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Epidemiological survey of *Cryptosporidium* spp. in companion and stray dogs in Kerman, Iran

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Summary

A cross-sectional survey was conducted to determine the prevalence of *Cryptosporidium* spp. infection in companion and stray dogs in Kerman, Iran. Faecal samples were randomly collected from 548 dogs (450 companion and 98 stray). *Cryptosporidium* oocysts were concentrated using the formalin ether sedimentation method according to the modified Ziehl-Neelsen staining technique. *Cryptosporidium* oocysts were identified in 2% (11/548) of samples. Faeces were classified according to the consistency as diarrhoeic (56/548) and non-diarrhoeic (492/548). Diarrhoea was recorded in 4 of the positive samples (7.14%). The prevalence of cryptosporidiosis was significantly higher in diarrhoeic dogs (7.14%) compared to the non-diarrhoeic dogs (1.4%) (p<0.05). There was no significant difference between the prevalence of *Cryptosporidium* spp. in stray dogs (4.08%) and companion dogs (1.55%). The age distribution of *Cryptosporidium* spp. in dogs below 1 year of age had a higher overall prevalence than dogs over 12 months of age (p<0.05). There was no significant difference in the prevalence between male (1.93%) and female (2.1%) dogs. The highest rate of infection was recorded during the autumn (3.9%) but this difference was not statistically significant (p>0.05). This study confirmed that dogs have a potential role in human cryptosporidiosis and faecal examination of dogs with persistent diarrhoea should be performed on a routine basis.

Keywords

Cryptosporidium, Dog, Prevalence, Iran, Parasite, Prevalence, Protozoa, Zoonosis.

Indagine epidemiologica su *Cryptosporidium* spp. in cani da compagnia e randagi a Kerman, Iran

Riassunto

Un' indagine trasversale (cross-sectional) è stata condotta per determinare la prevalenza di Cryptosporidium *spp. in cani da compagnia e randagi a Kerman, Iran. I campioni fecali sono stati raccolti casualmente da 548 cani (450 da compagnia e 98 randagi). Oocisti di* Cryptosporidium *sono state concentrate utilizzando il metodo di sedimentazione delle feci con formalina-etere secondo la tecnica di colorazione modificata Ziehl-Neelsen. Oocisti di* Cryptosporidium *sono stati identificate nel 2% (11/548) dei campioni. Le feci sono state classificate in base alla consistenza in diarroiche (56/548) e non diarroiche (492/548). La diarrea è stata registrata in 4 dei campioni positivi (7,14%). La prevalenza di criptosporidiosi era significativamente più alta nei cani diarroici (7,14%) rispetto ai non-diarroici (1,4%)* (p<0,05). *Non vi è alcuna differenza significativa tra la prevalenza di* Cryptosporidium *spp. nei cani randagi (4,08%) e cani da com-pagnia (1,55%). La distribuzione per età di* Cryptosporidium *spp. ha fatto registrare una maggiore prevalenza globale nei cani al di sotto di 1 anno di età rispetto ai cani di età superiore a 12 mesi* (p<0,05). *Nessuna differenza significativa è stata registrata nella*

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prevalenza tra maschi (1,93%) e femmine (2,1%) cani. Il più alto tasso di infezione è stato rilevato durante l'autunno (3,9%), anche se questa differenza non è statisticamente significativa (p>0,05). Il presente studio ha confermato il potenziale ruolo dei cani nella criptosporidiosi umana e l'esame fecale di cani con diarrea persistente deve essere eseguita su una base routinaria.

Parole chiave
Cryptosporidium, Cane, Iran, Parassita, Prevalenza, Protozoi, Zoonosi.

Introduction

Cryptosporidium spp. is a zoonotic protozoan parasite that causes cryptosporidiosis and is associated with foetal diarrhoea in animals and humans. *Cryptosporidium* species are widespread protozoa and are largely detected in environmental samples that infect both humans and animals. The parasite can cause acute and persistent disease in malnourished children and chronic, life-threatening infection in immunodeficient individuals, such as those affected by AIDS.

Transmission occurs through the he faecal-oral route, following direct or indirect contact with *Cryptosporidium* oocysts via person-to-person, zoonotic, waterborne, foodborne or airborne contact (7). Cryptosporidiosis is a significant cause of diarrhoeal disease in both developing and industrialised nations, but several epidemiological studies have demonstrated that *Cryptosporidium* is more prevalent in developing countries (5% to >10%) than in developed countries (<1% to 3%) (2). In the immunocompetent host, infection is self-limiting, lasting from a few days to 3 weeks, with possible morbidity in young animals (15, 19). In the immunocompromised host, infection may result in chronic debilitating diarrhoea with dehydration, malabsorption, wasting and death (15).

Methods to detect *Cryptosporidium* spp. in faeces usually involve microscopic examination of stained faecal smears (modified Ziehl-Neelsen, safranin methylene blue, auramine phenol), antigen detection (immunofluorescence, enzyme-linked immunosorbent assay [ELISA]) or genome detection (polymerase chain reaction [PCR] amplification of the 18S rRNA gene). Each varies in sensitivity and specificity and there is no universally accepted 'gold standard' (2).

There have been few reports of *Cryptosporidium* infection in dogs, with the majority of cases involving puppies aged less than six months. The first evidence of cryptosporidiosis in dogs was reported in 1981 by Tzipori and Campbell, as quoted in Morgan *et al.* (15) who detected *Cryptosporidium* antibodies in 16 of 20 canine serum samples (15). Morgan *et al.* also referred to the work by Wilson *et al.* who reported the first clinical case of canine cryptosporidiosis two years later, when life-cycle stages characteristic of *Cryptosporidium* were identified in a week-old puppy suffering from acute diarrhoea (15).

Cryptosporidium parvum is a well-known pathogen that affects humans and animals. Recent biological and genetic studies have also shown that *C. parvum* is also a complex of multiple genetically distinct genotypes. Humans may be infected with human, canine and bovine *Cryptosporidium* (1, 16). The monkey, mouse, pig, marsupial and ferret genotypes have been found only in their respective hosts and not in humans (1). However, the dog genotype was recently found in humans (1).

Numerous epidemiological studies of *Cryptosporidium* spp. in canines have been reported worldwide (1, 4, 5, 6, 15). There are no data available concerning the epidemiology and prevalence of *Cryptosporidium* spp. in companion and stray dogs in the State of Kerman, Iran. The main objective of our research was to determine the prevalence of *Cryptosporidium* spp. in dogs in Kerman.

Materials and methods

A cross-sectional survey was conducted to study the prevalence and intensity of infection with *Cryptosporidium* spp. in 548 companion and stray dogs in the urban areas of Kerman, south-east Iran. Kerman is located at 30°17′13″N and 57°04′09″E. The mean

elevation of the city is approximately 1 755 m above sea level. Kerman city has a hot and arid climate, with an average annual rainfall of 135 mm. Given the fact that it is located close to the Kavir-e lut, Kerman has hot summers. Based on climate, soil, and other geographic conditions, Kerman has varied vegetation and different types of agriculture.

Faecal samples from 548 dogs (450 companion and 98 stray dogs) were examined for the presence of parasites. Faecal samples were collected directly from the rectum and were stored in 10% formalin neutral buffer solution until examination.

We extended our trial to include acid-fast staining for coccidian parasites that may be associated with enteric disease. The dog's age, sex, season and consistency of faeces were noted. Details of the symptoms prompting the examination request were also noted. Faecal samples for routine ova and parasite examination were concentrated by using the formalin-ether sedimentation method. Thin smears of the concentrated pellet were prepared on glass slides and air-dried before modified Ziehl-Neelsen staining. Each slide was scanned at 1 000× magnification and confirmed under oil emersion. The faecal samples were carefully examined using the 100× lens, field by field covering the entire cover slip. Each oocyst observed was identified by using morphological characteristics. A dog was classified as positive if at least one oocyst was observed.

Data were analysed by Chi square test analysis. The significance level was $p<0.05$.

Results

In our study, of the 548 dogs examined, 11 faecal samples were positive for *Cryptosporidium* spp. oocysts and the prevalence of infection with *Cryptosporidium* spp. was 2%.

When the prevalence of *Cryptosporidium* spp. was analysed by age, it was observed that dogs below the age of 12 months (3.86%) had a higher overall prevalence than dogs over 12 months (1.1%) of age. Consequently, there was a significant difference in the prevalence of infection between the two age groups ($p<0.05$) (Table I).

There was no significant difference in prevalence between companion (1.55%) and stray (4.08%) dogs ($p>0.05$) (Table I). This was also the case for male (1.93%) and female (2.1%) dogs. The highest rate of infection occurred in the Autumn (3.9%) but this difference was not statistically significant ($p>0.05$, Table I).

Faeces were classified according to the consistency as diarrhoeic (56/548) and non-diarrhoeic (492/548). Diarrhoea was recorded in four of the positive samples (7.14%). Cryptosporidial prevalence was significantly higher in diarrhoeic (7.14%) versus non-diarrhoeic (1.4%) dogs ($p<0.05$) (Table I). This confirms the finding of Causape *et al*. (4) and Morgan *et al.* (15).

Discussion

In our study, *Cryptosporidium* oocysts were identified in 2% (11/548) of samples examined. According to the studies conducted in different countries worldwide, the estimate prevalence of canine *Cryptosporidium* oocysts vary different from 1% to 20% (9) and some factors, such as geographic location, status of animal ownership, sampling protocols, demographic factors, anthelmintic use and diagnostic techniques are responsible for the wide range of *Cryptosporidium* prevalence (3, 16).

With respect to *Cryptosporidium* spp., the infection rate of 2% is close to that observed by with Papazahariadou *et al*. (17) 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 that infection rates are variable, depending on the geographic area and range from 1.4% in the Czech Republic (5), 2.41% in Brazil (10), 1.4% in Uberlandia (Brazil) (16) and 2% in California (6).

Our findings were below levels reported from Tasmania (9.2%) by Milstein and Glodsmid (14), Osaka (9.3%) by Abe *et al.* (1), Ilam (Iran)

Table I
Prevalence of *Cryptosporidium spp*. in dogs according to sex, age, faecal consistency, season, and type of dog in Kerman, Iran

Sex	No. of dogs examined	Percentage abundance	No. of infections	
			No.	Percentage
Male	310	56.6	6	1.93
Female	238	43.4	5	2.1
Age				
0<1	181	33.3	7	3.86
0>1	362	66.7	4	1.1
Faecal consistency				
Diarrhoeic	56	10.2	4	7.14
Non-diarrhoeic	492	89.8	7	1.4
Season				
Spring	168	30.6	3	1.8
Summer	127	23.2	2	1.6
Autumn	127	23.2	5	3.9
Winter	126	23	1	0.8
Dogs				
Companion	450	82.1	7	1.55
Stray	98	17.9	4	4.08

(7.14%) by Bahrami *et al.* (3) and southern Victoria (0.6%-19.6%) by Johnston and Gasser (11), but higher than reports from Japan (1.4%) by Ugo *et al.* (20), and Edinburgh (0%) by Simpson *et al.* (18). This variation may be due to differences in management systems, health care and the degree of environmental contamination with infective stages.

The infection prevalence of *Cryptosporidium* spp. in our study in male and female dogs was 1.93% and 2.1%, respectively. There was no statistically significant difference (p>0.05) between the two sex categories; these results concur with those of Huber *et al*. (10) who reported on the situation in Rio de Janeiro. Hormonal factors and sex associated behaviour, such as roaming, may be contributing factors (12).

In this study, the prevalence of *Cryptosporidium* spp. was higher in dogs under the age of 12 months than in dogs over 12 months old which coincides with other studies (8, 13). In contrast, Causape *et al*. (4) found no statistical difference in prevalence between dogs below one year of age and dogs aged more than a year.

The seasonal variation in cryptosporidiosis has been reported in few studies. In our study, the highest rate of infection was observed in the Autumn (3.9%) which coincides with findings of Dubna *et al.* (5) but this difference was not statistically significant (p>0.05). Consequently, we did not consider that there was a seasonal effect on the prevalence of *Cryptosporidium* infection in dogs.

There was no significant difference between prevalence of *Cryptosporidium* spp. in stray dogs (4.08%) and companion dogs (1.55%). In addition, Causape *et al*. (4) found no statistical difference in prevalence between companion and stray dogs. Despite the fact that most companion dogs in our study received antiparasitic treatment regularly, the prevalence of *Cryptosporidium* spp. in companion dogs was almost the same as that in stray dogs. This may indicate resistance of *Cryptosporidium* spp. oocysts to treatment.

Results of measuring oocysts excreted by dogs (4.5 × 5.4) indicated that they were morphologically similar to those of *C. parvum* and were therefore considered to be zoonotic. This finding confirmed the report by Causape *et al*. (4).

Conclusions

Our study confirmed that dogs play potential role in human cryptosporidiosis and it is important that routine faecal examination is performed in dogs with persistent diarrhoea.

The presence of cryptosporidia in beef cattle and dogs suggested that both species could be a source of human infection. As *C. parvum* is a major waterborne protozoan pathogen, water contamination should be investigated to protect public health from the risk of transmission of the pathogen. In addition, these results also highlighted the importance of investigating the possibility that other animals may also act as reservoir hosts for *Cryptosporidium*.

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