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Study of *Trichinella* Spp in Rodents that Live Near Pig Farms in an Endemic Region of the Province of Buenos Aires, Argentina

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Abstract

Trichinellosis is a wide spread food borne zoonosis caused by species of the genus *Trichinella*. Until present *T. spiralis* is the only species usually found in porcine, sinantropics and wild animals from Argentina. Nevertheless, Krivokapich et al. [1] isolated a novel species (*Trichinella* T12) from a *Puma concolor*, *T. spiralis* can be transmitted and maintained in both a domestic and sylvatic cycle whereby rats, among others contribute to the spread of *T. spiralis* from domestic to sylvatic animals and vice versa. In this research we studied the presence of *Trichinella* infection in rodents which inhabit pig farms from General La Madrid, Buenos Aires, Argentina. For this purpose, 9 pig farms with different levels of sanitation and with or without *T. spiralis* infected pigs and a garbage dump were assessed between spring 2008 and winter 2009. A total of 150 rodents were captured. All the species belonged to the genus *Rattus species norvegicus*. The presence of *Trichinella* spp was tested by artificial digestion of each muscle sample. No positive *Trichinella* infection was found. Further assessment would be done so as to deal with the role of rats in the life cycle of *Trichinella* spp.

Keywords: *Trichinella*; Rodents; *Rattus norvegicus*; Trichinellosis; Argentina; Buenos aires

Introduction

Trichinellosis is a food borne zoonotic disease with an annual incidence of 10000 cases around the world [2].

In Argentina, trichinellosis is an endemic, re-emergent disease through 1990/2005. The parasitose was maintained between 100 and 200 annual cases, however there was a significant increase in infected people (5217 during 1990/1999 period), being the provinces of Buenos Aires, Santa Fe and Cordoba the most affected (89% of the cases) [3].

Raising pigs in Argentina is a very common practice among rural people. It basically consists of outdoor breeding where pigs can be fed with either balanced food or rubbish from garbage dumps depending on people economic incomes. Unlike other countries, in Argentina wastes from different sources such as animals or vegetables are not separated into so they are thrown mixed in garbage dumps. Therefore, there is a high risk for exposure animals to get infected with different diseases such as trichinellosis.

In 19th century Leuckart proposed the "rat theory", implicating rats as the major reservoir of trichinellosis for pigs. On the other hand, Zenker suggested that infection in rats was an indicator of the infection in pigs, being pig carcasses the true source of infection for both animals [4]. Although *T. spiralis* infection in pigs is associated with the infection of brown rats that cohabit abattoirs, farms or garbage dumps, there is no report proving the infection by *T. spiralis* in brown rats in places where pig population is negative. It may indicate that brown rats per se, without the external introduction of *T. spiralis* are not able to maintain the infection [4].

The aim of this research was studying rat species living in pig farms and analyzing rat muscles in areas where porcine trichinellosis is endemic.

Materials and Methods

Study area

Through epidemiological data on animal trichinellosis from Epidemiological Bulletins edited by the Health Ministry and Yearbooks of the National Service of Animal Health (SENASA 2006/2008) and human trichinellosis from bulletins edited by the National System of Epidemiological Surveillance (SINAVE 2006/2008) it was chosen General La Madrid (37° 15' 0" S, 61° 15' 0" W) Province of Buenos Aires, Argentina to make the fieldwork (Figure 1).

Rodent survey

Animal's capture was conducted during spring 2008 and Winter 2009 using Tomahawk live traps (31 cm lenght, 17 cm width and 14 cm hight) usually employed to capture *Rattus* spp. Traps were opened and baited with a mix of meat and cow fat.

Sampling was conducted in 9 pig farms and 1 garbage dump* classified with 2 criteria, sanitation level (low and good) and presence of *Trichinella* infection (positive/negative). Low sanitation meant no veterinary control of pigs slaughtered (slaughtered were performed by owners) and animals feeding with meat and vegetable garbage, and good sanitation level where pigs were sold to slaughterhouses were veterinary control was performed and animals were fed with balance foodstuffs. A total of 6 pig farms were classified as low sanitation and from them 2 had been positive for *Trichinella* infection twenty days before the current study. One pig farm with good sanitation level had been positive for *Trichinella* thirty-five days prior to this research.

Live traps were placed around the farms, making 4 lines of capture, each line consisting of 8 traps. They were left in each place for a period

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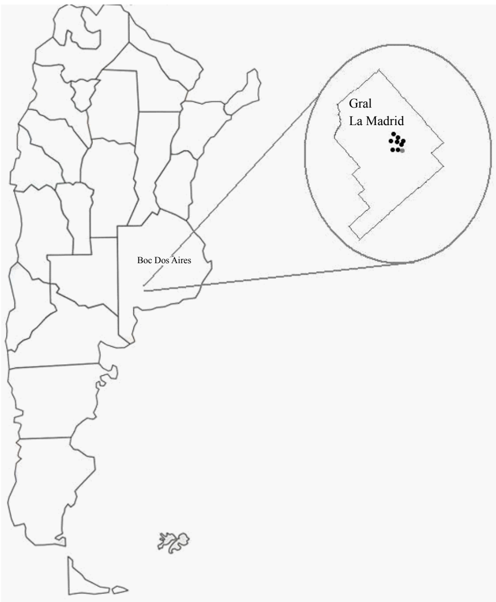

Figure 1: The map shows the district of Gral La Madrid, and the city with the same name where pig farms and the garbage dump were located.

of 3 nights, and checked daily. If an animal was found inside a trap, it was replaced by an empty one. Once animals were captured, they were identified and euthanized in order to take samples from different muscles: diaphragm, tongue, masseter, intercostal muscles, and the limbs. They were refrigerated at 4ºC until posterior analysis. Data about weight, sex, and sizes (total length, length of tail- length of body, shape of head, ear, and fur) were taken.

*The garbage dump is included in this classification. Although no pigs were being raised there at that time, that used to be the case.

Artificial digestion

Samples collected at necropsy were weighed, ground in a commercial meat grinder, and mixed with 1 l of artificial digestion fluid (1.0% pepsin [1:10,000 Sigma] and 1.0% hydrochloric acid [v/v]). Digests were mixed vigorously on a magnetic stir plate at 42-44° C for 2 h. At the conclusion of the 2 h, the digest was allowed to settle and the supernatant was decanted. The sediment was poured through an 80 µm mesh sieve into round-bottom pilsner glasses. Following settling for 20 min, the sediment was washed repeatedly in tap water. The samples were examined using a stereo microscope at a 10 X magnification [5].

Results

A total of 150 rodents 89 females and 61 males were captured. Through study of morphometric characteristics: total length, length of tail-length of body, shape of head, ear, fur (colour, length) it was determined that specimens found belonged to Family *Muridae* Subfamily *Murinae Genus Rattus* species *norvegicus*.

Samples were analyzed by artificial digestion with negative results. (No presence of *Trichinella* in any individual).

Discussion

Several studies relate trichinellosis to the association between pigs and rodents in pork farms with uncontrolled or deficient confinement and feeding [6]. These works aim to determine the source of infection in pigs and the relation existing with wild animals. Murrel et al. [6] established that a population of R. norvegicus can maintain the infection in an establishment through cannibalism, with 42% of the rodents infected. A study carried out in Croatia with 49 pigs from farms with deficient sanitary and hygienic conditions showed that *Trichinella* spp was present in 31.8% of the pigs and 2% of the rats, whereas in the farms with good sanitary conditions, the parasite was found neither in pigs nor in rats [7].

Pest control is one of the measures taken to reduce the rodent population. It is considered one of the bases for the control of trichinellosis [8]. Such control can be achieved through biological, mechanical or chemical methods. Though pest control is by definition a set of measures more complex than just the use of toxic baits, chemical control is predominant at present time. Smith et al. [9] studied the role of the brown rat as a vector of *T. spiralis* in herds of pigs in the Atlantic Provinces of Canada. A rat control program forced the rodents from *Trichinella*-infected herds to move to non-infected herds. A few months later, some of the pigs of the negative herd tested positive to the disease. On the other hand, Miroslav et al. demonstrated in a study in Croatia that the relative risk of trichinellosis infection in pigs in areas exposed to control programs with rat poison compared to non-exposed areas was 13.35 (IC 95%: 10.08 -7.68), $p < 0.05$. Pigs exposed to rodent control measures had 13% more chance to develop an infection than those in non-exposed areas. This shows a flaw in pest control to reduce the incidence of trichinellosis [8].

In Argentina, several authors have carried out studies in rodents. For example, Vázquez et al. [4] studied the prevalence of trichinellosis in rodents of risks areas in the city of Río Cuarto, Córdoba. All captured animals, a total of 1253, were controlled through artificial digestion and had negative results. Studies carried out in trichinellosis pork focus groups in the province of Río Negro found 15% of infected rodents [10], which matches the hypothesis claiming presence of infected pigs/infected rats. In another study, carried out in chicken farms in Exaltación de la Cruz, province of Buenos Aires, the absence of *T spiralis* in 152 individuals of *R. norvegicus* suggests the hypothesis of rodents as accidental hosts, which can acquire the infection through the consumption of infected pig carcasses. In this case, the high availability of rat food (chicken food) decreased the probability of eating infected pigs carcasses [11]. In the current research food availability could be an explanation why there was no positive rat captured in any of the pig farms studied (Figure 2).

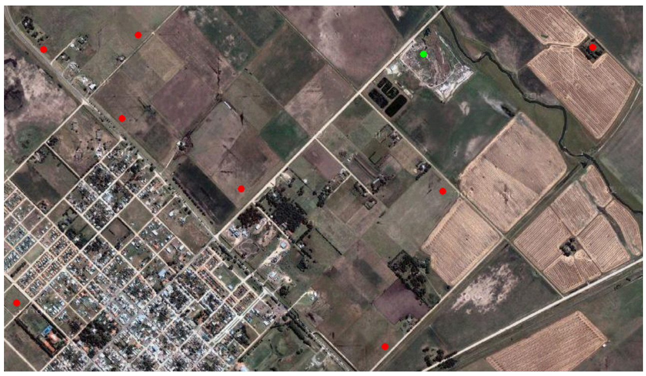

Figure 2: Shows the location of pig farms (red points) and the garbage dump (green point). Live traps were located around each pig farm making 4 lines of capture, each line consisting of 8 traps.

On the other hand, rodents present low symptomatology for trichinellosis, though the infection can cause alteration of their behavior, which would make them easier to capture either by pigs or other hosts, such as carnivorous mammals, whose bodies, once dead, could be ingested by pigs, thus resuming the cycle.

The role of *R. norvegicus* in the epidemiology of the disease is still a matter of debate. While in some studies it is argued it is a real natural reservoir, showing the presence of the infection in a habitat without the introduction of the parasite by other host species, others claim it primarily acts as a vector for domestic animals due to accidental infection.

As indicated by Hill et al. [12] in the absence of a significant source of *T. spiralis*-infected swine (wild or domestic), the risk of infection to wildlife hosts and the development of an independent sylvatic transmission cycle of *T. spiralis* is minimal.

Although rats captured resulted negative in this research, further researchers and many variables must be measured in order to deal with the role of rats in the life cycle of *Trichinella*. The presence of positive pigs in establishments where there is no occurrence of the disease in rodents could also indicate that there might be some natural reservoir that could play a decisive role in preserving the cycle but which has not been yet determined. Studies in wild animals such as wild boars, opossums, wild carnivores (mustelids, procyonidae, etc.) carried out in different parts of the world including Argentina [13-18] contribute to reconsider the existence of a natural reservoir other than the brown rat.

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