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Research Article

Prevalence of *Cryptosporidium* spp. in Dogs in The Aegean Region

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ABSTRACT

Cryptosporidium spp. is a protozoal parasite that can cause significant gastrointestinal diseases in humans and animals. Those parasites cause infection, especially in young and immunosuppressed animals in many mammals, poultry, reptiles, and humans. *Cryptosporidium spp*. localised in the digestive system, epithelial cells threaten human and animal health with their zoonotic properties. The study's animal material consisted of 200 dogs of different breeds, ages and sexes, including healthy (n=50) and diarrhoea (n=150). Stool samples from the dogs used in the study were stained using the modified Ziehl-Neelsen technique and examined under a microscope. The prevalence of *Cryptosporidium* spp. in faecal samples taken from all dogs was 15.5% and also it was 14% and 16% in healthy and diarrheal dogs, respectively. It was concluded that these results could be used as a reference for future studies on dogs.

Keywords: Cryptosporidium, dogs, prevalence

Ege Bölgesi'ndeki Köpeklerde *Cryptosporidium* spp.'nin Prevalansı

ÖZET

Cryptosporidium spp. insanlarda ve hayvanlarda önemli gastrointestinal hastalıklara sebep olabilen protozoal bir parazittir. İnsan dahil birçok memeli, kanatlı ve sürüngende sindirim sistemi epitel hücrelerine lokalize olan bu parazitler özellikle genç ve immun sistemi baskılanmış hayvanlarda enfeksiyona neden olmaktadır ve zoonotik özelliği ile insan ve hayvan sağlığını tehdit etmektedir. Araştırmanın hayvan materyalini farklı ırk, yaş ve cinsiyette sağlıklı (n=50) ve ishalli (n=150) olmak üzere 200 köpek oluşturdu. Çalışmada kullanılan köpeklerden dışkı örnekleri modifiye Ziehl-Neelsen tekniği ile boyandı ve mikroskopta incelendi. Tüm köpeklerden alınan dışkı örneklerinde *Cryptosporidium* spp. prevalansı %15,5, sağlıklı ve ishalli köpeklerde ise sırasıyla %14 ve %16 idi. Bu sonuçların köpekler üzerinde yapılacak ileri araştırmalar için referans olarak kullanılabileceği sonucuna varıldı.

Anahtar Kelimeler: Kriptosporidyum, köpekler, prevalans

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Introduction

Cryptosporidiosis; is a zoonotic infection caused by *Cryptosporidium* spp., a coccidian protozoan belonging to the class *Apicomplexa*, in many animal species and humans (Wright and Coop, 2007). Compared to other parasites belong in the coccidia family, *Cryptosporidium* spp. shares many similarities in its life cycle (having sexual and asexual forms) but differs from coccidia in some features (Divers and Peek, 2008). In this context, the sporulated form of *Cryptosporidium* oocysts are excreted in the faeces and can cause auto-infection in the animal (Hamnes et al., 2006; O'Handley and Olson, 2006). *Cryptosporidium* spp. and other coccidia parasites differ in their host specificity and size. *Cryptosporidium (C.) parvum* is less host-specific. *Cryptosporidium* spp. is much smaller, and it can not be readily determined by the faecal flotation method (Divers and Peek, 2008). In addition, Cryptosporidium is more resistant to external agents and has more distinct areas of localization regions in cells than other coccidia (Hamnes et al., 2007).

Cryptosporidium canis is a genotype of *Cryptosporidium spp*. that affects dogs (Fayer et al., 2001). The first report of *Cryptosporidium* spp. was published in England in 1983 (Wilson et al., 1983). Since then, several studies have been documented on the occurrence, prevalence and risk factors of cryptosporidiosis in dogs (Bajer et al., 2012). *C. canis* and its subgenotypes have been reported worldwide in dogs, foxes, coyotes, and humans (Fayer et al., 2001; Lucio-Forster et al., 2010; Elwin et al., 2012). It is generally species-specific, dogs are commonly infected with *C. canis*, and it has also been reported that they are infected with *C. parvum* (Scorza and Tangtrongsup, 2010; FitzGerald et al., 2011; Scorza et al., 2014). *C. canis* is often subclinical in dogs, and these dogs excrete oocysts without showing any clinical findings (Lindsay and Zajac, 2004; Scorza and Tangtrongsup, 2010;). Epidemiological studies in dogs with *Cryptosporidium spp*. have shown that disease prevalence varies by the geographic region, and there is no correlation between age groups or living conditions. Previous studies have shown that young and adult dogs can excretion of oocysts with their faeces (Huber et al., 2005). Chronic or intermittent diarrhoea, anorexia and wasting are the most common clinical findings in symptomatic dogs. Dogs with clinical findings are usually young animals (Lucio et al., 2016). The mechanism of diarrhoea, malabsorption and attenuation induced by *Cryptosporidium* is not fully understood (Lindsay and Zajac, 2004). Oocyst excretion has been shown to persist over a long period of time (more than 3-5 months) in experimentally infected animals. (Asahi et al., 1991). Cryptosporidiosis in dogs is transmitted directly or indirectly by the fecal-oral route. It occurs directly during coprophagy and indirectly through contaminated food and water (Baldursson and Karanis, 2011).

There are few studies on the prevalence of *Cryptosporidium* spp. in dogs. *Cryptosporidium spp*. prevalence was found to be 8% (5/62) in animal shelters and 10% (8/78) in veterinary clinics in Canada (Uehlinger et al., 2013). Epidemiological studies have shown that variation in prevalence depends primarily on the methods used and then on geographic location. It was reported as 0% in Austria (Bugg et al., 1999) and 5% in Argentina when the flotation method was used (Fontanarrosa et al., 2006). Percentages from the United Kingdom (Batchelor et al., 2008) and the Netherlands (Overgaauw et al., 2009) were reported as 0.6 and 8.7, respectively when the staining method was used. Data from prevalence studies with ELISA; Italy 1.7%, Canada 7.4%, Germany 23% (Bauer et al., 2004; Shukla et al., 2006; Rinaldi et al., 2008). A similar prevalence was 44% with the immunofluorescence technique in Norway (Hamnes et al., 2007). This high prevalence may be related to the commercial kit (Titilincu et al., 2010). It has been reported that the specificity and sensitivity of the ELISA kit are lower (94% and 71%, respectively) than immunofluorescence microscopy in *Cryptosporidium* spp. (Rimhanen-Finne et al., 2007). Parasites of dogs are not only crucial for animals but also for humans because of the zoonotic potential of some species and the need for frequent monitoring. While most human infections are caused by *C. parvum* and *C. hominis*, a certain percentage are caused by *C. canis* (Ryan et al., 2014). Several pathogens can cause mild intestinal diarrhoea in dogs. Therefore, native slide examination and faecal flotation are initially applied to the diagnostic approach (Mundim et al., 2007). Immunological methods are also used in the identification of the parasite. Direct Immunofluorescence Technique (DFA), Latex Agglutination Reaction, Reverse Passive Haemagglutination, Immunochromatography, Indirect Fluorescent Antibody Test (IFA), Enzyme-Linked Immunosorbent Antibody Test (ELISA) and rapid diagnosis kits are commercially available for the immunological investigation of *Cryptosporidium* in the stool (Fayer et al., 2001; Babac, 2014).

While there are several studies on the prevalence of *Cryptosporidium* spp. in humans and some animal species in our country, there are no studies on the prevalence of *Cryptosporidium spp*. in dogs. This study aimed to determine the prevalence of *Cryptosporidium spp*., which has a zoonotic feature, in dogs in the Aegean region.

Material and Methods

The research was started with Aydın Adnan Menderes University Veterinary Faculty Ethics Committee Ethics Committee (Date: 22.08.2017, Number: 64583101/2017/081). All stool samples were collected voluntarily by informing the owner. The material of the study was collected from dogs brought to Aydın Adnan Menderes University Veterinary Faculty Research and Application Hospital, polyclinics, some private veterinary clinics and animal hospitals in Aydın, İzmir, Manisa, Denizli and Muğla provinces between March 2018 and March 2019. The study included 200 dogs of different breeds, ages and sexes (50 healthy and 150 with diarrhoea). Stool samples were taken into sterile storage containers. After flotation in sucrose solution, the stool was stained using the modified Ziehl-Neelsen technique (Scorza and Lappin, 2012). The samples were prepared from the dyed materials by the smear method. They were examined

under the microscope at 100x magnification with a drop of immersion oil. This examination was performed to determine the status of the presence or absence status of *Cryptosporidium* spp. oocysts. They were defined as round structures with a diameter of 4-6 µm, which were heterogeneously stained in bright pink-red colour on a blue background, and most of them had black irregular granules.

The data obtained from the study were evaluated in the SPSS package program (SPSS© 22.0, IBM, Armonk, NY). The Shapiro-Wilk test determined whether it showed a normal distribution. Analysis of proportional data was evaluated by chi-square analysis. The level of error (*P*) was taken as 0.05.

Results

The prevalence of *Cryptosporidium* spp. in stool samples taken from dogs was 15.5% (31/200). At the same time, 24 samples out of 150 dogs (16%) with diarrhoea and 7 samples from 50 healthy dogs(14%) were positive for *Cryptosporidium* spp. (Table 1).

Table 1. Distribution of *Cryptosporidium* spp. in healthy and diarrheal dogs (Number, %).

		Samples		Total
		Positive	Negative	
Condition	Healthy	7 (14%)	43 (86%)	50 (100%)
	Diarrheal	24 (16%)	126 (84%)	150 (100%)
Total		31 (15.5%)	169 (84.5%)	200 (100%)

X^2=0.115 P<0.05

When the rate of Cryptosporidium positivity according to gender was examined, it was determined at 16.4% in male dogs and 14.6% in female dogs (Table 2).

Table 2. *Cryptosporidium* spp. incidence rates by gender (Number, %).

		Samples		Total
		Positive	Negative	
Sex	Male	15 (16.4%)	76 (83.5%)	91 (100%)
	Female	16 (14.6%)	93 (85.3%)	109 (100%)
Total		31 (15.5%)	169 (84.5%)	200 (100%)

X^2=1.802 P<0.05

Cryptosporidium positive rate by age group; was seen at 11.3% in dogs aged 0-6 months, 24.1% in dogs aged 7-24 months, and 14.3% in dogs 25 months and older (Table 3).

14% positive in dogs with solid stool, 20.3% positive in dogs with pasty stool, 14.3% positive in dogs with liquid stool; this rate was found to be 5.6% in dogs with hemorrhagic stools when evaluated by stool consistency groups (Table 4).

Discussion

Cryptosporidium, which is localised to the epithelial cells of the digestive system in many mammals, including humans, poultry and reptiles, is a protozoal parasite that can cause significant gastrointestinal diseases (Mundim et al., 2007). These parasites cause infection in immunocompromised patients. In addition to the chemical and physical characteristics of the agent in the spread of the parasite, the presence of many hosts, the ease of spread of the parasite and its ability to infect a small number of oocysts increase its importance to animal and human health (Scorza and Lappin, 2012).

It has been reported that the parasite is naturally found in the flora. *Cryptosporidium* spp. infections are generally observed in asymptomatic form in dogs (Hall and Day, 2017). *Cryptosporidium* spp. infects the organism based on the developmental stages in the epitelium of the middle and last duodenal mucosa and the epithelial parts of the head and middle jejunal mucosa (Wilson et al., 1983; Greene et al., 1990). It causes villus atrophy and inflammation in the affected area (Koudela and Jiri, 1997). As a result, malabsorption and maldigestion develop and cause various symptoms in the organism. It is reported that the affected area in the organism differs depending on the *Cryptosporidium* species (Plutzer and Karanis, 2009). Although four different *Cryptosporidium* species have been identified in dogs, *C. canis, C. parvum* and C*. meleagridis* have been reported to settle in the small intestine and *C. muris* in the stomach (Cuia et al., 2018). It has been reported that the affected area in the organism differs according to Cryptosporidium species (Plutzer and Karanis, 2009). In this study, *Cryptosporidium* spp. was determined in symptomatic and asymptomatic dogs without species identification.

Laboratory diagnosis of cryptosporidiosis involves microscopic, serologic, and molecular methods. Diagnosis can be supported by direct microscopic examination using various staining methods, immunofluorescence methods, ELISA, and PCR methods. IFA is an essential but expensive technique for diagnosis in the early stages of the disease. Specific anti-*Cryptosporidium* spp. IgG and IgM can be detected by the ELISA method. Recently, PCR techniques have been developed for diagnostic purposes. However, their use in diagnosis is limited because they are expensive. The use of more than one

Table 3. *Cryptosporidium* spp. incidence rates by age groups (Number, %).

		Samples		Total
		Positive	Negative	
Age Groups	0- 6 months	11 (13.3%)	86 (88.7%)	97 (100%)
	7-24 months	13 (24.1%)	41 (75.9%)	54 (100%)
	≤25 month	7 (14.3%)	42 (85.7%)	49 (100%)
Total		31 (15.5%)	169 (84.5%)	200 (100%)

X^2= 0.113 P<0.05

method in combination with microscopic methods for diagnosis increases the reliability of the results (Bennett et al., 1985). It has been reported that many different prevalence studies have been performed in dogs using other methods (Lindsay and Zajac, 2004). *Cryptosporidium spp*. oocysts were determined in stool samples collected in this study, as in many other studies, using the Ziehl-Neelsen staining technique.

Although it has been reported that cases of cryptosporidiosis in dogs generally occur in young individuals, epidemiological studies have reported that there is no relationship between age and infection in *Cryptosporidium* spp. infections (Moreira et al., 2018). However, some authors noted that the infection rate is higher in young dogs (Pivoto et al., 2013; Olabanji et al., 2016), while some researchers referred to adult dogs (Bresciani et al., 2008). While Pivoto et al. (2013) argue that there is no relationship between age and infection, they report that the percentage of oocysts (25%) in animals under one year old is slightly higher than the percentage of animals older than one year (23.2%). The researchers emphasise that age is not a risk factor. The prevalence of infection is higher in dogs three to six months of age. In contrast, Bresciani et al. (2008) reported a higher prevalence of infection in animals one to four years of age. Thompson et al. (2005) note that although infections are recurrent in adult animals, disease frequency is higher in young animals. Noordeen et al. (2001) and Bajer et al. (2012) emphasise that prevalence is higher in young animals other than dogs. In this study, in agreement with many authors, although no correlation was found between Cryptosporidium infection and age, the highest prevalence (24.1%) in the age group between 7-24 months was in agreement with the results of Gbemisola et al.(2016).

Although sex has been reported to play no role in *Cryptosporidium* spp. infection (Mundim et al., 2007), it is said to be relatively more common in female dogs than in males. Zelalem and Addis (2012) reported that the prevalence of *Cryptosporidium* was higher in males (79.2%) than in females (76.8%). It has been reported that the higher incidence in females may be related to decreased immunity during certain periods due to physiological cycle.

When evaluating the sex distribution of infected animals in this study, the disease incidence was found to be 16.4% in males and 14.6% in females. Previous studies reported that there was no sex predisposition in *Cryptosporidium* spp. parasite infections (Mundim et.al., 2007). There was no association between Cryptosporidium prevalence and the sex of dogs, suggesting that both sexes may have an equal chance of becoming infected when exposed to an infected or contaminated substance.

Table 4. *Cryptosporidium* spp. incidence rates according to stool consistency groups (Number, %).

		Samples		Totally
		Positive	Negative	
Stool Consistency	Solid	7 (14%)	43 (86%)	50 (100%)
	Pasty	14 (20.3%)	55 (79.7%)	69 (100%)
	Fluid	9 (14.3%)	54 (85.7)	63 (100%)
	Hemorrhagic	1 (5.6%)	17 (94.4%)	18 (100%)
Totally		31 (15.5%)	169 (84.5%)	200 (100%)

X^2=0.463 P<0.05

The prevalence of *Cryptosporidium* infection is reported to be higher in pure breeds. Different breeds of dogs were used in this study. For this reason, it was assumed that it would not give a healthy result and was not involved in the statistical analysis.

The prevalence of Cryptosporidium in dogs is range from 0% and 44.8% in many studies conducted worldwide (Lindsay and Zajac, 2004). The prevalence depends on the determination method, geographic location, care, feeding, environmental conditions, and cleaning-disinfection (Cirak and Bauer, 2004; Shukla et al., 2006). In the prevalence study conducted by ELISA, the prevalence was found to be 1.7% in Italy, 7.4% in Canada, and 23% in Germany (Cirak and Bauer, 2004; Shukla et al., 2006). A similar prevalence was 44% by immunofluorescence technique in Norway (Hamnes et al., 2007).

Reported rates on direct microscopic examination of faecal samples using various staining methods in dogs range from 2% to 17% in the USA; 8.8% in Brazil; 2.2% in Argentina; 6.3% in Spain; 9.7% in Korea, and 11% in Australia (Scorza and Tangtrongsup, 2010). The prevalence of *Cryptosporidium* spp. was found to be 8% (5/62) in shelters and 10% (8/78) in veterinary clinics in Canada (Uehlinger et al., 2013). There is no study on the prevalence of *Cryptosporidium* spp. in our country. In this study, the prevalence of *Cryptosporidium* spp. in dogs in the Aegean region was 15.5% (31/200).

Conclusion

According to the consistency of the stool, classification was defined as healthy in animals with solid stool status. As a result of the diagnostic applications, it was determined that the incidence of *Cryptosporidium* was 14% in animals classified as healthy and 16% in animals with diarrhoea. This situation supports that the agent can widely be asymptomatic. It was determined that age, gender and faeces consistency were not crucial in the prevalence of *Cryptosporidium.* At the same time, it is thought that taking these factors into account can be used as a reference for future studies on dogs.

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This study was summarised by the first author's Master Thesis.

Conflict of interest

The authors declare that they have no conflict of interest.

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