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Presence of Toxoplasma gondii infection in wild boar in southern Brazil

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Abstract: Previous studies on toxoplasmosis carried out in Brazil have shown that Toxoplasma gondii infections are relatively frequent in domestic animals and man. However, little is known on the impact of toxoplasmosis in wildlife in that country. In this study, presence of Toxoplasma gondii in wild boar in the southern Brazilian State of Rio do Sul was assessed by bioassay. Thirty-four wild boars were killed by hunters and samples of boar tissues (heart and brain) were used to inoculate mice. Later, diverse murine tissue samples were obtained for microscopic diagnosis. Five mice were positive by bioassay (toxoplasmosis prevalence = 14.28 %). Interestingly, Toxoplasma cysts were found in the liver of one mouse. All mice fed with boar tissues remained alive at the end of the experiment, which suggests that Toxoplasma isolates from wild boar were mild pathogens. Nevertheless, infected rodents showed a noticeable immune response, all showing antibody titers of 1:16. The present data suggest that consumption of undercooked wild boar meat or entrils are a potential source of toxoplasmosis for both animal and man in Brazil.
Keywords: Sus scrofa; Toxoplasma gondii; wild boars; bioassay; antibody

INTRODUCTION

Wild boars **(**Sus scrofa, Linnaeus, 1758**)** are indigenous in many countries in the world. This species was introduced into Argentina and Uruguay in early 20th century [3]. Wild boars soon invaded Brazilian territory through the Southwest region (namely in the border between the state of Rio Grande do Sul and Uruguay, as pointed out by [9]. If not properly managed, this exotic mammal has the potential of transmitting diverse infections to livestock (especially domestic swine). Besides, it may cause extensive damage to native wildlife, due to its ability to modify habitats. In addition, boars may feed on crops, thus depleting agricultural resources [4, 10]. Toxoplasma gondii is a zoonotic parasitic protozoa that infects wildlife, domestic animals and humans [2, 8, 10, 12]. Mammalian hosts become infected by eating undercooked or raw meat containing cysts, or by ingesting food or water contaminated with sporulated oocysts. Interestingly, hunters may be infected when handling carcasses. Cats, including both domestic and free-ranging felids, are the definitive hosts of this parasite [5 ,13, 16]. T. gondii is common in most areas of the world and is of veterinary and medical importance, because it may cause abortion or congenital disease in its intermediate hosts, including humans. Domestic pigs are considered a relevant source of T. gondii infection in humans. In Brazil, pork meat and handmade sausages (either uncooked or undercooked) are often sources of T. gondii infections. On the other hand, wild game meat consumption has been highlighted as an emerging risk factor for T. gondii infection in humans. In Brazil, previous studies have shown that T. gondii infections in animals and man are relatively frequent. [2, 15, 18]. In the Rio Grande do Sul state in southern Brazil, the prevalence of the parasite in humans is unusually high, reaching 74.5% in some areas [1]. However, prevalence of toxoplasmosis in wildlife reservoirs in this region is unknown. By these reasons, the present study was aimed at determining prevalence of T. gondii infection in free-range wild boar. Furthermore, pathogenicity of protozoal isolates was tested by bioassay in mice. Wild boars were selected for the study as they are susceptible to fatal toxoplasmosis and, moreover, they are important game animals whose meat is frequently consumed by people.

MATERIALS AND METHODS

The present study was approved by the Local Commission for Ethics in Animal experiments (Decision No. 2297/2013). Thirty four wild boars were killed by authorized hunters (Brazilian National Environmental Agency nº 28810-1) in the State of Rio Grande do Sul (Southern Brazil). The study area spanned five municipalities in the Rio Grande do Sul state, southern Brazil: Piratini, Herval, Capão do Leão, Pedro Osório and Jaguarão , which are located between latitudes 31º 21" and 31 º 46" S and meridians 53 º 06" and 51º 58" W. Tissue samples (heart and brain) of all wild boars were collected for bioassays. Brains and hearts of wild boars were minced, homogenized, and prepared using peptic digestion, as described by Dubey (1998). Twenty-five grams of both boar organs were ground and a total of 50 g of mixed tissue were

homogenized in an electric blender with 125 vol (v/v) of aqueous 0.85% NaCl (saline). The homogenate was mixed with 5 vol. of acidic pepsin (pepsin, 2.6g NaCl, 5.0g; HCl 7,0 mL; enough distilled water to 500 mL solution-pH 1.1 - 1.2), and incubated in a shaking water bath for 1 h at 37º C. The digested tissue was filtered through two layers of gauze and centrifuged. After centrifugation, supernatant was discarded, and a neutralizing solution (1.2% sodium bicarbonate, pH ~ 8.3) was added to the sediment. The pellet was resuspended in saline and centrifuged again. The supernatant obtained was discarded. Five to ten ml of sterile saline solution, containing 1,000 IU penicillin and 100 mg of streptomycin per ml, were added to the pellet for resuspension. Two 1-mL doses of this tissue homogenate were inoculated intraperitoneally into five mice at an interval of 24 hours. Swiss Webster female mice with an average age of two months from the Central Animal Facility of the Faculty of Veterinary Medicine at the Federal University of Pelotas were used in experimental infection assays. Inoculated animals were observed daily, and lung, brain, and liver imprints of the mice that died by natural causes were examined microscopically for presence of T. gondii tachyzoites and/or tissue cysts ([5]. The surviving animals were bled on day 45 post-inoculation (pi), and a 1:16 dilution of serum from each mouse was tested for T. gondii antibodies by IFAT [3]. Finally, all mice were sacrificed at day 60 p.i., and their heart, brain and liver squashes were examined microscopically for tissue cysts [5]. For such purpose, tissue samples (brain, lung and liver) were individually collected and fixed in 10% of buffered formal saline (NaCl 0,15M), sectioned and stained with hematoxylin–eosin. Microscopic glass slides were examined by optical microscopy for T. gondii cyst detection. Mice were considered to be infected with T. gondii when tachyzoites or tissue cysts were found in any biological sample.

RESULTS AND DISCUSSION

The prevalence of T. gondii in feral pigs by bioassay was 14.28% (5 positives out of 34 animals). To the best of our knowledge, this study represents the first report of T. gondii infection in Brazilian wild boar. According to [7], successful isolation of T. gondii depends on the type of bioassay, the amount of tissue fed and finally, the concentration of protozoa in tissue samples. Interestingly, parasitic cysts were observed in liver smears of one Toxoplasma-infected mice. Murine liver cysts of Toxoplasma showed the typical morphology: varied in size and containing bradyzoite agglomerates (Figure 1).

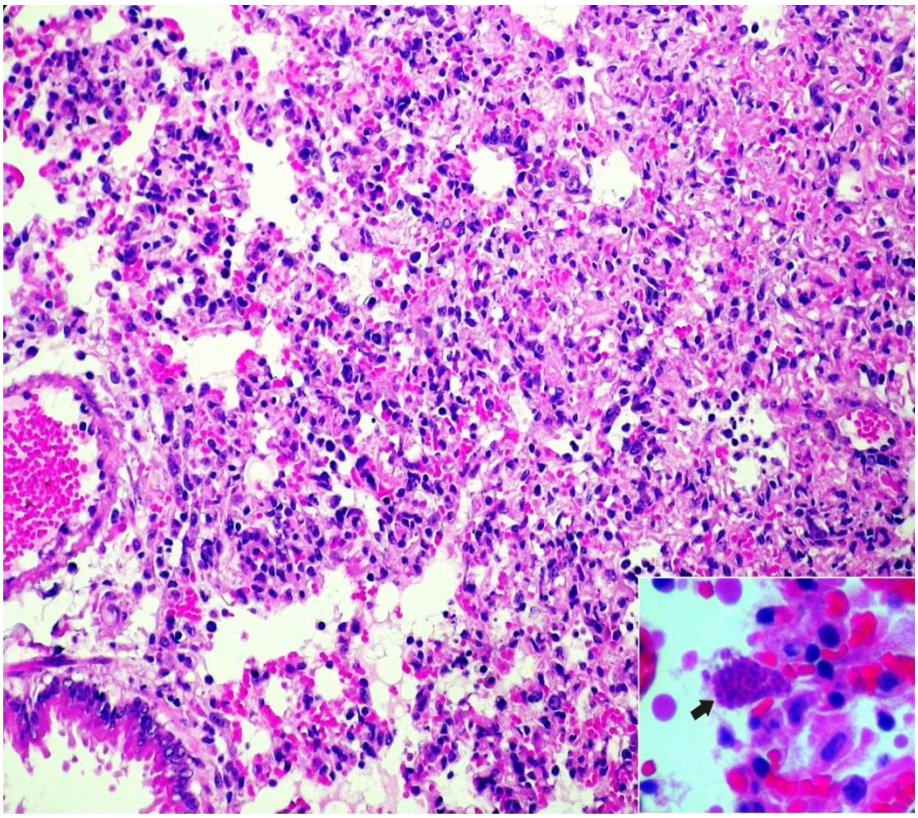

Fig 1: Lung tissue with marked diffuse necrotizing pneumonia with intralesional Toxoplasma gondii cyst (arrow) formed by bradizoites.

The frequency of toxoplasmosis in Rio Grande do Sul is different from those reported for domestic pigs and farmed boars in Brazil. Thus, antibodies to T. gondii were found in 36.0% of domestic pigs in Rio Grande do Sul [2] and 4.5% in farmed boars in the State of São Paulo (Fornazari et al., 2009). According to a recent revision [4], prevalence of toxoplasmosis in feral pigs in other countries ranges from 0.5 to 100%.

However, when the bioassay method was used for diagnosis, prevalence ranged from 2-17% [14] which is in agreement with data in the present study.

None of the five protozoa isolates derived from boars killed the bioassayed mice. This suggests that Toxoplasma type II is present in Brazilian feral pigs, as such genotypes are mild pathogens [9, 20]. Further molecular analysis of the feral pig isolates will be neccesary to confirm such hypothesis. In spite of the low pathogenicity of the boar isolates of T. gondii, infected mice showed a noticeable immune response, with high antibody titers (1:16). Occurrence of chronic toxoplasmosis following experimental infection may be due to several causes: low virulence of T.gondii strains, presence of a small number of cysts in inoculated tissue, or subsistence of viable bradyzoites after peptic digestion [7, 10].

Exposure of wild boar to domestic and wild felids faeces in their habitat (through the ingestion of contaminated food or water), carrion feeding or cannibalism are the main causes for T. gondii infection in wildlife [6, 9, 17]). Consumption of meat from wild and farmed wild boar has been highlighted as an emerging risk factor for T. gondii infection in humans [21]. In Southern Brazil, wild boars show a wide geographical distribution. Moreover, due to its abundance and the damage caused to agriculture in rural areas is considered a mammalian pest. By this reason, wild pigs may be killed by hunters, while hunting other wildlife species is absolutely prohibited in this Brazilian region [15].

CONCLUSIONS

This is the first report of Toxoplasma gondii in free range wild boars in Brazil. Hence, consumption of undercooked boar meat is a health risk and may be a potential source of infection for humans.

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