

Detection of the B1 gene of *Toxoplasma gondii* by PCR in the feces of domestic cats

Copro-PCR do gene B1 para diagnóstico de *Toxoplasma gondii* em fezes de gatos domésticos

Isa Marianny Ferreira Nascimento Barbosa de Souza¹; Samuel Felipe Netzlauff²; Amanda Cristina Corrêa Fleury²; Victor da Silva Siqueira³; Gabriela Katrinny Avelar Oliveira¹; Thais Santos Anjo Reis¹; Stéfanne Rodrigues Rezende⁴; Vanessa Bridi^{4*}; Ludimila Paula Vaz Cardoso⁵; Hanstter Hallison Alves Rezende^{6*}

Highlights

Determinação da prevalência de *Toxoplasma gondii* em fezes de gatos.

Avaliação da frequência de positividade entre os grupos de gatos testados.

Uso de técnicas moleculares para melhor sensibilidade na detecção parasitária.

Abstract

Toxoplasma gondii is an obligate intracellular parasite that has a heteroxenic life cycle, with felines as its definitive host, a fact that culminates in the maintenance of the parasitic life cycle. The aim of this study was to determine the accuracy of polymerase chain reaction (PCR) in identifying *Toxoplasma gondii* in the feces of cats, as well as to evaluate the frequency of *T. gondii* positivity, comparing categories of cats (stray vs. domestic, male vs. female, and castrated vs. intact). Fecal samples, collected from 120 cats, were subjected to spontaneous sedimentation. After 24 hours, DNA was extracted from the samples using a commercial kit, with adaptations. After DNA extraction, PCR was performed with primers that amplify the B1 gene of *T. gondii* and electrophoresis was performed on a 6% polyacrylamide gel. Among the 120 fecal samples analyzed, *T. gondii* was identified by PCR in 17 (14.1%), whereas none of the samples tested positive in the parasitological examination. The *T. gondii* positivity rate was higher among the stray cats than among the domestic cats. There was no significant difference in relation to

¹ M.e in Applied Health Sciences from the Postgraduate Program in Applied Health Sciences, PPGCAS, Universidade Federal de Jataí, UFJ, Jataí, GO, Brazil. E-mail: isaa656@gmail.com; enfgabriela26@gmail.com; thais_santosarjo@hotmail.com

² Students of the Undergraduate Course in Biomedicine, UFJ, Jataí, GO, Brazil. E-mail: samuelnetzlauff@discente.ufj.edu.br; amandafleury@discente.ufj.edu.br

³ Student of the Doctoral Course of the Postgraduate Program in Tropical Medicine and Public Health, IPTSP, Universidade Federal de Goiás, UFG, Goiânia, GO, Brazil. E-mail: victordasilva20122@hotmail.com

⁴ Students of the Master's Course of the Postgraduate Program in Applied Health Sciences, PPGCAS, UFJ, Jataí, GO, Brazil. E-mail: stefannerezende@discente.ufj.edu.br; vanessabridi@hotmail.com

⁵ Prof^a Dr^a, Medicine Course, UFJ, Jataí, GO, Brazil. E-mail: ludimilacardoso@ufj.edu.br

⁶ Prof. Dr., Biomedicine Course, UFJ, Jataí, GO, Brazil. E-mail: hanstterhallison@ufj.edu.br

* Author for correspondence

sex and castrated or non-castrated animals. It seems that fecal PCR has high sensitivity for detection of *T. gondii*, which it detected even in samples that tested negative in the parasitological examination, and that stray cats are more likely to be infected with *T. gondii* than are domestic cats.

Key words: Fecal PCR. Oocysts. Animal toxoplasmosis.

Resumo

Toxoplasma gondii é um parasita intracelular obrigatório que possui um ciclo de vida heteroxênico, tendo como hospedeiro definitivo os felinos, fato que culmina na manutenção do ciclo de vida parasitário. O objetivo deste estudo foi determinar a prevalência de *Toxoplasma gondii* em fezes de gatos por meio da Copro-PCR, bem como avaliar a frequência de positividade entre gatos errantes e domiciliados, machos e fêmeas e castrados e não castrados. Para tal, foram coletadas 120 amostras fecais de gatos que, posteriormente, foram submetidas à sedimentação espontânea. Após 24 horas extraiu-se o DNA das amostras com um kit comercial, com adaptações. Após a extração, realizou-se a PCR com os primers que amplificam o gene B1, seguida da eletroforese em gel de poli-acrilamida a 6%. Foi possível obter uma prevalência para *T. gondii* de 14,1% (17/120) na Copro-PCR ao passo que o Exame Parasitológico de Fezes não detectou nenhuma amostra positiva. Além disso, os gatos errantes obtiveram maior frequência de positividade quando comparado aos domiciliados. Não houve diferença significativa em relação ao sexo e aos animais castrados ou não castrados. Foi possível concluir que a copro-PCR do Gene B1 utilizada na detecção de *T. gondii* possui uma alta sensibilidade, detectando até mesmo amostras negativas no Exame Parasitológico de Fezes e que os gatos errantes possuem maior probabilidade de se infectarem com *T. gondii* do que os domiciliados.

Palavras-chave: Copro-PCR. Oocistos. Toxoplasmose animal.

Introduction

Toxoplasma gondii is an obligate intracellular parasite that belongs to the phylum Apicomplexa and is the cause of the disease known as toxoplasmosis. It has a heteroxenic cycle in which the asexual phase occurs in intermediate hosts, consisting of humans and other warm-blooded animals, and the sexual phase occurs in definitive hosts, which are felines (Hutchison et al., 1969; Aguirre et al., 2019; Attias et al., 2020).

The domestic cat (*Felis catus*) is of great epidemiological importance for maintaining the *T. gondii* life cycle in the

urban environment (Shapiro et al., 2019). Cats excrete *T. gondii* oocysts in their feces 7–14 days after infection (Fritz et al., 2012; Portilho & Carvalho, 2019). The oocysts are excreted in their immature form, becoming sporulated within 5 days if the conditions (humidity and temperature) are right. The sporulation process involves the formation of two sporocysts, each containing four sporozoites (Shapiro et al., 2019). Sporulation is thought to increase oocyst resistance to environmental insults, because the inner wall of the sporocyst is rich in glycoproteins and lipids (Bushkin et al., 2012; Asghari et al., 2021).

When pregnant women and immunocompromised individuals acquire toxoplasmosis by accidentally ingesting water or food contaminated with oocysts, they can develop severe forms of the infection, such as neurotoxoplasmosis, and infected women can transmit the infection to their newborns (Dupont et al., 2021; Smith et al., 2021; Wehbe et al., 2022). Therefore, contamination must be avoided by employing tools for disseminating prophylactic measures in society, as well as by carrying out preventive diagnosis in cats (Almeria & Dubey, 2021).

Typically, oocysts are identified in feces through a parasitological examination, mainly by the Hoffman–Pons–Janer–Lutz spontaneous sedimentation technique, as described by Rezende et al. (2015). However, Salant et al. (2007) found that polymerase chain reaction (PCR) is highly sensitive and specific for the detection of *T. gondii* DNA in fecal samples, being capable of detecting such DNA even if there are only 1–2 oocysts within 200 µg of feces. In addition, Igreja et al. (2021) found that PCR was able to identify *T. gondii* in fecal samples that tested negative in parasitological examinations. Therefore, there is a need for molecular techniques for the identification of oocysts.

The primary aim of the present study was to determine the accuracy of PCR for detecting *T. gondii* in the feces of cats. A secondary aim was to evaluate the frequency of *T. gondii* positivity, comparing stray cats with domestic cats, male cats with female cats, and castrated cats with intact cats.

Material and Methods

Experimental design

The study was approved by the Animal Research Ethics Committee of the Federal University of Jataí (Reference no. 009/2021). The cats were divided into stray and domestic groups. The domestic cats were recruited through posts on social media and direct contact with their owners, whereas the stray cats were obtained from nongovernmental organizations, where they remained trapped in individual, comfortable cages, with the collection carried out by volunteers from the institution, who soon after collection, they contacted the researchers to remove the fecal material. In addition, the animals were stratified by sex (male and female) and reproductive capacity (castrated and intact). Fecal samples were collected between June and December of 2021. For molecular diagnosis, the samples were sent to the Federal University of Jataí Laboratory for Clinical Biochemistry and Analysis of Body Fluids.

Processing of fecal samples

The fecal samples were subjected to the spontaneous sedimentation test (Hoffman et al., 1934; Lutz, 1919) for a period of 24 hours. This test was carried out with the aim of concentrating the parasites present in the fecal sediment of the samples and verifying their positivity using this technique. After this, the sediment obtained was separated for submission to molecular tests. The results obtained in both methods were compared at the end of the experiments.

DNA extraction from fecal samples

We extracted DNA from sediment samples obtained through spontaneous sedimentation. The DNA extraction was carried out by using a commercial kit (Mebeq Bioscience/Ludwig Biotecnologia, Alvorada, RS, Brazil), in accordance with the manufacturer's instructions, although with one adaptation: initially, the samples were boiled in water for twenty minutes and allowed to sit overnight at 37°C, in order to rupture the oocyst wall (Igreja et al., 2021). After that, the extraction protocol was applied in accordance with the manufacturer's recommendations.

Fecal PCR

Each PCR was performed in a final volume of 25 µL containing 10 mM of Tris-HCl (pH 9.0), 3.5 mM of MgCl₂, 0.2 U of Taq DNA Polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA), 0.5 mM of each deoxynucleotide (dATP/dTTP/dGTP/dCTP; Sigma Chemical Co., St. Louis, MO, USA), 50 pmol of each reaction primer (Invitrogen Life Technologies), and 5 µL of template DNA. The reactions were performed in a thermal cycler (GeneMax; Hangzhou Bioer Technology, Hangzhou, China), as described by Igreja et al. (2021).

The amplification consisted of an initial denaturation at 94°C for five minutes, 35 cycles of denaturation at 94°C for one minute, annealing at 62°C for one minute, an extension at 72°C for one minute, and a final extension at 72°C for ten minutes. 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 GTA3'), both of which amplify the base sequence of the B1 gene of *T. gondii* (Igreja et al., 2021).

As positive controls, we used positive fecal samples and peritoneal lavage fluid from mice infected with the RH strain of *T. gondii*, whereas we used negative fecal samples as negative controls. The PCR-amplified products that were ≥ 110 bp in size were visualized by electrophoresis, on 6% polyacrylamide gels, with silver staining.

Statistical analysis

To compare the frequencies of positivity between the groups, we used the chi-square test and the software Rstudio, version 9.1.191.26 (<http://www.rstudio.com/>). Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

We collected 120 fecal samples, of which 17 (14.1%) tested positive for *T. gondii* on PCR. That finding is of considerable importance, given that the parasitological examination did not identify any positive samples. There are two hypotheses that could explain the superiority of PCR for the detection of *T. gondii*. The first is that, when there is acute visceral infection or reinfection in older cats, the DNA originates from the asexual forms of the parasite. In both cases, it would be necessary to know the ages of the cats (Davis & Dubey, 1995), which were not known in the present study. The second

hypothesis is that the DNA originates from the oocysts, a low rate of oocyst excretion therefore decreasing the sensitivity of the conventional test (Mancianti et al., 2010; Poulle et al., 2016).

Over the years, molecular techniques have been widely used to detect *T. gondii* in the feces of cats, in order to complement the diagnosis (Salant et al., 2007; Schares et al., 2008; Montoya et al., 2009; Mancianti et al., 2010; Herrmann et al., 2011; Poulle et al., 2016; Veronesi et al., 2017; Hanafiah et al., 2018; Nasiru Wana et al., 2020; Igreja et al., 2021; Karakavuk et al., 2021, 2022). In a study carried out in Brazil, Igreja et al. (2021) used the same molecular markers employed in the present study and demonstrated that 18 (75%) of the 24 samples that were positive for *T. gondii* on PCR tested negative in the parasitological examination. These results highlight the high sensitivity of PCR amplification of the B1 gene of *T. gondii* in the diagnosis of toxoplasmosis in cats. In Italy, Mancianti et al. (2010) obtained a similar result, reporting that all 50 of the fecal samples evaluated tested negative for *T. gondii* in the parasitological examination, whereas eight (16.0%) of those samples tested positive on PCR.

Molecular markers other than the B1 gene have been used in fecal PCR and have presented similar sensitivity. For example, Miura et al. (2021) compared the accuracy of the Sheather technique with that of quantitative PCR of the repetitive 529-bp DNA fragment. The authors concluded

that quantitative PCR of that marker is an alternative to the Sheather technique for the detection of *T. gondii*. In another study employing fecal PCR, Nasiru Wana et al. (2020) compared the sensitivity of the B1 gene with that of the repetitive 529-bp fragment and found that both were able to identify positivity in 17 (8.5%) of the 200 samples evaluated.

Of the 120 fecal samples tested, 64 were collected from domestic cats and 56 were collected from stray cats. On PCR, three (4.69%) of the domestic cat group samples tested positive, compared with 14 (25.0%) of the stray cat group samples (Figure 1). The difference between the two groups was statistically significant ($p = 0.01$), suggesting that stray cats are more likely to be infected with *T. gondii* than are domestic cats, which could be explained by the fact that domestic cats receive prophylactic care, such as the administration of anthelmintic and antiprotozoal agents, from their owners (Campos et al., 2015). In addition, domestic cats receive a balanced diet and do not hunt wild prey, unlike stray cats, which, when performing predatory activities, have greater contact with tissues potentially contaminated with *T. gondii* (Majid et al., 2021). Our data corroborate those obtained in an epidemiological survey carried out by Dubey et al. (2020), in which the reported prevalence of toxoplasmosis, in various countries, was higher in stray cats than in domestic cats.

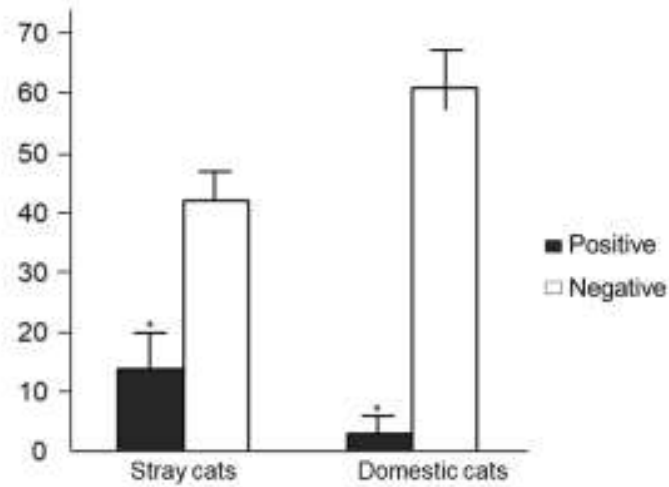


Figure 1. Frequency of PCR positivity among stray and domestic cats. *p < 0,05.

Of the 120 cats in our sample, 65 were male and 55 were female. Of the 65 fecal samples collected from the male cats, 10 (15.38%) tested positive on PCR, compared with seven (12.72%) of the 55 collected from the female cats (Figure 2). However,

the difference between the two sexes was not statistically significant. That is similar to what was reported by Majid et al. (2021), who found no significant difference between male and female cats in terms of the frequency of positivity for *T. gondii*.

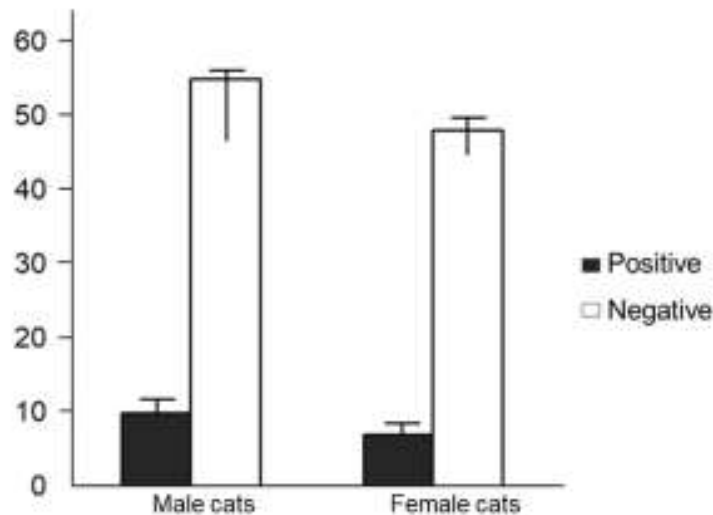


Figure 2. Frequency of PCR positivity among male and female cats.

Of the 120 fecal samples tested, 61 were collected from castrated cats and 59 were collected from intact cats. On PCR, seven (11.48%) of the castrated cats tested positive, compared with 10 (16.94%) of the intact cats (Figure 3). We found no statistical difference between those two

groups. Nevertheless, it is known that castration is a protective factor against *T. gondii* infection, because intact cats exhibit agonistic behavior that can directly influence their eating habits, leading them to engage in more hunting activity (Afonso et al., 2010; Mitsui et al., 2020; Vitale, 2022).

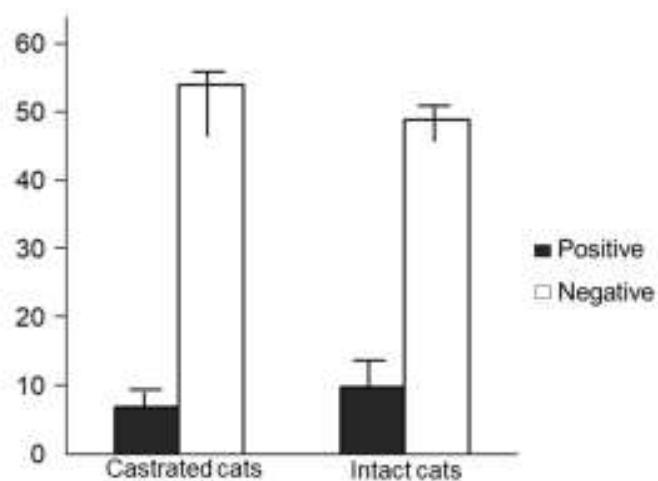


Figure 3. Frequency of PCR positivity in castrated and intact cats.

Conclusions

Amplification of the B1 gene of *T. gondii* by fecal PCR has high sensitivity for the detection of the parasite, as evidenced by the fact that, in our study, samples testing negative in the parasitological examination tested positive on PCR. In addition, the prevalence of *T. gondii* infection appears to be high in cats in general, the positivity rate being higher in those that are strays. Therefore, early diagnosis is extremely important.

References

- Afonso, E., Thulliez P., & Gilot-Fromont, E. (2010). Local meteorological conditions, dynamics of seroconversion to *Toxoplasma gondii* in cats (*Felis catus*) and oocyst burden in a rural environment. *Epidemiology & Infection*, *138*(8), 1105-1113. doi: 10.1017/S0950268809991270
- Aguirre, A. A., Longcore, T., Barbieri, M., Dabritz, H., Hill, D., Klein, P. N., Lepczyk, C., Lilly, E. L., McLeod, R., Milcarsky, J., Murphy, C. E., Su, C., VanWormer, E., Yolken, R., & Sizemore, G. C. (2019). The

- one health approach to toxoplasmosis: epidemiology, control, and prevention strategies. *EcoHealth*, 16(2), 378-390. doi: 10.1007/s10393-019-01405-7
- Almeria, S., & Dubey, J. P. (2021). Foodborne transmission of *Toxoplasma gondii* infection in the last decade: an overview. *Research in Veterinary Science*, 135, 371-385. doi: 10.1016/j.rvsc.2020.10.019
- Asghari, A., Majidiani, H., Nemati, T., Fatollahzadeh, M., Shams, M., Naserifar, R., & Kordi, B. (2021). *Toxoplasma gondii* tyrosine-rich oocyst wall protein: A closer look through an in silico prism. *Biomed Research International*, 1(1), 1-13. doi: 10.1155/2021/1315618
- Attias, M., Teixeira, D. E., Benchimol, M., Vommaro, R. C., Crepaldi, P. H., & Souza, W. de. (2020). The life-cycle of *Toxoplasma gondii* reviewed using animations. *Parasites & Vectors*, 13(1), 1-13. doi: 10.1186/s13071-020-04445-z
- Bushkin, G. G., Motari, E., Magnelli, P., Gubbels, M. J., Dubey, J. P., Miska, K. B., & Samuelson, J. (2012). β -1, 3-glucan, which can be targeted by drugs, forms a trabecular scaffold in the oocyst walls of *Toxoplasma* and *Eimeria* MBio, 3(5), 1-9. doi: 10.1128/mBio.00258-12
- Campos, D. R., Ouza, H. J. M., & Carrasco, L. P. S. (2015). Verminoses em gatos. *Bayer Veterinary*, 2(7), 1-8.
- Davis, S. W., & Dubey, J. P. (1995). Mediation of immunity to *Toxoplasma gondii* oocyst shedding in cats. *The Journal of Parasitology*, 81(6), 882-886. doi: 10.2307/3284034
- Dubey, J. P., Cerqueira-Cézar, C. K., Murata, F. H. A., Kwok, O. C. H., Yang, Y. R., & Su, C. (2020). All about toxoplasmosis in cats: the last decade. *Veterinary Parasitology*, 283, 109-145. doi: 10.1016/j.vetpar.2020.109145
- Dupont, D., Fricker-Hidalgo, H., Brenier-Pinchart, M. P., Garnaud, C., Wallon, M., & Pelloux, H. (2021). Serology for *Toxoplasma* in immunocompromised patients: still useful?. *Trends in Parasitology*, 37(3), 205-213. doi: 10.1016/j.pt.2020.09.006
- Fritz, H. M., Bowyer, P. W., Bogyo, M., Conrad, P. A., & Boothroyd, J. C. (2012). Proteomic analysis of fractionated *Toxoplasma* oocysts reveals clues to their environmental resistance. *PLoS One*, 7(1), e29955. doi: 10.1371/journal.pone.0029955
- Hanafiah, M., Prastowo, J., Hartati, S., Aliza, D., & Nurcahyo, R. W. (2018). Detection of *Toxoplasma gondii* copro-prevalence by polymerase chain reaction using repetitive 529 bp gene in feces of pet cats (*Felis catus*) in Yogyakarta, Indonesia. *Veterinary World*, 11(9), 1338. doi: 10.14202/vetworld.2018.1338-1343
- Herrmann, D. C., Maksimov, A., Pantchev, N., Globokar, M., Vrhovec, F. J. C., & Schares, G. (2011). *Toxoplasma gondii* oocysts in cat faeces. *Ber Münch Woch*, 124(11/12), 497-502. doi: 10.2376/0005-9366-124-497
- Hoffman, W. A., Pons, J. A., & Janer, J. L. (1934). The sedimentation concentration method in schistosomiasis mansoni. *The Puerto Rico Journal of Public Health and Tropical Medicine*, 9, 283-291.

- Hutchison, W. M., Dunachie, J. F., Siim, J. C., & Work, K. (1969). Life cycle of *Toxoplasma gondii*. *British Medical Journal*, 4(5686), 806. doi: 10.1136/bmj.4.5686.806-b
- Igreja, J. A. S. L. D., Rezende, H. H. A., Melo, J. D. O., Garcia, J. L., Martins, F. D. C., & Castro, A. M. D. (2021). Copro-PCR in the detection and confirmation of *Toxoplasma gondii* oocysts in feces of stray and domiciled cats. *Revista Brasileira de Parasitologia Veterinária*, 30(2), e000621. doi: 10.1590/S1984-29612021022
- Karakavuk, M., Can, H., Karakavuk, T., Gül, A., Alak, S. E., Gül, C., & Döşkaya, A. D. (2022). Rapid detection of *Toxoplasma gondii* DNA in cat feces using colorimetric loop-mediated isothermal amplification (LAMP) assays targeting RE and B1 genes. *Comparative Immunology, Microbiology and Infectious Diseases*, 81, 101745. doi: 10.1016/j.cimid.2022.101745
- Karakavuk, M., Can, H., Selim, N., Yeşilsiraz, B., Atlı, E., Şahar, E. A., Demir, F., Gul, A., Ozdemir, H. G., Alan, N., Yalçın, M., Ozkurt, O., Aras, M., Çelik, T., Can, S., Döşkaya, A. D., Guruz, A. Y., Döşkaya, M. (2021). Investigation of the role of stray cats for transmission of toxoplasmosis to humans and animals living in İzmir, Turkey. *Journal of Infection Developing Countries*, 15(1), 155-162. doi: 10.3855/jidc.13932
- Lutz, A. O. O. (1919). *Schistosoma mansoni* e a schistosomatose segundo observações, feitas no Brasil. *Mem Inst Oswaldo Cruz*, 11(1), 121-155. doi: 10.1590/S0074-02761919000100006
- Majid, A., Ahmad, N., Haleem, S., Zareen, S., Taib, M., Khan, S., & Hussain, R. (2021). Detection of toxoplasmosis in pets and stray cats through molecular and serological techniques in Khyber Pakhtunkhwa, Pakistan. *BMC Veterinary Research*, 17(1), 1-7. doi: 10.1186/s12917-021-03064-9
- Mancianti, F., Nardoni, S., Ariti, G., Parlanti, D., Giuliani, G., & Papini, R. A. (2010). Cross-sectional survey of *Toxoplasma gondii* infection in colony cats from urban Florence (Italy). *Journal of Feline Medicine and Surgery*, 12, 351-354. doi: 10.1016/j.jfms.2009.09.001
- Mitsui, K., Sato, S., & Kakuma, Y. (2020). Effects of the community cats program on population control, migration and welfare status of free-roaming cats in Tokyo, Japan. *Animals*, 10(3), 461-473. doi: 10.3390/ani10030461
- Miura, A. C., Barros, L. D., Minutti, A. F., Martins, T. A., Sasse, J. P., & Nino, B. S. L. (2021). Evaluation of quantitative polymerase chain reaction for the detection of *Toxoplasma gondii* oocysts shed by cats. *Brazilian Journal of Parasitology Veterinary*, 30(4), e016621. doi: 10.1590/S1984-29612021091
- Montoya, A., Miro, G., Mateo, M., Ramírez, C., & Fuentes, I. (2009). Detection of *Toxoplasma gondii* in cats by comparing bioassay in mice and polymerase chain reaction (PCR). *Veterinary Parasitology*, 160(1-2), 159-162. doi: 10.1016/j.vetpar.2008.10.029
- Nasiru Wana, M., Mohd Moklas, M. A., Watanabe, M., Zasmy Unyah, N., Alhassan Abdullahi, S., Ahmad Issa Alapid, A., & Abd Majid, R. (2020). Molecular detection and genetic diversity of *Toxoplasma gondii* oocysts in cat faeces from Klang Valley,

- Malaysia, using B1 and REP genes in 2018. *Pathogens*, 9(7), 576. doi: 10.3390/pathogens9070576
- Portilho, M. B. F., & Carvalho, A. V. de. (2019). A toxoplasmose em felinos: parasitologia, imunologia e diagnóstico animal. *Ag Liber*, 1(1), 1-11. doi: 10.6008/CBPC2674-6476.2019.001.0001
- Pouille, M. L., Forin-Wiart, M. A., Josse-Dupuis, É., Villena, I., & Aubert, D. (2016). Detection of *Toxoplasma gondii* DNA by qPCR in the feces of a cat that recently ingested infected prey does not necessarily imply oocyst shedding. *Parasite*, 23(29), 1-4. doi: 10.1051/parasite/2016029
- Rezende, H. H. A., Avelar, J. B., Storchilo, H. R., Vinaud, M. C., & Castro, A. M. D. (2015). Evaluation of the accuracy of parasitological techniques for the diagnosis of intestinal parasites in cats. *Revista Brasileira de Parasitologia Veterinária*, 24(4), 471-474. doi: 10.1590/S1984-29612018004
- Salant, H., Markovics, A., Spira, D. T., & Hamburger, J. (2007). The development of a molecular approach for coprodiagnosis of *Toxoplasma gondii*. *Veterinary Parasitology*, 146(3-4), 214-220. doi: 10.1016/j.vetpar.2007.02.022
- Schares, G., Vrhovec, M. G., Pantchev, N., Herrmann, D. C., & Conraths, F. J. (2008). Occurrence of *Toxoplasma gondii* and *Hammondia hammondi* oocysts in the faeces of cats from Germany and other European countries. *Veterinary Parasitology*, 152(1-2), 34-45. doi: 10.1016/j.vetpar.2007.12.004
- Shapiro, K., Bahia-Oliveira, L., Dixon, B., Dumètre, A., Wit, L. A. de, VanWormer, E., & Villena, I. (2019). Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. *Food and Waterborne Parasitology*, 1(15) e00049. doi: 10.1016/j.fawpar.2019.e00049
- Smith, N. C., Goulart, C., Hayward, J. A., Kupz, A., Miller, C. M., & van Dooren, G. G. (2021). Control of human toxoplasmosis. *International Journal for Parasitology*, 51(2-3), 95-121. doi: 10.1016/j.ijpara.2020.11.001
- Veronesi, F., Santoro, A., Milardi, G. L., Diaferia, M., Morganti, G., Ranucci, D., & Gabrielli, S. (2017). Detection of *Toxoplasma gondii* in faeces of privately owned cats using two PCR assays targeting the B1 gene and the 529-bp repetitive element. *Parasitology Research*, 116(3), 1063-1069. doi: 10.1007/s00436-017-5388-z
- Vitale, K. R. (2022). The social lives of free-ranging cats. *Animals*, 12(1), 126. doi: 10.3390/ani12010126
- Wehbe, K., Pencole, L., Lhuair, M., Sibiude, J., Mandelbrot, L., Villena, I., & Picone, O. (2022). Hygiene measures as primary prevention of toxoplasmosis during pregnancy: a systematic review. *Journal of Gynecology Obstetrics and Human Reproduction*, 51(3), 102300. doi: 10.1016/j.jogoh.2021.102300