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Prevalence of Stray Dogs with Intestinal Protozoan Parasites

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Abstract: Problem statement: Intestinal protozoan parasites are important enteropathogens in dogs. Moreover, several canine intestinal protozoan parasites are zoonotic and are considered important to public health. This study investigates the level of intestinal protozoan parasites in stray dogs, in Kerman city, Iran. **Approach:** Determination of the prevalence of infections was based on faecal examination. Stool samples (n = 98) collected from dogs of different ages and gender were analyzed using five techniques, i.e., centrifugal flotation in sucrose solution, centrifugal flotation in 33% Zinc solphate solution, Ziehl-Neelsen staining, trichrome staining and iodine staining. **Results:** The overall prevalence of parasitism was 13 (13.26%) dogs. The parasites most frequently detected were: *Giardia* spp. (7.14%), *Isospora* spp. (5.1%) and *Cryptosporidium* spp. (4.08%). Single parasitic infection was present in 11 (11.22%) dogs. There was no significant difference in the prevalence between male (13.3%) and female (13.2%) dogs ($p>0.05$). There was significantly ($p<0.05$) greater prevalence of parasites in dogs less than 1 year old. **Conclusion/Recommendations:** The results of this research showed that stray dogs are reservoirs for zoonotic intestinal protozoan parasites and should be considered important to public health. So that, it is imperative for human to avoid faecal contamination in streets, public gardens and parks. Also stray dogs should be euthanized in dog population control program in Iran.

Key words: Intestinal protozoan parasites, *Cryptosporidium*, *Giardia*, *Isospora*, Stray dogs, Kerman

INTRODUCTION

Intestinal protozoan parasites are important enteropathogens in dogs. Moreover, several canine intestinal protozoan parasites are zoonotic and are considered important to public health, e.g., *Cryptosporidium*, *Giardia*, *Entamoeba* and *Isospora*. All these intestinal protozoan parasites have an oral-fecal transmission cycle and a major component for the spread of these parasites is the shedding of oocysts or cysts into the environment (Claerebout *et al*., 2009). It is common to observe intestinal protozoan parasites in canine of all ages, but the prevalence of infection is usually high in puppies, mainly due to the fact that certain modes of transmission are exclusive to the newly whelped or neonates and also, because young dogs have not yet acquired immunity to parasites (Ramirez-Barrios *et al*., 2004). The infective stages of protozoan parasites are cysts and oocysts passed in the faeces and are capable of prolonged survival in the environmental. Infection and re-infection of human, domestic animals or wildlife can occur when the cysts or oocysts are ingested via contamination water, food materials or through host to host (Leonhard *et al*., 2007). The clinical signs of protozoan infection are variable and occasionally some infected animals will be asymptomatic. However, severe clinical cases in young dogs will lead to diarrhea, anemia and death (Ramirez-Barrios *et al*., 2004). Previous studies have shown that intestinal protozoan parasites are common in dogs (Dubna *et al*., 2007; Mundim *et al*., 2007; Oliveira-Sequeira *et al*., 2002; Palmer *et al*., 2008; Papazahariadou *et al*., 2007; Ramirez-Barrios *et al*., 2004; Rimhanen-Finne *et al*., 2007). However, these studies were conducted in relatively limited geographic areas and resulted cannot necessarily be extrapolated to other regions. Understanding the epidemiology of the different parasites infections in a specific canine population is a useful tool for the veterinarian practitioner when he/she has to provide a clinical diagnostic (Ramirez-Barrios *et al*., 2004). Considering aspects related to public and animals' health, study of the prevalence of parasite infection in dogs should, therefore, be continuous task, with the most relevant aim being the establishment of control measures (Oliveira-Sequeira *et al*., 2002). The aim of our investigation was to determine the prevalence of protozoan infections in stray dogs in Kerman city, Iran.

MATERIALS AND METHODS

Samples collected: Free roaming stray dogs were randomly selected among the animals that were euthanized in dog population control program (according to OIE Guidelines), which was organized by municipality. A total of 98 stray dogs faecal specimens were collected randomly from Kerman city of Iran, in 2009, situated in the southern part of the national capital of Iran. The current population of Kerman is 2,652,413 (2006). The area is approximately 180726 km^2 (the second largest prefecture in Iran). About 700,000 people live in the prefecture capital of Kerman, with the remainder dispersed over other 15 cities. Each faecal sample consisted of approximately 5 g of fresh stool, collected from rectum of the stray dogs and was accompanied by information about the gender and age of dogs. Then, the samples were immediately processed in the parasitology diagnostic laboratory of the Veterinary School of Shahid Bahonar University of Kerman.

Parasitological procedure: Faecal specimens were concentrated by the formalin-ether sedimentation method. Faecal smears of the sediment (20 µL) were made and stained by the modified Ziehl-Neelson technique. The complete surface of the smear was examined for *Cryptosporidium* oocysts (Causape *et al*., 1996). Smear of the faeces was prepared and stained with trichrome and iodine Stain to detect cysts or trophozoites of *Giardia* and *Entamoeba* (Tanyuksel and Petri Jr, 2003). Also, samples were examined for the presence of *Giardia* cysts and trophozoites by centrifugal flotation in 33% Zinc solphate solution (Mundim *et al*., 2007). Additionally, faecal flotation in Sheathers sugar solution (500 g of sugar, 320 mL of water, 6.5 g of phenol), with a specific density of 1.3 g mL^{-1}, were examined by light microscopy for *Isospora* spp. (Lindsay *et al*., 1997).

Analysis of results: The data analysis was performed separately by grouping the animals by age (≤1 year and >1 year) and gender (male and female). In each case, the general prevalence for all intestinal protozoan parasites and the prevalence of each particular parasite were analyzed by using x^2 test and 17 version of SPSS software.

RESULTS

Intestinal protozoan parasites were detected in feces of 13 dogs (13.26%). Four samples (4.08%) contained *Cryptosporidium* spp. oocysts, 7 (7.14%) contained *Giardia* spp. cysts and 5 (5.1%) contained *Isospora* spp. oocysts (Table 1). *Entamoeba* spp. was not found in samples. Seven (13.2%) of the female dogs were eliminating cysts or oocysts of intestinal protozoan in their faeces, while six (13.3%) of the male dogs were infected. In this study, no statistical differences were found between infection by intestinal protozoan parasites and sex of the dogs ($p>0.05$). When the general prevalence analyzed by gender, no statistical differences in prevalence of *Cryptosporidium*, *Giardia* and *Isoapora* occurred between female dogs and male dogs ($p>0.05$). With respect to the age of the

Table 1: Prevalence of individual protozoan parasites in 98 dogs

Parasite	No. of infected dogs	Relative percentage[1]	Percentage of total dogs[2]
Cryptosporidium	4	30.77	4.80
Giardia	7	53.85	7.14
Isospora	5	38.46	5.10
Entamoeba	0	0.00	0.00
Total[3]	16		

[1]: Percentages were calculated as the number possessing an individual parasite divided by the total positive dogs (13); [2]: Percentages were calculated as the number possessing 1, 2 and 3 parasites species divided by the total dogs (98); [3]: This total is greater than 13 because of multiple parasitism

Table 2: Relationship of age and gender to prevalence of intestinal protozoan parasites in 98 dogs

	Gender				Age			
	Female		Male		1≥0		1<0	
Parasite	No. of infected dogs	Percentage[1]	No. of infected dogs	Percentage[1]	No. of infected dogs	Percentage[1]	No. of infected dog	Percentage[1]
Cryptosporidium	2	3.80	2	4.44	2	11.1	2	2.50
Giardia	4	7.54	3	6.70	2	11.1	5	6.25
Isospora	2	3.80	3	6.70	3	16.6*	2	2.50
Entamoeba	0	0.00	0	0.00	0	0.0	0	0.00
Total parasitized dogs[2]	7	13.20	6	13.33	5	27.8*	8	10.00
Total[3]	53		45		18		80	

[1]: Percentages were calculated by dividing the number of dogs possessing an individual parasite by the total number of individual in the group. These figures total greater than 100% because of multiple parasitism; [2]: The percentages were calculated by dividing the number of positive dogs by the total number on individual in the age and gender group; [3]: Total number of dogs in each age and gender group; *: $p< 0.05$

dogs, five (27.77%) of the puppies and eight (10%) of the adults were infected by intestinal protozoan parasites. Statistical differences were found between infection by intestinal protozoan parasites and age of the dogs ($p<0.05$). When the general prevalence analyzed by age, statistical differences in prevalence of *Isoapora* occurred between dogs ≤1 years old and 1<dogs ($p<0.05$), but no statistical differences in prevalence of *Cryptosporidium* and *Giardia* occurred between this two groups ($p>0.05$), (Table 2).

DISCUSSION

There have many studies of the general prevalence of intestinal protozoan parasites in dogs population worldwide (Dubna *et al*., 2007; Little *et al*., 2009; Mundim *et al*., 2007; Oliveira-Sequeira *et al*., 2002; Palmer *et al*., 2008; Papazahariadou *et al*., 2007; Ramirez-Barrios *et al*., 2004; Rimhanen-Finne *et al*., 2007). Prevalence is variable and depended on a number of factors including age, living conditions, diagnostic methodology employed and region studied (Mundim *et al*., 2007). In the present study, the overall prevalence of intestinal protozoan parasites in dogs was 13.26%. The overall prevalence of *Giardia* infection (7.14%) identified in this study is approximately similar to that previously reported in Australia (9.4%) (Palmer *et al*., 2008), Greece (4.3%) (Papazahariadou *et al*., 2007), Finland (5%) (Rimhanen-Finne *et al*., 2007), United States (4%) (Little *et al*., 2009). Although the overall prevalence of this parasite has been reported in Brazil (12.2%) (Oliveira-Sequeira *et al*., 2002). Also Papini *et al*. (2005) and Szenasi *et al*. (2007) found high prevalence of infection of 55.2 and 58.8% in kenneled dogs respectively. It may be attributed to climate conditions. In this study, no statistical differences between infection by *Giardia* spp. and sex of the dogs were found. This confirms the finding of Kirkpatrick (1988); Bugg *et al*. (1999) and Huber *et al*. (2005). No statistical bias for *Giardia* infection due to age of dogs was found in the present study. This confirms the finding of Huber *et al*. (2005). The presence of *Giardia* spp. cysts was statistically associated with the presence of other intestinal protozoan. In the present study, *Giardia* spp. was mainly associated with *Isospora* spp. (3 cases) and this is in agreement with Oliveira-Sequeira *et al*. (2002), who found a greater prevalence of *Giardia* spp. associated with *Isospora* spp. than with other parasites.

With respect to *Cryptosporidium* spp., the infection rate of 4.08% is approximately in agreement with Papazahariadou *et al*. (2007), who found 2.8% of faecal samples from dogs collected in the Serres Prefecture, Northern Greece to contain oocysts of *Cryptosporidium* spp. Epidemiological studies on the prevalence of *Cryptosporidium* in dogs showed the infection rates are variable according to geographic area and range from 1.4% in Czech (Dubna *et al*., 2007), 2.41% in Brazil (Huber *et al*., 2005), 1.4% Uberlandia (Mundim *et al*., 2007), 2% in California (El-Ahraf *et al*., 1991). The likelihood of finding a source of oocyst could explain differences in prevalence between different areas. Other researchers suggested that prevalence may be highest in dogs from rural environments, since Cryptosporidiosis is primarily associated with farm livestock (Causape *et al*., 1996). Grimason *et al*. (1993) and coworker found 1% of the faecal specimen collected in seven public parks in Scotland contained *Cryptosporidium* oocysts, with a prevalence in individual parks ranging from 0-2.4%. In this study, no statistical differences between infection with *Cryptosporidium* spp. and sex of the dogs were found. This confirms the finding of Huber *et al*. (2005). No statistical differences between infection with *Cryptosporidium* spp. and age of the dogs were found. Also, Causape *et al*. (1996) found no statistically differences in prevalence occurred between dogs under 1 year of age and dogs over 1 year old.

Another very common parasite found in the evaluated dogs was *Isospora* spp. (5.1%), which shows that these coccidia are the main intestinal protozoa found in these pets, mostly in younger animals, as indicated by Ramirez-Barrios *et al*. (2004); Visco *et al*. (1977) and Vanparijs *et al*. (1991). In the presence study, we found statistical differences between infection by *Isospora* and age of the dogs ($p<0.05$). *Isospora* spp. was the most common enteric protozoan of stray dogs in our study (5.1%), that similar results were obtained by Vanparijs *et al*. (1991), who observed *Isospora* spp. prevalence of 5.2% in dogs in Belgium. Although, prevalence is lower than in previous surveys in Venezuela by Ramirez-Barrios *et al*. (2004), in Zaragoza, Spain by Causape *et al*. (1996) and in Sao Paulo State, Brazil by Oliveira-Sequeira *et al*. (2002), who found 8.1, 9.9 and 8.5% prevalence, respectively.

Frequency of intestinal protozoan parasites in the studied stray dogs was high. *Giardia* spp. and *Isospora* spp. were the most frequent parasites.

CONCLUSION

The results of this research showed that stray dogs are reservoirs for zoonotic intestinal protozoan parasites and should be considered important to public health. Stray dogs may have an important role in the transmission of some diseases and understanding the pathogenicity and epidemiology of potential zoonotic

agents in this and other animals closely associated with human is fundamental to public health. So that, it is imperative for human to avoid faecal contamination in streets, public gardens and parks. Also stray dogs should be euthanized in dog population control program in Iran.

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