Original article

Antigenic detection of *Giardia duodenalis* in companion dogs of Ahvaz area, south-west of Iran

Bahman Mosallanejad*[1], Reza Avizeh[1], Mohammad Hossein Razi Jalali[2], Ali Reza Alborzi[2]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

How to cite this article:
Mosallanejad B, Avizeh R, Razi Jalali MH, Alborzi AR. Antigenic detection of *Giardia duodenalis* in companion dogs of Ahvaz area, south-west of Iran. Jundishapur J Microbiol. 2010; 3(4): 187-93.

Received: May 2010 **Accepted:** June 2010

Abstract

Introduction and objective: *Giardia duodenalis* is a ubiquitous protozoan parasite in several animal species and humans. The objective of the present survey was to investigate the prevalence of *G. duodenalis* in the fecal samples of companion dogs in Ahvaz area, south-western Iran.

Materials and methods: A total of 150 companion dogs of different ages were examined for antigenic detection of *G. duodenalis* in fecal samples by a commercial *Giardia* Antigen Test Kit. Fecal centrifugation-flotation technique was also used for identification of cyst by microscopic examination. The studied dogs were selected from those referring to Veterinary Hospital of Chamran University Ahvaz from June 2007 to January 2010. They were divided into two groups clinically (diarrheic and non-diarrheic) and based on age into three groups (<6 months, 6 months-3 years and >3 years).

Results: Six out of 150 fecal samples (4%) were positive for antigen of *G. duodenalis* by immunochromatography assay. Prevalence was significantly higher in young dogs less than 6 months (11.6%) compared with adult dogs 6 months-3 years (1.6%) (P=0.041). The infection was more common in diarrheic dogs (18.5%) compared with non-diarrheic dogs (0.8%) and the difference was significant (P=0.001). Microscopic examination on fecal samples showed that 2.7% (4 out of 150) of the studied dogs were positive.

Conclusion: The infection rate of giardiasis in companion dogs' particularly in diarrheic dogs is considered to be critical from the viewpoint of public health. Our results indicate that this parasite is a zoonotic infection in Ahvaz district.

Keywords: *Giardia duodenalis*, Immunochromatography assay, Dog, Ahvaz

Address for correspondence:
Bahman Mosallanejad, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; Tel: +989177911452; Fax: +98611 3360807; Email: bmosallanejad@scu.ac.ir

Introduction

Giardia duodenalis (Synonyms: *G. intestinalis*, *G. lamblia*) is a protozoan parasite found in the intestinal tract of humans, domestic and wild animals throughout the world. *Giardia* has two morphologic forms (trophozoite and cyst). Transmission is fecal-oral route by ingestion of feces or fecal-contaminated water, food or fomites. Most infections are subclinical or show only transient softening of the stool early in the infection, although diarrhea may be acute and short-lived, chronic, or intermittent in dogs and cats [1,2,3]. The prevalence ranges 5 to 80% according to the age of dogs, and colony size [3,4].

The diagnosis of *Giardia* infection traditionally depends on microscopic identification of trophozoites or cysts in feces of infected animals. Although flotation is a reference method for the detection of *Giardia* cysts, it is suggested that an alternative test is also needed because microscopic examination is time consuming and needs an experienced microscopist. Many artifacts (e.g., grass pollen, yeast) mimic to varying degrees the morphology of *Giardia* cysts, and care must be exercised in differentiating these from *Giardia* [3,5].

A commercially available direct immunofluorescence assay can be used to facilitate the diagnosis of *Giardia*; however, this test requires a fluorescent microscope for detection of the cysts. Several laboratory methods have been developed to detect antigen in the feces of infected dogs such as PCR, ELISA and molecular techniques. Though these tests are more sensitive, specific and more reproducible, they are expensive and generally take time to be analyzed by a specialized laboratory. Recently, a commercial *Giardia* Antigen Test Kit was released for detection of *G. duodenalis* antigen in canine feces. This test is a rapid enzyme immunoassay that can be conducted on fresh feces, previously frozen feces, or feces stored at 2°C to 7°C for up to 7 days. Immunochromatography assay is one of the most common rapid field diagnostic methods used in clinical practice.

Sensitivity and specificity for kits of *Giardia* Ag Test were 95.6% and 100%, respectively [6]. In Iran, regarding to the cultural and religious believes, having pet is less common. However, lack of consideration to the health of these animals can decrease the health of humans. Nevertheless large numbers of dogs are found roaming residential streets and increasing the risk of public health for other animals and humans.

Some studies carried out in Iran showed a low relatively prevalence of *Giardia* infection in dogs. For example, a prevalence study of *Giardia* in Isfahan showed 3.33% (4 out of 120) in stray dogs [7]. In another study in Shiraz, 0.68% of stray dogs (1 out of 147) were positive for *Giardia* [8]. Since there is not any information about this parasite in Ahvaz, south-western Iran, the objective of the present survey was to investigate the prevalence of *G. duodenalis* in the fecal samples of companion dogs in this area.

Materials and methods

Study area and sample population

This study was performed in Ahvaz area, south-west of Iran that is located at an elevation of 12 meters above sea level and the climate is warm-humid. In the present study, a total of 150 companion dogs of different ages were examined for fecal antigens of *G. duodenalis* by immunochromatography assay to detect cyst or trophozoite in feces by microscopic examination (flotation method). The dogs used in this study were referred cases to

Veterinary Hospital of Chamran University of Ahvaz from June 2007 to January 2010. Most of the dogs were apparently healthy and had referred for other reasons mostly for vaccination. Three stool samples were collected from each animal at 48h intervals, producing a total of 450 stool samples. Samples were stored in an ice chest and transported to the Parasitology Laboratory of Veterinary Faculty to be processed.

Information about companion dogs was taken from their owners. The studied dogs were divided into two groups (diarrheic and non-diarrheic) and based on age into three groups (group 1: <6 months, group 2: 6 months-3 years and group 3: >3 years). Classification was also made by sex, breed and season. Breeds of the studied dogs were German Shepherd-Doberman Pincher-Terrier- Pekingense- Spitz and Mixed. All data were entered and recorded in a computerized database. Age was estimated by dental formulary and owner information's.

Laboratory methods

Two methods were employed in order to detect *Giardia*: Fecal centrifugation-flotation technique and immunochromatogr-aphy assay.

Fecal centrifugation-flotation technique

Fecal samples (1g) of 150 dogs (three times with 48h interval) were examined macroscopically for the presence of *G. duodenalis* cysts by sucrose gradient centrifugal flotation in 33% zinc sulphate solution (specific density 1.27). It was filtered through gauze, and centrifuged in a 15ml tube at 400g for 10min. A drop of the float from the meniscus was examined microscopically at 400x magnification for the presence of *G. duodenalis* cyst [9]. As trophozoites will not be detected by floatation techniques because, the floatation solution lyses the trophozoites, so direct fecal smears were carried out for demonstration of trophozoites. Multiple smears over time were done because of the intermittent nature of *Giardia* shedding.

Immunochromatography assay and interpretation of the test

We added a volume of one full spoon (5g) of fecal sample into the buffer diluent. Then the vial was closed and shaken for homogenization. We opened the lid of the sample tube for identification and took out the sample spoon that was provided with the tube. The strip stand was left one minute in the solution. Then the strip was removed and placed on a flat and horizontal surface for running.

Rapid detection of soluble *G. duodenalis* cyst antigens (BVT Co., Ltd, Lion) is a qualitative test. The result is read at five minutes of migration. A positive result indicates the presence of *Giardia* cysts in the feces. One blue and one colored line are positive. One blue colored line is negative (Fig. 1). If the animal does not show any clinical symptoms at the time of sampling, but it is infected by *Giardia*, this animal should be considered as a healthy carrier, and thus is a source of contamination in group housing situation. Speed *Giardia* test helps us detecting the cyst presence for a concentration higher than 80 cysts per gram of feces [9].

Fig. 1: Positive (above) and negative (below) sample of rapid *Giardia* Ag test

Statistical analysis

Dogs were grouped by age, sex, breed, season, and diarrheic or non-diarrheic. To determine whether these factors were associated with *G. duodenalis* infection, we used Chi-squared analysis, Fisher's exact test and Z test. Statistical comparisons were carried out using SPSS 16.0 statistical software. Differences were considered significant when $P< 0.05$.

Results

Six out of 150 fecal samples (4%) were positive for antigen of *G. duodenalis* by immunochromatography assay. Prevalence was significantly higher in young dogs less than 6 months (11.6%; 5(3 male, 2 female) out of 43) compared with adult dogs group 6 months- 3 years (1.6%; 1(male) out of 62). All dogs above 3 years were negative (0.0%; 0 out of 45). The infection was more common in diarrheic dogs (18.5%; 5 of 27) compared with non-diarrheic dogs (0.8%; 1 of 123). Prevalence was higher in male dogs (4.8%; 4 out of 83) than females (2.98%; 2 out of 67) and in the summer (5.3%; 2 out of 38), was higher than other seasons. Prevalence rate in winter, spring and autumn was 3.2% (1 out of 31), 4.2% (2 out of 47) and 2.9% (1 out of 34), respectively. Microscopy examination showed that 2.7% (4 out of 150) of fecal samples were positive. All of the affected dogs had access to open environment.

Discussion

The present study that is the first report on prevalence of *G. duodenalis* in companion dogs in Ahvaz district revealed that the overall prevalence of the infection was 4% and 2.7% by using immunochromatography assay and fecal centrifugation-flotation technique, respectively. Our data indicated that young dogs less than 6 months were significantly more susceptible to *Giardia* infections compared with adult dogs ($P=0.041$).

Our results were similar to those described by Kirkpatrick [10], le Nobel *et al*. [11], Itoh *et al.* [12], Pullola *et al.* [13], Mundim *et al.* [14], Martinez-Carrasco *et al.* [15], and Palmer *et al.* [16]. It means that young dogs are at the risk of exposure to infection, shedding cysts and so have potential to transmit the infection to people. This may suggest that specific immunity to the parasite develops with age, probably as a consequence of one or more exposures. Puppy behavior, particularly the habit of biting and licking objects which could be contaminated with *Giardia* cysts may also be a significant contributing factor.

The results of the present study appeared that diarrhea is an important sign of infection in dogs, because the prevalence of infection was 18.5% in diarrheic dogs, compared with non-diarrheic dogs (0.8%). There was evidence of a statistically

significant association between the presence of *Giardia* and diarrhea (P=0.001). Generally, a centrifugation-flotation technique is regarded as more sensitive and accurate than flotation alone to detect protozoan cysts [17].

In a study, only six of 27 participants could identify *Giardia* cysts using flotation techniques on a known positive sample [18]. For the detection of *Giardia* in dog samples, immunochromatography is more sensitive than centrifugation-flotation [6]. For these reasons, feces of dogs were examined by the immunochromatography technique in the present survey. It is shown that the estimated amount of *Giardia* antigens can range above 80 cysts in one gram feces in positive samples by immunochromatography assay. As a consequence, Rapid *Giardia* test kit appears to be sufficiently sensitive to detect cases of giardiasis when fairly high levels of antigen are shed.

Surveys in many countries have shown that infection with *Giardia* is common and widespread in crowded and open environments, such as kennels and shelters [3,19-23]. An association was shown between giardiasis and contact with farm animals and pets [24]. In our study, all of the affected dogs had access to open environment.

In our survey, a higher prevalence was seen in male companion dogs than females. It can be explained by the habitat associated with males, as they have a wider area of operation than females, though the difference was not significant between different sexes (P>0.05). Our results were in agreement with findings of Kirkpatrick [10] and Huber *et al.* [25], although Coggins [21] found a higher prevalence among females. As a general, it doesn't seem sex to be a determining factor of infection as other studies have concluded.

Regarding seasonal variation in the prevalence of *Giardia*, seasonal effects on the infection rate may reflect climatic changes, which affect the parasite itself, as well as changes in the photoperiod which may, in turn, influence the host's physiology [18]. In the present study, there was not significant difference with regard to season changes (P>0.05). The prevalence of Giardiasis has been studied in some different areas of Iran. Compared to former surveys conducted in Iran, the infection rate in our study (4%) is slightly higher than other studies done by Razmi [26], (1.1%; 2 out of 174), Jafari Shoorijeh *et al.* [8] (0.68%; 1 out of 147) and Shirani *et al*. [7], (3.33%; 4 out of 120 pet dogs of Isfahan). There is no obvious explanation for these differences. These may be due to geographical variation or to differences in the number of animals and type of population surveyed, or may be attributed to different sensitivity of the diagnostic procedure used.

There are many studies of the general prevalence of giardiasis in dog populations worldwide. Results of prevalence have been recorded 4.5-39% in the United States [21], 18.1-39.1% in the Czech Republic [23], 5.3-37% in Western Australia [22], 6.7-59.3% in Japan [12], 14.4% in Yugoslavia [27], 1.9% in Germany [28], 19.04% and 19.6% in Italy [18,29], 20.7% in Norway [30].

Excretion of *G. duodenalis* cysts is intermittent in symptomatic and asymptomatic animals [25], so one negative fecal exam may not necessarily mean that the animal is not infected by this protozoan. Diagnosis can be improved by repeating examinations whenever possible. Mundim *et al.* [14] found that examination of three samples from the same animal increased the likelihood of positive results. In the present study, three samples were collected from each animal to increase accuracy of the

sampling from the point of statistical analysis.

There were two samples that were positive on the immunochromatography test, but negative by microscopy examination. These results show that sensitivity of immunochromatography is higher and more accurate. Due to close contact of dogs with human and this fact that children play outdoors on the soil, dogs can be an important potential source of transmission of zoonotic parasite such as *Giardia* and have an important role in contamination of environment to cyst or trophozoite.

Conclusion

Our results indicated that *Giardia* is a zoonotic infection in Ahvaz district, south-west of Iran. It suggests that climatic condition in this area (warm and humid) is relatively suitable for the spread and survival of the cysts. The genetics of parasites in genus *Giardia* is still poorly understood. Our results will be the basis of further studies that will permit to deepen our knowledge of the epidemiology of giardiasis. Further studies in various areas will be necessary to survey the overall epidemiological status of giardiasis in companion and stray dog populations.

Acknowledgements

We would like to greatly appreciate Research Council of Shahid Chamran University of Ahvaz for the financial support (Grant no 70170).

References

1) Adam RD. Biology of *Giardia Lamblia*. *Clin Microbiol Rev*. 2001; 14(3): 447-75.
2) Ponce-Macotela M, Peralta-Abarca GE, Martínez-Gordillo MN. *Giardia intestinalis* and other zoonotic parasites: prevalence in adult dogs from the southern part of Mexico City. *Vet Parasitol*. 2005; 131(1-2): 1- 4.
3) Barr SC. Enteric protozoal infections: Giardiasis. In: Greene CE, (ed), *Infectious diseases of the dog and cat*. 3rd ed, Philadelphia, WB Saunders, 2006; 736-52.
4) Nolan TJ, Smith G. Time series analysis of the prevalence of endoparasitic infections in cats and dogs presented to a veterinary teaching hospital. *Vet Parasitol*. 1995; 59(2): 87-96.
5) Naoyuki I, Kazutaka K, Yasutomo H, Fumio H, Seiichi H. Prevalence of *Giardia intestinalis* and other zoonotic intestinal parasites in private household dogs of the Hachinohe area in Aomori prefecture, Japan in 1997, 2002 and 2007. *J Vet Sci*. 2009; 10(4): 305-8.
6) Geurden T, Berkvens D, Casaert S, Vercruysse J, Claerebout E, Bayesian A. Evaluation of three diagnostic assays for the detection of *Giardia duodenalis* in symptomatic and asymptomatic dogs. *Vet Parasitol*. 2008; 157(1-2): 14-20.
7) Shirani D, Khalili MR, Meshgi B. The prevalence of *Giardia* sp. among the pet dogs of Isfahan. *J Vet Res*. 2006; 61(2): 161-3.
8) Jafari Shoorijeh S, Sadjjadi SM, Asheri A, Eraghi K. *Giardia* spp. and *Sarcocystis* spp. status in pet dogs of Shiraz, southern part of Iran. *Trop Biomed*. 2008; 25(2): 154-9.
9) Dryden MW, Payne PA, Smith V. Accurate diagnosis of *Giardia* spp. and proper fecal examination procedures. *Vet Ther*. 2006; 7(1): 4-14.
10) Kirckpatrick CE. Epizootiology of endoparasitic infections in pet dogs and cats presented to a veterinary teaching hospital. *Vet Parasitol*. 1988; 30(2): 113-24.
11) le Nobel WE, Robben SR, Dopfer D, Hendrikx WM, *et al.* Infections with endoparasites in dogs in Dutch animal shelters. *Tijdschr Diergeneesk*. 2004; 129(2): 40-4.
12) Itoh N, Muraoka N, Saeki H, Aoki M, Itagaki T. Prevalence of *Giardia intestinalis* infection in dogs of breeding kennels in Japan. *J Vet Med Sci*. 2005; 67(7): 717-8.

13) Pullola T, Vierimaa J, Saari S, Virtala AM, Nikander S, Sukura A. Canine intestinal helminths in Finland: prevalence, risk factors and endoparasite control practices. *Vet Parasitol*. 2006; 140(3-4): 321-6.
14) Mundim MJ, Rosa LA, Hortencio SM, Faria ES, Rodrigues RM, Cury MC. Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in dogs from different living conditions in Uberlândia, Brazil. *Vet Parasitol*. 2007; 144(3-4): 356-9.
15) Martinez-Carrasco E, Berriatua M, Garijo J, Martinez D, Ruiz Y. Epidemiological study of non-systemic parasitism in dogs in southeast Mediterranean Spain assesses by coprological and post-mortem examination. *Zoonoses Public Health*. 2007; 54: 195-203.
16) Palmer CS, Thompson RCA, Traub RJ, Rees R, Robertson ID. National study of the gastrointestinal parasites of dogs and cats in Australia. *Vet Parasitol*. 2008; 151(2-4): 181-90.
17) Payne PA, Ridley RK, Dryden MW, Bathgate G, Miliken GA, Stewart PW. Efficacy of a combination febantel-praziqauntel-pyrantel product, with or without vaccination with a commercial *Giardia* vaccine, for treatment of dogs with naturally occurring giardasis. *J Am Vet Med Assoc*. 2002; 220(3): 330-3.
18) Bianciardi P, Papini R, Giuliani G, Cardini G. Prevalence of *Giardia* antigen in stool samples from dogs and cats. *Vet Med Rev*. 2004; 155(8-9): 417-21.
19) Swan JM, Thompson RCA. The prevalence of *Giardia* in dogs and cats in Perth, Western Australia. *Aust Vet J*. 1986; 63(4): 110-12.
20) Hahn NE, Glaser CA, Hird DW, Hirsh DC. Prevalence of *Giardia* in the feces of pups. *J Am Vet Med Assoc*. 1988; 192(10): 1428-29.
21) Coggins JR. Effect of season, sex and age on prevalence of parasitism in dogs from southeastern Wisconsin. *J Helminthol Soc Wash*. 1998; 65: 219-24.
22) Bugg RJ, Robertson ID, Elliot AD, Thompson RCA. Gastrointestinal parasites of urban dogs in Perth, Western Australia. *Vet J*. 1999; 157(3): 295-301.
23) Svobodova V. Parasitic infections in an animal shelter. *Acta Vet Brno*. 2003; 72: 415-20.
24) Warburton AR, Jones PH, Bruce J. Zoonotic transmission of giardiasis: a case control study. *Commun Dis Rep*. 1994; 4(3): 32-6.
25) Huber F, Bomfim TCB, Gomes RS. 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. *Vet Parasitol*. 2005; 130: 69-72.
26) Razmi GhR. Survey of dogs' parasites in Khorasan Razavi province, Iran. *Iran J Parasitol*. 2009; 4(4): 48-54.
27) Nikolic A, Dimitijevic S, Djurkovic DO, Bobic B, Maksimonic O. Giardiasis in dogs and cats in the Belgrade area. *Acta Vet Beograd*. 2002; 52(1): 43-7.
28) Solarczyk P, Majewska AC. A survey of the prevalence and genotypes of *Giardia duodenalis* infecting household and sheltered dogs. *Parasitol Res*. 2010; 106(5): 1015-19.
29) Rinaldi L, Maurelli MP, Musella V, *et al.* *Giardia* and *Cryptosporidium* in canine faecal samples contaminating an urban area. *Res Vet Sci*. 2008; 84(3): 413-15.
30) Hamnes IS, Gjerde BK, Robertson LJ. A longitudinal study on the occurrence of *Cryptosporidium* and *Giardia* in dogs during their first year of life. *Acta Vet Scand*. 2007; 11(49): 22.