https://doi.org/10.19052/mv.vol1.iss44.4

Indirect Hemagglutination Test in the Detection of Antibodies against *Toxoplasma gondii* in Venezuelan Felids*

Israel Cañizales[1]

Abstract

Current knowledge of *Toxoplasma gondii* infection in Venezuelan ecosystems is limited. 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 environmentally resistant oocysts. Other carnivores can be infected consuming 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 employing the technique of indirect hemagglutination. 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 found in 21 of 22 (95.45 %) stray cats' titers 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 four of six (66.67 %) ocelots' titers of 1:64 in one, 1:256 in one, 1:1024 in one, and one with titers 1:2048. In three of four (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 = 8.413, p = 0.015).

Keywords: antibodies, cats, jaguar, South America, Toxoplasma *gondii*.

Prueba de hemaglutinación indirecta en la detección de anticuerpos contra *Toxoplasma gondii* en félidos venezolanos

Resumen

El conocimiento actual de la infección por *Toxoplasma gondii* en los ecosistemas venezolanos es limitado. Los mamíferos y las aves son hospedadores intermedios y los félidos son hospedadores definitivos. En la mayoría de los hábitats alterados por el hombre, el gato doméstico es el hospedador definitivo predominante. Los gatos son importantes en la epidemiología de la infección por *T. gondii* porque son los únicos hospedadores que pueden excretar los ooquistes resistentes al medio ambiente. Otros carnívoros pueden infectarse por el consumo de quistes tisulares al alimentarse de animales infectados y por la ingestión incidental de ooquistes procedentes de la contaminación ambiental. Este estudio tuvo como objetivo cuantificar los valores de anticuerpos para *T. gondii* en el suero sanguíneo de algunas especies de félidos mediante la técnica de hemoaglutinación

* Research article.

1 Médico veterinario. Doctor en Ciencias del Instituto de Zoología y Ecología Tropical. Facultad de Ciencias, Universidad Central de Venezuela. Apartado Postal 47058. Caracas 1041-A, Venezuela.
✉ israel.canizales@ciens.ucv.ve
http://orcid.org/0000-0001-6553-9494

Cómo citar este artículo: Cañizales I. Indirect Hemagglutination Test in the Detection of Antibodies against Toxoplasma gondii in Venezuelan Felids Rev Med Vet. 2022;(44): 25-31. Available from: https://doi.org/10.19052/mv.vol1.iss44.4

Recibido: 10 de mayo de 2021. Aprobado: 15 de junio de 2021. Versión Online First: 26 de octubre de 2021. Publicación final: 24 de enero 2022

indirecta. En el presente estudio se determinó la seropositividad de *T. gondii* en el suero de 35 animales (22 gatos callejeros y 13 felinos silvestres) de Venezuela, Sudamérica. Los anticuerpos contra *T. gondii* se encontraron en 21 de 22 (95,45 %) gatos callejeros con títulos de 1:64 en cuatro, 1:128 en cuatro, 1:256 en uno, 1:512 en uno, 1:1024 en tres y 1:2048 o más en ocho. En cuatro de seis (66,67 %) ocelotes con títulos de 1:64 en uno, 1:256 en uno, 1:1024 en uno, y uno con títulos 1:2048. En 3 de 4 (75,00 %) jaguares con títulos de 1:512 en uno, y dos con títulos 1:2048. La prueba de *Kruskal-Wallis* mostró una diferencia estadísticamente significativa entre las especies (H = 8,413, p = 0,015).

Palabras clave: anticuerpos, gatos, jaguar, Sudamérica, *Toxoplasma gondii*.

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 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, 3-5).

In Venezuela, previous studies of exposure to *T. gondii* in captive neotropical wild felid species there are not known, and reports in domestic or stray animals are scarce. The aim of the present study was to determine the occurrence of anti-*T. gondii* in a) wild felids that live in confinement in two zoological institutions, and b) stray cats from a private animal shelter using the technique of indirect hemagglutination.

Materials and methods

Animal sample size

In both the zoos and the animal shelter, the total number of animals that could be sampled was chosen and defined by those directly responsible for each facility. This study does not evaluate in any way the management or housing protocols of the animals sampled. Although no information of feeding protocols available was observable on site, it was noted that all animals were fed with a variety of uncooked meat of different species (cattle, horse, sheep, pork, chicken, pigeon, fish, mice, rat, guinea pigs, rabbit), and commercial food.

Sample collection

Blood samples were collected by venipuncture from jaguars (*Panthera onca Linnaeus*, 1758), ocelots (*Leopardus pardalis* Linnaeus, 1758), and pumas (*Puma concolor*

Linnaeus, 1771) kept in the zoos Metropolitano del Zulia, Zulia State, and Paraguaná, Falcón State, in northwestern Venezuela. As well as from a population of stray cats (*Felis catus* Linnaeus, 1758) kept in an animal shelter located in La Vela de Coro, Falcón State. 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.

Serological test

The presence of *T. gondii* antibodies was detected by an indirect hemagglutination test (Toxotest-HAI® Wiener Lab), which recognize antibodies (IgG, IgM) against *T. gondii*. The results are interpreted as negative when button-shaped sediment or regular edge ring is present, 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. To determine the presence of both heterophile antibodies and IgM, the positive sera were subjected to 2-mercaptoethanol (MO) titration, so it could be indicated if we are facing an acute or chronic infection.

Statistical analysis

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

Results

A total of 35 animals (4 jaguars, 3 pumas, 6 ocelots, and 22 stray cats) were evaluated by IHT. Any serum above the cut-off point was considered positive. The results revealed 80 % of seropositivity for *T. gondii*. Jaguars (75.00%), ocelots (66.67%), and stray cats (95.45%). The pumas were all negatives (see table 1).

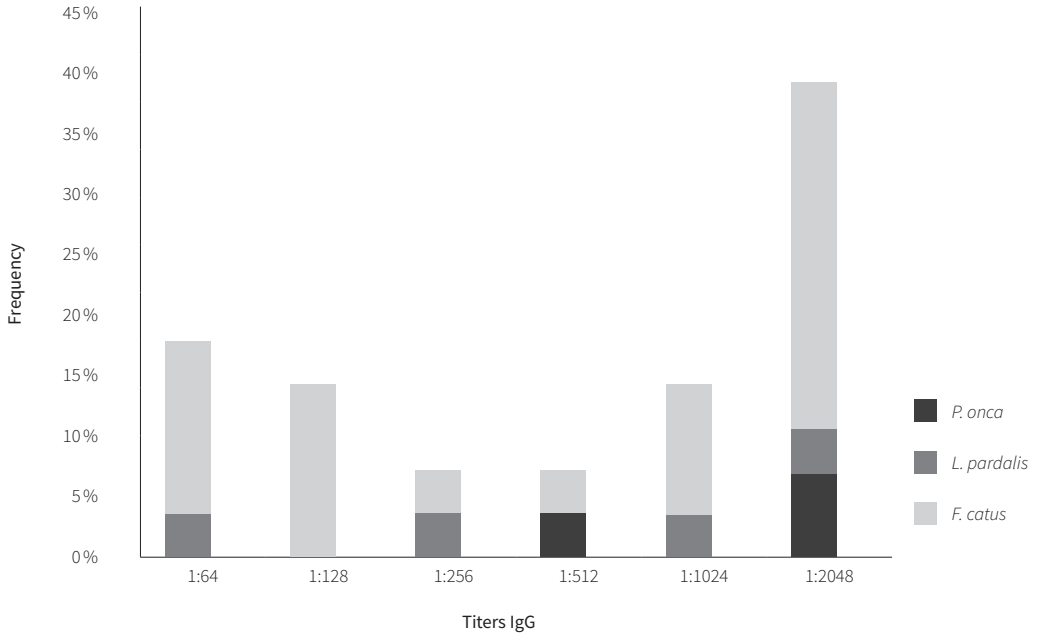
Of the total seven positive sera of wild cats, in jaguars, one showed titer 1:512, and two titers 1:2048, while in ocelots one showed titers of 1:64, one titer 1:256, one titer 1:1024, and one with titers 1:2048. Of the total of 21 positives sera from stray cats, 4 showed titers of 1:64, 4 titers of 1:128, 1 titer of 1:256, 1 titer of 1:512, 3 titers of 1:1024, and 8 titers of 1:2048 (Figure 1).

Table 1. Data of species, age, and sexes of animals included in the study

Subfamily			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
Source: own work

Figure 1. Anti-*Toxoplasma gondii* IgG titration. The bars represent the frequency of positive cases



Source: own work

High titers of 1:1024 or higher do not always correspond with acute infection. In sera from animals treated with 2-mercaptoethanol, the titers decreased by at least two dilutions, corresponding to the elimination of IgM; consequently, a total of eleven animals showed chronic infection (two jaguars, one ocelot, and eight stray cats). The *Kruskal-Wallis* test showed a statistically significant difference between the three species (H = 8.413, p = 0.015). However, between jaguars and ocelot, the seropositivity showed no differences (H = 0.508, *p* = 0.476).

Discussion

According to the author's knowledge, this study reports for the first time the detection of antibodies against *T. gondii* in jaguars and ocelots in Venezuela. Similar studies have been conducted for both captive and free-living jaguars in French Guiana (6) and Brazil (7-9). The overall seropositivity for *T. gondii* in jaguars in the present study was lower than those other studies. For ocelots, the overall seropositivity (66.67%) was slightly different from the previous report in Bolivia (10), Brazil (11, 12), and Mexico (13). On the other hand, anti-*T. gondii* antibodies for *T. gondii* in stray cats in this study were significantly higher than those reported in stray cats (45.20%) in Bogota, Colombia (14), and in pet cats (48.30%) in San Carlos, Chile (15). However, it might not be possible to compare results because of the sample size and the serologic test used.

For South America, the global seropositivity observed for wild felids ranges from 41% up to 91% (16). Whereas for jaguars' ranges from 63.5% up to 84%, ocelots from 58.1% up to 73.8% (17). For domestic cats, it has been observed from 29% up to 45% (16) and from 34 % up to 49.6% (17).

It is important to note that both in the two zoos and the shelter tested wild birds (pigeons, vultures), dogs and stray cats, rodents (rats and mice), and insects (flies and cockroaches), can enter and leave without restriction. The above-mentioned insects are known to be defined as transport hosts for *T. gondii*, as they can spread the oocysts present in contaminated fecal material (18). Likewise, birds and rodents are described as intermediate hosts of great importance, which constitute the favorite living prey of cats (19). Additionally, all animals receive raw meat in their diet, which also provides an opportunity for infection with *T. gondii*. This can explain the high seroprevalence stated in this study among the tested cats (95.45%).

The difference in titers depends on the antigen profiles presented in each test for antibody detection. Under normal conditions, Ig is produced after infection and a cell-mediated response occurs, and those can be considered as markers of the acute or chronic phase of the infection (20). 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 recent infections. A cat with active toxoplasmosis will have a 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 appears at three weeks and a very high titer persists for a long time, especially in the case of cats that can remain up to five 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.

In the current study, 50 % of jaguars, 16.67% of ocelots, and 63.64% of stray cats were IgM positive, which may indicate a recent infection. Because IgM is occasionally also detected in the serum of animals with chronic infection, or as a false-positive IgM caused by autoimmune diseases (20), it is likely that not all tested animals with anti-*T. gondii* IgM had been recently infected.

The toxoplasma indirect hemagglutination test, reported in this study, can be used as a rapid screening test for toxoplasmosis because it has high sensitivity and specificity, is not very laborious, and is free of contamination risks.

Acknowledgments

The author would like to thank Paraguana and Metropolitano del Zulia zoos for allowing to take samples of their animals.

Ethical statement

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 of 1970 and approved by Animal Research Ethical Committee.

Conflict of interest

The author declares that there is no conflict of interest.

Funding

This study did not receive funds or sponsorship from any public or private organization.

References

1. Dubey JP. Toxoplasmosis of Animals and Humans (2nd ed.). Boca Raton, Florida: CRC Press; 2010.
2. Radostits O, Gay C, Hinchcliff K, Constable P. (eds.). Veterinary Medicine. (10th ed.). A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. Saunders Ltd; 2006.
3. Bahnass MM, El-Shahawy IS, Fawzi EM. Comparative analysis of toxoplasmosis in farm animals by indirect hemagglutination assay and enzyme linked immunosorbent assay. Alexandria J Vet Sci. 2015;(46): 15-19. Available from: https://doi.org/10.5455/ajvs.184502
4. Fernandes SB, Brilhante-Simões P, Coutinho T, Cardoso L, Dubey JP, Lopes AP. Comparison of indirect and modified agglutination tests for detection of antibodies to Toxoplasma gondii in domestic cats. J Vet Diagnostic Invest. 2019;(31): 774-777. Available from: https://doi.org/10.1177/1040638719868753
5. Yang YX, Chen YK, Wei SJ, Song RH. Efficiency of three methods for detecting Toxoplasma IgG antibody. Chinese J Schist Ctrl. 2014;26(1): 109-110.
6. Demar M, Ajzenberg D, Serrurier B, Dardé M, Carme B. Atypical Toxoplasma gondii strain from a free-living jaguar (Panthera onca) in French Guiana. Am J Trop Med Hyg. 2008;78(2): 195-197. Available from: https://doi.org/10.4269/ajtmh.2008.78.195
7. André MR, Adania CH, Teixeira RHF, Silva KF, Jusi MMG, Machado STZ, de Bortolli CP, Falcade M, Sousa L, Alegretti SM, Felippe PAN, Machado RZ. Antibodies to Toxoplasma gondii and Neospora caninum in captive neotropical and exotic wild canids and felids. J Parasit. 2010;96(5): 1007-1009. Available from: https://doi.org/10.1645/GE-2502.1
8. Onuma SSM, Melo Tomé AL, Zanella Kantek DL, Crawshaw Jr. PG, Goncalves Morato RG, Pacheco May-Júnior JA, dos Anjos Pacheco T, de Aguiar DM. Exposure of free-living jaguars to Toxoplasma gondii, Neospora caninum, and Sarcocystis neurona in the Brazilian Pantanal. Rev Bras Parasitol Vet. 2014;23(4): 547-553. Available from: https://doi.org/10.1590/s1984-29612014077
9. Silva JC, Ogassawara S, Marvulo MF, Ferreira-Neto JS, Dubey JP. Toxoplasma gondii antibodies in exotic wild felids from Brazilian zoos. J Zoo Wild Med. 2001;32(3): 349-351. Available from: https://doi.org/10.1638/1042-7260(2001)032[0349:TGAIEW]2.0.CO;2
10. Fiorello CV, Robbins RG, Maffei L, Wade SE. Parasites of free-ranging small canids and felids in the Bolivian Chaco. J Zoo Wildl Med. 2006;37(2): 130-134. Available from: https://doi.org/10.1638/05-075.1 PMID: 17312790.
11. Ferraroni JJ, Reed SG, Speer CA. Prevalence of Toxoplasma antibodies in humans and various animals in the Amazon. Proc Helminthol Soc Wash. 1980;47(1): 148-150.
12. Ramos Silva JC, Ogassawara S, Adania CH, Ferreira F, Gennari SM, Dubey JP, et al. Seroprevalence of Toxoplasma gondii in captive neotropical felids from Brazil. Veterinary Parasitology. 2001;102(3): 217-224. Available from: https://doi.org/10.1016/s0304-4017(01)00523-4
13. Rendón-Franco E, Caso-Aguilar A, Jiménez-Sánchez NG, Brousset Hernández-Jauregui DM, Sandoval-Sánchez AL, et al. Prevalence of Anti-Toxoplasma gondii Antibody in Free-ranging Ocelots (Leopardus pardalis) from Tamaulipas. J Wildl Dis. 2012;48(3): 829–831. Available from: https://doi.org/10.7589/0090-3558-48.3.829
14. Dubey JP, Su C, Cortes JA, Sundar N, Gomez-Marin JE, Polo LJ, Zambrano L, et al. Prevalence of Toxoplasma gondii in cats from Colombia, South America, and genetic characterization of *T. gondii* isolates. Vet Parasitol. 2006;(141): 42-47. Available from: https://doi.org/10.1016/j.vetpar.2006.04.037
15. Troncoso Toro IE, Uribe Henríquez PA, Arrué Brenet KC, Valenzuela Contreras AA, Fischer Wiethuchter C. Seroprevalencia de Toxoplasma gondii en gatos (Felis catus, Linnaeus 1758) residentes en San Carlos, Chile. Rev Med Vet. 2015;(29): 23-31. Available from: https://doi.org/10.19052/mv.3443
16. Montazeri M, Mikaeili Galeh T, Moosazadeh M, Sarvi S, Dodangeh S, Javidnia J, et al. The global serological prevalence of Toxoplasma gondii in felids during the last five decades (1967–2017): a systematic review and meta-analysis. Parasites Vectors. 2020;(13):82. Available from: https://doi.org/10.1186/s13071-020-3954-1
17. Hatam-Nahavandi K, Calero-Bernal R, Rahimi MT, Pagheh AS, Mehdi Z, Dezhkam A, et al. Toxoplasma

gondii infection in domestic and wild felids as public health concerns: a systematic review and meta-analysis. Sci Rep. 2021;(11):9509. Available from: https://doi.org/10.1038/s41598-021-89031-8

18. Afonso E, Lemoine M, Poulle ML, Ravat MC, Romand S, Thulliez P, et al. Spatial distribution of soil contamination by Toxoplasma gondii in relation to cat defecation behavior in an urban area. Internat J Parasitol. 2008;(38): 1017-1023. Available from: https://doi.org/10.1016/j.ijpara.2008.01.004
19. Baker PJ, Bentley AJ, Ansell RJ, Harris S. Impact of predation by domestic cats Felis catus in an urban area. Mammal Review. 2005;(35): 302-312. Available from: https://doi.org/10.1111/j.1365-2907.2005.00071.x
20. Tizard IR. Veterinary immunology. St. Louis: Elsevier Health Sciences; 2013.