

**Prevalence of Antibodies to *Trypanosoma cruzi*, *Leishmania infantum*, *Encephalitozoon cuniculi*, *Sarcocystis neurona*, and *Neospora caninum* in Capybara, *Hydrochoerus hydrochaeris*, from São Paulo State, Brazil**

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## PREVALENCE OF ANTIBODIES TO *TRYPANOSOMA CRUZI*, *LEISHMANIA INFANTUM*, *ENCEPHALITOZOOM CUNICULI*, *SARCOCYSTIS NEURONA*, AND *NEOSPORA CANINUM* IN CAPYBARA, *HYDROCHOERUS HYDROCHAERIS*, FROM SÃO PAULO STATE, BRAZIL

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**ABSTRACT:** Little is known about the importance of capybara, *Hydrochoerus hydrochaeris*, as reservoirs for parasites of zoonotic or veterinary importance. Sera from 63 capybaras, from 6 counties in the state of São Paulo, Brazil, were examined for antibodies to *Trypanosoma cruzi*, *Leishmania infantum*, *Encephalitozoon cuniculi*, *Sarcocystis neurona*, and *Neospora caninum* using an indirect immunofluorescent antibody test. Five (8%) of the 63 capybaras had antibodies to *T. cruzi* epimastigotes. None of the samples from capybara reacted positively with *L. infantum* promastigotes or with spores of *E. cuniculi*. Two (3%) of the serum samples were positive for antibodies to *S. neurona* merozoites, and 2 (3%) of the serum samples were positive for antibodies to *N. caninum* tachyzoites. A serum sample from 1 capybara was positive for antibodies to both *T. cruzi* and *N. caninum*. None of the remaining 62 samples reacted with more than 1 parasite.

The capybara (*Hydrochoerus hydrochaeris*) is the largest rodent in the Americas. It is widespread in Central and South America and lives in a great variety of habitats including forests, seasonally flooded savannas, and mangrove swamps (Mones and Ojasti, 1986; Moreira and MacDonald, 1997). Capybaras have a strong affinity for aquatic habitats, which they use for mating and to avoid predators (Escobar and González-Jiménez, 1976; MacDonald, 1981; Schaller, 1983). Free-living populations are commercially harvested for their meat and leather in Colombia, Venezuela, and Argentina (Ojasti, 1991). Even though capybaras are rodents, and have high reproductive rates, there is concern that legal and illegal harvesting is not sustainable (Ojasti, 1991).

Little is known of the prevalence of antibodies to protozoa of zoonotic and veterinary importance in these rodents. The prevalence of *Toxoplasma gondii* has been examined in capybaras from Brazil; from 40% to 77% of animals examined were antibody positive (Cañón-Franco et al., 2003; Yai, Ragozo, Aguiar et al., 2008). Several different *T. gondii* genotypes are present in capybaras from Brazil (Yai et al., 2009). A single study of *Neospora caninum* antibodies in capybara from Brazil revealed a prevalence of 9% (Yai, Ragozo, Cañón-Franco et al., 2008). In the present study, we examined sera from capybaras for antibodies to the important human parasites *Trypanosoma cruzi*, *Leishmania infantum*, and *Encephalitozoon cuniculi*. Additionally, we examined the sera from these animals for antibodies to *Sarcocystis neurona* and *Neospora caninum*, both of which are parasites of economic importance in veterinary medicine.

## MATERIALS AND METHODS

### Capybara samples

For the present study, 63 capybaras serum samples were collected from 6 counties (Cordeirópolis, n = 9; Valparaíso, n = 7; Andradina, n = 10; Cosmorama, n = 15; São Paulo, n = 9; and Ribeirão Preto, n = 13) in São Paulo State (Yai, Ragozo, Aguiar et al., 2008). These counties are located between 65 and 627 km from each other. Thirty-seven capybaras were female (59%), 25 were male (41%), and the sex of 1 animal was not recorded. The capybaras had different ages; those greater than 1-yr-old were considered adults. From the 63 samples, 42 (67%) were considered as adults and 21 (33%) as juveniles. Serum samples were stored at –20 C and subsequently sent to the Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia–Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia for serologic testing.

### Parasite culture and antigen production

Epimastigotes of the Brazil strain of *T. cruzi*, and promastigotes of the LIVT-1 strain (Rosypal et al., 2003) of *L. infantum*, were grown in Grace's insect medium containing 30% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin (antibiotics). *Encephalitozoon cuniculi* (ATCC 50502 "canine subtype," American Type Culture Collection, Manassas, Virginia) was grown in human foreskin fibroblasts (Hs68, ATCC CRL1635) that had developed to confluence in 75-mm<sup>2</sup> tissue culture flasks. Growth media consisted of 10% FBS (v/v) in RPMI 1640 medium, supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin. After monolayers had reached confluence, the growth medium was removed and replaced with a maintenance medium that was identical to the former, except that the volume of FBS was 2% (v/v). Flasks were incubated at 37 C in a humidified incubator containing 5% CO<sub>2</sub> and 95% air. *Encephalitozoon cuniculi* spores were harvested from the supernatant. Merozoites of the SN-37R isolate (Sofaly et al., 2002) of *S. neurona*, and tachyzoites of the NC-1 strain (Dubey et al., 1988) of *N. caninum*, were grown and maintained in African green monkey (*Cercopithecus aethiops*) kidney cells (CV-1, ATCC CCL-70, American Type Culture Collection) using techniques identical to those described for Hs68 cells.

### Indirect fluorescent antibody tests

Approximately 0.5 to 1 × 10<sup>4</sup> stages of *T. cruzi*, *L. infantum*, *S. neurona*, and *N. caninum*, or 5 × 10<sup>5</sup> spores of *E. cuniculi* in 30 μl phosphate-buffered saline (PBS), were separately air-dried onto 12-well, Teflon-coated, indirect fluorescent antibody test (IFAT) slides (Fisher Scientific, Pittsburgh, Pennsylvania). Antigen-containing slides were then dried in air for 4–12 hr. Slides containing air-dried spores of *E. cuniculi* were fixed in 100% acetone for 30 sec, while antigen slides containing the other parasites were not fixed in acetone. Antigen IFAT slides were stored at –20 C until

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used. Additionally, we made IFAT slides containing tachyzoites of the RH strain of *T. gondii*. In the absence of defined anti-sera to the parasites in the present study, these slides were used with known-positive and -negative anti-*T. gondii* capybara sera from a previous study on *T. gondii* (Yai, Ragozo, Aguiar et al., 2008) in order to validate the performance of the reagents used in our IFAT.

Capybara sera were diluted 1:25 in PBS and 25 µl were added to a test well. The diluted sera were incubated with antigen for 30 min at room temperature, and the slide was then washed twice in PBS. Fluorescence-labeled goat anti-capybara (Yai, Ragozo, Aguiar et al., 2008) was diluted 1:200 in PBS, and 30 µl were added to each well and incubated at room temperature for 30 min. Slides were washed twice in PBS, mounted in Fluoromount-G (Southern Biotechnology Associates, Inc., Birmingham, Alabama), and viewed with an Olympus BX60 epifluorescent microscope (Olympus America Inc., Center Valley, Pennsylvania) equipped with differential contrast optics. Only samples that exhibited fluorescence of the entire parasite surface were considered positive.

## RESULTS

Five (8%) of the 63 capybara had antibodies to *T. cruzi*; 2 were from juvenile males from São Paulo County, 1 from a juvenile female from Cosmorama County, and 2 were from adult females from Ribeirão Preto County. None of the samples reacted positively with *L. infantum* or *E. cuniculi* antigens.

Two (3%) of the 63 samples had antibodies to *S. neurona*; 1 was from an adult male from Ribeirão Preto County and the other was an adult female from Cordeirópolis County. Two (3%) of the 63 samples were positive for antibodies to *N. caninum*; 1 was from an adult female from Ribeirão Preto County and the other sample was from São Paulo County, but its age and sex were not recorded.

The sample from 1 adult female from Ribeirão Preto County was positive for antibodies to both *T. cruzi* and *N. caninum*. None of the other samples reacted with more than 1 parasite.

## DISCUSSION

*Trypanosoma cruzi* and *L. infantum* are closely related hemoflagellates that are endemic in Brazil. *Trypanosoma cruzi* is the etiologic agent of American trypanosomiasis, or Chagas' disease, and is vectored by hematophagous triatomine arthropods (Dias et al., 2002). Five (8%) of the 63 capybara examined in the present study had antibodies to *T. cruzi*. We are not aware of any case reports, or serological surveys, for antibodies to *T. cruzi* in capybara. However, capybaras are considered a reservoir for the salivarian-transmitted parasite *Trypanosoma evansi*. *Trypanosoma evansi* is present in domestic and wild animals in Brazil (Rademaker et al., 2009). Cross-reactivity of *T. cruzi* antisera with *T. evansi* has been demonstrated using human sera in an ELISA with crude *T. evansi* antigens (Desquesnes et al., 2007). The prevalence of 8% in the samples positive for antibodies to *T. cruzi* epimastigotes in our study may represent a portion that is cross-reactive with *T. evansi*. Food-borne transmission of *T. cruzi*, via the ingestion of raw meat, has been suggested as a potential means of *T. cruzi* transmission in various animals, including humans (see Pereira et al., 2009). Recent studies indicate that this probably does not occur (Roellig et al., 2009) and that the consumption of capybara meat most likely does not pose a human health problem.

Visceral leishmaniasis (VL) is a potentially fatal disease caused by infection with protozoan parasites in the *L. donovani* complex, which includes *L. infantum* (syn. *L. chagasi*; Mauricio et al., 2000). In Latin America, vertebrate hosts become infected with *L.*

*infantum* by infected, blood-feeding phlebotomine sand flies, primarily *Lutzomyia longipalpis*. Studies have examined the prevalence of *Leishmania* species in rodents from Brazil and have determined that rodents can be reservoirs of species that cause the 3 clinical forms of leishmaniasis (Ready et al., 1983). We are not aware of any case reports or surveys documenting *Leishmania* species detection or prevalence in capybaras, and none of the animals examined in the present report had detectable antibodies. Cross-reaction between *T. cruzi* and *Leishmania* species in dogs (*Canis familiaris*) can occur using the IFAT (Duprey et al., 2006), but this was not observed in the present study and all capybara were considered negative for *Leishmania* species.

*Encephalitozoon cuniculi* is well recognized as a parasite of rabbits (*Oryctolagus cuniculus*), rodents, dogs, and occasionally, of humans. It has a fecal-oral transmission pattern. The parasite is not recognized as a major human or animal health problem in Brazil. However, serological studies indicate that the parasite is present in Brazil; antibodies to *E. cuniculi* were found in 79 (14%) of 559 horses (Goodwin et al., 2006) and in 9 (14%) of 63 dogs (Lindsay et al., 2009) from Brazil.

*Sarcocystis neurona* is the cause of equine protozoal myeloencephalitis, an important neurological disease of horses in the Americas (see Dubey, Lindsay, Saville et al., 2001). It has a wide range of intermediate hosts consisting of cats, raccoons (*Procyon lotor*), nine-banded armadillos (*Dasypus novemcinctus*), striped skunks (*Mephitis mephitis*), and sea otters (*Enhydra lutris*), which develop sarcocysts in their muscles. Horses are aberrant hosts for the parasite. Opossums (*Didelphis* spp.), including *D. albiventris*, which is present in Brazil, are definitive hosts for *S. neurona* and excrete sporocysts in their feces (Dubey, Lindsay, Kerber et al., 2001). Opossums excrete sporocysts in their feces after ingesting tissues of intermediate hosts that harbor the sarcocysts. Antibodies to *S. neurona* were demonstrated in the sera from 669 (70%) of 961 horses from Brazil (Hoane et al., 2006), indicating a high environmental contamination with the parasite. Only 2 (3%) of the 63 capybara samples examined had antibodies to *S. neurona*, demonstrating a low prevalence of the parasite in these animals, despite *S. neurona* potentially being in the environment.

*Neospora caninum* is a protozoan parasite that can cause paralysis and death in dogs and is a major cause of bovine abortion worldwide. Dogs and coyotes (*Canis latrans*) are the only recognized definitive hosts for *N. caninum*; they excrete unsporulated oocysts in their feces (McAllister et al., 1998; Gondim et al., 2004). *Neospora caninum* has been isolated from the tissues of naturally infected calves (Locatelli-Dittrich et al., 2003; García-Melo et al., 2009), sheep (*Ovis aries*) (Pena et al., 2007), water buffaloes (*Bubalus bubalis*) (Rodrigues et al., 2004), and dogs (Gondim et al., 2001) from Brazil. Using serological (Jenkins et al., 2007) and PCR-based detection methods, rodents have been implicated as an intermediate host for *N. caninum* (Huang et al., 2004; Hughes et al., 2006; Ferroglio et al., 2007; Jenkins et al., 2007). The result of the present study, which found that 2 (3%) of 63 capybaras had antibodies to *N. caninum*, is lower than the 9% prevalence in capybara examined by Yai, Ragozo, Cañón-Franco et al. (2008). Additional research is needed on potential wildlife intermediate hosts for *N. caninum* in Brazil, and in other countries, to determine the significance of the sylvatic cycle (Rosypal and Lindsay, 2005) in maintenance of the parasite and its transmission to domestic animals.



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