Article

Prevalence of Potentially Zoonotic Endoparasites in Domestic Dog Puppies

Gisele Moraes dos Santos Reginaldo , Giovanni Widmer , Sandra Valéria Inácio , Jancarlo Ferreira Gomes , Walter Bertequini Nagata , Gabriela Pinheiro Tirado Moreno , João Alfredo Biagi Camargo Neto , Wagner Luis Ferreira , Alexandre Xavier Falcão , Katia Denise Saraiva Bresciani *

Posted Date: 22 January 2025

doi: 10.20944/preprints202501.1622.v1

Keywords: helminths; Protozoa; One Health; dogs; diagnosis

Article

Prevalence of Potentially Zoonotic Endoparasites in Domestic Dog Puppies

Gisele Moraes dos Santos Reginaldo[1], Giovanni Widmer[2], Sandra Valeria Inácio[3], Jancarlo Ferreira Gomes [4], Walter Bertequini Nagata [3], Gabriela Pinheiro Tirado Moreno [5], João Alfredo Biagi Camargo Neto[3], Wagner Luis Ferreira [3], Alexandre Xavier Falcão [6] and Katia Denise Saraiva Bresciani [3,*]

[1] São Paulo State University (Unesp), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil
[2] Department of Infectious Diseases and Global Health, Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts, 01536, USA
[3] Faculdade de Medicina Veterinária de Araçatuba, FMVA/ São Paulo State University -UNESP. 793, Clóvis Pestana Street. Zip Code 16050-680. Araçatuba, Brazil
[4] School of Medical Sciences, University of Campinas, São Paulo, 13083-887, Brazil. jgomes@ic.unicamp.br
[5] School of Veterinary Medicine and Zootechnics, São Paulo, Brazil
[6] Laboratory of Image Data Science, Institute of Computing, University of Campinas, São Paulo, 13083-852, Brazil
* Correspondence: katia.bresciani@unesp.br; Phone number: +551836361370

Simple Summary: Gastrointestinal parasites are common in domestic dogs around the world. Many of these parasites are potentially zoonotic and are important pathogenic agents in Public Health. This is the first study of the occurrence of gastrointestinal parasites in domesticated puppies under six months of age. Samples were collected from 100 randomized animals of both sexes and the occurrence of *Toxocara* spp., *Isospora* spp., *Ancylostoma* spp., *Giardia* spp. was found. Toxocariasis in asymptomatic dogs highlights the risk of zoonotic transmission..

Abstract: Despite the existence of therapeutic and prophylactic measures, gastrointestinal parasites are common in pets. Due to the zoonotic potential of some species, parasitic protozoa and helminths are of great importance to public health. In this study, we investigated the occurrence of the main gastrointestinal parasites in domestic dog puppies in the city of Araçatuba, São Paulo, Brazil. One hundred fecal samples were collected from dogs up to six months of age. Parasites were diagnosed using three methods; Willis', Faust's and malachite green coproparasitological techniques. Parasite prevalence as determined by Willis and/or Faust diagnostic techniques was as follows*: Toxocara* spp. 34%, *Isospora* spp. 28%, *Ancylostoma* spp. 22% and, *Giardia* spp. 8%. These prevalence rates were calculated by considering an animal to be positive if Willis' or Faust's, or both tests returned a positive result. Diagnosis with malachite green was negative for all samples. Infection with *Toxocara* spp., the most prevalent pathogen in this survey, was not limited to dogs with abnormal fecal consistency. The occurrence of asymptomatic parasitized dogs increases the risk of zoonotic transmission.

Keywords: helminths; Protozoa; One Health; dogs; diagnosis

1. Introduction

Some gastrointestinal parasites are of interest from a One Health perspective because of their veterinary and public health relevance. Visceral Larva Migrans and Cutaneous Larva Migrans caused by *Toxocara* spp. and *Ancylostoma* spp., respectively, are examples of such parasites [1–4]. Helminth eggs and protozoan cysts excreted in the environment by domestic animals should be a matter of concern to their owners due to the importance that animals can have for emotional support and their

 doi:10.20944/preprints202501.1622.v1

role in social and physical development of people, particularly children and the elderly [5–8]. Close co-habitation of humans and domestic animals, which are sometimes view as members of the family, favors zoonotic transmission [9–11].

Various parasite species are diagnosed with moderate to high frequency in companion animals, despite the existence of therapeutic and prophylactic measures [8,12]. Diarrhea resulting from parasitic infections and from other causes is one of the most frequent disorders in canine puppies and negatively interferes with their growth [8,13]. Thus, it is essential to prevent or minimize parasite transmission. Screening for the presence of gastrointestinal parasites [2,14] informs treatment options and the adoption of environmental management practices for infection control [10,13,15]. In contrast with surveys which include a wide age range, the aim of our study was to investigate the occurrence of gastrointestinal parasites in domestic dogs up to six months of age.

2. Materials and Methods

2.1. Study Population

The minimum sampling required for the execution of this project, at the 95% confidence level and with absolute precision of 10%, was determined to be 96 samples, based on a prevalence of 50% [16]. Thus, we collected fecal samples from 100 randomly selected dogs, 60 males and 40 females, all domestic and of mixed breed, aged less than six months. Within this age range, 20 animals were one to two months old, 44 three to four months old and 36 five to six months old. Puppies aged between 2 and 3, and 4 and 5 months were not represented in the sample. The study was approved by the animal use ethics committee of the Faculty of Dentistry of Araçatuba - São Paulo, under protocol number FOA-00312-2016.

2.2. Degree of Dehydration and Fecal Consistency

The degree of dehydration was assessed as not apparent, mild, moderate, severe and shock [17]. Fecal consistency was assessed visually and defined as liquefied, pasty, semi-solid and solid, according to Coelho et al., 2012 [18].

2.3. Collection of Fecal Material and Parasite Detection

With the aid of urethral catheter (nº 6 - 8 - 10) and a 10-mL syringe, feces were collected directly from the rectal ampulla. Samples were stored in sterile vials and refrigerated between 4º and 8ºC until processed. Subsequently, Faust's flotation technique in saturated sodium chloride solution [19] and Willis' centrifuge-flotation technique in zinc sulfate [20] were applied. The presence of parasites eggs and cysts was also examined by negative malachite green staining [21]. All samples were examined with the three diagnostic techniques.

3. Results

Based on at least one positive test, 34% of dogs were positive for Toxocara spp., 28% for Isospora spp., 22% for Ancylostoma spp. and eight for Giardia spp.. In 21% of the animals mixed infections were detected. One or more parasite species were detected in 62% of dogs with at least one technique. Among the 100 dogs evaluated in the study, with the Willis technique we detected 32% positive animals for Toxocara spp., 19% for Ancylostoma spp. and 16% for Isospora spp., 16% of which presented mixed infections with Ancylostoma spp., Isospora spp. and Toxocara spp. Faust's technique identified 30% of dogs with Toxocara spp., 26% with Isospora spp., 10% with Ancylostoma spp. and eight with Giardia. Based on this technique, 21% of the animals were infected with more than one parasite. For 82% of the samples, Faust and Willis gave concordant results in the sense that both tests were negative or both were positive. Of the 18 (18%) discordant results, 12 were positive according to Willis and negative according to Faust. For 6 samples the results were reversed (Chi-square=39.0, $p<0.001$). Six of 6 Willis-negative/Faust-positive sample were Faust-positive for Isospora

 doi:10.20944/preprints202501.1622.v1

spp., indicating that the Willis technique may underestimate the prevalence of this parasite. Conversely, of the 12 samples positive by Willis and negative by Faust, Ancylostoma spp. was detected by itself or in combination with a second parasite in 10 samples, suggesting that the Faust method may undercount this nematode. Of the 44 double-positive samples, 12 were diagnosed with different parasites or a different combination of parasites where more than one pathogen was detected. The diagnosis for the remaining 32 samples was fully concordant.

Dehydration was not apparent in 50 dogs and the same number was found to be mildly dehydrated. Regarding the consistency of fecal samples, 43 samples were scored as liquefied, 7 as pasty, 8 as semi-solid and 42 as solid. Dogs excreting liquefied and solid feces presented more positive than dogs with pasty and semi-solid feces. In particular, it is important to note that 40 of 53 puppies excreting solid feces were positive for gastrointestinal parasites (Table 1). There was no significant association between fecal consistency and presence of parasite (Fisher's Exact Test, p=0.8589).

Table 1. Number of domestic dogs positive for gastrointestinal parasites by fecal consistency.

Parasite	Consistency of faecal samples				
	Liquefied	Pasty	Semi-Solid	Solid	Total
***Toxocara* spp.**	14	3	5	15	37
***Ancylostoma* spp.**	8	1	2	11	22
***Giardia* spp.**	5	0	0	3	8
***Isospora* spp.**	12	0	5	11	28
No parasites	19	3	3	13	38

No association between age and parasite prevalence was observed using linear regression (r=0.05, p=0.56). Similarly, sex and infection were not significantly associated. A total of 65% of males and 57.5% of females were positive by at least one diagnostic technique (Chi-square=0.30, p=0.58). Toxocara was more prevalent in the 2 younger age groups (45% and 34%) and equally prevalent as Ancylostoma spp. and Isospora spp. in the oldest group (28%) (Table 2). An opposite trend was observed for Ancylostoma spp. which increases in prevalence with age from 5% to 25% and 28%.

Table 2. Number of positive domestic dogs for gastrointestinal parasites according to age*.

Age range (months)	Gastrointestinal parasites															
	Toxocara spp.				*Ancylostoma* spp.				*Giardia* spp.				*Isospora* spp.			
	Positive	%	Negative	%	Positive	%	Negative	%	Positive	%	Negative	%	Positive	%	Negative	%
1-2	9	26,5	11	16,7	1	4,5	19	24,4	2	25	18	19,6	8	28,6	12	16,7
3-4	15	44,1	29	43,9	11	50,0	33	42,3	4	50	40	43,5	10	35,7	34	47,2
5-6	10	29,4	26	39,4	10	45,5	26	33,3	2	25	34	36,9	10	35,7	26	36,1
Total	**34**	**100**	**66**	**100**	**22**	**100**	**78**	**100**	**8**	**100**	**92**	**100**	**28**	**100**	**72**	**100**

* Positive by one or both diagnostic methods.

4. Discussion

Our results extend the epidemiological study of common gastrointestinal helminths and protozoa of dogs to puppies up to six months of age. Typically, coproparasitological surveys include animals of any age [2,14,22]. Other studies have reported the occurrence of gastrointestinal parasites, but they differ from this study since they frequently examine stray dogs regardless of age [15,23–25].

The main finding of our survey is the frequent detection of *Isospora* spp., *Giardia* spp., *Toxocara* spp. and *Ancylostoma* spp. in puppies with varied fecal consistencies, including animals showing no symptoms typically associated with intestinal parasites. This observation is relevant to public health

 doi:10.20944/preprints202501.1622.v1

as the latter three species are potentially zoonotic [3,4,26–29]. As puppies are more likely to excrete gastrointestinal parasites [23,28] deworming and other measures to reduce transmission are particularly important to reduce the risk of infection, which can have severe consequences in immunocompromised children and adults. Such measures are also expected to benefit the health of puppies [23,38]. Mixed infections with two, or even three, parasites were relatively common in our survey. This observation emphasizes the need for adequate medications to treat helminth and protozoan co-infections.

The use of two flotation techniques supports the conclusion that both methods have similar sensitivity. This observation is consistent with the fact that both methods concentrate parasite eggs and cysts by flotation on a high-density salt solution. The difference in spp. and *Isospora* spp. prevalence based on Faust and Willis raises interesting questions about the buoyant properties of these eggs and cysts and may justify the use of both methods where the presence of these parasites is suspected [2,14,30]. This recommendation should be easy to implement as fecal flotation is cheap and easy to perform [14]. Veterinarians should make owners aware of the importance of diagnosing these parasites, particularly given the high prevalence of *Toxocara* spp. and its potential for zoonotic transmission [11,31].

Malachite green staining did not reveal the presence of *Cryptosporidium* spp. oocysts. This stain has the advantage of being cheaper and easier to perform than immunological and molecular assays [32], but its disadvantage is low sensitivity, with the possibility of false negative results [33]. The oocysts have small dimensions, being hardly observed in fecal smears after staining, which requires time and experience from the examiner [34].

5. Conclusions

We investigated for the first time the occurrence of gastrointestinal parasites in domestic puppies less than six months of age. The high prevalence of intestinal helminths, and the diagnosis of *Toxocara* spp. in asymptomatic dogs highlights the risk of zoonotic transmission.

Author Contributions: Conceptualization, G.M.S.R. and K.D.S.B. W.L.F. prepared the intial draft of the manuscript. G.M.S.R.; K.D.S.B. and GW reviewed and edited the manuscript, G.M.S.R., K.D.S.B., W.L.F., S.V.I., J.F.G., W.B.N., G.P.T.M., J.A.B.C.N., W.B.N., G.W. and A.X.F. participated in compiling and analyzing the data. All authors have read and agreed to the final version of the manuscript.

Funding: This research was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Thematic Project Process Nº 2014/12236-1. G.W. was partially supported by the National Institute of Allergy and Infectious Diseases, grant R21AI144521-02S1.

Institutional Review Board Statement: The study was approved by the animal use ethics committee of the Faculty of Dentistry of Araçatuba - São Paulo, under protocol number FOA-00312-2016.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Pedrassani, D.; Viera, A.M.; Thiem, B.E.M. Contamination by Toxocara spp. and Ancylostoma spp. in areas of leisure from Canoinhas County, Santa Catarina state. *Arch. Vet. Sci*, **2008**, 13, 110-117.
2. Lima, V.F.S.; Cringoli, G.C.; Rinaldi, L.; Monteiro, M.F.M.; Calado, A.M.C.; Ramos, R.A.N.; Meira-Santos, P.O.; Alves, L.C. A comparison of mini-FLOTAC and FLOTAC with classic methods to diagnosing intestinal parasites of dogs from Brazil. *Parasitol. Res*. **2015**, 114,3529–3533, doi: 10.1007 / s00436-015-4605-x.
3. Rodriguez-Morales, A.J.; González-Leal, N.; Montes-Montoya, M.C. Cutaneous Larva Migrans. *Curr Trop Med Rep*., **2021**, 8, 190–203. https://doi.org/10.1007/s40475-021-00239-0
4. Narayan, K.G.; Sinha, D.K.; Singh, D.K. Larva Migrans. In: *Handbook of Management of Zoonoses*. Springer, Singapore, **2024**, 1051-1059. https://doi.org/10.1007/978-981-99-9885-2_110

5. Silva, A.M.B.; Bouth, R.C.; Costa, K.S.; Carvalho, D.C.; Hirai, K.E.; Prado, R.R.; Araújo, S.G.; Pereira, A.C.L.; Ribeiro, K.T.S. Ocorrência de enteroparasitoses em comunidades ribeirinhas. *Rev. Pan-Amaz Saude*. **2014**, 5, 45-51, doi: 10.5123/S2176-62232014000400006.
6. Kohansall, M. H.; Fazaeli, A.; Nourian, A.; Hanilool, A.; Kamali, K. Dogs' gastrointestinal parasites and their association with public health in Iran. *J. Vet. Res*. **2017**, 61, 189-195, doi:10.1515/jvetres-2017-0024.
7. Otranto, D.; Dantas-Torres, F.; Mihalca, A.D.; Traub, R.J.; Lappin, M.; Baneth, G. Parasitas zoonóticos de cães abandonados e abrigados na era da crise econômica e política global. *Trends in Parasitology*, **2017**, 33, 813–825, doi: 10.1016 / j.pt.2017.05.013.
8. Heilmann, R.M.; Grellet, A.; Grützner, N.; Cranford, S.M.; Suchodolski, J.S.; Chastant-Maillard, S.; Steiner, J.M. Effect of selected gastrointestinal parasites and viral agents on fecal S100A12 concentrations in puppies as a potential comparative model. *Parasit Vectors*, **2018**, 11, 252, doi: 10.1186/s13071-018-2841-5.
9. Coelho, N.M.D.; Coelho, W.M.D.; Gomes, J.F.; Meireles, M.V.; Nagata, W.B.; de Lima, V.M.F.; Santos-Doni, T.R.; Silva, V.B.; da Silveira Neto, L.; Nakamura, A.A.; Evidence of the Zoonotic Transmission of Cryptosporidium among Children and Pets. *Pathogens*, **2023**, 12, 1393. https://doi.org/ 10.3390/pathogens12121393
10. Souza, J.B.B.; Silva, Z.M.d.A.; Alves-Ribeiro, B.S.; Moraes, I.d.S.; Alves-Sobrinho, A.V.; Saturnino, K.C.; Ferraz, H.T.; Machado, M.R.F.; Braga, Í.A.; Ramos, D.G.d.S. Prevalence of Intestinal Parasites, Risk Factors and Zoonotic Aspects in Dog and Cat Populations from Goiás, Brazil. *Vet. Sci*. **2023**, 10, 492. https://doi.org/10.3390/ vetsci10080492
11. Merigueti, Y.F.F.B.; Giuffrida, R.; Silva, R.C.d.; Kmetiuk, L.B.; Santos, A.P.D.; Biondo, A.W.; Santarém, V.A. Dog and Cat Contact as Risk Factor for Human Toxocariasis: Systematic Review and Meta-Analysis. *Front. Public Health,* **2022**, 10:854468. doi: 10.3389/fpubh.2022.854468
12. Santos, I.F.; Nhantumbo, B.; Alho, P. Ocorrência de casos de Ancylostoma caninum e Toxocara canis no Hospital Veterinário escola (HEV) (2001-2010) – Maputo - Moçambique . *Rev. Eletrônica de Med. Vet.,* **2013,** 21.
13. Grellet, A.; Chastant-Maillard, S.; Robin, C.; Feugier, A.; Boogaerts, C.; Boucraut-Baralon, C.; Polack, B. Fatores de risco de diarreia ao desmame em cachorros alojados em canis de reprodução. *Prev. Vet. Med*., **2014**. 117, 260–265, doi: 10.1016 / j.prevetmed.2014.07.016.
14. Táparo, C.V.; Perri, S.H.V.; Serrano, A.C.M.; Ishizaki, M.N.; Costa, T.P.; Amarante, A.F.T.; Bresciani, K.D.S. Comparison between coproparasitological techniques for the diagnosis of helminth eggs or protozoa oocysts in dogs. *Rev. Bras. Parasitol. Vet*. **2006**, 15, 1-5.
15. Barutzki, D.; Schaper, R. Age-dependant prevalence of endoparasites in young dogs and cats up to one year of age. *Parasitol. Res.* **2015**, 112: 119–131, doi: 10.1007 / s00436-013-3286-6.
16. Lwanga, S.K.; Lemeshow, S. Sample Size Determination in health studies: a practical manual. *World Health Organization*. Geneva, **1991**.
17. Elwood, C.; Devauchelle, P.; Elliott, J. Emesis in dogs: a review. *Journal of Small Animal Practice*, **2010**, 51, 4–22.
18. Coelho, W.M.D.; Amarante, A.F.T.; Perri, S.H.V.; Coelho, N.M.D.; Apolinário, J.C;, Teixeira, W.F.P.; Bresciani, K.D.S. Coccidiose em cães e gatos do município de Andradina, estado de São Paulo, Brasil. *Braz. J. Vet. Res. Anim. Sci*, **2012**, 49, 162-166, doi: 10.11606/issn.2318-3659.v49i2p162-166.
19. Faust, E.C.; D'Antoni, J.S.; Odom, V.; Miller, M.J.; Peres, C.; Sawitz, W.; Thomen, L.F.; Tobie, J.; Walkern, J.H. A critical study of clinical laboratory techinics for the diagnosis of protozoan cysts and helminth eggs in feces. I: preliminary communication. *The American Journal of Tropical Medicine and Hygiene*, **1938,** 18, 169-183, doi: https://doi.org/10.4269/ajtmh.1938.s1-18.169.
20. Willis, H.H. A simple levitation method for the detection of hookworm ova. *Med. J. Aust.,* **1921**, 8, 375-376.
21. Elliot, A.; Morgan, U.M.; Thompson, A.R.C. Improved staining method for detecting Cryptosporidium oocysts in stools using malachite green. *J. Gen. Appl. Microbiol.,* **1999**, 45, 139-142, doi: 10.2323 / jgam.45.139.
22. Johnson, S.A.M.; Gakuya, D.W.; Mbuthia, P.G.; Mande, J.D.; Maingi, N. Prevalence of gastrointestinal helminths and management practices for dogs in the Greater Accra region of Ghana. *Heliyon*, **2015**. 1, doi: 10.1016/j.heliyon.2015.e00023.

23. Zanzani, S.A.; Gazzonis, A.L.; Scarpa, P.; Berrilli, F.; Manfredi, T.M. Intestinal Parasites of Owned Dogs and Cats from Metropolitan and Micropolitan Areas: Prevalence, Zoonotic Risks, and Pet Owner Awareness in Northern Italy. *Biomed Res International*. **2014**. doi: 10.1155/2014/696508.
24. Torres-Chablé, O.M.; García-Herrera, R.A.; Hernández-Hernández, M.; Peralta-Torres, J.A.; Ojeda-Robertos, N.F.; Blitvich, B.J.; Baak-Baak, C.M.; García-Rejón, J.E.; Machain-Wiliams, C.I. Prevalence of gastrointestinal parasites in domestic dogs in Tabasco, southeastern Mexico. *Braz. J. Vet. Parasitol*., **2015**, 24, 432-437, doi: 10.1590/S1984-29612015077.
25. Moskvina, T.V.; Ermolenko, A.V. Helminths infections in domestic dogs from Russia. *Vet. World*., **2016**, 9, 1248-1258, doi: 10.14202/vetworld.2016.1248-1258
26. Uehlinger, F.D.; Naqvie, S.A.; Greenwood, S.J.; Mcclure, J.T.; Conboy, G.; O'Handley, R.; Barkema, H.W. Zoonotic potential of Giardia duodenalis and Cryptosporidium spp. and prevalence of intestinal parasites in young dogs from different populations on Prince Edward Island, Canada. *Vet. Parasitol*., **2013,** 196, 509–514, doi: 10.1016/j.vetpar.2013.03.020.
27. Seva, A.P.; Pena, H.F.J.; Nava, A.; Souza, A.O.S.; Holsback, L. Soares, R.M. Endoparasites in domestic animals surrounding an Atlantic Forest remnant, in São Paulo State, Brazil. *Brasil. J. Vet. Parasitol.*, **2018**, 27, 12-18, doi: 10.1590/s1984-29612017078.
28. Sanchez-Thevenet, P.; Carmena, D.; Adell-Aledo, M.; Dacal, E.; Arias, E.; Sougar, J.M.; Rodriguez, E.; Dea-Ayuela, M. High Prevalence and Diversity of Zoonotic and Other Intestinal Parasites in Dogs from Eastern Spain. *Vector Borne Zoonotic Dis*, **2019**, 19, 915-922, doi:10.1089 / vbz.2019.2468.
29. Duijvestijna, M.; Mughini-Grasa, L.; Schuurmana, N.; Schijfa, W.; Wagenaara, A.J.; Egberinka, H. Enteropathogen infections in canine puppies: (Co-)occurrence, clinical relevance and risk factors. *Vet. Microbio*., **2016,** 195, 115–122, doi: 10.1016 / j.vetmic.2016.09.006.
30. Ferreira, J.I.; Pena, H.F.; Azevedo, S.S.; Labruna, M.B.; Gennari, S.M. Occurrences of gastrointestinal parasites in fecal samples from domestic dogs in São Paulo, SP, Brazil. *Rev. Bras. Parasitol. Vet.*, **2016**, 25, 435–440, ISSN: 1984-296.
31. Overgaauw, P.A.M.; Knapen, F.V. Veterinary and public health aspects of Toxocara spp. *Vet. Parasitol*., **2013**, 193, 398-403, doi: 10.1016/j.vetpar.2012.12.035.
32. Weisel, T.; Dittrich, S.; Möhl, I.; ADusu, E.; Jelinek, T. Evaluation of seven commercial antigen detection tests for Giardia and Cryptosporidium in stool samples. *Clinical Microbiology and Infection*, **2006**, 12, 656-659.
33. Casemore, D.P. Laboratory methods for diagnosing cryptosporidiosis. *Journal of Clinical Pathology*, **1991**, 44, 445-451.
34. Fayer, R. Taxonomy and species delimitation in Cryptosporidium. *Experimental Parasitology*, **2010**, 124, 1, 90-97.