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Low occurrence of *Cryptosporidium canis* in feces of dogs seroreactive for *Leishmania* spp.

Baixa ocorrência de *Cryptosporidium canis* em fezes de cães soropositivos para *Leishmania* spp.

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Highlights

Cryptosporidium canis oocyst found in feces of a dog seroreactive for *Leishmania* spp.
Asymptomatic *C. canis* infection in a dog.
100% genetic identity with *C. canis* previously found in human feces.

Abstract

In this study, we investigated the occurrence and characterized molecularly *Cryptosporidium* oocysts in feces of dogs seroreactive for *Leishmania* spp. We hypothesized that the clinical staging of canine visceral leishmaniasis (CVL) influences the occurrence and intensity of *Cryptosporidium* spp. oocyst shedding in feces from the canine host due to the immunosuppression induced by *Leishmania infantum.* Fecal samples from 101 dogs with anti-*Leishmania* spp. antibodies detected by Dual-Path Platform (DPP®) immunochromatographic test (Biomanguinhos) and/or enzyme-linked immunosorbent assay (Biomanguinhos) were concentrated by the centrifuge-sedimentation method in water-ethyl acetate. The sediments were submitted to the technique of negative staining with malachite green and observed under an optical microscope (400× magnification). The occurrence was 0.99% (95 confidence interval 0.00%-2.93%). A single *Cryptosporidium* oocyst was found in one of the samples examined by microscopy, and the result was confirmed by nested-PCR. The amplicon sequence showed 100% genetic identity with *Cryptosporidium canis*. The dog presented the following clinical signs suggestive of CVL: cachexia, generalized alopecia, pale colored gingival mucosa, splenomegaly, and onychogryphosis. We conclude that there is no evidence that the oligosymptomatic and symptomatic clinical staging of dogs seroreactive

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for *Leishmania* spp. influenced the occurrence and intensity of *C. canis* oocyst shedding in the canine host.
Key words: Cryptosporidiosis. Epidemiology. Leishmaniasis. One health. Zoonosis.

Resumo

Neste estudo, nós investigamos a ocorrência e caracterizamos molecularmente oocistos de *Cryptosporidium* em fezes de cães sororreativos para *Leishmania* spp. Nossa hipótese era que o estadiamento clínico da leishmaniose visceral canina (LVC) influenciasse na ocorrência e intensidade de eliminação de oocistos de *Cryptosporidium* spp. em fezes do hospedeiro canino devido à imunossupressão induzida por *Leishmania infantum.* Amostras fecais de 101 cães com anticorpos anti-*Leishmania* spp. detectados pelo teste imunocromatográfico *Dual-Path Platform* (DPP®) (Biomanguinhos) e/ou ensaio imunoenzimático (Biomanguinhos) foram concentrados pelo método de centrífugo-sedimentação em água-acetato de etila. Os sedimentos foram submetidos à técnica de coloração negativa por verde malaquita e observados ao microscópio óptico (ampliação de 400x). A ocorrência foi de 0,99% (intervalo de confiança de 95%: 0,0 - 2,93%). Um único oocisto de *Cryptosporidium* foi encontrado em uma das amostras examinadas por microscopia, e o resultado foi confirmado por *nested*-PCR. A sequência do *amplicon* apresentou 100% de identidade genética com *Cryptosporidium canis.* O cão apresentava os seguintes sinais clínicos sugestivos de LVC: caquexia, alopecia generalizada, mucosa gengival pálida, esplenomegalia e onicogrifose. Concluímos que não há evidências de que o estadiamento clínico oligossintomático e sintomático de cães sororreativos para *Leishmania* spp. influencie na ocorrência e intensidade de excreção de oocistos de *C. canis* em fezes do hospedeiro canino.
Palavras-chave: Criptosporidiose. Epidemiologia. Leishmaniose. Saúde única. Zoonose.

Introduction

Cryptosporidium spp. is an important protozoan for One Health. The pathogen has been studied more since the 1980s because it causes severe and potentially fatal chronic diarrhea in patients with acquired immunodeficiency syndrome (AIDS) (Fayer, 2008). In 1993, in Milwaukee, the pathogen was responsible for the largest outbreak of waterborne disease in the history of the United States of America, where about 403,000 people were infected (Mac Kenzie et al., 1994). Subsequently, in 2004, cryptosporidiosis was included in the Neglected Diseases Initiative of the World Health Organization due to its severity and close relationship with the deficiency of basic sanitation and the low purchasing power of the population at risk (Assis et al., 2013; Savioli et al., 2006). On the other hand, the relevance of *Cryptosporidium* spp. for companion animals is still uncertain.

Dogs are mainly infected by *Cryptosporidium canis*, while *Cryptosporidium* parvum and *Cryptosporidium hominis* are responsible for > 90% of cryptosporidiosis in humans (Feng et al., 2018). Furthermore, from a clinical point of view, the majority of infected dogs remain asymptomatic dogs remain (Abe et al., 2002; Cui et al., 2018; R. C. A. Thompson et al., 2008), but immunosuppression seems to be a predisposing factor for the clinical manifestation of cryptosporidiosis in the canine host (Cui et al., 2018).

Canine visceral leishmaniasis (CVL) is a disease with a high prevalence in Brazil. In endemic areas, anti-*Leishmania* spp. antibodies are detected in up to 40% of the canine population (Peixoto et al., 2015). *Leishmania infantum* infection can lead to immunosuppression in dogs, especially in advanced stages of the disease (Gonçalves et al., 2019; Toepp & Petersen, 2020). We hypothesized that this immunosuppression could contribute to *Cryptosporidium* spp. infection in dogs. Our objective was to evaluate the occurrence and to characterize *Cryptosporidium* spp. oocysts in fecal samples from dogs seroreactive for *Leishmania* spp. and to compare them with results obtained from epidemiological surveys conducted in geographic regions not endemic for CVL.

Material and Methods

Ethics statement

This research was approved by the Ethics Committee on Animal Experimentation of the Universidade Federal do Tocantins, under protocol 23101.002955-2018-0.

Sampling and procedure

The non-probabilistic convenience sampling method was used in this study. Fecal samples from 101 adult dogs (< 1 year old) domiciled or stray captured by the Zoonosis Control Center (ZCC) of the Municipality of Gurupi, Tocantins, Brazil, were collected in 2018. All animals showed anti-*Leishmania* spp. detected by the Dual-Path Platform immunochromatographic test (DPP®, Biomanguinhos) and/or enzyme linked-immunosorbent assay (ELISA, Biomanguinhos). Dogs were classified into two groups: oligosymptomatic (n = 23) and symptomatic (n = 78), according to criteria by Mancianti et al. (1988). The following clinical signs were considered suggestive of CVL: weight loss or cachexia, pale mucous membranes, lymphadenomegaly, splenomegaly, hepatomegaly, onychogryphosis, alopecia, hyperkeratosis, skin ulcers, seborrheic dermatitis, epistaxis, uveitis, and keratoconjunctivitis sicca (Koutinas & Koutinas, 2014).

Immediately after euthanasia of the seroreactive dogs, as recommended by the Brazilian Ministry of Health (Ministério da Saúde [MS], 2014), fecal samples were collected directly from the rectum, by enterotomy, and separated into two aliquots. One aliquot was maintained in 5% (mass/volume) potassium dichromate at 4ºC for microscopy and the other was frozen at -20 ºC in a 1.5 mL DNAse and RNAse-free microcentrifuge tube for molecular analysis.

Microscopy

Fecal samples maintained in 5% potassium dichromate were concentrated and purified using the water-ether centrifugation technique (Meloni & Thompson, 1996). Ethyl acetate was used instead of ethyl ether because it is cheaper and less toxic. Subsequently, a drop of the sediment was submitted to the technique of negative staining by malachite green (Elliot et al., 1999) and observed under a conventional optical microscope with 400× total magnification. Only the sample that was positive based on microscopy was analyzed molecularly.

DNA extraction and nested-PCR (nPCR)

Genomic DNA from the microscopy-positive fecal sample was extracted using the commercial GenElute® Stool DNA Isolation Kit (Sigma-Aldrich, USA), according to the manufacturer's instructions. nPCR was used to amplify a ~800 bp fragment of the 18S rRNA gene from *Cryptosporidium* sp. (Xiao et al., 2000). The positive and negative controls for the reaction were *C. parvum* genomic DNA and ultrapure water, respectively. The amplified fragments were visualized by electrophoresis on a 1.5% agarose gel stained with GelRed® (Biotium, USA). Reactions were performed using Platinum® PCR Supermix (Invitrogen, USA), with 200 nM of each primer and 2.5 µL of target DNA. The samples were subjected to initial DNA denaturation at 94°C for 3 minutes, followed by 39 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 60 seconds; there was a final extension at 72°C for 7 minutes. The same reaction conditions were used in the primary and secondary reaction.

Amplicon purification and molecular characterization

Amplicons were purified with the ExoSAP-IT™ PCR Product Cleanup Reagent kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions, and subsequently sequenced using the ABI Prism® DyeTerminator 3.1, in an ABI 3730XL automatic sequencer (Applied Biosystems, USA). The sequencing reactions were performed in one direction, using the forward primer of the secondary reaction.

Alignment and translation of nucleotide sequences

Consensus sequences were aligned with CLUSTAL_X (J. D. Thompson et al., 1997) and curated with the software BioEdit Sequence Alignment Editor, using as reference homologous 18SrRNA sequences downloaded from GenBank accession number AF112576 (Fayer et al., 2001).

Results and Discussion

The main clinical signs of CVL in our study were lymphadenomegaly (89.1%), splenomegaly (68.3%), hepatomegaly and onychogryphosis (both at 59.4%), alopecia (52.5%), skin lesions (51.5%), hyperkeratosis (40.6%), skin ulcers (38.6%), eye injury (53.6%), cachexia (30.7%), seborrheic dermatitis (28.7%), and keratoconjunctivitis sicca (8.9%).

A single *Cryptosporidium* oocyst was found in the fecal sample of only 1 of the 101 dogs examined. The identity was confirmed with nPCR (Figure 1). The amplicon sequence showed 100% genetic identity with *C. canis.* The dog was adult, female, and showed the following clinical signs of CVL: cachexia, alopecia in thorax, pale gingival mucosa, splenomegaly, and onychogryphosis (Figure 2). The consistency of the stool was pasty. Although cross-reactions between the antigen used for the DPP and anti-Babesia canis vogeli antibodies and between the antigen used for ELISA and anti-*B. canis vogeli*, anti-*Trypanosoma cruzi,* anti-*Ehrlichia canis,* and anti-*Neospora caninum* antibodies (Laurenti et al., 2014) can occur, the dog with the *C. canis* oocyst in its feces had clinical signs strongly suggestive of CVL.

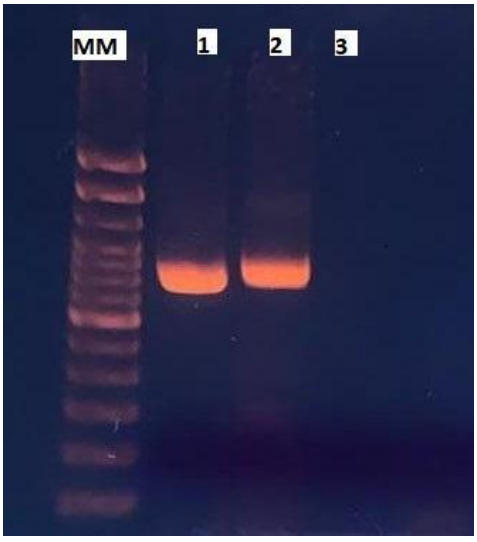

Figure 1. Detection of the 18S ribosomal RNA (18S rRNA) gene of *Cryptosporidium canis* obtained by nested-PCR. Lane identities: MM, 100 bp molecular weight marker; column 1: positive control; column 2: positive sample; column 3: negative control.

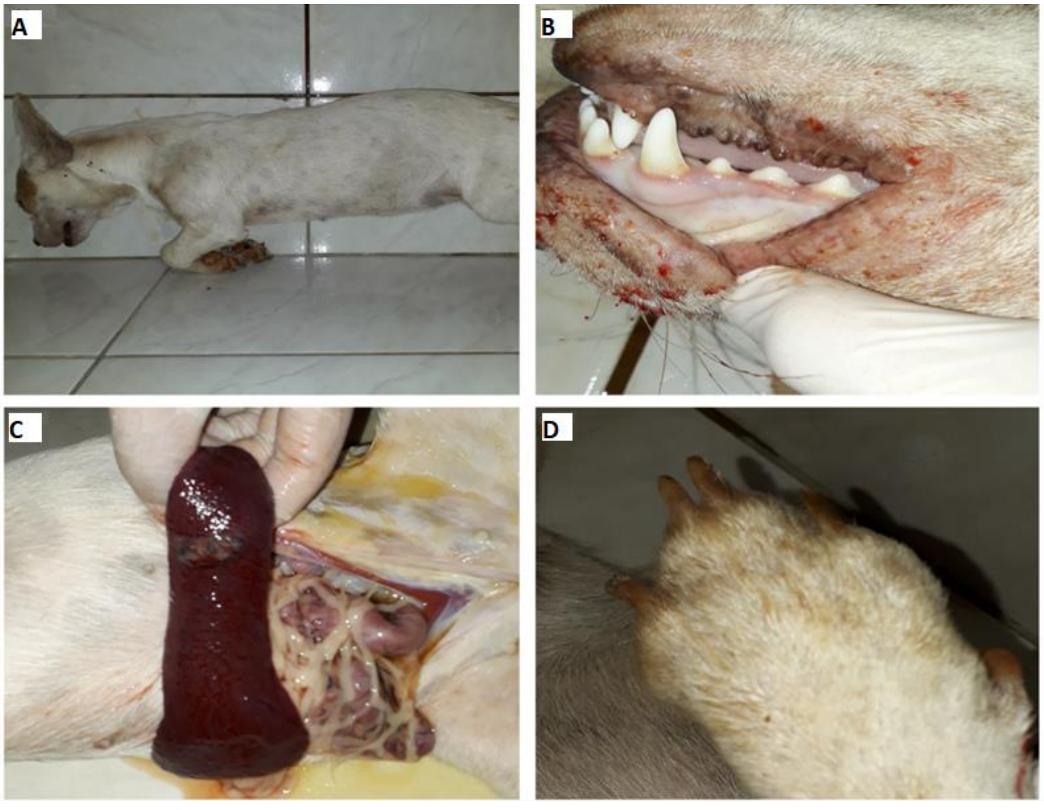

Figure 2. Macroscopic alterations in a dog with anti-*Leishmania* spp. antibodies detected by the Dual-Path Platform immunochromatographic test (DPP®) (Biomanguinhos) and/or enzyme-linked immunosorbent assay (Biomanguinhos) and infected by *Cryptosporidium canis:* A) cachexia and fur loss; B) pale gingival mucosa; C) splenomegaly; D) onychogryphosis.

In this study, the presence of anti-*Leishmania* spp. antibodies was used as an inclusion criterion, because *L. infantum* can lead to immunosuppression canines (Almeida et al., 2013; Reis et al., 2009; Silva et al., 2013), especially in advanced stages of the disease (Gonçalves et al., 2019; Toepp & Petersen, 2020). We hypothesized that oligosymptomatic and symptomatic dogs seropositive for *Leishmania* spp. have a greater chance of excreting *Cryptosporidium* oocysts. However, the occurrence of this parasite in 0.99% (95 confidence interval 0.00%-2.93%) is below the average of 14% found in Brazil, as reported in a systematic review with meta-analysis (Taghipour et al., 2020). The occurrence in this study is similar or lower than the occurrence of the parasite found in many epidemiological surveys carried out in regions not endemic for CVL (Asano et al., 2004; el-Ahraf et al., 1991; Gennari et al., 1999; Huber et al., 2005; Palmer et al., 2008; Santos et al., 2007; Satoh et al., 2006).

In an experiment conducted in China, healthy dogs were immunosuppressed by treatment with dexamethasone (6 mg/dog) for 5 consecutive days and subsequently experimentally infected by *C. canis* oocysts. The onset of oocyst shedding and clinical signs, such as diarrhea, opaque fur, and lethargy, occurred on days 3 and 8 post-infection, respectively. On the other hand, challenged immunocompetent dogs did not show any clinical signs nor shed oocysts in feces. This confirms that immunosuppression is a risk factor for the clinical manifestation and oocyst shedding by dogs (Cui et al., 2018). Nevertheless, the dexamethasone dose used may have a greater immunosuppressive effect than that induced by *L. infantum* (Poot et al., 2005). Hence, the immunosuppression in dogs with CVL might not be enough to increase the occurrence and intensity of *C. canis* oocyst shedding under uncontrolled conditions.

The type of diagnostic technique used to detect *Cryptosporidium* spp. oocysts in feces can interfere with the results. Researchers have reported divergent results between microscopy and ELISA (Bresciani et al., 2008), between microscopy and immunochromatography test (Shukla et al., 2006), between microscopy and immunofluorescence antibody test (Miambo et al., 2019), and between microscopy and PCR (Alves et al., 2018; Lallo & Bondan, 2006). However, we have only compared our results to epidemiological surveys that also used microscopy as the diagnostic technique.

Comparisons among studies should be interpreted with caution, because some variables may interfere with the results. Although there is still no consensus, young dogs (Jian et al., 2014; Tangtrongsup et al., 2020) immunosuppressed by coinfections (Miller et al., 2003; Willard & Bouley, 1999), fed a natural diet (Miller et al., 2003; Turnwald et al., 1988; Willard & Bouley, 1999), and living in kennels (Itoh et al., 2019; Olabanji et al., 2016) or in rural areas (Frizzo et al., 2016; Sevá et al., 2010) may be more susceptible to infection by *Cryptosporidium* spp. The owner's socioeconomic vulnerability has also been reported as a possible risk factor for infection in the canine host canino (Ederli et al., 2008). The dogs included in our study came from the ZCC and there was no detailed information about how they were cared by their owners.

The number of samples collected from the same dog can also interfere on results, because *Cryptosporidium* spp. oocysts are

shed intermittently. The collection of three fecal samples on consecutive or alternate days can increase the sensitivity of the diagnostic technique (Fayer et al., 2000). However, this procedure was not feasible in our study, because dogs seroreactive for *Leishmania* spp. were euthanized immediately due to the inadequate conditions of the ZCC: Animals cannot be kept for longer than 2 days.

The nucleotide sequence obtained in our study showed 100% genetic identity with *C. canis,* which has also been found in feces from children in Peru (Xiao et al., 2007) and Mexico (González-Díaz et al., 2016) and patients infected by the human immunodeficiency virus (HIV) in Brazil (Lucca et al., 2009). Although *C. canis* is one of the five species found in human feces, > 90% of cryptosporidiosis cases in humans are caused by *C. hominis* and *C. parvum* (Feng et al., 2018).

The risk infection by *Cryptosporidium* spp. in dogs living in uncontrolled conditions probably has multiple causes and depends on the interaction between variables related to environmental contamination, parasite species, and canine host health. Prospective studies with a larger sample size allowing researchers to evaluate more dog feces samples with laboratory analyses in order to quantify the immunosuppression caused by *L. infantum* could be conducted in the future to test the hypothesis that CVL increases the risk of *Cryptosporidium* spp. infection in the canine host. Our results suggest that there is an association between *Leishmania* spp. mediated immunosuppression and Cryptosporidium spp. infection.

Conclusion

We conclude that there is no evidence that the oligosymptomatic and symptomatic clinical staging of dogs seroreactive to *Leishmania* spp. influences the occurrence and intensity of *C. canis* oocyst shedding in the canine host.

References

Abe, N., Sawano, Y., Yamada, K., Kimata, I., & Iseki, M. (2002). *Cryptosporidium* infection in dogs in Osaka, *Japan. Veterinary Parasitology, 108*(3), 185-193. doi: 10.1016/S0304-4017(02)00204-2

Almeida, B. F. M., Narciso, L. G., Bosco, A. M., Pereira, P. P., Braga, E. T., Avanço, S. V., Marcondes, M., & Ciarlini, P. C. (2013). Neutrophil dysfunction varies with the stage of canine visceral leishmaniosis. *Veterinary Parasitology, 196*(1-2), 6-12. doi: 10.1016/j.vetpar.2013.02.016

Alves, M. E. M., Martins, F. D. C., Bräunig, P., Pivoto, F. L., Sangioni, L. A., & Vogel, F. S. F. (2018). Molecular detection of *Cryptosporidium* spp. and the occurrence of intestinal parasites in fecal samples of naturally infected dogs and cats. *Parasitology Research, 117*(9), 3033-3038. doi: 10.1007/s00436-018-5986-4

Asano, K., Suzuki, K., Matsumoto, T., Sakai, T., & Asano, R. (2004). Prevalence of dogs with intestinal parasites in Tochigi, Japan in 1979, 1991 and 2002. *Veterinary Parasitology, 120*(3), 243-248. doi: 10.1016/j.vetpar.2004.01.009

Assis, D. C., Resende, D. V., Cabrine-Santos, M., Correia, D., & Oliveira-Silva, M. B. (2013). Prevalence and genetic characterization of *Cryptosporidium* spp. and *Cystoisospora belli* in HIV-infected patients. *Revista do Instituto de Medicina Tropical, 55*(3), 149-154. doi: 10.1590/S0036-46652013000300002

Bresciani, K. D. S., Amarante, A. F. T., Lima, V. M. F., Marcondes, M., Feitosa, F. L. F., Táparo, Serrano, A. C. M., Ishizaki, M. N., Tome, R. O., Perri, S. H. V., & Meireles, M. V. (2008). Infecções por *Cryptosporidium* spp. em cães de Araçatuba, Brasil. *Veterinária e Zootecnia, 15*(3), 466-468. https://rvz.emnuvens.com.br/rvz/article/view/396/213

Cui, Z., Dong, H., Wang, R., Jian, F., Zhang, S., Ning, C., & Zhang, L. (2018). A canine model of experimental infection with *Cryptosporidium canis. Experimental Parasitology, 195*(12), 19-23. doi: 10.1016/j.exppara.2018.09.019

Ederli, B. B., Ederli, N. B., Oliveira, F. C. R., Quirino, C. R., & Carvalho, C. B. (2008). Fatores de risco associados à infecção por *Cryptosporidium* spp. em cães domiciliados na cidade de Campos dos Goytacazes, Estado do Rio de Janeiro, Brasil. *Revista Brasileira de Parasitologia Veterinária, 17*(1), 260-266. https://rbpv.org.br/download/17supl.12008/Protozool014

el-Ahraf, A., Tacal, J. V., Sobih, M., Amin, M., Lawrence, W., & Wilcke, B. W. (1991). Prevalence of cryptosporidiosis in dogs and human beings in San Bernardino County, California. *Journal of the American Veterinary Medical Association, 198*(4), 631-634.

Elliot, A., Morgan, U. M., & Thompson, R. C. A. (1999). Improved staining method for detecting *Cryptosporidium* oocysts in stools using malachite green. *The Journal of General and Applied Microbiology, 45*(3), 139-142. doi: 10.2323/jgam.45.139

Fayer, R. (2008). General biology. In R. Fayer, & L. Xiao (Orgs.), *Cryptosporidium and Cryptosporidiosis* (2nd ed., Cap. 1, pp. 1-42). CRC Press.

Fayer, R., Morgan, U., & Upton, S. J. (2000). Epidemiology of Cryptosporidium: transmission, detection and identification. *International Journal for Parasitology, 30*(12-13), 1305-1322. doi: 10.1016/s0020-7519(00)00135-1

Fayer, R., Trout, J. M., Morgan, U. M., Lal, A. A., & Dubey, J. P. (2001). *Cryptosporidium canis* n. sp. from domestic dogs. *The Journal of Parasitology, 87*(6), 1415-1422. doi: 10.1645/0022-3395(2001)087[1415:CCNSFD]2.0.CO;2

Feng, Y., Ryan, U. M., & Xiao, L. (2018). Genetic diversity and population structure of Cryptosporidium. *Trends in Parasitology, 34*(11), 997-1011. doi: 10.1016/j.pt.2018.07.009

Frizzo, C., Schmidt, A. P., Wagner, G., & Müller, G. A. (2016). Intestinal parasites present in canine fecal samples collected in Rural Areas of municipalities in the Midwest of Santa Catarina, Brazil. *Revista de Patologia Tropical, 45*(2), 227. doi: 10.5216/rpt.v45i2.41774

Gennari, S. M., Kasai, N., Pena, H. F. J., & Cortez, A. (1999). Ocorrência de protozoários e helmintos em amostras de fezes de cães e gatos da cidade de São Paulo. *Brazilian Journal of Veterinary Research*

and Animal Science, 36(2), 87-91. doi: 10.1590/S1413-95961999000200006

Gonçalves, A. A. M., Leite, J. C., Resende, L. A., Mariano, R. M. da S., Silveira, P., Melo, O. A. de O., Jr., Ribeiro, H. S., Oliveira, D. S. de, Soares, D. F., Santos, T. A. P., Marques, A. F., Galdino, A. S., Martins, O. A., Fº., Dutra, W. O., Silveira-Lemos, D. da, & Giunchetti, R. C. (2019). An overview of immunotherapeutic approaches against Canine Visceral Leishmaniasis: What has been tested on dogs and a new perspective on improving treatment efficacy. *Frontiers in Cellular and Infection Microbiology, 9*(12), 427. doi: 10.3389/fcimb.2019.00427

González-Díaz, M., Urrea-Quezada, A., Villegas-Gómez, I., Durazo, M., Garibay-Escobar, A., Hernández, J., Lihua, X., & Valenzuela, O. (2016). *Cryptosporidium canis* in two Mexican toddlers. In *Pediatric Infectious Disease Journal, 35*(11), 1265-1266. doi: 10.1097/INF.0000000000001287

Huber, F., Bomfim, T. C. B., & Gomes, R. S. (2005). Comparison between natural infection by *Cryptosporidium* sp., Giardia sp. in dogs in two living situations in the West Zone of the municipality of Rio de Janeiro. *Veterinary Parasitology, 130*(1-2), 69-72. doi: 10.1016/j.vetpar.2005.03.012

Itoh, N., Tanaka, H., Iijima, Y., Kameshima, S., & Kimura, Y. (2019). Molecular prevalence of Cryptosporidium spp. in breeding kennel dogs. *Korean Journal of Parasitology, 57*(2), 197-200. doi: 10.3347/kjp.2019.57.2.197

Jian, F., Qi, M., He, X., Wang, R., Zhang, S., Dong, H., & Zhang, L. (2014). Occurrence and molecular characterization of *Cryptosporidium* in dogs in Henan Province, China. *BMC Veterinary Research, 10*(26), 10-13. doi: 10.1186/1746-6148-10-26

Koutinas, A. F., & Koutinas, C. K. (2014). Pathologic mechanisms underlying the clinical findings in canine leishmaniosis due to *Leishmania infantum/chagasi. Veterinary Pathology, 51*(2), 527-538. doi: 10.1177/0300985814521248

Lallo, M. A., & Bondan, E. F. (2006). Prevalência de Cryptosporidium sp. em cães de instituições da cidade de São Paulo. *Revista de Saúde Pública, 40*(1), 120-125. doi: 10.1590/S0034-89102006000100019

Laurenti, M. D., Santana Leandro, M. V. de, Tomokane, T. Y., De Lucca, H. R. L., Aschar, M., Souza, C. S. F., Silva, R. M., Marcondes, M., Matta, V. L. R. da. (2014). Comparative evaluation of the DPP® CVL rapid test for canine serodiagnosis in area of visceral leishmaniasis. *Veterinary Parasitology, 205*(3-4), 444-450. doi: 10.1016/j.vetpar.2014.09.002

Lucca, P. de, Gaspari, E. N., Bozzoli, L. M., Funada, M. R., Silva, S. O. S., Iuliano, W., & Soares, R. M. (2009). Molecular characterization of *Cryptosporidium* spp. from HIV infected patients from an urban area of Brazil. *Revista do Instituto de Medicina Tropical de São Paulo, 51*(6), 341-343. doi: 10.1590/S00 36-46652009000600007

Mac Kenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. A., Peterson, D. E., Kazmierczak, J. J., Addiss, D. G., Fox, K. R., Rose, J. B., & Davis, J. B. (1994). A massive outbreak in Milwaukee of *Cryptosporidium*

infection transmitted through the public water supply. *The New England Journal of Medicine, 331*(3), 161-167. doi: 10.1056/NEJM199407213310304

Mancianti, F., Gramiccia, M., Gradoni, L., & Pieri, S. (1988). Studies on canine leishmaniasis control. 1. Evolution of infection of different clinical forms of canine leishmaniasis following antimonial treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 82*(4), 566-567. doi: 10.1016/0035-9203(88)90510-X

Meloni, B. P., & Thompson, R. C. A. (1996). Simplified methods for obtaining purified oocysts from mice and for growing *Cryptosporidium parvum in vitro. The Journal of Parasitology, 82*(5), 757-762.

Miambo, R. D., Laitela, B., Malatji, M. P., Afonso, S. M. S., Pondja, A., Jr., Lindh, J., & Mukaratirwa, S. (2019). Prevalence of *Giardia* and *Cryptosporidium* in young livestock and dogs in Magude district of Maputo Province, Mozambique. *Onderstepoort Journal of Veterinary Research, 86*(1), 1-6. doi: 10.4102/ ojvr.v86i1.1709

Miller, D. L., Liggett, A., Radi, Z. A., & Branch, L. O. (2003). Gastrointestinal cryptosporidiosis in a puppy. *Veterinary Parasitology, 115*(3), 199-204. doi: 10.1016/S0304-4017(03)00237-1

Ministério da Saúde (2014). *Manual de vigilância e controle da leishmaniose visceral.* Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Brasília, DF: Ministério da Saúde.

Olabanji, G. M., Maikai, B. V., & Otolorin, G. R. (2016). Prevalence and risk factors associated with faecal shedding of *Cryptosporidium* oocysts in dogs in the Federal Capital Territory, Abuja, Nigeria. *Veterinary Medicine International, 2016*(1), 4591238. doi: 10.1155/2016/4591238

Palmer, C. S., Traub, R. J., Robertson, I. D., Devlin, G., Rees, R., & Thompson, R. C. A. (2008). Determining the zoonotic significance of *Giardia* and *Cryptosporidium* in Australian dogs and cats. *Veterinary Parasitology, 154*(1-2), 142-147. doi: 10.1016/j.vetpar.2008.02.031

Peixoto, H. M., Oliveira, M. R. F., & Romero, G. A. S. (2015). Serological diagnosis of canine visceral leishmaniasis in Brazil: systematic review and meta-analysis. *Tropical Medicine and International Health, 20*(3), 334-352. doi: 10.1111/tmi.12429

Poot, J., Rogers, M. E., Bates, P. A., & Vermeulen, A. (2005). Detailed analysis of an experimental challenge model for *Leishmania infantum* (JPC strain) in dogs. *Veterinary Parasitology, 130*(1-2), 41-53. doi: 10.1016/j.vetpar.2005.03.002

Reis, A. B., Martins-Filho, O. A., Teixeira-Carvalho, A., Giunchetti, R. C., Carneiro, C. M., Mayrink, W. Taguri, L. W., Corrêa-Oliveira, R. (2009). Systemic and compartmentalized immune response in canine visceral leishmaniasis. *Veterinary Immunology and Immunopathology, 128*(1-3), 87-95. doi: 10.1016/j.vetimm.2008.10.307

Santos, F. A. G., Yamamura, M. H., Vidotto, O., & Camargo, P. L. (2007). Ocorrência de parasitos gastrintestinais em cães

(*Canis familiaris*) com diarreia aguda oriundos da região metropolitana de Londrina, Estado do Paraná, Brasil. Semina: *Ciências Agrárias, 28*(2), 257. doi: 10.5433/1679-0359.2007v28n2p257

Satoh, M., Matsubara-Nihei, Y., Sasaki, T., & Nakai, Y. (2006). Characterization of *Cryptosporidium canis* isolated in Japan. *Parasitology Research, 99*(6), 746-748. doi: 10.1007/s00436-006-0216-x

Savioli, L., Smith, H., & Thompson, A. (2006). Giardia and Cryptosporidium join the "Neglected Diseases Initiative." *Trends in Parasitology, 22*(5), 203-208. doi: 10.1016/j.pt.2006.02.015

Sevá, A. P., Funada, M. R., Souza, S. O., Nava, A., Richtzenhain, L. J., & Soares, R. M. (2010). Occurrence and molecular characterization of *Cryptosporidium* spp. isolated from domestic animals in a rural area surrounding Atlantic dry forest fragments in Teodoro Sampaio municipality, State of São Paulo, Brazil. *Revista Brasileira de Parasitologia Veterinária, 19*(4), 249-253. doi: 10.1590/s1984-29612010000400011

Shukla, R., Giraldo, P., Kraliz, A., Finnigan, M., & Sanchez, A. L. (2006). *Cryptosporidium* spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario. *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne, 47*(12), 1179-1184. https://www.ncbi. nlm.nih.gov/pmc/articles/PMC1636587/pdf/cvj47pg1179.pdf

Silva, K. L. O., Melo, L. M., Perosso, J., Oliveira, B. B., Santos, P. S. P. dos, Eugênio, F. de R., & Lima, V. M. F. de. (2013). CD95 (FAS) and CD178 (FASL) induce the apoptosis of CD4+ and CD8+ cells isolated from the peripheral blood and spleen of dogs naturally infected with *Leishmania* spp. *Veterinary Parasitology, 197*(3-4), 470-476. doi: 10.1016/j.vetpar.2013.07.012

Taghipour, A., Olfatifar, M., Bahadory, S., Godfrey, S. S., Abdoli, A., Khatami, A., Javanmar, E., & Shahrivar, F. (2020). The global prevalence of *Cryptosporidium* infection in dogs: a systematic review and meta-analysis. *Veterinary Parasitology, 281*(3), 109093. doi: 10.1016/j.vetpar.2020.109093

Tangtrongsup, S., Scorza, A. V., Reif, J. S., Ballweber, L. R., Lappin, M. R., & Salman, M. D. (2020). Seasonal distributions and other risk factors for *Giardia duodenalis* and *Cryptosporidium* spp. infections in dogs and cats in Chiang Mai, Thailand. *Preventive Veterinary Medicine, 174*(1), 104820. doi: 10.1016/j.prevetmed.2019.104820

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*(24), 4876-4882. doi: 10.1093/nar/25.24.4876

Thompson, R. C. A., Palmer, C. S., & O'Handley, R. (2008). The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Veterinary Journal, 177*(1), 18-25. doi: 10.1016/j.tvjl.2007.09.022

Toepp, A. J., & Petersen, C. A. (2020). The balancing act: immunology of leishmaniosis. *Research in Veterinary Science, 130*(6), 19-25. doi: 10.1016/j.rvsc.2020.02.004

Turnwald, G. H., Barta, O., Taylor, H. W., Kreeger, J., Coleman, S. U., & Pourciau, S. S. (1988). Cryptosporidiosis associated with immunosuppression attributable to distemper in a pup. *Journal of the American Veterinary Medical Association, 192*(1), 79-81.

Willard, M. D., & Bouley, D. (1999). Cryptosporidiosis, coccidiosis, and total colonic mucosal collapse in an immunosuppressed puppy. *Journal of the American Animal Hospital Association, 35*(5), 405-409. doi: 10.5326/15473317-35-5-405

Xiao, L., Alderisio, K., Limor, J., Royer, M., & Lal, A. A. (2000). Identification of species and sources of *Cryptosporidium* oocysts in storm waters with a small-subunit rRNA-based diagnostic and genotyping tool. *Applied and Environmental Microbiology, 66*(12), 5492-5498. doi: 10.1128/AEM.66.12.5492-5498. 2000

Xiao, L., Cama, V. A., Cabrera, L., Ortega, Y., Pearson, J., & Gilman, R. H. (2007). Possible transmission of *Cryptosporidium canis* among children and a dog in a household. *Journal of Clinical Microbiology, 45*(6), 2014-2016. doi: 10.1128/JCM.00503-07