{C}$  until the moment of use. Serum samples were analysed using an ELISA Kit (PrioCHECK® *Trichinella* Ab, Prionics AG, Schlieren-Zurich, Switzerland) following the manufacturer's instructions for duplicate testing.

#### **2.4 Muscle Juice**

After euthanasia, tissue samples from the tongue, diaphragm, upper foreleg, and upper hind limb were collected in conical containers and frozen at  $-20\text{ }^{\circ}\text{C}$  for 24 h to obtain the muscle juices. Afterwards, the samples were let to thaw at  $4\text{ }^{\circ}\text{C}$  for 18 - 24 h. Muscle juice samples were kept at  $-20\text{ }^{\circ}\text{C}$  until immunoassay was performed using the ELISA Kit.

#### **2.5 Statistical Analysis**

All analyses have been conducted using R (R Core team, 2018). ML recovery was analysed using Kruskal Wallis' variance analysis ( $P < 0.050$ ). Optical density (OD) values from sera and muscle juice were compared using the Spearman correlation test ( $\rho$ ). A repeated measure design was applied for the evaluation of the antibody kinetics with the three *Trichinella* species ( $P < 0.050$ ).

### 3. Results

The haematological parameters analysed were normal according to species (Casas-Díaz et al., 2015; Harapin et al., 2003). The RBC count was  $7.22 \pm 0.98 \times 10^{12}/L$ , and the WBC count was  $8.15 \pm 2.06 \times 10^9/L$ . No wild boars had anti-*Trichinella* antibodies prior to the experimental infection. In addition, parasites were not found in the faecal test performed.

*T. patagoniensis* ML were recovered from three out of five infected animals. ML were found primarily in the upper foreleg, diaphragm and tongue, although the larval load was the lowest in comparison to the larval load of the other *Trichinella* species used in this study.

The highest larval load was 1,812 lpg for *T. spiralis*, 134.3 lpg for *T. pseudospiralis* and 0.087 lpg for *T. patagoniensis*. The main muscles infected for the three *Trichinella* spp considered were tongue and diaphragm (Table 1). All *Trichinella* species under study showed moderate larval load in the pork shoulder, upper foreleg and tenderloin muscles. Statistical differences were found for the different muscle groups in the ML recovery from *T. spiralis*, *T. patagoniensis* and *T. pseudospiralis* ( $P < 0.050$ ).

The serological response was detected at different times pi in the wild boars. Seropositivity was observed at 2-4 weeks pi for *T. patagoniensis*, at 2 weeks pi for *T.*

*pseudospiralis* and at 3-4 weeks pi for *T. spiralis* (Fig. 1). All animals, except the control group, remained above the cut-off value until the end of the experiment (week 19 pi). Statistical differences were observed in OD values in all three groups at week 2 pi; also at week 2 pi, there were differences between *T. pseudospiralis* and *T. patagoniensis*, and between *T. pseudospiralis* and *T. spiralis* at week 3 pi, and at week 19 pi, between *T. patagoniensis* and *T. spiralis* ( $P < 0.050$ ).

Significant positive correlations between ELISA values from sera and muscle juice originating from the four muscle groups were demonstrated. The correlation was independent of the origin of the muscle juice ( $P < 0.050$ ). The mean rho values for the serum and diaphragm muscle juice was 0.99 for *T. patagoniensis*, 0.91 for *T. spiralis*, and 0.94 for *T. pseudospiralis*. The mean rho values for the serum and the tongue muscle juice was 0.97 for *T. patagoniensis*, 0.91 for *T. spiralis*, and 0.91 for *T. pseudospiralis*. The mean rho values for the serum and the upper foreleg muscle juice was 0.87 for *T. patagoniensis*, 0.95 for *T. spiralis*, and 0.89 for *T.*

*pseudospiralis*. The mean rho values for the serum and the upper hind limb muscle juice was 1 for *T. patagoniensis*, 0.93 for *T. spiralis*, and 0.92 for *T. pseudospiralis*.

One wild boar infected with *T. spiralis* and another one with *T. pseudospiralis* died unexpectedly between weeks 2 and 4 pi. No lesions associated to *Trichinella* infection were found in the respective necropsies.

#### **4. Discussion**

The present study has recorded the experimental infection of wild boars with *T. patagoniensis* for the first time and has shown the ability of this parasite to develop in this host. Nevertheless, *T. patagoniensis* showed low infectivity in wild boars. Similar results were found in pigs which were infected with *T. patagoniensis* (Krivokapich et al., 2012).

*T. patagoniensis* was first found in a cougar in Argentina (Krivokapich et al., 2012) and further studies showed its high capacity to infect domestic cats (Ribicich et al., 2013). Based on these observations, prior studies have concluded that carnivores may be suitable hosts for *T. patagoniensis* (Krivokapich et al., 2012; Pasqualetti et al., 2014; Pozio and Zarlenga, 2013, 2005; Reichard et al., 2017; Ribicich et al., 2013; Webster et al., 2002). *T. patagoniensis*' low infectivity in pigs seems to be more related to *T. nativa* and *T. murrelli* (Krivokapich et al., 2012). These last two species also showed a low level of infectivity in rats, pigs (Murrell et al., 2000; Pozio and Zarlenga, 2005) and wild boars (Kapel, 2001).

In wild boars infected with *T. patagoniensis*, ML were mainly recovered from diaphragm, tongue, and upper foreleg and no ML were found in masseters, nor intercostal muscle in any of the animals analysed. In a study with *T. patagoniensis* experimentally infected pigs (20,000 ML), Krivokapich et al. (2012) showed that the main infected muscle was the tongue. In the present study, diaphragm and tongue showed similar larval distribution in wild boars infected with *T. pseudospiralis* and *T. spiralis*. Comparable results were found in experimentally infected pigs, using

different infection doses and different *Trichinella* species (Kapel et al., 1998; Nöckler et al., 2005; Smith, 1988).

The ML distribution of the three *Trichinella* spp. had no substantial differences in these infected wild boars. Similar results were observed in wild boars, pigs and horses infected with different *Trichinella* spp. (Kapel, 2001; Kapel et al., 2005; Kapel and Gamble, 2000), which seem to confirm the hypothesis that the distribution of ML is mainly influenced by the host species (Kapel et al., 1998). Moreover, all analysed species showed moderate larval load in the tenderloin, upper foreleg and pork shoulder muscles. Similar results were found in experimentally infected wild boars and pigs (Kapel, 2001; Kapel et al., 2005).

Although wild boar seroconverted at different time following the infection with the three *Trichinella* species, all seroconverted before 29 days pi. Comparable results were found in pigs inoculated with 10,000 larvae (Kapel and Gamble, 2000) and wild boars experimentally infected with the 20,000 larvae (Kapel, 2001) of sylvatic and domestic *Trichinella* species. In another study carried out on specific pathogen-free (SPF) pigs infected with 20,000 ML of *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*, all the animals seroconverted before 40 days pi (Nöckler et al., 2005). Throughout the study wild boars infected with *T. spiralis* and *T. patagoniensis* showed higher antibody levels than wild boars infected with *T. pseudospiralis*. This was also observed in wild boars infected with *T. patagoniensis*, in which no ML were found. An explanation for that maybe that *T. pseudospiralis* is

a non-encapsulated genotype, so stimulation of the immune system might differ (Kapel, 2001), but further studies are needed to understand the differences in these biological characteristics. There was a difference regarding the rapid antibody response in wild boars inoculated with *T. pseudospiralis*, in contrast to what was found by Kapel, (2001), in experimentally infected wild boars with this same *Trichinella* spp., in which the antibody increase was delayed in comparison to other species.

*T. patagoniensis* showed a decline in the OD values during the study, even when it always remained above the cut-off value. After reaching the OD maximum level, both *T. spiralis* and *T. pseudospiralis* remained in a plateau. Similar results were observed for *T. spiralis* in several studies (Bolas- Fernandez et al., 1993; Kapel, 2001; Kapel and Gamble, 2000).

The OD values in sera and muscle juice showed a positive correlation. This finding is in line with results obtained in other studies that show that muscle juice might be useful for epidemiological research, especially because it has a further advantage: it can be obtained from dead animals and can be stored for long periods of time (Gamble and Patrascu, 1996; Kapel et al., 1998; Nöckler et al., 2005). In addition, this could be a significant tool for epidemiological surveillance of *T. patagoniensis* as the larval load could be very low in wild boars and pigs.

## **5. Conclusions**

For the first time, this study has shown the ability of *T. patagoniensis* to develop and complete its life cycle in wild boars. The animals infected with *T. patagoniensis* showed a persistently high antibody level, while the muscle parasitic load was very low. These findings suggest that wild boars may not act as a significant source of infection for other animal species. However, the persistent antibody levels observed in these animals' sera reinforce the importance of using serological tools for epidemiological surveillance in *Trichinella* spp. in wild animals.

### Credit author contributions

**Clara Bessi:** Conceptualization, Methodology, Validation, Investigation.

**Mariano E. Ercole:** Conceptualization, Methodology, Validation, Investigation.

**Fernando A. Fariña:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - Review & Editing.

**M Mabel Ribicich:** Conceptualization, Methodology, Investigation, Supervision, Funding acquisition.

**Montalvo Francisco:** Investigation, Resources.

**Acerbo Marcelo:** Resources.

**Krivokapich, Silvio J.:** Resources.

**Mariana I. Pasqualetti:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - Review & Editing, Supervision, Funding acquisition.

### **Ethics approval**



The present study was approved under permit number 2015/16 by the Institutional Committee for Use and Care of Laboratory animals of the Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina (CICUAL) .

### **Declaration of Competing Interest**

None.

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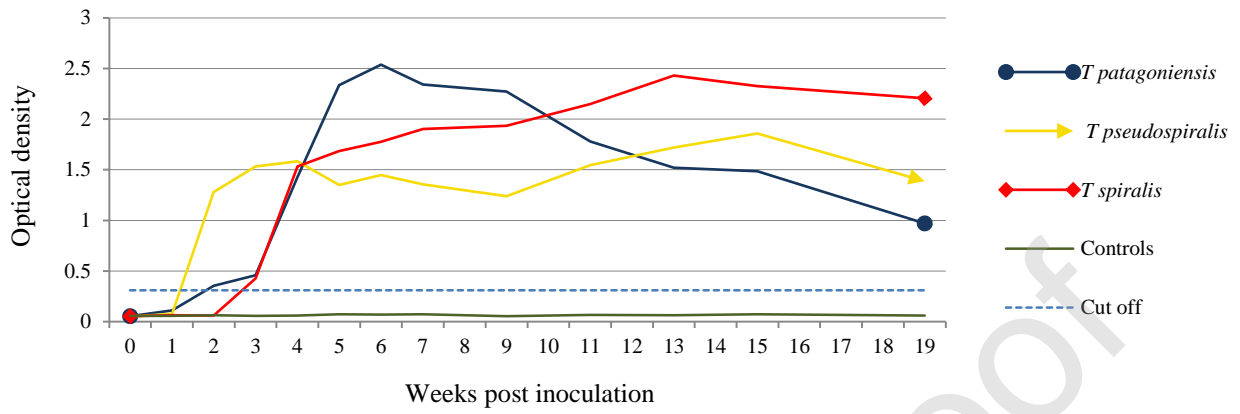
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**Fig. 1. Antibody dynamics.** ELISA antibody values obtained from the infected wild boars with 20000 ML *Trichinella* species. The OD values represent the mean of each sample date.





**Table 1. Larval load.**

The larval load of *Trichinella spiralis*, *T. patagoniensis* and *T. pseudospiralis* of experimentally infected wild boars. The larval load was determined by percentage (%) using as reference the muscle with the highest number of larvae per gram (lpg) as 100%.

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Relative larval load (%)	<i>Trichinella spiralis</i>				<i>Trichinella patagoniensis</i>					<i>Trichinella pseudospiralis</i>			
	1	2	3	4 <sup>a</sup>	1	2	3	4	5	1	2	3	4 <sup>a</sup>
Diaphragm	50.	97.		50.	22.9	100	0	0	66.6	23.3	97.7	100	100
Oesophagus muscle	22.		23.	15.	75.9	0	0	0	0	1.7	23	8.4	4.7
Intercostal muscles	24.	34.	13.		0	0	0	0	0	1.7	18.8	11.5	10.8
Tenderloin	4	9	5	8.7	28.		35.	30.		22.9	0	0	33.3
Tongue	3	3.1	3	3	74.					10.3	67.4	47.5	27.5
Masseters	100	100	5	100	100	25	0	0	100	100	100	21.9	49.3
Pork shoulder	45.	62.	18.		0	0	0	0	0	5.4	45.3	0	9.1
Upper foreleg	9	2	8	43	23.	39.	34.			22.9	0	0	0
Upper hindlimb	5	9	6	21	59.		47.	29.		7.8	59.8	36.6	21.1
	6	2.3	5	2	22.9	25	0	0	33.3	3.3	15.5	14.4	6.6
	18.		23.	12.									
	6	1.5	5	4	22.9	0	0	0	0	11.1	25.1	13.1	21.1

<sup>a</sup> Only 4 wild boars of *T. spiralis* and *T. pseudospiralis* are seen in this table because one wild boar of each group died unexpectedly during the study.