Veterinary Parasitology 169 (2010) 362–366

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Trichinella infection in wild boars (*Sus scrofa*) from a protected area of Argentina and its relationship with the presence of humans

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ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form 30 October 2009
Accepted 5 January 2010

Keywords:
Trichinellosis
Epidemiology
Wild boar
Serological diagnosis
Artificial digestion
Argentina

ABSTRACT

In Argentina, *Trichinella* infection has been documented in humans and animals of several provinces since 1930. This zoonotic parasite infection has been recently detected in humans and pigs of a region historically considered as *Trichinella*-free, suggesting the spread of these pathogens. The aim of the present work was to investigate the presence of *Trichinella* infection in wild boars (*Sus scrofa*) and in the human population living in a protected area. *Trichinella* infection has been investigated by serology (in humans and wild boars) and by artificial digestion of wild boar muscles. The isolated *Trichinella* larvae have been identified at the species level by multiplex PCR. A geographical information system has been used to collect environmental data. The results showed the circulation of *Trichinella spiralis* in wild boars with a low parasite burden, and suggest the influence of human behavior on the transmission. The transplacental passage of parasite is postulated. It follows that the declaration of region as *Trichinella*-free should be carefully established by means of extensive monitoring programs, not only in humans and domestic animals but also in wildlife.

1. Introduction

Trichinellosis is a worldwide zoonotic disease caused by nematode worms of the genus *Trichinella* (Dupouy-Camet and Murrell, 2007). These parasites circulate among domestic pigs and wild animals (both carnivores and omnivores) and can reach the human beings through the consumption of raw or undercooked meat bearing larvae of *Trichinella* spp.

In Argentina, *Trichinella spiralis* is the most prevalent etiological agent found in domestic pigs and synanthropic animals (e.g. brown rats and armadillos); however, little is known about its circulation among wildlife, even when outbreaks of trichinellosis caused by the consumption of meat from wild animals have been documented (Tesón et al., 1997; Huici et al., 1999; Pozio, 2000; Ribicich et al., 2005).

Today in Argentina, there are endemic, non-endemic and *Trichinella*-free provinces (Bolpe and Boffi, 2001). The criterion adopted by the local health authorities for this classification is based on the presence or absence of human or porcine outbreaks or the detection of isolated infections.

The presence of a transmission cycle of the parasite among domestic animals in a given region is frequently associated with the presence of infection in wildlife (Pozio et al., 1996; Pozio, 1998). In Europe and in non-European countries, the wild boar plays an important role as reservoir for *Trichinella* spp., mainly *T. spiralis* and it is

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doi:10.1016/j.vetpar.2010.01.005

also one of the most important sources of infections for humans (Pozio, 2007; Pozio et al., 2009).

Recently, some of us have shown the presence of *Trichinella* infections in humans and pigs of an area which had been previously considered to be free from these zoonotic parasites (Costantino et al., 2009). Taking into account the high percentage of humans and swine that were serologically positive in this area, we looked forward to detect the presence of these parasites in wild boars. The aims of the present work were (1) to detect *Trichinella* infection in wild boars and humans by means of parasitological and/or serological methods, (2) to identify the parasite species circulating in the area, and (3) to establish the geographical distribution of the wild boar infection and its relationship with the presence of human settlements.

2. Materials and methods

2.1. Area under study

The area under study belongs to a natural protected habitat belonging to National Park Administration, located in the center-east of the Entre Ríos province of Argentina, along the occidental riverside of the Uruguay River (31°51′44″S; 58°13′57.4″W). The park has an extension of 8500 ha featured by a slightly undulating plain landscape. The climate is variable and the vegetation consists mainly of low woods and savannahs alternating with hard grasslands and palm trees. The fauna consists of native species such as rodents (plains viscacha, *Lagostomus maximus*; capybara, *Hydrochoerus hydrochaeris*), birds (rheas, *Rhea americana* and *R. pennata*) and several species of lizards which co-inhabit with exotic species such as the axis deer (*Cervus axis*), several antelope species and wild boars.

The park is divided by a creek into two areas: the Northern area, where a recreational zone (with camping, restrooms, a drugstore, barbecues, a restaurant, the governor's office and a beach) is located, and the Southern area where hunting activities are permitted only for authorized persons.

The nearest human settlement is 12 km far from the entrance of the park. This village has 2334 inhabitants (INDEC, 2001) most of them devoted to agricultural activities, and being some others employees at the park. Some of these inhabitants are authorized hunters and the game meat originating from the hunting activity is served in communal canteens of the village. Hunters coming from other villages or towns are allowed to take away part of the game meat for themselves. An important feature of the village is the presence of a huge open air garbage dump located 6 km far from the park, separated by the N°14 National Route and to which it is fairly easy to gain access.

2.2. Samples

Muscle samples originated from 112 adult wild boars, a 3-day old wild boar and from a fetus all of them hunted at random in 2007. Serum samples were collected from 48 wild boars by puncture of the femoral vein. In addition, serum samples were also collected from 44 people (31 men and 13 women). Written consent was obtained from all the blood donors. These people (hunters, rangers and park personnel) belong to a human settlement located in the park. All serum samples were kept at −20 °C until used.

2.3. Collection of epidemiological data

To evaluate the alimentary habits at risk for this parasite infection, a questionnaire was given to enrolled people to get information about the frequency of game consumption and how the meat was consumed (raw, semi-raw, etc.). In addition, age, gender, occupation, and the possible occurrence of signs and symptoms of trichinellosis after game consumption, were included in the questionnaire.

2.4. Parasitological test

Samples of skeletal muscle (diaphragm, tongue, masseters and hind quarters) weighing 85 ± 22 g were tested from each wild boar ($n = 114$) by the artificial digestion (AD) according to a previous published protocol (Gamble et al., 2000). After digestion and sedimentation, *Trichinella* larvae were collected, washed in saline and counted by a light microscope and by two independent observers. Results were expressed as number of muscle larvae per gram of digested muscles (ML/g). Larvae were preserved in absolute ethanol and sent to the International *Trichinella* Reference Centre at the Istituto Superiore di Sanitá (Rome, Italy) to be identified at the species level by Multiplex PCR according to a published protocol (Pozio and La Rosa, 2003).

2.5. Detection of anti-Trichinella antibodies in sera

The detection of anti-*Trichinella* antibodies in human serum samples was carried out by ELISA, indirect immunofluorescence (IIF) and Western blot (WB), as previously described (Nuñez et al., 2000; Calcagno et al., 2005). Similar tests, developed for the detection of anti-*Trichinella* antibodies in porcine serum samples, were used to detect anti-*Trichinella* antibodies in sera from wild boars, with slight modifications (Venturiello et al., 1998; Nuñez et al., 2000). Briefly, all anti-swine IgG (H + L) sera were previously tested to corroborate their reactivity with wild boar sera. For the IIF, serum samples were employed at a dilution of 1/10 in saline with 0.1% Tween and an anti-swine γ-globulin serum conjugated to fluorescein isothiocyanate (FITC, Dako Corporation, Carpintería, CA, USA) diluted 1/70 in 1/30000 Evans blue. Those samples rendering fluorescence of the cuticle of the parasite were considered positive. In the WB, an anti-swine γ-globulin serum conjugated to biotin (Vector Laboratories, Burlingame, CA, USA), diluted 1/70 was employed followed by the addition of a macromolecular complex of avidin and biotinylated peroxidase (ABC, Vector Laboratories) according to the manufacturer's instructions. Those samples developing the characteristic bands corresponding to the molecular weights of 45 and 55 kDa were considered positive. For ELISA, an anti-swine γ-globulin serum conjugated to biotin (Vector Laboratories) diluted 1/