

Short Paper

Antigenic detection of *Cryptosporidium parvum* in urban and rural dogs in Ahvaz district, southwestern Iran

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Summary

Cryptosporidium parvum is a zoonotic protozoan parasite with a wide range of vertebrate hosts. The present study was conducted to determine the prevalence of *Cryptosporidium parvum* in urban and rural dogs of the Ahvaz area. Faecal samples were collected randomly from 93 dogs between May 2005 and September 2007. The studied dogs were divided into two groups (urban and rural) and based on age into three groups (<6 months, 6 months–3 years and >3 years). The results were analyzed by using Chi-square analysis and Fischer's exact test. Prevalence to *Cryptosporidium parvum* antigens was 4.3% (4 of 93) by means of ELISA, indicating that this antigen is present in the ecosystem. The infection was more prevalent in rural dogs (6.4%; 3 of 47) in comparison with urban dogs (2.17%; 1 of 46), nevertheless, there were no significant differences between the different groups ($P>0.05$), but the infection was more prevalent in diarrheic dogs (17.65%; 3 of 17) compared with non-diarrheic dogs (1.3%; 1 of 76), and the difference was significant ($P=0.019$). Infection was not significant in the different age groups ($P>0.05$). Concurrent detection of *Cryptosporidium parvum* with *canine distemper* (one sample) and *parvovirus* (one sample) were shown in the studied dogs. Modified Ziehl-Neelsen staining was also carried out and the prevalence of infection was 2.15% (2 of 93). The use of ELISA allowed the detection of more positive cases than light microscopy. This study showed that *Cryptosporidium parvum* can be a risk factor, particularly for those dogs in contact together in the population of urban and rural dogs.

Key words: *Cryptosporidium parvum*, ELISA, Dog, Ahvaz, Iran

Introduction

Parasites belonging to the genus *Cryptosporidium* are an important and widespread cause of enteric disease in over 170 different host species, as *Cryptosporidium parvum* causes the majority of mammalian infections. In the immunocompetent host, infection is self-limiting, with possible mortality in young animals. It may result in chronic debilitating diarrhea with dehydration, malabsorption, wasting, and death (Dubey and Greene, 2006; Katagiri and Oliveira-Sequeira, 2008). Dogs play an important role as an infection source of human cryptosporidiosis. The first

evidence of cryptosporidiosis in dogs was reported by Tzipori and Campbell (1981). Wilson *et al.* (1983) reported the first clinical case of canine cryptosporidiosis in a 1-week-old pup 2 years later. Case-control studies conducted in the United States have suggested only a weak association between the occurrence of cryptosporidiosis in human immunodeficiency virus positive persons and contact with dogs. In contrast, contact with dogs or cats is not a risk factor for cryptosporidiosis in England and is actually a protective factor in Australia (Tilley and Smith, 2000; Palmer *et al.*, 2008). Ranges for the prevalence of faecal oocysts in various populations have been 0-

44.8% in dogs (Dubey and Greene, 2006).

The diagnosis of the etiological agent can only be performed in the laboratory because clinical signs do not allow differentiating between the different microorganisms. It is possible to identify *Cryptosporidium parvum* by means of floating or staining techniques (Modified Ziehl Neelsen). Although acid-fast staining is a reference method for the detection of *C. parvum* oocysts, it is suggested that an alternative test is also needed because microscopic examination is time consuming and user-dependent, requires an experienced microscopist, and detecting oocysts in subclinical infections is difficult. These classical techniques can be replaced by the ELISA, PCR and molecular technology that are sensitive, specific and reproducible, and can be carried out in specialized laboratories (Thomaz *et al.*, 2007; Lupo *et al.*, 2008). The present study was undertaken to investigate the prevalence of *Cryptosporidium parvum* infection in dogs in the Ahvaz district, southwestern Iran. This survey, which is the first report about the prevalence of *C. parvum*, provides preliminary information about cryptosporidiosis in the Ahvaz district, southwestern Iran. In this study, ELISA technique and Modified Ziel-Neelsen (MZN) were used to investigate the presence of *C. parvum* antigen in the faeces of urban and rural dogs in this area.

Materials and Methods

Study area and sample population

This study was conducted in Ahvaz city, southwestern Iran, located at an elevation of 12 meters above sea level, having a hot, humid climate. A total of 93 (47 rural and 46 urban dogs) faecal specimens were obtained randomly from urban and rural dogs in the Ahvaz district between May 2005 and September 2007. The urban dogs (companion) were selected from referred dogs to the Veterinary Hospital of Ahvaz University. The rural dogs were randomly selected from villages of different regions of Ahvaz (Gamboeh, krishan, Large Jasanieh, Khobineh, Mashroohat and Biooz) (7-8 samples each village). Faecal samples were placed on ice in a nonbreakable container.

The studied dogs were divided into two groups (urban and rural) and based on age into three groups (<6 months, 6 months–3 years and >3 years). Samples were obtained either directly from the rectum or from the ground immediately after defecation. The animals' identification, age, sex, breed, health status, the date of collection, the name of the village and faecal consistency were recorded. Age was estimated by dental formulary. All data were entered and stored in a computerized database.

Laboratory methods

ELISA Test Kit (code L11113 Fomat) was used to investigate the presence of *C. parvum* antigen in the faecal samples. The sensitivity and specificity of the ELISA kit were above 98% for the detection of *Cryptosporidium parvum*. The test used 96-well microtitration plates sensitized by specific antibodies for an antigenic determinant of *Cryptosporidium parvum*. Briefly, approximately 1 g faecal samples were diluted in buffer. The diluted samples were added to the wells of the microplate, which was coated with a specific antibody of *C. parvum*. The plate was incubated at room temperature for 1 h and rinsed with the washing solution. The diluted conjugate was then dropped into each well, and incubated at room temperature for 1 h. The plate was rinsed with the washing solution again. Then the indicator solution [chromogen (tetramethylbenzidine) + substrate (hydrogen peroxide)] was dropped into each well. The plate was incubated for 10 min at room temperature. The stop solution was added to each microwell, and the results read using a plate reader with a 450 nm filter. The samples that yielded a difference in optical density greater than or equal to 0.150 were considered positive, and those with an optical density less than 0.150 were considered negative.

The microscopic examination was performed with Modified Ziehl-Neelsen staining also. For preparation, thin faecal smears were dried at room temperature and fixed in methanol for 2 min, then flooded with basic fuchsin solution for 5 min and rinsed in 50% ethanol. Following washing, the slides were pooled in methylene blue solution for 1 min, washed and air dried, and

examined under a 100 X objective. In each positive slide, the size of at least ten *Cryptosporidium* oocyst (4-5 μ m) was measured by micrometric eyepiece. If no oocysts were seen the sample was recorded as negative (Anon., 1991).

Statistical analysis

Dogs were grouped by age, sex, breed and geographic area (urban and rural) to determine whether these factors were associated with *Cryptosporidium* infection, using fisher's exact test and Chi-square analysis. Statistical comparisons were carried out using SPSS 16.0 statistical software. Differences were considered significant when $P < 0.05$.

Results

Prevalence to *Cryptosporidium parvum* antigens in these dogs was 4.3% (4 of 93) by means of ELISA Test Kit, indicating that this antigen is present in the ecosystem. The infection had greater prevalence in rural dogs (6.4%; 3 of 47) in comparison with urban dogs (2.17%; 1 of 46), nevertheless, there was no significant difference between the different groups ($P > 0.05$), but the infection was more prevalent in diarrheic dogs (17.65%; 3 of 17) compared with non-diarrheic dogs (1.3%; 1 of 76) (by ELISA technique) and the difference was significant ($P = 0.019$). Infection was not significant in the different age groups. The age of the affected dogs to cryptosporidiosis ranged from 2.5 months to 7 years. In relation to sex, 5.6% (3 of 54) of males and 2.6% (1 of 39) of females carried the infection. Although the prevalence of infection was more significant in the males compared with the females, the difference was not significant ($P > 0.05$). Concurrent detection of *Cryptosporidium parvum* with *canine distemper* (one sample) and *parvovirus* (one sample) were shown in the studied dogs. Prevalence of infection was obtained 2.15% (2 of 93) by Modified Ziehl-Neelsen staining also. Ziehl-Neelsen positive specimens were also positive with ELISA. Two specimens (2.15%) that were negative with staining, were positive with ELISA. Results are summarized in Tables 1 and 2.

Table 1: Prevalence of *Cryptosporidium parvum* infection in urban and rural dogs of different age and sex in Ahvaz district, Iran by ELISA Kit, 2005-2007

Sex	Age					
	<6 months		6 months-3 years		>3 years	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Male	16	1	18	1	17	1
Female	13	0	13	1	12	0
Total = 93	29	1	31	2	29	1

Table 2: Prevalence of *Cryptosporidium parvum* infection in urban and rural dogs of different age and sex in Ahvaz district, Iran by Modified Ziehl-Neelsen staining, 2005-2007

Sex	Age					
	<6 months		6 months-3 years		>3 years	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Male	16	1	18	1	18	0
Female	13	0	14	0	12	0
Total = 93	29	1	32	1	30	0

Discussion

Our study revealed that prevalence to *Cryptosporidium parvum* antigens was 4.3% by means of ELISA in the Ahvaz district, southwest Iran. The low prevalence of *Cryptosporidium parvum* (2.17%) suggests a limited risk from urban dogs in the Ahvaz district as a source of environmental contamination, but the higher prevalence of infection in rural dogs (6.4%) indicates that this protozoan parasite is a potentially greater environmental concern. The use of ELISA allowed the detection of more positive cases (4.3%) than light microscopy (2.15%). In this study, the observation of the low numbers of oocysts by microscopical examination may indicate that the intensity of the infection is slight; nevertheless, environmental contamination with small numbers of infective oocysts can be dangerous for public health (Dubey and Greene, 2006).

The reported results confirm that the prevalence of *Cryptosporidium* infection in dogs is different not only between countries, but also between different areas of each country. The prevalence of faecal oocysts were reported 2% in California, 3.8% in Colorado (Dubey and Greene, 2006), 8.8 and 9.5% in Brazil by light microscopy examination and PCR techniques respectively (Lallo and Bondan, 2006), 0.6% (29/4526) in the UK (Batchelor *et al.*, 2008),

0% in Ontario (Lefebvre *et al.*, 2005), 0.23% (5/2193) in Southern Greater Buenos Aires (Fontanarrosa *et al.*, 2006), 2.41% (4/166) in Rio de Janeiro (Huber *et al.*, 2005), 3.8% (5 of 130) in north-central Colorado (Hackett and Lappin, 2003), 9.3% (13/140) in Osaka city, Japan (Abe *et al.*, 2002), 44.1% (128/290) in Norway (Hamnes *et al.*, 2007), 2.8% (8 of 281) in the Serres Prefecture, Northern Greece (Papazahariadou *et al.*, 2007), 1.5% in Bangkok (Inpankaew *et al.*, 2007), 7.4% in the Niagara region (Shukla *et al.*, 2006), 1.4% in the Prague city centre (Dubna *et al.*, 2007), 1.4% (6 of 433) (Mundim *et al.*, 2007), and 3.3% in central Italy (Giangaspero *et al.*, 2006). A different prevalence rate (1.6-7%) has been reported in previous studies in Iran (Ranjbar Bahadori and Fattahi Boorani, 2005).

In the present survey, *Cryptosporidium* oocysts have been observed in clinically healthy dogs as well as in diarrheic cases with more prevalence. In our study, the infection rate in dogs with diarrhea was higher (17.65%; 3 of 17) than that in asymptomatic animals (1.3%; 1 of 76) ($P < 0.05$). Causape *et al.* (1996) and Giangaspero *et al.* (2006) reported the prevalence was significantly higher in diarrheic versus non-diarrheic dogs. We conclude that cryptosporidiosis is a cause for diarrhea in dogs in Ahvaz, southwestern Iran. *Cryptosporidium* oocysts are only 4-6 μm in size, which can evade traditional water filtration methods. The size of oocysts was 4-5 μm in our study. One study has suggested that these characteristics might be the reason for the 1994 outbreak in Las Vegas, Nevada (Goldstein *et al.*, 1996).

There have been few reports of *Cryptosporidium* infection in dogs, with the majority of cases involving pups less than 6 months of age. Fontanarrosa *et al.* (2006), Hamnes *et al.* (2007) and Papazahariadou *et al.* (2007) reported the highest level of prevalence in dogs <6-month-old, and declining with age ($P < 0.05$). In contrast, young animals showed a lower frequency (5.5%) compared to adults (10.1%) with no significant difference (Lallo and Bondan, 2006). In our study, infection was not significant between the different age groups, of course the number of the affected dogs was too small.

Huber *et al.* (2005) observed a statistically significant difference between the shelter dogs (45%) and the household pets (12.3%). Some seasonality was detected with a higher prevalence of *Cryptosporidium* oocyst shedding found from October to December (Batchelor *et al.*, 2008). In our survey, there was no significant difference in the overall prevalence between the origin of the dogs (urban = 2.17 and rural = 6.4%), genders (female = 2.6%, male = 5.6%), seasonality and breeds.

Cryptosporidiosis has been described in immunosuppressed pups with *canine distemper* and in dogs with gastrointestinal lymphoma. In two dogs, with concurrent *parvovirus* infection or immune-mediated thrombocytopenia, the dogs had hemorrhagic diarrhea (Dubey and Greene, 2006). Previous documented cases of *canine cryptosporidiosis* have involved symptomatic and asymptomatic animals, often with other concurrent infections. An incidental finding of *Cryptosporidium* infection in a 3-month-old pup with *canine distemper* virus was seen. Similarly, *Cryptosporidium* oocysts were detected in the faeces of a 6-month-old female pup with *canine distemper* virus. In our survey, concurrent detection of *Cryptosporidium parvum* with *canine distemper* (one sample) and *parvovirus* (one sample) were seen in the studied dogs.

In conclusion, the results of this study indicate that further biological studies are required to fully determine the prevalence, transmission, zoonotic capacity and species status of the *Cryptosporidium* dog genotype, especially in immunocompromised hosts. People should practice good sanitation and hygiene to minimize environmental contamination and contact with the infectious oocysts that may be shed by their pets.

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