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Authors: Ferreira, R. A., Mineo, J. R., Duarte, J. M., Silva, D. A. O., and Patarroyo, J. H.

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Toxoplasmosis in Naturally Infected Deer from Brazil

R. A. Ferreira,[1] J. R. Mineo,[1] J. M. Duarte,[2] D. A. O. Silva,[1] and J. H. Patarroyo,[3] [1]Universidade Federal de Uberlândia, Laboratory of Immunology, Department of Pathology, Uberlândia-MG, Brazil; [2]Universidade Estadual Paulista (UNESP), Department of Animal Genetic Improvement, Jaboticabal-SP, Brazil; [3]Universidade Federal de Viçosa, Laboratory of Biology and Control of Hemoparasites, Viçosa-MG, Brazil

ABSTRACT: Serum samples from 107 cervids were examined for *Toxoplasma gondii* antibodies using indirect hemagglutination (IHA), indirect immunofluorescence (IFA), enzyme linked immunosorbent assay (ELISA) and Dot-ELISA. Samples were obtained from 66 marsh deer (*Blastocerus dichotomus*) in the State of São Paulo (Brazil) and from 41 pampas deer (*Ozotocerus bezoarticus*) in the State of Goiás (Brazil). Antibodies to *T. gondii* were found in 23 (22%) of the deer, with 18 and 5 positive samples, respectively, for *B. dichotomus* and *O. bezoarticus*. The highest prevalence of *T. gondii* antibodies were young adults (32%), following by adults (27%) and fawns (13%). Only one serum sample (8%) from a newborn fawn was positive in the serological tests. The convenience of the Dot-ELISA test is obvious when compared with other serological tests for both laboratory or field surveys, mainly due to its features of practicability and reagent stability.

Key words: Antibodies, *Blastocerus dichotomus*, enzyme-linked immunosorbent assay (Dot-ELISA, ELISA), haemagglutination, immunofluorescence, marsh deer, *Ozotocerus bezoarticus*, pampas deer, *Toxoplasma gondii*.

Toxoplasmosis, a zoonosis transmitted by the parasite *Toxoplasma gondii*, occurs in many mammals and birds, including domestic and wild animals. Human infections are widespread but they are assymptomatic in a majority of the individuals. While domestic and wild felidae are the hosts in which the life cycle of *T. gondii* is completed, the tissue phase of the parasite is carried as natural infections in many other species which are natural reservoirs for the infection acquired through ingestion of their viscera and meat.

Although surveys indicate that wild animals are often serologically positive for *T. gondii* (Dubey and Beattie, 1988; Dressen, 1990), the role of wildlife in the transmission of *T. gondii* is not well understood (Humphreys et al., 1995; Lindsay et al., 1991). It is well known that carnivores are frequently infected, but there is less information on infection in herbivores (Smith and Frenkel, 1995). The present study investigated the prevalence of *T. gondii* in the marsh deer, (*Blastocerus dichotomus*) and pampas deer (*Ozotocerus bezoarticus*) in order to understand the epidemiology of toxoplasmosis in these natural occurring cervid populations.

Serum samples were collected from 41 pampas deer in Pantanal region of Mato Grosso do Sul (19°50'S to 56°33'W) and Emas National Park (18°15'S to 52°53'W), Goiás, Brazil, from July to December 1995, and from 66 marsh deer, captured in the flooding of a portion of Tietê River (22°15'S to 52°38'W), São Paulo, Brazil from April to September 1990. All 107 samples were analyzed for *T. gondii* antibodies by indirect hemagglutination (IHA), indirect immunofluorescence (IFA), enzyme linked immunosorbent assay (ELISA) and Dot-ELISA.

Methods for cervid capture included the net-gun, drive-net (black polypropylene, diameter: 2.5 mm; mesh: 20 cm—UNESP, Jaboticabal, São Paulo, Brazil) and manual immobility. The animals were aged based on biometrical information such as size and weight and they were imprinted with an ear-tag and a radio-collar was placed on each animal. Blood samples were collected and the serum stored at −20 C until used. Difference in antibody prevalence across age groups was determined using the student *t* test (Kuzma, 1992). The level of significance was at $P < 0.05$.

Formalin treated human type O Rh negative red blood cells were treated with tannic acid for IHA and coated with *T. gondii* soluble antigen which was obtained by freezing-and-thaw procedure (Camargo et al., 1989). All serum samples were ini-

tially tested at 1:16 and 1:32 dilutions. Positive sera were then titered, using two-fold serial dilutions from 1:16 to 1:4,096. To 50 μl of diluted serum, 25 μl of treated cells were added and the agglutination pattern was determined after incubation of 1 hr at room temperature. As a control, all serum samples were simultaneously added to uncoated cells at a dilution of 1:16.

Slides were prepared with the RH strain of *T. gondii* tachyzoites for IFA as described by Camargo (1964). Two-fold serial dilutions of the serum samples, starting at a dilution of 1:8 through 1:1,024 were added to the slides. Fluorescein isothiocyanate (Sigma Chemical Co., St. Louis, Missouri, USA) was conjugated to rabbit anti-*B. dichotomus* IgG, according to described protocol (Camargo, 1964). This conjugate was utilized on appropriated dilution and incubated for 30 min at 37 C. All reactions had reference samples as control. The preparations were observed using an epifluorescence microscope (Olympus, Model BH2, Tokyo, Japan).

The ELISA and Dot-ELISA were performed using polyvinyl microplates and nitrocellulose discs, respectively. The microplates were incubated with *T. gondii* tachyzoites (1×10^5/well) and the nitrocellulose discs were incubated with the same soluble antigen described for IHA. For Dot-ELISA, the treated membranes were incubated for 10 min with a solution of 0.03% H_2O_2 to block endogenous peroxidase. The immunosorbents were incubated with 2-fold serial dilutions of the cervid serum samples, starting at a dilution of 1:8 until 1:512. After incubation of 60 min at room temperature, the immunosorbents were washed again four times in a 0.01 m phosphate buffered saline−0.05% tween 20. Protein-A-peroxidase conjugate (Sigma Chemical Co.) was added as secondary reagent for 30 min at room temperature. For ELISA, the plates were incubated with substrate solution consisting of H_2O_2 (Ibian Chimica Ltd., São Paulo, Brazil) and o-phenylenediamine (Merck, Müchen, Germany) in 0.1M citrate-Na_2HPO_4 buffer (pH 5.5) for 15 min at room temperature. The reaction was stopped with 2N H_2SO_4 and the absorbance was read at 492 nm in a microwell reader system (Titertek Multiskan, Flow Laboratories Inc., McLean, Virginia, USA). For Dot-ELISA, the membranes were incubated for 25 min with substrate solution, consisting of H_2O_2 (Ibiza Química Ltda., Guarulhos, São Paulo, Brazil), diaminobenzidine (Sigma Chemical Co.), $NiCl_2$ (Química Fina Ltda., São Paulo, Brazil) and Trizma-HCl (Sigma Chemical Co.). Antigen discs were finally washed four times with distillate water and the positive reactions were determined by the appearance of clearly defined dark dots.

Table 1 shows the distribution of the titers in the cervid serum samples for the serological tests. The titer 16 was considered cut-off for IHA and ELISA, while the titer 8 was the cut-off for IFA and Dot-ELISA. Antibodies to *T. gondii* were found in 23 (22%) of the deer, with 18 (27%) and 5 (12%) positive samples, respectively, for *B. dichotomus* and *O. bezoarticus*. Titers ranged from 16 to 2,048 by IHA. Serum samples were reactive by IFA presenting titers ranging from 8 to 512. Titers ranged from 8 to 128 by Dot-ELISA and from 16 to 256 by ELISA.

Taken into account that the samples were considered positive when showing reactivity in at least two tests, the presence of antibodies to *T. gondii* was found in 23 of 107 cervids (22%). Table 2 demonstrates the distribution of these results according to host species, sex and age. When analyzing the data based on age distribution, the major difference in antibody prevalence to *T. gondii* was found in the adult and newborn fawn ($P < 0.002$). Only 1 (8%) newborn fawn was serologically positive for *T. gondii* antibodies. Twelve of 57 female serum samples and 11 of 46 male serum samples were seropositive for *T. gondii* (Table 2).

The ubiquitous nature of *T. gondii,* has been well documented (Dressen, 1990). Nearly all animal species are susceptible to

TABLE 1. Comparison of antibody titers to *Toxoplasma gondii* as determined by IHA, IFA, ELISA and Dot-ELISA, in 23 serum samples of marsh deer *(B. dichotomus)* and pampas deer *(O. bezoarticus)*. A titer of 16 was considered cut-off for IHA and ELISA, while the titer 8 was the cut-off for IFA and Dot-ELISA.

Species and sample number	Antibody Titers			
	IHA	Dot-ELISA	IFA	ELISA
O. bezoarticus				
11	512	16	128	16
13	256	64	128	64
30	2,048	16	64	256
35	2,048	128	512	256
11/02	2,048	NR[a]	256	256
B. dichotomus				
16	16	8	NR	NR
134	16	64	128	128
127	16	NR	8	32
04	64	8	128	NR
128	64	8	64	NR
15	16	128	64	NR
08	16	64	32	32
110	64	64	16	128
103	16	NR	8	NR
02	NR	64	16	32
141	NR	16	8	NR
17	NR	128	16	64
144	NR	128	NR	16
27	NR	16	8	16
99	NR	128	NR	16
118	NR	64	32	128
113	NR	16	16	16
106	NR	NR	8	16

[a] NR = Non Reactive.

TABLE 2. Seroprevalence of *Toxoplasma gondii* antibodies in marsh deer and pampas deer from Brazil.

	Number tested	Positive (%)	Negative (%)
Species			
B. dichotomus	66	18 (27)	48 (73)
O. bezoarticus	41	5 (12)	36 (88)
Sex			
Male	46	11 (24)	35 (76)
Female	57	12 (21)	45 (79)
NA[a]	4	0 (0)	4 (100)
Age			
Newborn fawn	12	1 (8)	11 (92)
Fawn	15	2 (13)	13 (87)
Young-adult	13	3 (23)	10 (77)
Adult	63	17 (27)	46 (73)
NA	4	0 (0)	4 (100)
Total	107	23 (22)	84 (79)

[a]NA = Not Available.

T. gondii and there are different rates of seropositivity and clinical signs of toxoplasmosis in different hosts (Dubey and Beattie, 1988). In Brazil, Sogorb et al. (1977) examined 10 species of mammals for the presence of *T. gondii* antibodies by the Sabin-Feldman test, which was positive in nine species, but the majority of the serum samples presented titers ≤1:256.

In the United States, there is serological evidence that deer may serve as reservoirs for the tissue cyst stages of *T. gondii*. In California, antibody prevalence of 7% has been reported in black-tailed deer (Chomel et al., 1994). The prevalence of antibodies to *T. gondii* increases with age in various mammal populations (Franti et al.,

1975). The present study confirms that *T. gondii* prevalence is higher in adult and young-adult animals. Infection may result from exposure to oocysts in the soil, contaminated water or forage (Oertley, 1981). Depending on soil conditions, moisture, and temperature, oocysts may survive for as long as 18 mo (Frenkel et al., 1975). Previous serological surveys demonstrated significant differences in the frequency of seropositive animals among three species of cervids (Kapperud, 1978). These observations may be due to differences in climate and in the presence of cats, which affect the prevalence of oocysts and their survival.

The present study shows that, although the higher titers of antibodies were observed in the IHA and the highest number of reactive samples was detected by ELISA, the Dot-ELISA may be easier to use for diagnosis of toxoplasmosis in cervids or other animals. We emphasize the convenience of this test when compared with other serological tests for both laboratory or field surveys, mainly due to its features of practicability, reagent stability and the possibility of evaluation by visual reading.

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