Prevalence of *cryptosporidium* spp. in domestic companion animals of elderly population in Teresópolis, Rio de Janeiro, Brazil

Prevalência de **cryptosporidium** *spp. em animais domésticos de companhia da população idosa em Teresópolis, Rio de Janeiro, Brasil*

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Abstract

Objective: This study aims to highlight the prevalence of cryptosporidiosis in domestic companion animals. *Method:* Eligible for study were all elderly (over 60 years of age) of both sexes who have dogs and / or cats at home, living in the city of Teresópolis and who attended a vaccination post in the municipality during the period of national campaigns vaccination against influenza in 2007 and 2008. *Results:* It was identified the presence of one or more oocysts in fecal material (positive for *Cryptosporidium* spp.) in 29.0% (87) of these animals and 28.7% had about 2 or more oocysts per field. The prevalence by history of diarrhea among the 300 animals examined was 27%, reaching 29.5% in dogs and 24.7% in cats showed no statistically significant difference between species. *Conclusion:* This fact indicates gaps to be more detailed, since there are few studies that explore the cryptosporidiosis relationship in the human population with companion animals. The results show the importance of conducting periodic parasitological examination in dogs with or without diarrhea for the specific treatment and the implementation of prophylaxis and control methods.

Key words: *Cryptosporidium.* Prevalence. Health of the elderly. Domestic Animal.

Resumo

Objetivo: Este estudo visa a destacar a prevalência da criptosporidiose em animais de companhia doméstica. *Método:* Foram elegíveis para o estudo todos os idosos (acima de 60 anos de idade) de ambos os sexos que tenham cães e / ou gatos em casa, vivendo na cidade de Teresópolis e que foram a um posto de vacinação no município durante o período das campanhas nacionais de vacinação contra a

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gripe em 2007 e 2008. *Resultados:* Em 29,0% dos animais pesquisados detectou-se a presença de oocistos e em 28,7% foram observados 2 ou mais oocistos por campo. A prevalência de história de diarreia entre os 300 animais examinados foi de 27%, atingindo 29,5% em cães e 24,7% em gatos, não mostrando diferença estatisticamente significativa entre as espécies. *Conclusão*: Este fato indica lacunas a serem mais aprofundadas, uma vez que são poucos estudos que exploram a relação da criptosporidiose com os animais de companhia na população humana. Os resultados demonstram a importância de realização periódica de exames parasitológicos em cães com e sem diarreia para tratamento específico e adoção medidas de controle e profilaxia.

INTRODUCTION

The cryptosporidiosis is an important zoonosis responsible for clinical signs of diarrhea and gastroenteritis, associated with abdominal pain in humans and domestic animals caused by opportunistic protozoan of the genus *Cryptosporidium*.[1] It is transmitted between individuals through oocysts that are already eliminated in the infectious form, being the main transmission routes the direct contact (person to person), oral-fecal or indirectly by ingestion of contaminated food or water (cysts and oocysts).[2]

Its distribution is cosmopolitan and presents various hosts where *C. muris* was the first species described in 1907 by Tyzzer, being its endogenous development restricted to glands of the rodent stomach. *C. parvum*, also described by Tyzzer (1912) occurs mainly in the small intestine of various mammals, including humans.[3]

The protozoan parasite *Cryptosporidium* has emerged as an important water contaminant, responsible for several outbreaks of cryptosporidiosis, affecting until half of 2001, approximately, 427,000 people worldwide. Several *Cryptosporidium* species have been described, but only *C. parvum* have been associated with gastrointestinal illnesses.[4] The cryptosporidiosis can be fatal in immunocompromised patients and can severely debilitate immunocompetent individuals. Another problem is given by the fact that *Cryptosporidium* oocysts can survive for several months in the aquatic environment and are also resistant to disinfection by chlorine used in conventional water treatment.[5]

The importance of a study on the occurrence of *Cryptosporidium* spp. in the aquatic environment has been reinforced by Brazilian regulations in Health Environmental Surveillance associated to Drinking Water Quality Manual (Act n° 518, Ministry of Heath)[6] that developed and implemented regulatory standards for *Cryptosporidium* in drinking water. Currently, monitoring of *Cryptosporidium* in drinking water system that supplies cities between 10,000 and 100,000 inhabitants is worldwide recommended.[7] However, the methods commonly used in the determination of oocysts in water are highly variable and inefficient, for example, the identification of false-positive by interference with algae and others protozoan species.[8]

The amount and the quality of water are important factors for the establishment of health benefits related to the incidence reducing and prevalence of several diseases, including diarrheal illness.[9] Brazil is one of the countries with high incidence of diarrhea, which directly affects the rates of infant mortality and with greater severity among the elderly.[10,11]

C. parvum has been recognized worldwide as one of the major contaminants of the water supply.[12] The description of hydric contamination by oocysts presence, probably due to human and animal origin, are frequently associated with diarrhea outbreaks and consequently to high morbidity and mortality levels, preferably affecting immunocompromised and children, but also immunocompetent and animals.[13] Although of endemic characteristics, diarrhea can present cases related to itself (clinical, spatial-temporal distribution, source of infection) that are able to characterize an outbreak.

The *Cryptosporidium* transmission through the environment is gaining recognition, especially, after the occurrence of numerous outbreaks associated with consumption or contact with contaminated waters.[14] Some biological factors and characteristics of *Cryptosporidium* facilitate disease transmission through water.[13] There is no specific therapy to treat the high number of oocysts excreted by infected individuals, around 10^9 to 10^{10} oocysts, as well as a wide variety of hosts that act as reservoirs of infection or promote the cross-transmission or increase the potential dissemination of cryptosporidiosis.[5,9] The oocysts excretion may or may not coincide with the period of symptomatic disease; there may be uncertainties as to its actual occurrence, by the lack of further information on the incidence in asymptomatic individuals.[10,13]

The elderly population presents a greater susceptibility to cryptosporidiosis.[15-17] The number of fatal cases by specific enteric pathogens present from 10 to 100 times higher in elderly than in the general population.[18,19] Mirzaei[20] observed that in different age groups of Iran population a prevalence of *C. parvum* in individuals over 51 years ranged from 25.6% with diarrhea and it was more expressive than in cases of non-diarrhea (3.7%).

Studies detach the role of pets, especially dogs and cats, indicating significant benefits for people and for society. They would contribute for the physical, social and emotional development of the children and with well-being of its proprietors, particularly the elderly.[16] However, companion animals can be an important source of infection for humans, determining diseases generically known as zoonosis, as cryptosporidiosis.[16,21] Aggravating case related to this infection is given by the fact that most of the infected animals are asymptomatic.[22,23]

This still little explored scenario motivated this paper, that aims to present the prevalence of cryptosporidiosis in companion animals, in a sample of the elderly population from the city of Teresópolis, Rio de Janeiro, Brazil.

METHODS

Local Study

According to the Demographic Census,[24] Teresópolis has a total of 138,081 inhabitants with 115,198 (83.5%) inhabitants living in the urban areas and 22,883 (16.5%) inhabitants living in rural areas, with a density of 158.7 inhabitant/km^2 and urbanization rate of 83.9%.

Population target

All elderly (60 years or older) of both sexes who have dogs at home and / or cats, living in Teresópolis and who hat attended a municipal health unit during the national influenza vaccination campaign in 2007 and 2008 were eligible for the study.

We identified 300 elderly in these two campaigns who agreed with the term consent for the anamnesis, carried out through a questionnaire answered by the research participants, with the aim to gather information about residence location and conditions of handling the animal in the household.

Were collected fecal samples of companion animals (dogs and cats). The fecal samples obtained from the animals were kept in phormol acetic acid 10% and submitted to refrigeration until laboratorial analysis.[7] Two coprological methods for diagnosis were used. As first method it was done direct fecal smear, and these stained by modified Ziehl-Neelsen,[25,26] after which it was carried out the microscopic observation of smears. As second method it was weighed two grams of each stool sample which diluted in 15.0 ml PBS (Phosphate-buffered saline), filtered and placed in centrifuge tube. The filtrate was centrifuged at 750 x g per 7 minutes, discarding the supernatant. In pellet was added 10.0 ml of sucrose solution saturated with a specific gravity of 1.2 and centrifuged under the same conditions. Supernatant sample was collected with the aid of a bacteriological loop being prepared smears on a glass microscopic slide. Drying and fixation

with methanol were followed by staining using the modified Ziehl-Neelsen technique. The stained slides were observed and *Cryptosporidium* oocysts were identified by ocular micrometer ×1000 using a Zeiss Axioskop microscope.

It was considered as relevant for prevalence study age data owner's grouped by age band (60 to 64 years, 65 to 69 years, 70 to 74 years and above 75 years), sex of owner, number of domestic animals for household, its distribution according to species, its domiciliation inside or outside the residence, and origin as the location (urban or rural) as well as the diarrhea presence or absence in both species.

Statistical analysis

Descriptive statistics included the calculation frequency measures and prevalence for diarrhea for internal and external residence variables, the total number of dogs and cats in residence, presence of cysts and number of cysts per field observed in the laboratory, using *SPSS* software for windows version *15.0* (SPSS Inc. Chicago, III., USA).

Ethical issues

The ethical issues were respected according to the Diretrizes and Normas Regulamentadoras - Resolution # 196/96, through a Free Informed Consent Term and by the consent of the institution's Ethical Committee where this research was conducted.

RESULTS

The average age of the 300 elderly, owners of companion animals who participated in this study was 68.9 years with standard deviation (SD) of 6.9 and a median of 68 years, being the minimum age of 58 years and maximum of 90 years. The age group 70 years and more concentrated 43.3% of participants and there was a predominance of elderly females (67.7%). The animal of choice for company was preferentially the dog (71.7%), cats (12.0%) and in 16.3% both coexisted. Whether dogs or cats, the majority of owners (59.3%) had only one pet per household and 78.3% lived in urban areas (table 1).

Table 1 - Distribution of the elderly population and animals according to predetermined variables, Teresópolis, RJ, 2007-2008.

Variables		
Owners age	Number	%
60 to 64 years	102	34.0
65 to 69 years	68	22.7
70 to 74 years	64	21.3
> 75 years	66	22.0
Total	300	100.0
Owners sex	Number	%
Female	203	67.7
Male	97	32.3
Total	300	100.0
Domestic animal per residence	Number	%
Canine	215	71.7
Feline	36	12.0
Both	49	16.3
Permanence of the animal inside the residence	Number	%
Yes	172	57.3
No	128	42.7
Number of animals per residence	Number	%
1	178	59.3
2 to 4	103	34.3
5 or more	19	6.3
Origin of animal residence	Number	%
Urban	235	78.3
Rural	65	21.7
Total	300	100.0

We identified the presence of one or more oocysts in fecal material (positivity for *Cryptosporidium* spp.) In 29.0% (87) of these animals and 28.7% had about 2 to 10 oocysts per field. Prevalence of diarrhea among the 300 animals examined was 27% to 29.5% in dogs and 24.7% in cats, showing no statistically significant difference between species (table 2).

Tabel 2 - Animal distribution in data on diarrhea, presence of oocysts in laboratory tests and number of oocysts per field, Teresópolis, RJ, 2007-2008.

Variables		
Diarrhea in total examined animals	Number	%
Yes	81	27.0
No	219	73.0
Diarrhea in canines	Number	%
Yes	78	29.5
No	186	70.5
Diarrhea in felines	Number	%
Yes	64	75.3
No	21	24.7
Cryptosporidium spp. oocysts presence	Number	%
Yes	87	29.0
No	213	71.0
Number of *Cryptosporidium* spp. oocysts per field	Number	%
1	62	71.3
2	16	18.4
3	5	5.7
4 or more	4	4.6

The prevalence ratio of diarrhea in the animals researched for oocysts presence showed a strong association with statistical significance with P-value of 0.0077 (x^2 = 7.09), PR = 5.49 (CI 95%: 3,66-8,21) and when was stratified to oocysts number per field showed a dose response with increasing effect measures reaching a PR of 6.39 (CI 95%: 3,25-12,56) when the oocysts number was 4 or more per field. It was observed a protective effect with 27% reduction in the probability of diarrhea in animals when they lived inside the home. Considering as reference category (OR = 1) that only one companion animal per residence, no significant difference was found when compared with the prevalence of diarrhea in animals living until a total of 4 per residence (OR = 1.004), but the probability of diarrheic events was 2.6 times higher when living 5 or more animals (table 3).

Tabel 3 - Prevalence ratio of diarrhea in animals for oocysts presence, oocysts number per field, residence type, animals number per residence, Teresópolis, RJ, 2007-2008.

Variables	Diarrhea			PR	CI (95%)
	Yes N° (%)	No N° (%)	Total N° (%)		
Oocysts presence					
Yes	56 (64.37)	31 (35.63)	87 (100)	5.49	3.66 – 8.21
No	25 (11.74)	188 (88.26)	213 (100)		
Oocysts number per field					
1	38 (61,29)	24 (38,71)	62 (100)	5,22	3,43 – 7,94
2	11 (68,75)	5 (31,25)	16 (100)	5,86	3,57 – 9,61
3	4 (80)	1 (20)	5 (100)	6,82	3,84 – 12,10
4 or more	3 (75)	1 (25)	4 (100)	6,39	3,25 – 12,56
Residence type					
Inside	39 (22,67)	133 (77,33)	172 (100)	1.00	
Outside	42 (32.81)	86 (67.19)	128 (100)	0.73	0.48 – 1.10
Animals number per residence					
1	43 (24.15)	135 (75.85)	178 (100)	1.00	
2 to 4	26 (25.24)	77 (74.76)	103 (100)	1.04	0,68 – 1,59
5 or morte	12 (63.15)	7 (36.85)	19 (100)	2.61	1.69 – 4.03
				χ^2= 7.09; P = 0.0077	

PR = Prevalence ratio; CI = Confidence interval; x^2 = Chi square; P = P-value

DISCUSSION

The 300 elderly residents in Teresópolis who attended the national influenza vaccination campaign in 2007 and 2008 owned at least one domestic animal. T population sample studied was predominantly female, preferred dogs as companion animal and most of them kept the animals inside the residence.

The study performed in a population of dogs and cats (300) showed a high global prevalence of positivity for *Cryptosporidium* spp. (29.0%), higher than the one reported among other canine populations in the literature. Lallo and Bondan,[23] in a study conducted with dogs from a university hospital and two private kennels, reported a positivity rate of 8.85% when the light microscopy technique was used, and 9.5% with polymerase chain reaction (PCR) and a similar proportion (10.2%) was observed by Newman and colleagues[27] with an animal population in northwestern Brazil. An investigation conducted with 263 faeces samples collected from healthy dogs from the city of Lavras and Viçosa, Minas Gerais state, Brazil, found a prevalence of 1.85% for *C. parvum.*[22] Slightly higher proportion was reported by Gennari and colleagues, in São Paulo State,[28] analyzed 160 dogs' faeces samples with the presence od diarrhea , getting a global frequency of 2.83% of excreted oocysts, with no significant difference between the two groups. A study done in Zaragoza, Spain, in 81 dog, registered positivity of 7.4%,[29] while El-Ahraf and colleagues,[30] testing 200 dogs in San Bernadino, California, found 2%. Some studies conducted in Finland did not reveal oocysts excretion in adult dogs' faeces.[31-33]. A study conducted in São Paulo, Brazil, showed that oocysts in feces is shedding.[23]

Prevalence of *Cryptosporidium* spp. in the canine population and those with companion animals (dogs and cats) are sparse and the prevalence rates reported are very different; some factors may be pointed to justify these differences, especially the degree of environmental sanitation, the type of fecal collection and technical analysis.

The high prevalence of *Cryptosporidium* in Teresópolis region is consistent with the findings of Pereira and colleagues[26], who detected high contamination (100%) of this protozoan in vegetables consumed by the population in Teresópolis, a worrying data for water and sludge treatment, due to non-sanitation actions.

The elderly population, most of the time, is psychologically dependent on companion animals, including medical prescription. Infectious diseases are common causes of increased morbidity and mortality in elderly patients and show avery frequent problem in daily geriatrics practice.[34] Infections in elderly people are different from the ones presented by the young population, differences that can be due to immunological changes or organic malfunction (which decline with age).[16]

A positive for *Cryptosporidium* in animals living with elderly people aged of 70-74 and 75 or more (43.9%) was very high, setting a higher exposure probability for this in more susceptible population groups . A study conducted in Iran [20] with 400 individuals the group above 51 years who had history of diarrhea had a prevalence of 25% in positivity for *Cryptosporidium* spp.

It can also be noticed that the canine population for one animal per residence compared to cases of overcrowding (more than 5 animals), showed a lower diarrhea prevalence, probably as a result of minor contact with other animals (oral / faecal route) avoiding cross-contamination. It was also observed that animals that lived inside the houses had a protective prevalence rate, with reduced probability of presenting diarrhea, maybe due to the fact that the inner environment means less exposure to *Cryptosporidium* infection.

Domestic dogs and / or cats are sources of asymptomatic *Cryptosporidium* infection, housing them in their intestinal tract and feeding viable oocysts to the environment through their faeces.[22] The concern is that the high prevalence of cryptosporidiosis is only clinically manifested in diarrheal events in cases of weakness of the animal.[23] The prevalence of *Cryptosporidium* spp. increases with higher population density of companion animals per residence. This fact points to the need of further deepening of this research, since there are few studies that explore the cryptosporidiosis relationship between human population and animals. So it should improve the assessment of the level that this relationship conforms, that is, elderly companion animals, by the potential cycle of exposure of the elderly, thus justifying the attempt to know what happens in this particular group of population, here preliminarily studied.

CONCLUSION

Domestic companion animals provide valuable assistance to the physical and mental health of their owners. It was found that the close relationship to these animals, besides benefits, may bring risks to public health, exacerbated by the potential present of parasites in their animals. Therefore, owners need to be aware of the risks of human infection through faeces of infected dogs and thus have a greater concern with regular vermifugation of animals (deworming program), in order to provide better health conditions for animals and avoid the risk of transmission to the owners and for the general population.

We consider relevant the findings of this research, which are important to show the zoonotic relationship of *Cryptosporidium* spp. in fecal samples of companion animals of the the elderly populationbeing studied.

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