Detection of DNA and anti-*Toxoplasma gondii* antibodies in errant cats (*Felis catus domesticus*, Linnaeus, 1758) captured by the Zoonoses Control Center of Goiânia, state of Goiás, Brazil

Hanstter Hallison Alves Rezende

Universidade Federal de Jataí, Câmpus Cidade Universitária, BR 364, km 195, n. 3800, 75801-615, Jataí, Goiás, Brasil, hanstter@gmail.com

Jaqueline Ataíde Silva Lima

Antonio Roberto Gomes-Júnior

Jade de Oliveira Melo

Heloísa Ribeiro Storchio

Taynara Cristina Gomes

Marina Clare Vinaud

Ana Maria de Castro

Universidade Federal de Goiás, Instituto de Patologia Tropical e Saúde Pública, Laboratório de Estudos da Relação Parasito-Hospedeiro, Goiânia, Goiás, Brasil, amaria.ana@gmail.com

Abstract: The purpose of this study was to verify the seropositivity of IgG anti-*T. gondii* antibodies in serum samples and to detect *T. gondii* DNA detection in tissue from stray cats captured by the Zoonosis Control Center (ZCC)of Goiânia, state of Goiás, during 2016. Antibodies were analyzed by means of an indirect hemagglutination assay (IHA), while DNA was detected by polymerase chain reaction (PCR), using primers that amplify the B1 gene in specimens of brain, muscle and heart from the animals. The serological analysis revealed that 87.4% (21/24) of the cats were positive and the molecular test indicated a positivity rate of 75% (18/24). These high rates of serological and molecular detection are worrisome for public health because they confirm the important role cats play in environmental contamination, and hence, in the transmission of toxoplasmosis to humans.

Keywords: Toxoplasmosis, cats, hemagglutination, polymerase chain reaction, gene.

Detecção de DNA e anticorpos anti-*Toxoplasma gondii* em gatos errantes (*Felis catus domesticus*, Linnaeus, 1758) capturados pelo Centro de Controle de Zoonoses de Goiânia, Goiás, Brasil

Resumo: O objetivo deste estudo foi verificar a soropositividade de anticorpos IgG anti-*T. gondii* em amostras de soro e detectar DNA do parasito em tecidos de gatos errantes capturados pelo Centro de Controle de Zoonose de Goiânia, Goiás, durante 2016. Para a análise dos anticorpos foi utilizada a

técnica de Hemaglutinação Indireta (HAI) e para a pesquisa de DNA foi utilizada a Reação em Cadeia da Polimerase (PCR) com *primers* que amplificam o gene B1 em amostras de cérebro, músculo e coração dos gatos. A análise sorológica identificou 87,4% (21/24) de animais soropositivos e a análise molecular identificou 75% (18/24). A elevada taxa de detecção sorológica e molecular são aspectos preocupantes em saúde pública, pois comprova o importante papel desses animais na contaminação ambiental e consequentemente pela transmissão da toxoplasmose para o ser humano.

Palavras-chave: Toxoplasmose, gatos, hemaglutinação, Reação em Cadeia da Polimerase (PCR), gene.

Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*, whose definitive hosts are domestic and wild cats and whose intermediary hosts are a variety of birds and mammals, including humans (Dubey et al., 2013).

Cats in urban areas can release millions of oocysts in their feces. Therefore, stray cats that defecate in public areas are major factors in the perpetuation and transmission of this parasite in cities. Both young and adult cats may return to the acute stage of infection and release oocysts, particularly when they are co-infected with the feline immunodeficiency virus (Brown et al., 2003).

The *T. gondii* can be transmitted via several mechanisms, including infection resulting from the accidental ingestion of sporulated oocysts in contaminated water and/or food, and raw or undercooked meat or sausages (Dubey et al., 2013). The acquired form of the infection is related mainly to eating habits and may occur in the early years of life (Rezende et al., 2017).

In the municipality of Goiânia, Goiás, surveys conducted at two public maternity hospitals revealed a 51.85% prevalence rate of human toxoplasmosis in chronically infected mothers (Avelar, 2013). Out of a total of 10,316 screened mothers, 67.7% were IgG positive and 32.2% were IgG negative but are at risk of infection (Sartori et al., 2011).

There are few reports about the seroprevalence rate of *T. gondii* in cats and their shedding of oocysts. Rezende (2015) reported that 13.6% of stray cats captured by the Zoonosis Control Center in the metropolitan region of Goiânia were shedding *T. gondii* oocysts and that the seroprevalence rate in cats was 64% (Rezende, 2015).

The purpose of this study was to verify the seropositivity of IgG anti-*T. gondii* antibodies in serum samples and to detect *T. gondii* DNA detection in tissue from stray cats captured by the Zoonosis Control Center (ZCC) of Goiânia, state of Goiás, during 2016.

The experimental procedures of this study were carried out as specified by the National Council for the Control of Animal Experimentation (CONCEA). Moreover, the study was approved by the Ethics Committee on Animal Use of the Federal University of Goiás (CEUA/UFG), under Protocol No. 024/2016.

Description of the animals of this study - A total of 24 stray cats *(Felis catus domesticus*) captured by the ZCC of the Municipal Secretariat for Epidemiological Surveillance of Goiânia were analyzed during 2016. Due to operational constraints, we were unable to determine the capture site of the cats. However, it should be noted that these animals had been slated for euthanasia due to signs of illness. Healthy stray cats are routinely sent to animal shelters and offered for adoption.

Collection of biological samples - Sick animals were euthanized with an overdose of sodium thiopental by the head veterinary physician at the ZCC. A sample of 3 mL of blood was then drawn from each animal via intracardiac puncture and stored in a sterile tube without anticoagulant. Each animal was necropsied and samples of brain, heart and thigh muscle tissue were collected. The tissue samples were put into plastic bags and stored in an icebox.

The blood and tissue samples were sent to the Laboratory for Host-Parasite Relationship Studies at the Institute of Tropical Pathology and Public Health, Federal University of Goiás (LAERPH/IPTSP-UFG). The whole blood was centrifuged at 2500g for 15 minutes to obtain serum. The tissue samples from each cat were sliced with a sterile scalpel and homogenized in a food processor. The pooled tissues were stored in microtubes at -20 ºC for later DNA extraction.

Indirect Hemagglutination Assay (IHA) - The serum samples were analyzed by means of the indirect hemagglutination assay (IHA),

using a commercial Toxotest Wiener Lab® kit. Samples showing titers of ≥ 32 were considered reactive. Positive and negative controls were used in all the reactions, and the reactive samples were subjected to titration up to a titer of ≥ 1024.

Polymerase Chain Reaction (PCR)- DNA was extracted from the pooled tissues using the protocol of the BIOPUR® commercial kit for DNA tissue extraction. The DNA samples extracted from the homogenates were subjected to B1 gene PCR. The reactions were performed in a final volume of 25 µL containing 17.3 µL of sterile Milli-Q H_2O, 1.0 µL of $MgCl_2$, 2.5 µL of 10X Buffer (Invitrogen®), 0.2 µL of Taq DNA Polymerase (Invitrogen®), 0.5 mM of each deoxynucleotide (dATP/ dTTP/ dGTP/ dCTP, Sigma®), 50 pmol of each reaction primer (Invitrogen®) and 2µL of extracted DNA. The primer pairs used were: Toxo-B5 (5'-TGA AGA GAG GAA ACA GGT GGT CG-3') and Toxo-B6 (5'-CCG CCT CCT TCG TCC GTC GTA-3').

The PCR amplification program consisted of initial denaturation at 94 ºC (5 min), followed by 35 denaturation cycles at 94 ºC (1 min), annealing at 62 ºC (1 min) and extension at 72 ºC (1 min), and ending with a final extension at 72 ºC (10 min). The PCR amplification products with a size of 110 pb were examined using silver-stained 6% polyacrylamide gel electrophoresis.

Statistical analysis - A statistical analysis was made of the concordance between the positivity detected by IHA and PCR by Pearson's chi-squared test (X^2). In addition, an evaluation was made of the Kappa coefficient, which varies from -1 (complete discordance) to +1 (total concordance).

The serological analysis indicated that 87.4% (21/24) of the cats had anti-*T. gondii* antibodies, and the titer most frequently found was 64 (Tab. 1).

The seroprevalence rate of stray cats found in this study was higher than the 64% detected previously by Rezende (2015) in Goiânia. High seroprevalence rates in cats have also been reported in other regions of Brazil. For instance, in the state of Rondônia, in northern Brazil, a prevalence rate of 87.3% was reported (Cavalcante et al., 2006), while in the state of Paraná, in the south, the reported prevalence rate was 84.4% (Dubey et al., 2004). In the state of Pernambuco, northeastern Brazil, a seroprevalence rate of 72% was reported in the archipelago of Fernando de Noronha (Costa et al., 2012), and the state of Rio de Janeiro, in southeastern Brazil, showed the same prevalence rate of 72%, high seroprevalence rates in cats are indicative of high environmental contamination with *Toxoplasma gondii* oocysts (Mendes-de-Almeida et al., 2007).

In addition to cases of *T. gondii* infection in humans, the high infection rates among cats is also worrisome, given the large numbers of oocysts that have been detected in the feces of adult animals. In an analysis of 149 samples of cat feces, 65 from stray cats and 84 from domestic cats, a prevalence rate of 27.7% (18/65) was found in stray cats and of 3.6% (3/84) in domestic cats. These prevalence rates underscore the important role of stray cats in the epidemiology of toxoplasmosis in urban areas (Lima, 2016).

The analysis of the presence of *T. gondii* DNA in the tissues of cats revealed a positivity rate of 75% (18/24). In the PCR analysis, one positive sample was detected that had tested negative by IHA, while four IHA positive samples tested negative by PCR (Tab. 2).

This difference in the results obtained by the IHA and PCR techniques is attributed to the fact that immunological tests detect antibodies that are dissolved in the serum, whereas molecular assays are directly related to the presence of the parasite in the biological material

Tab. 1. Serological analyses of anti-*Toxoplasma gondii* antibodies detected by IHA in stray cats in Goiânia, Goiás, Brazil during 2016*.

Antibody Titers	Stray cats	
	Absolute frequency	Relative frequency
<32	3*	12.5%
32	3	12.5%
64	11	45.8%
128	4	16.6%
256	1	4.2%
512	1	4.2%
≥1024	1	4.2%
Total	**24**	**100%**

*Only samples presenting titers of ≥ 32 were considered positive.

Tab. 2. Comparison of the IHA results of antibody titers in serum of stray cats and the PCR results from the pool of tissues from stray cats in Goiânia, Goiás, Brazil.

Antibody Titers	Stray cats		
	PCR Positive	PCR Negative	Total
<32	1	2	3
32	3	0	3
64	9	2	11
128	2	2	4
256	1	0	1
512	1	0	1
≥1024	1	0	1
Total	**18**	**6**	**24**

under analysis. Hence, according to the literature, only one cyst of the parasite is found in each 100g of tissue (Aigner et al., 2010).

In conclusion, this study found a high prevalence rate of antibodies and DNA of *T. gondii* in tissue samples from stray cats captured by the ZCC in Goiânia. These findings confirm the role of cats in the transmission cycle of infection in this region, which may explain the high prevalence of toxoplasma infection in humans in the metropolitan region of Goiânia.

References

Aigner, C. P., A. V. Da Silva, F. Da Sandrini, P. S . Osório, L. Poiares & A. Largura. 2010. Real-time PCR-based quantification of *Toxoplasma gondii* in tissue samples of serologically positive outdoor chickens. Mem. Inst. Oswaldo Cruz. 105 (7): 935–937.

Avelar, J. B. 2013. Toxoplasmose crônica em gestantes: Avaliação da prevalência, fatores de risco e acompanhamento de um grupo de recém-nascidos em Goiânia – Goiás. 105 f. Tese de Doutorado em Medicina Tropical e Saúde Pública – Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia.

Brown, R. R., T. H. Elston, L. Evans, C. Glaser, M. L. Gulledeg, L. Jarboe, M. R. Lappin & L. C. Marcus. 2003. American Association of Feline Practitioners. Compendium. 25 (12): 948–996.

Cavalcante, G. T., D. M. Aguiar, D. Chiebao, J. P. Dubey, V. L. A. Ruiz, R. A. Dias, L. M. A. Camargo, M. B. Labruna & S. M. Gennari. 2006. Seroprevalence of *Toxoplasma gondii* Antibodies in Cats and Pigs From Rural Western Amazon, Brazil. J. Parasitology. 92 (4): 863–864.

Costa, D. G. C., M. F. V. Marvulo, J. S. A. Silva, S. C. Santana, F. J. R. Magalhães, C. D. F. Lima Filho, V. O. Ribeiro, L. C. Alves, R. A. Mota, J. P. Dubey & J. C. R. Silva. 2012. Seroprevalence of *Toxoplasma gondii* in domestic and wild animals from the Fernando de Noronha, Brazil. J. Parasitology. 98 (3): 679–680.

Dubey, J. P., S. Choudhary, G. Tilahun, N. Tiao, W. A. Gebreyes, X. Zou & C. Su. 2013. Genetic diversity of *Toxoplasma gondii* isolates from Ethiopian feral cats. Vet. Parasitology. 196 (1): 206–208.

Dubey, J. P., P. G. Parnell, C. Sreekumar, M. C. B. Vianna, R. W. De Young, E. Dahl & T. Lehmann. 2004. Biologic and Molecular Characteristics of *Toxoplasma gondii* Isolates From Striped Skunk (*Mephitis mephitis*), Canada Goose (*Branta canadensis*), Black-Winged Lory (*Eos cyanogenia*), and Cats (*Felis catus*). J. Parasitology. 90 (5): 1171–1174.

Lima, J. A. S. 2016. Comparação da prevalência de parasitos entéricos em gatos errantes e domiciliados em Goiânia, Goiás, análise da acurácia de técnicas parasitológicas e avaliação da copro-PCR para o diagnóstico de Toxoplasma gondii. 67 f. Dissertação de Mestrado em Medicina Tropical e Saúde Pública – Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia.

Mendes-De-Almeida, F., M. M. O. Mattos & N. Labarthe. 2007. *Giardia* spp. in fecal samples of domestic cats from Rio de Janeiro, RJ. Acta Sci. Vet. 35 (2): 469–480.

Rezende, H. H. A. 2015. Prevalência de parasitos intestinais em gatos errantes em Goiânia – Goiás: ênfase no diagnóstico de *Toxoplasma gondii* e avaliação da acurácia de técnicas parasitológicas. 101 f. Dissertação de Mestrado em Medicina Tropical e Saúde Pública – Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia.

Rezende, H. H. A., H. R. Storchilo, J. A. S. Lima, A. R. Gomes-Júnior, T. C. Gomes, J. Y. Souza, M. M. Avelino, W. N. Amaral, M. C. Vinaud & A. M. Castro. 2017. Nursing infant with acquired toxoplasmosis in the first months of life – a case report. Rev. Inst. Med. Trop. São Paulo. 59 (2): 265–274.

Sartori, A. L., R. Minamisava, M. M. Avelino & C. A. Martins. 2011. Triagem pré-natal para toxoplasmose e fatores associados à soropositividade de gestantes em Goiânia, Goiás. Rev. Bras. Ginecol. Obstet. 33 (2): 93–98.

Submitted in 12.XII.2018
Accept in 27.XI.2019