Indirect Hemagglutination Test for Detection of Antibodies to *Toxoplasma gondii* in Venezuelan felids

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Short Report

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Abstract

Current knowledge of *Toxoplasma gondii* infection in Venezuelan ecosystems is limited. *T. gondii* is a ubiquitous intracellular protozoan parasite. Mammals and birds are intermediate hosts and felid species are definitive hosts. In most human altered habitats, the domestic cat is the predominant definitive host. Cats are important in the epidemiology of *T. gondii* infection because they are the only hosts that can excrete the environmentally resistant oocysts. Other carnivores can be infected by the consumption of tissue cysts when feeding on infected animals and by incidental ingestion of oocysts from environmental contamination. This study aimed to quantify the values of antibodies for *T. gondii* in blood serum of some felids species by means of the technique of Indirect Hemoagglutination. In the present study, seropositivity of *T. gondii* was determined in serum of 35 animals (22 stray cats and 13 wild cats) from Venezuela, South America. Antibodies to *T. gondii* were assayed by the indirect hemagglutination test and found in 21 of 22 (95.45 %) stray catstiters of 1:64 in four, 1:128 in four, 1:256 in one, 1:512 in one, 1:1024 in three, and 1:2048 or higher in eight. In 4 of 6 (66.67 %) ocelots titers of 1:64 in one, 1:256 in one, 1:1024 in one, and one with titers 1:2048. In 3 of 4 (75.00 %) jaguars titers of 1:512 in one, and two with titers 1:2048. The Kruskal-Wallis test showed a statistically significant difference between species ($H = 6.983$, $p = 0.03$).

Introduction

Toxoplasmosis is a parasitic disease, whose etiological agent is *Toxoplasma gondii,* an obligatory intracellular parasite of worldwide distribution, which infects almost all animal species (birds and mammals), including man. The definitive hosts are domestic and wild cats, all those non-feline hosts are intermediaries [1]. Humans and other animals can become infected by ingesting tissue cysts from undercooked meat or from food or drink contaminated with oocysts shed in cat feces. It is a disease of difficult parasitological diagnosis, since it is not easy to demonstrate the etiological agent and to establish the relationship between the infection and the disease; for this reason, the use of serological tests as indirect indicators of the infection is indispensable to make the diagnosis of the etiological agent, based on the presence of antibodies type immunoglobulin G or M (IgG or IgM), equivalent to chronic or acute infections, respectively.

The indirect hemagglutination test (IHT) is used as a routine clinical test in veterinary hospitals because of its level of sensitivity and ease of use [2]. The test is based on the property of anti-*T. gondii* immunoglobulins to produce agglutination in the presence of cytoplasmic antigen-sensitized red blood cells and the parasite's membrane. It is considered a reliable method for the determination of specific immunoglobulins with values of sensitivity 89.80% to 92.85%, specificity 96,60 % to 100% and efficiency 94.80% [1, 3, 4, 5]. Due to the role of the feline in the cycle of the parasite and in consideration to the scarce studies of prevalence in animals in Venezuela, it was proposed to quantify the values of antibodies for *Toxoplasma gondii* in blood serum of some felids species by means of the technique of Indirect Hemoagglutination.

Materials And Methods

Sample collection. Blood samples were collected according to ethical rules, by venipuncture from a population of stray cats (*Felis catus* Linnaeus, 1758) kept in an animal shelter located in La Vela de Coro, Falcón State, and from ocelots (*Leopardus pardalis* Linnaeus, 1758) pumas (*Puma concolor* Linnaeus, 1771) and jaguars (*Panthera onca* Linnaeus, 1758) kept in the zoos of Paraguaná, Falcón State, and Metropolitano del Zulia, Zulia State, in northwestern Venezuela. A volume of 3 ml of blood was obtained from each animal. The blood samples were transported, within 2 h of collection, to the research laboratory and centrifuged for 10 min at 1500 g. The separated sera were stored frozen at (−20°C) until analysis. The presence of *T. gondii* antibodies was analyzed by indirect hemagglutination test (Toxotest-*HAI*® Wiener Lab), which detects antibodies (IgG, IgM) against *T. gondii* and where the reading is interpreted as negative when the presence of a button-shaped sediment or regular edge ring, and positive with the formation of a film or mantle covering 50% or more of the bottom of the wells, equivalent to values ≥1:16 (cut-off point suggested by the manufacturer). It was taken as cut-off titer ≥1:64 modifying the cut-off value. On the other hand, the kit offers the possibility to use 2-Mercaptoethanol (MO), a reagent that allows to differentiate the presence of IgG or IgM, so it could be indicated if we are facing an acute or chronic infection.

Statistical analysis. Since this was a descriptive study was estimated the relative frequency as percentage by analyzing the number of animals of each species reacting to toxoplasma. To determine whether significant differences existed between species, the Kruskall-Wallis test was applied, with a significance $p \leq 0.05$.

Results

In the present study, a total of 22 stray cats, six ocelots, four jaguars and three pumas were examined. Any serum above the cut-off point was considered positive. Based on this sample population seropositivity for *T. gondii* was 21 (95.45 %) for the stray cats, four (66.67 %) for ocelots and three for jaguars (75.00 %). The three pumas were all negatives (Table 1).

Table 1. Data of species, age and sexes of animals include on the study.

Family			males		females	
Species	*n*	age (yr)	+	-	+	-
Felinae						
Felis catus Linnaeus, 1758	22	>2 <6	10	0	11	1
Leopardus pardalis Linnaeus, 1758	6	>5 <9	2	1	2	1
Puma concolor Linnaeus, 1771	3	>5 <9	0	1	0	2
Pantherinae						
Panthera onca Linnaeus, 1758	4	>5 <9	2	0	1	1
Total	35		14	2	14	5

n = sample size, + = positives, - = negatives

Of the total 21 positives sera from stray cats; four showed titers of 1:64, four titers 1:128, one titers 1:256, one titers 1:512, three titers 1:1024, and eight with titers 1:2048. Of the total 7 positives sera of wild cats, in ocelots one showed titers of 1:64, one titers 1:256, one titers 1:1024, and one with titers 1:2048, in jaguars one showed titers 1:512, and two titers 1:2048 (Fig. 1).
High titers of 1:1024 or higher do not always correspond with acute infection. In sera from animals treated with 2-mercaptoethanol the titres decreased by at least two dilutions, corresponding to the elimination of IgM, consequently, a total of 11 animals showed chronic infection. The Kruskal-Wallis test showed a statistically significant difference ($H = 6.983$, $p = 0.03$).

Discussion

To detect seroprevalence of *T. gondii* in domestic and stray cats as well as wild cats, researchers have used different laboratory methods in different countries. The Toxoplasma Indirect Hemoagglutination Test, reported in this study, can be used as a rapid screening test for toxoplasmosis because it has a high sensitivity and specificity, is not very laborious and is free of contamination risks.

Under normal conditions, Ig are produced after infection and a cell-mediated response occurs and can be considered as markers of the acute or chronic phase of the infection [6]. Classically, IgM is considered as a marker of the acute phase of a disease, because it is the first to appear. However, it is known that IgM titers can remain detectable for many months or even years after a first infection. The absence of IgM therefore rules out a recent infection. A cat with active toxoplasmosis will have high IgM titer. The second marker is IgG. Its presence implies that the animal has been in contact with the parasite at some point in its life. Specific IgG appear at three weeks and very high titer persist for a long time, especially in the case of cats can remain up to 5 years. Therefore, this fact indicates that when only one IgG titer is observed, active toxoplasmosis cannot be suspected. This only indicates the presence of the antigen in the patient and not the disease. To determine the presence of both heterophile antibodies and IgM, characteristic of the acute period of the parasitosis, the positive sera were subjected to 2-mercaptoethanol titration.

It is important to note that both in the shelter and in the two zoos insects (flies and cockroaches), wild birds (pigeons, vultures), rodents (rats and mice), dogs and stray cats can enter and leave without restriction. The above-mentioned insects are known to be defined as transport hosts for *T. gondii*, as they are capable of spreading the oocysts present in contaminated fecal material [7]. Likewise, birds and rodents are described as intermediate hosts of great importance, which constitute the favorite living prey of cats [8].

In the present study, the overall seropositivity for *T. gondii* in stray cats was significantly higher than in other studies in Latin America. In Bogota, Colombia [9] reported 45.20% stray cats; whereas [10] found 48.30% positivity in pet cats in San Carlos, Chile. On the other hand, anti-*T. gondii* antibodies have been reported in both captive and free-living jaguars in Brazil [11-13]. The overall seropositivity for *T. gondii* in jaguars was lower in comparison with the aforementioned studies. According to the author's knowledge,

this article reports for the first time the detection of antibodies against *T. gondii* in ocelots and jaguars in Venezuela.

Declarations

To carry out the study, approval was obtained through an informed consent read and signed by the administrative representatives of each facility. All protocols followed good practices and animal welfare principles set forth in the Law on the Practice of Veterinary Medicine Official Gazette No. 28,737 dated 24 September 1968 and the Law on the Protection of Wild Fauna Official Gazette No. 28,289 dated 11 August 1970 and approved by Animal Research Ethical Committee. All efforts were made to utilize only the minimum number of animals necessary to produce reliable scientific data.

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Conflict of interest

The author declares that there is no conflict of interest.

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Figures

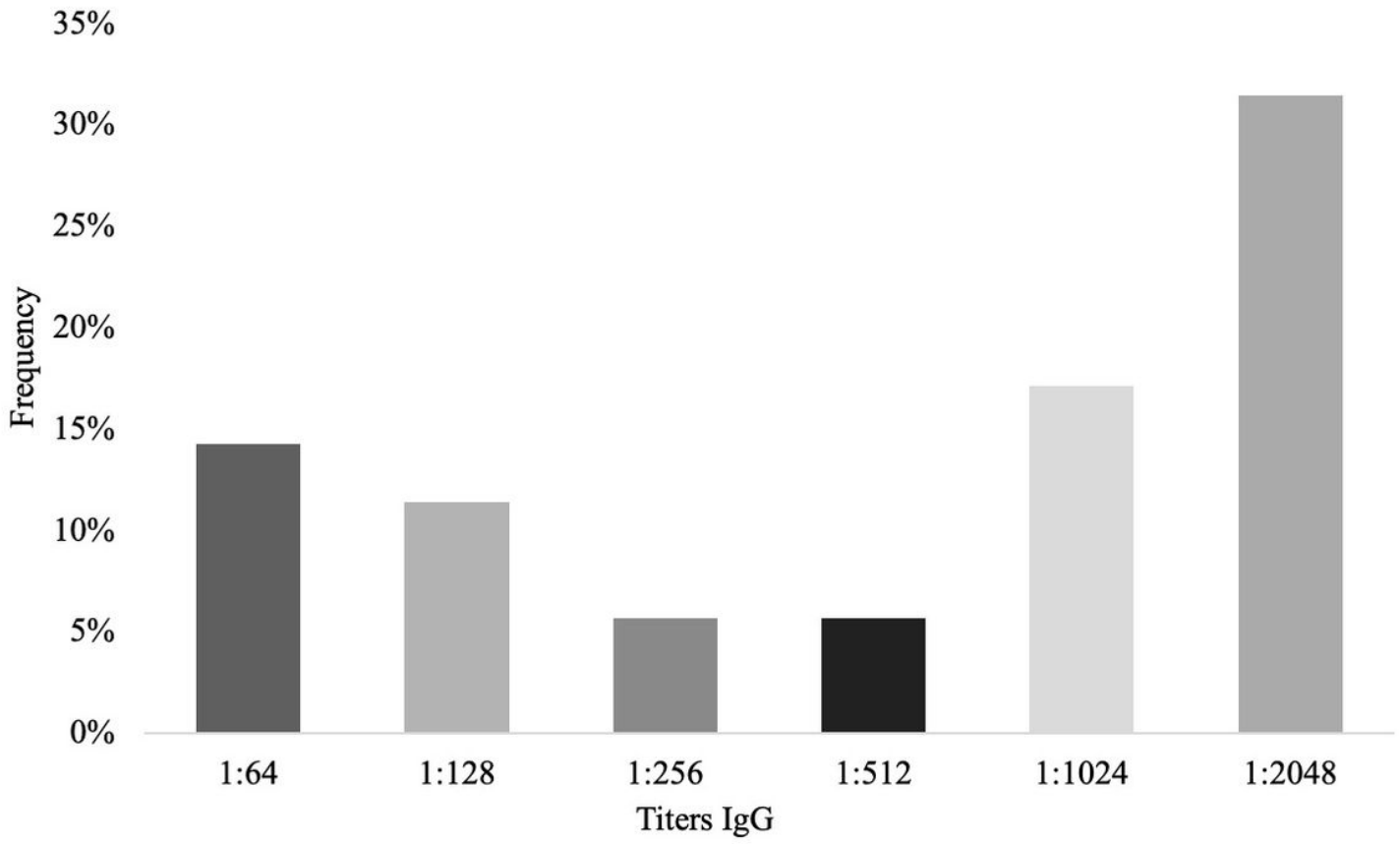

Figure 1

Anti-Toxoplasma gondii IgG titration. The bars represent the frequency of positive cases.