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First record of *Toxoplasma gondii* in *Chaetophractus villosus* in Argentina

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Abstract

Toxoplasma gondii is an obligate intracellular apicomplexan parasite that causes abortion and reproductive disorder in domestic animals. *T. gondii* is a common worldwide disease in homeothermic animals, including birds and humans. The aim of the present study was to determine the presence of antibodies against *T. gondii* in the armadillo *Chaetophractus villosus* in the province of La Pampa, Argentina. Serum samples were collected from 150 individuals (70 males and 80 females). For serological detection of *T. gondii*, a latex agglutination test was first performed and then positive sera were confirmed with an indirect hemagglutination test, using 1:4 to 1:64 dilutions. Results showed that 27% (41) of the samples presented titers for antibodies against *T. gondii*. There were not significant differences between the presence of antibodies against *T. gondii* and age or sexes of the armadillos. Results show that presence of *T. gondii* antibodies in armadillos were associated with presence of pigs, and sheep, however there was not association with chickens and dairy cattle in capture site. *T. gondii* has an important presence in *C. villosus* population, suggesting a potential zoonotic risk for humans and wildlife animals when *C. villosus* meats are consumed raw or undercooked. This is the first record of the presence of antibodies against *T. gondii* in *C. villosus*.

Keywords

Antibodies, *Toxoplasma gondii*, *Chaetophractus villosus*, zoonosis, Argentina-La Pampa

Introduction

Toxoplasmosis is a worldwide distributed zoonosis that affects man and most warm-blooded animals, with a great economic impact in animal and public health.

Toxoplasma gondii is an obligate intracellular apicomplexan parasite associated with abortions and reproductive disorders in domestic animals. In sheep, toxoplasmosis causes fetal resorption, abortion at any stage of pregnancy, fetal mummification, stillbirth, or birth of live but weak offspring in sheep and goats (Caldas *et al*. 2006). For pigs disease, in general courses as subclinical however in some cases weak born animals or stillborn (Basso and Venturini 2014) may be observed.

Animals and humans become infected with this protozoan after ingesting oocysts present in the environment, in food contaminated with infected feces, or in tissues of intermediate hosts. Then *T. gondii* can be transmitted between domestic and wild animals through ingestion of infected carcasses further favored by the overlap of animal distribution. In this way armadillos infected with these protozoa represent a potential risk, whether as a reservoir or spillover of disease.

Taking into account the economic loss caused in domestic livestock and the problems they cause to human health, armadillos infected with these protozoa represent a potential risk.

An important risk factor in rural areas in Argentina is the consumption of meat of wild animals such as armadillos (Kawazoe, 2009). Popularly, their meat is prized and is part of the rural population diet. Hereafter the importance of investigates the presence of antibodies to *T. gondii* in *C. villosus* (Xenarthra). Antibodies against *T. gondii* have been investigated from various species of Xenarthra from Florida (Burridge *et al*. 1979), French Guiana (Carme *et al*. 2002; Thoisy *et al*. 2003), Brazil (Shaw and Lainson 1973; Schenk *et al*. 1976; Sogorb *et al*. 1977; Salata *et al*. 1985; Vieira da Silva *et al*. 2006; Costa da Silva *et al*. 2008) and Bolivia (Deem *et al*. 2009).

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In Argentina studies about Xenarthra infected with *T. gondii* are scarce, where levels of prevalence were 0% in *Chaetophractus vellerosus* and *Zaedyus pichiy* and 6.25% for *Dasypus novemc*inctus (Ramírez *et al*. 1984; Superina 2007). However, there is no information about *T. gondii* in C. *villosus*.

Chaetophractus villosus is a member of the superorden Xenarthra, and its distribution range extends from the arid Gran Chaco region, which is located between Bolivia, Paraguay and northern Argentina, to as far south as the Argentine Tierra del Fuego and Magallanes in Chile (Gardner, 2005). Is an omnivore that feeds on insects, invertebrates, seeds, small vertebrates and habitually carrion (pigs, sheep, cattle, chickens) of infected animals. Tissue cysts of *T. gondii* contained in meat from domestic animals that died may be important sources of infection for *C. villosus*. However the number of tissue cysts produced varies with the intermediate host species. In livestock, *T. gondii* tissue cysts are most frequently observed in various tissues of infected pigs, sheep and goats, and less frequently in infected poultry, rabbits, dogs and horses. By contrast, tissue cysts are found only rarely in skeletal muscles of cattle or buffaloes (Tenter, 2009).

Considering the importance of toxoplasmosis in wildlife and the lack of epidemiological information in Argentina, this study aimed to determine the prevalence of infection caused by *T. gondii* in *C. villosus* in the province of La Pampa, Argentina, as well as to identify risk factors associated to the infection.

Materials and Methods

Chaetophractus villosus were manually captured with the authorization of the Ministry of Production, Secretariat of Agricultural and Natural Resources Directorate of La Pampa province, and with the permission of the farm owners. The capture sites are located in central La Pampa (R1: 36°47′37″S to 64°10′03″W; R2: 36°29′37″S to 64°15′44″W; R3: 36°22′16″S to 65°02′48″W; R4: 36°51′41″S to 64°29′16″W; R5: 36°41′34″S to 64°11′10″W; R6: 36°46′18″S to 64°06′54″W; R7: 36°32′32″S to 63°59′26″W.)

Blood was extracted from the caudal vein of 150 armadillos captured between 2007 and 2010 in the province of La Pampa, Argentina. The blood samples were centrifuged for 15 minutes at 2,500 rpm. The sera were separated and stored at –20°C until the time of analysis and testing for the presence of *T. gondii* antibodies.

For the serological detection to *T. gondii*, we first performed an agglutination test with Toxotest latex (Wiener Lab., Argentina). This test has 91.0% sensitivity and 96.4% specificity. Then positive sera to latex were confirmed with an indirect haemagglutination test (IHA, Wiener Lab.). Each serum sample was diluted 2-fold in a diluting buffer and 25 µL of each diluted test sample was re-diluted with an equal volume of buffered saline to obtain serial 2-fold dilutions from 1:4 to 1:64. Whereas serum samples had been positive in the Latex test for IHA, serum dilutions of 1:4 or above were regarded as positive.

Statistical analysis of *T. gondii* seroprevalence in the animals was performed by Chi-square tests. Statistical significance in this study was defined at the $p \leq 0.05$ levels (Epi Info 6.0.4 software).

Results

Of 150 sera samples analyzed, 41 (27%, $CI_{95\%}$: 20.4–35.2) were positive for *T. gondii* (latex and IHA ≥ 4) with titers ranging from 1:4 to 1:64 for IHA. Four (10%) *C. villosus* had a titer of 1:4, 12 armadillos (29%) of 1:8, seventeen (41%) of 1:16, four (10%) of 1:32 and four armadillos (10%) had a titer of 1:64.

The prevalence found in males was 26% (18/70), in females 29% (23/80), in young 21.7% (5/23) and in adult 28.3% (36/127). Presence of *C. villosus* with *T. gondii* antibodies was 22.7% (10/44) and 29.2% (31/106) in captures sites with dairy and beef herds respectively. In places where free-range chickens were present 32% (31/97) had *T. gondii* antibodies while in places without free-range chickens the prevalence was 18.8% (10/53). There were no statistical differences between sex ($p = 0.716$), age ($p = 0.617$), presence of dairy ($p = 0.103$) and chickens at the capture site ($p = 0.086$).

Presence of *C. villosus* with *T. gondii* antibodies was 55.5% (10/18) and 23.5% (31/132) in captures sites with pigs presence or not respectively. In places where sheep were not present 17.2% (10/58) had *T. gondii* antibodies while in places with sheep the prevalence was 33.7% (31/92). A significant statistical differences has been observed for the presence of pigs ($p = 0.004$, OR: 4.073) and sheep ($p = 0.028$, OR: 2.439) in the capture site.

Discussion

The presence of antibodies against *T. gondii* is a good indicator of exposure of the animal to the parasite. The results of this study show that *C. villosus* is exposed to protozoa, constituting the first record of antibodies against *T. gondii*.

C. villosus showed a higher seraprevalence for *T. gondii* than those recorded for *D. novemcinctus* in São Paulo, in Minas Gerais (Brazil) and in Florida (USA) where the prevalence ranged from 13% to 19% (Costa da Silva *et al*. 2008; Schenck *et al*. 1976; Burridge *et al*. 1979) whereas in French Guiana (Carme *et al*. 2002) reported a seropositive rate of 46% in *D. novemcinctus*. This higher prevalence may be attributable to a greater abundance of oocysts in the environment. Oocysts are highly resistant to environmental conditions and contaminate water, soil, dust, vegetables and fruits (Hill and Dubey 2002).

Differences found among authors can be related also to laboratory techniques, and to positivity criteria established in each study.

In Argentina the prevalence found in Xenarthra for *T. gondii* ranged from 0% in *C. vellerosus* and *Z. pichiy* to

6.25% in *D. novemcinctus* (Ramírez *et al*. 1984; Superina 2007), both being lower than that found in *C. villosus*. However these two species are essentially insectivores, on the contrary *C. villosus* in La Pampa region feeds habitually carrion (pigs, sheep, cattle, chickens) of infected animals.

Prevalence of antibodies against *T. gondii* in free-range chickens from Argentina was 65.5% (Dubey *et al*. 2003). However in this study the presence of chickens was not significantly associated with *T. gondii* in *C. villosus*, probably because *T. gondii* tissue cysts are less frequently observed in infected chickens (Tenter 2009).

The prevalence of antibodies against *T. gondii* in domestic pigs from La Pampa was 58.7% (Venturini *et al*. 2004). Similar prevalence of *T. gondii* antibodies was recorded in *C. villosus* (55.5%) captured in sites with pigs presence, conversely the prevalence was significantly lower (23.5%) in places with absence of pigs. For sheep was the same, in places where sheep were present the prevalence was significantly higher ($p = 0.028$, OR: 2.438). At this point it is important to note that the organotropism of *T. gondii* and the number of tissue cysts produced in a certain organ vary with the intermediate host species. In livestock, *T. gondii* tissue cysts are most frequently observed in various tissues of infected pigs, sheep and goats, and less frequently in infected poultry, rabbits, dogs and horses. By contrast, tissue cysts are found only rarely in skeletal muscles of cattle (Tenter 2009). Considering that a significant part of *C. villosus* diet is carrion (pigs, sheep, cattle, chickens) of infected animals, it is easy to understand that the presence of pigs and sheep predisposes to *C. villosus* toxoplasmosis and conversely not the presence of cattle or chickens.

The presence of cats was not evaluated as a factor risk because in all places, where the capture was conducted the presence and/or circulation of domestic and wild cats was frequent.

The route of infection with *T. gondii* in man and animals is by incidental ingestion of oocysts from the feces of cats and the oocysts are highly resistant to environmental conditions. However, infection through the ingestion of tissue cysts in meat is considered one of the main sources of infection to humans. Between 30% and 60% of pregnant women who consumed inadequately cooked meat may suffer from acute toxoplasmosis (Cook *et al*. 2000).

The findings reported here have important public health implications as they suggest that *C. villosus* meat is a source of contamination with great potential for transmission of *T. gondii*. Based on the results of this study, it may be assumed that men should be careful when eating armadillos meat, since these animals may transmit toxoplasmosis by means of the ingestion of raw or undercooked meat.

Conclusion

The presence of antibodies against *T. gondii* in *C. villosus* has been registered for the first time. Thus, it represents a relevant contribution to expand the scarce knowledge about the health state of wild Xenarthra.

Forty one of the 150 (27.3%) *C. villosus* in this study were antibody positive to *T. gondii*, suggesting that this species may be a health threat to humans if undercooked armadillo meat, harboring cysts, was consumed.

The seroprevalence observed suggest frequent contamination in the environment, which may indicate that *C. villosus* is common participant in the cycle of *T. gondii*, and could thus be a source of infection for other animals and humans.

The presence of pigs and sheep predisposes to *C. villosus* toxoplasmosis. Further studies should be carried out to determine the importance of this parasite from the point of view of the conservation of wildlife populations and their potential risk to human health mostly in areas where hunting and ingestion of their meat is a frequent habit.

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