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ORIGINAL ARTICLE / ARTÌCULO ORIGINAL 9 FECAL EXAMINATIONS OF DOGS AND CATS RESCUED AND ADOPTED FROM 10 THE FLOOD IN PORTO ALEGRE, RIO GRANDE DO SUL, BRAZIL 11 12 EXÁMENES FECALES DE PERROS Y GATOS RESCATADOS Y ADOPTADOS DE LA INUNDACIÓN EN PORTO ALEGRE, RIO GRANDE DO SUL, BRASIL 13 Sandra Márcia Tietz Marques^{1*}; Rafael Gustavo Tonin² & Elissandra da Silveira³ 14 1 Laboratório de Helmintoses, Departamento de Patologia Clínica Veterinária, Faculdade de 15 16 Veterinária, UFRGS, Porto Alegre, RS, Brasil. Av. Bento Goncalves, 9090, Porto Alegre. 17 2 Hospital de Clínicas Veterinárias, Faculdade de Veterinária, UFRGS, Porto Alegre, RS, Brasil. 3. Base Aérea de Canoas, Força Aérea Brasileira. R. Augusto Severo, 1700 - Nossa Sra. das 18 Graças, Canoas - RS, Brasil. CEP: 92110-390 19 20 * Corresponding author: E mail: santietz@gmail.com 21 Titulillo: Fecal examination of dogs and cats 22 Margues et al. 23 Sandra Márcia Tietz Margues: https://orcid.org/0000-0002-7541-9717 24 Rafael Gustavo Tonin: https://orcid.org/0000-0002-9619-2144 Elissandra da Silveira: https://orcid.org/0000-0003-3582-4723 25 26

27 ABSTRACT

The objective of this work is to report gastrointestinal parasitism in dogs and cats 28 29 abandoned during the May 2024 flood, rescued and adopted in Porto Alegre, Rio Grande do Sul, Brazil. From June to October 2024, fecal samples from 93 pets (15 dogs and 78 30 cats) were analyzed using five parasitological techniques. Of the total number of pets 31 (15 dogs and 78 cats), the frequencies of positive samples were 46.6% (7/15) and 43.6% 32 (34/78), respectively, for dogs and cats. The parasites present in dogs were: 33 34 Ancylostoma caninum (Ercolani 1859), Trichuris vulpis (Frolich 1789), Cystoisospora felis (Wenyon, 1923 Frenkel 1977) and Toxocara canis (Werner, 1782); in cats they 35 were: T. canis, C. felis, Giardia sp., larvae of Aelurostrongylus abstrusus (Railliet, 1898) 36 and Strongyloides sp., A. caninum, Dipylidium caninum (Linnaeus, 1758) and 37 Dioctophyma renale (Goeze, 1782). These results demonstrate the importance of 38 parasitological diagnosis based on the identification of parasites that can affect not only 39 40 animal health, but also human health.

41 Keywords: emerging zoonoses – parasitic zoonoses – pet animal – prevention –
42 helminth

43

44 RESUMEN

El objetivo de este trabajo es reportar el parasitismo gastrointestinal en perros y gatos 45 46 abandonados durante la inundación de mayo de 2024, rescatados y adoptados em Porto Alegre, Rio Grande do Sul, Brasil. De junio a octubre de 2024 se analizaron muestras 47 48 fecales de 93 mascotas (15 perros y 78 gatos) mediante cinco técnicas parasitológicas. 49 Del total de mascotas (15 perros y 78 gatos), las frecuencias de muestras positivas fueron del 46,6% (7/15) y del 43,6% (34/78), respectivamente, para perros y gatos. Los 50 parásitos presentes en los perros fueron: Ancylostoma caninum (Ercolani, 1859), 51 52 Trichuris vulpis (Frolich, 1789), Cystoisospora felis (Wenyon, 1923 Frenkel, 1977) y Toxocara canis (Werner, 1782); en gatos fueron: T. canis, C. felis, Giardia spp., larvas 53 de Aelurostrongylus abstrusus (Railliet, 1898) y Strongyloides sp., A. caninum, 54 Dipylidium caninum (Linnaeus, 1758) y Dioctophyma renale (Goeze, 1782). Estos 55 resultados demuestran la importancia del diagnóstico parasitológico basado en la 56 identificación de parásitos que pueden afectar no sólo la salud animal, sino también la 57 58 salud humana.

59 Palavras clave: animal de companhia – helminto – prevención– zoonosis emergentes
 60 – zoonosis parasitarias

61

62 **INTRODUCTION**

Gastrointestinal parasites that more frequently occur in dogs in Brazil are nematodes of the genera *Ancylostoma* spp., *Toxocara* spp., *Trichuris* spp. and *Strongyloides* spp., followed by the cestode of the genus *Dipylidium* (Berenger *et al.*, 2021; Lima *et al.*, 2021; Lopes *et al.*, 2021; Souza *et al.*, 2024). All these genera, with the exception of *Trichuris* spp., contain species with zoonotic potential of transmission, especially in the presence of poor hygiene and sanitation conditions (Chomel, 2008) to control active infections in the environment (Henke *et al.*, 2023).

Infections caused by Toxocara spp. and Ancylostoma spp. can cause the zoonoses 70 Visceral Larva Migrans (VLM) and Cutaneous Larva Migrans (CLM), respectively. 71 72 Cutaneous Larva Migrans (CLM) is an infectious syndrome caused by several Ancylostoma species; it is more commonly transmitted by animal feces depositing eggs 73 74 in the soil, with larvae entering humans through direct contact with skin (Maxfield & 75 Crane, 2024). Visceral Larva Migrans (VLM) occurs due to the migration of the second 76 stage larvae of nematodes through the human body's viscera. It is an underdiagnosed 77 condition that usually affects the liver with eosinophilic abscesses and that appears as coalescent and conglomerate cavities in imaging exam (Kashyap et al., 2024). They 78 present a rare, but grave risk, to humans (Huynh et al., 2024). Migrans cutaneous larva 79 is unlike the cutaneous manifestation of the infection by Strongyloides stercoralis 80 (Bavay, 1876) called larva currens, which demonstrates fast movement through the skin 81 (Patil et al., 2024), and the intestinal strongyloidiasis that affects about 100 million people 82 worldwide, being endemic in tropical and subtropical regions (Merman et al., 2016). 83

Prevalence of parasitic infection in dogs, confirmed through stool samples, in European
countries are variable, with *Toxocara* spp. (1.7% – 22.4%) and *Ancylostoma* spp. (2.0%
- 8.6%), *Dipylidium caninum* (0.2% – 1.25%), *Cystoisospora felis* (4.6% – 22.3%) and *Giardia* spp. (3.8% – 51.6%) (Barutzki & Schaper, 2003; Riggio *et al.*, 2013; Kostopoulou *et al.*, 2017; Bourgoin *et al.*, 2022), while in Morocco, the general prevalence was of 58%,
with *Ancylostoma* spp. (31.9%), *Toxocara canis* (27.1%), *Cystoisospora* spp. (13.4%)
and *Giardia* spp. (7.2%) (Idrissi *et al.*, 2022).

In cats, the most frequent helminthiasis in Brazil is caused by Ancylostoma spp., with a 91 92 prevalence greater than 40% (Melo et al., 2021), but Toxocara spp. is the most common worldwide parasite in cats, especially in young animals (Marques et al., 2020). 93 94 Prevalences in cats in Brazil vary: in the Southern region, an occurrence from 21% to 76.9% is reported (Margues et al., 2017; Mósena et al., 2019); in the Southeast, it varies 95 96 from 18.1% to 50.64% (Ramos et al., 2020) and in the Northeast rates vary from 13.65% to 100% (Silva et al., 2017). Aelurostrongylus abstrusus (Railliet, 1898) is the most 97 important nematode that affects the respiratory system of domestic cats, therefore being 98 an important differential diagnosis in respiratory diseases (Crisi et al., 2019; Fagundes-99 Moreira et al., 2023). The conditions that favor the development of both eggs and larvae 100 (exogenous development stages) in the environment are similar to all helminths that 101 infect dogs and cats in tropical and subtropical regions and, despite biological and 102 transmission particularities of each species, mixed infections occur frequently in these 103 104 hosts (Silva et al., 2017).

Giardia spp. Is the most frequent protozoosis in dogs and cats and is included among the "Neglected Tropical Diseases" of the World Health Organization (WHO), due to its connection to poverty and poor sanitation conditions. It is part of a group of diarrheal diseases that cause serious socioeconomic problems in Least Developed Countries (Souza *et al.*, 2024). *Cystoisospora* spp. frequently causes diarrhea, fever, dehydration,

and a decline in immunity. The contamination of the environment combined with the lack 110 111 of basic sanitation becomes an important factor that contributes to the occurrence of this 112 disease. Furthermore, its control is made difficult given how easily the etiologic agent can spread to dogs and cats; prevalence was of 24.73% in household cats that had 113 114 access to the outdoors in Porto Alegre (Klimpel et al., 2010; Margues et al., 2017). The goal of this investigation was to determine, through stool samples, the parasite infections 115 116 present in adopted dogs and cats, based on the unknown sanitation history, thus being able to guide treatment and epidemiologic care. 117

118

119 MATERIAL AND METHODS

Stool samples of 15 dogs (4 males, 11 females, 3 puppies and 12 adults) and of 78 cats (68 males, 10 females, 3 kittens and 75 adults), with a total of 93, were analyzed from June to October 2024. These animals were rescued after being abandoned during the flooding that destroyed and damaged many districts of Rio Grande do Sul in May of 2024.
Fig. 1 shows some examples of dogs and cats that were rescued.



126

Figure 1. Examples of cats and dogs being rescued in the 2024 flooding in Porto
 Alegre, Rio Grande do Sul, Brazil.

129 Samples were collected in two community shelters in the city of Porto Alegre, Rio Grande do Sul, Brazil. The animals underwent veterinary screening and necessary exams before 130 being put for adoption. Cats and dogs sampled had their stool collected and sent to the 131 Laboratório de Parasitologia da Faculdade de Veterinária da Universidade Federal do 132 133 Rio Grande do Sul (Laboratory of Parasitology of the Veterinary College of the Federal University of Rio Grande do Sul), in Porto Alegre, where they were kept in a temperature 134 of 39.2 °F and processed in a period no longer than 24 hours with the following 135 136 techniques: Willis-Mollay, Lutz, Faust, Baermann and direct parasitological examination. 137 The identification of the parasite genre was conducted based on the morphologic 138 characteristics of the eggs, cysts, oocysts and larvae (Zajac & Conboy, 2012). The microscopy for each sample was observed with the use of a binocular optical microscope 139

Olympus® BX 41 with an amplification of 100 × and, if necessary, 400 × to confirm the
protozoan parasites.

142 **Ethic aspects**: For this study formal consent is not required.

143 **RESULTS AND DISCUSSION**

In the study period, 465 stool sample exams were performed with the previously mentioned techniques. From the total of 93 animals rescued and adopted (15 dogs and 78 cats), the frequency of positive samples for dogs was of 46,6% (7/15) and of 43,6% (34/78) for cats. Table 1 describes the parasites detected in the specific methodologies for visualization and identification of eggs, larvae, cysts and/or oocysts in stool samples.

Table 1. Parasites diagnosed in stool samples of dogs and cats rescued in the flooding

150 (May/2024) in Porto Alegre, Rio Grande do Sul, Brazil.

Species	Identified parasite	N (%)
Canine	Ancylostoma caninum	3 (20)
	Ancylostoma caninum + Trichuris vulpis	2 (13.3)
	Ancylostoma caninum + Cystoisospora felis	1 (6.7)
	Ancylostoma caninum + Toxocara canis	1 (6.7)
	Positive Samples	7 (46.7)
	Negative Samples	8 (53.3)
	Total	15
Feline	Toxocara canis	10 (12.8)
	Cystoisospora felis	6 (7.7)
	Giardia spp.	4 (5.1)
	Aelurostrongylus abstrusus	3 (3.8)
	Strongyloides sp. larvae	2 (2.6)
	Ancylostoma caninum	3 (3.8)

	93
Total	78
Negative samples	44 (56.4)
Positive samples	34 (43.6)
Dioctophyma renale	1 (1.3)
Dipylidium caninum	2 (2.6)
Ancylostoma caninum + Toxocara canis	1 (1,3)
Giardia spp. + Toxocara canis	1 (1.3)
Giardia spp. + Cystoisospora felis	1 (1,3)

Total

152 The presence of endoparasites in dogs and cats involves matters of public health, since 153 some parasite genera have significant zoonotic potential. All seven infected dogs (46.7%) presented eggs of potentially zoonotic agents. In the stool samples of the 78 154 155 cats, the frequency of gastrointestinal parasites was of 46.3%, with 22 animals 156 presenting zoonotic parasites. Prevalence rates in the range of 50% in this relevant study impact on how important parasitological diagnosis is both for human and animal health. 157 158 In some cases, it was not possible to determine the origin of the animals since they were taking shelter wherever they could in order to escape the powerful flooding (over 4ft of 159 water). The catastrophic situation did not allow many of these animal's tutors to stay with 160 them, since many of these people had no home to return to. 161

Prevalence of gastrointestinal parasites in dogs and cats in Brazil vary, ranging up to 80%. Nevertheless, this report is in accordance with other investigations (Marques *et al.*, 2017; Silva *et al.*, 2017; Mósena *et al.*, 2019; Ramos *et al.*, 2020; Berenger *et al.*, 2021) for more important zoonotic helminths. The reality of frequencies also applies to the same helminths and prevalence rates, as published in other countries (Barutzki *et al.*, 2003; Riggio *et al.*, 2013; Kostopoulou *et al.*, 2017; Bourgoin *et al.*, 2022; Idrissi *et al.*, 2022). Aelurostrongylus abstrusus is the most important nematode that affects the respiratory system of domestic cats, thus being an important differential diagnosis in diseases that affect the respiratory tract (Fagundes-Moreira *et al.*, 2023). Diagnosis through the Baermann technique is reliable and low cost.

Strongyloides sp. larvae were present in the stool samples of two cats, which is unusual in the routine of exams of this laboratory. In literature, there are few reports of infection by the currens larva, whose clinical signs are, frequently, unspecific. Patil *et al.* (2024) report that the proper diagnosis and treatment with ivermectin is necessary, especially when the patient is immunocompromised and at higher risk of hyperinfection syndrome and disseminated disease. This recent case justifies giving the due attention to the presence of this parasite in animals in close proximity to humans.

Giardiasis is a routine result in the laboratory as stool samples of dogs and cats are run, 179 and it can manifest with typical signs such as feces with mucus or bloody and smelly 180 diarrhea, with or without vomiting. Prevalence is high in young animals and can reach up 181 182 to 50%. There are 8 different genotypes (assemblages) (A - H). C and D are the most 183 common ones in dogs and the F assemblage is more frequent in cats. Nonetheless, 184 animals can also be infected by the zoonotically efficient A and B assemblages or have multiple infections. The adoption of specific hygiene habits is necessary and, in case the 185 pet presents with clinical signs, or lives in the same household as high-risk patients, 186 187 medication is recommended (Kansky et al., 2023). Kurnosova et al. (2024) have investigated stool samples of 2761 dogs and 1579 cats in order to determine infection 188 rates by G. duodenalis, that resulted in 18.2% (215/1182) in dogs from 1 to 12 months 189 190 and 3.8% (60/1579) in dogs older than 12 months; for cats the infection rate was of 7.8% 191 (48/615) in cats from 1 to 12 months and 3.35% (33/994) in cats >12 months. The rate 192 of positive animals in this study was of 7.7% (6/78) in cats, and even though our sampling 193 is not big and cannot be compared to that of the previously mentioned study, the zoonotic

aspect should be considered. In dogs, prevalences vary uniformly in many studies, and
in this report with a sampling of 15 dogs, not one has tested positive for giardiasis in the
one stool sample collected. In our routine practice, though, the collection of three stool
samples per animal is recommended.

In Rio Grande do Sul, a prevalence of endoparasites of 47.1% has been observed in
household and shelter cats, with *T. canis* and *Giardia* spp. being the biggest occurrences
(Pivoto *et al.*, 2013). In the city of Porto Alegre-RS, a *T. canis* prevalence of 26,8% in the
stool samples of cats has been reported (Marques *et al.*, 2017; Sarmento *et al.*, 2021).
High prevalence rates for *Toxocara* spp. have been reported in Andradina-SP, 43.1%
(Coelho *et al.*, 2009) and Lages-SC, 49.4% (Quadros *et al.*, 2014).

204 Henke et al. (2023) have described in a comprehensive review that growing evidence suggests a potential association between toxocariasis and chronic diseases such as 205 206 of cognitive behavioral disturbance asthma. cases and (schizophrenia, neurodegenerative diseases and epilepsy), making the investigation of the physio 207 208 pathological interactions between host and parasite in the pathogenesis of many 209 systemic disorders a true research challenge. This observation is particularly important when we take into consideration that helminthiases are the etiology of millions of deaths, 210 and take part in the estimate to determine the number of Disability Adjusted Life Years 211 (DALY), that associate these diseases as the cause for a lack in productivity of 6 to 212 213 35.3% in those affected. Therefore, feces of infected dogs make parks, squares, 214 playgrounds, litter boxes and beaches the main source of transmission to humans. E 215 Huynh et al. (2024) have described a case of hepatic larva migrans in a young person 216 caused by T. canis. The lack of specific clinical signs was what stood out in general 217 practice, which leads to the importance of considering toxocariasis as a differential 218 diagnosis in hepatic abscesses, especially in regions of high seroprevalence, highlighting the need of corroborative evidence, including invasive procedures such as hepaticbiopsy, for the precise diagnosis of hepatic toxocariasis.

221 Unlike the treatment dispensed to cats and dogs with parasitic diseases, treatment in 222 humans varies according to clinical symptoms and the location of larvae. Molecular 223 technologies can also help demonstrate the importance of T. canis in public health, 224 providing new evidence to support the launching of control programs, by national 225 initiatives, that still need to be developed. Many countries have developed programs of reproductive control in household and stray dogs to reduce the number of young dogs-226 227 more prevalent-in the population. The growth in human and canine populations and, more recently, the population of cats in the homes of Brazilians, populational movements 228 229 as well as climate changes will help increase the importance of this zoonosis 230 (Macpherson, 2013).

Toxocariasis continues to be an important neglected parasitic disease, as it is one of the 231 most common zoonotic infections caused by the parasite T. canis or, less frequently, T. 232 233 cati. The disease's epidemiology is complex due to its transmission route by accidental 234 ingestion of eggs or embryonated larvae of Toxocara from domestic or wild paratenic host's tissue. Although the World Health Organization and the Centers of Disease 235 Control have classified toxocariasis among the six main parasitic infections of priority to 236 public health, global epidemiological data on the relationship between seropositivity and 237 238 toxocariasis is limited.

Infection by cutaneous larva migrans, caused by the helminth of the genus *Ancylostoma* in humans, occurs when the skin gets into contact with fomites contaminated with the feces of dogs or cats. L3 stage has the capacity to penetrate skin tissue through the action of an enzyme known as hyaluronidase or through the hair follicle, sweat glands and skin tears, popularly known as "geographic worm" in Brazil due to its creeping

eruptions (Soares et al., 2018; Lima et al., 2021), and it can cause a series of risks both 244 245 for animal and human health, with severe complications for both species. As a 246 consequence, there is a great need to control this disease in order to interrupt its 247 biological cycle. Many options can be taken to prevent the transmission of this zoonosis 248 and among them we can quote the administration of anthelmintic drugs, that can also be 249 used therapeutically in infected animals. The drug resistance found in *A. caninum* is an 250 emerging and serious problem (Lima et al., 2021). Despite anthelmintic drugs being 251 efficient in deworming, the fast reinfection in endemic areas occurs due to the organism's inability to develop immunological anti-parasite memory, thus requiring treatment in 252 infected hosts to be repetitive and resulting in helminth resistance to the available drugs 253 (Wang et al., 2010). Prevention strategies include educational programs, changes in 254 behavior and hygiene habits, enhancement of the role of the veterinarians, and 255 anthelmintic protocols to control active infections (Margues et al., 2020; Henke et al., 256 257 2023).

Gastrointestinal parasitic diseases are one of the main causes of infections in humans (Hall *et al.*, 2008). They usually affect populations that do not have access to basic sanitation, education, and are more socioeconomic challenged, therefore accounting for a public health problem in Brazil (Cirne & Cabrera, 2019). Every year, about 3.5 billion of people are affected by these diseases, and around 65 thousand people die, especially in Least Developed Countries (Zamprogno *et al.*, 2015).

264 Control and prevention of emerging parasitic zoonoses poses complex challenges that 265 demand an integrated and multidisciplinary approach. Therefore, ecological and 266 environmental modifications need to be implemented in order to reduce not only parasite 267 load, but also the risk of parasite transmission. In conclusion, the climate tragedy that 268 took place in part of the state of Rio Grande do Sul, affecting millions of people and 269 animals, has confirmed the importance of One Health actions to prevent and control

- 270 zoonotic parasitic diseases, promote programs to spay, neuter and deworm pets, public
- 271 policies and educational programs directed to the more vulnerable communities.

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- 279 **Data curation**: SMTM, RGT, ES
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- 282 Investigation: SMTM
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