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Abstract: Faecal samples of 172 diarrhoeic and 130 non-diarrhoeic cattle were examined for the presence of *Cryptosporidium* oocysts. The prevalence of infection in diarrhoeic and non-diarrhoeic cattle was 63.3% and 69.2%, respectively. The infection was prevalent in cattle over three years old, in both diarrhoeic and non-diarrhoeic animals. In both groups, oocyst sheeding level of (+) was the most common.

Cryptosporidium-positive faecal specimens were examined for the presence of *Escherichia coli* K 99 and rotavirus. *E. coli* K 99 was isolated from the faeces of 35 (32.1%) diarrhoeic and 23 (25.5%) non-diarrhoeic animals. Rotavirus was not detected in any of the faecal samples.

Key Words: *Cryptosporidium*, prevalence, cattle, *E. coli* K 99 and rotavirus

Ankara ve civarındaki sığırlarda *Cryptosporidium* enfeksiyonunun prevalansı ve diğer enterik patojenlerle (*Escherichia coli* K 99 ve rotavirus) ilişkisi

Özet: Toplanan 172 ishalli ve 130 normal sığır dışkı örneğinde *Cryptosporidium* oosistlerinin varlığı araştırılmış, enfeksiyonun prevalansı ishalli ve normal sığırlarda sırasıyla %63.3 ve %69.2 olarak saptanmıştır. Enfeksiyonun üç yaşın üstündeki ishalli ve normal sığır gruplarında daha yaygın olduğu gözlenmiştir. Dışkıdaki oosist sayısının her iki grupta (+) düzeyinde yoğunlaştığı belirlenmiştir.

Cryptosporidium pozitif örnekler *Escherichia coli* K 99 ve rotavirus etkenlerinin varlığı yönünden incelenmişlerdir. *E. coli* 35 ishalli (%32.1) ve 23 normal (%25.5) dışkıdan izole edilmiştir. *Cryptosporidium* pozitif dışkı örneklerinde rotavirus saptanamamıştır.

Anahtar Sözcükler: *Cryptosporidium,* yaygınlık, sığır, *E. coli* ve rotavirus

Introduction

Cryptosporidium spp. are protozoan parasites of worldwide distribution, with infection reported in wild and domestic animal species (1, 2), including dairy and beef calves in many parts of the world (3-5). Infection has been reported in domestic animals in Turkey (6, 7). Cryptosporidiosis, alone or in combination with other agents such as *Escherichia coli* K 99 and rotavirus, is recognized as a contributing factor in diarrhoea (8, 9). *Cryptosporidium* spp. oocysts, and *E. coli* K 99, however, have been observed in clinically healthy animals as well (10-12). The infection is generally seen in young calves (13, 14); however, *Cryptosporidium* spp. have also been found in cattle over two years of age (15). The infection is diagnosed by identifying the oocyst stage of the parasite in host faeces (16, 17) or in histological sections taken during necropsy (18, 19). Detection of parasites in faecal specimens is enhanced by the use of concentration procedures (20, 21). The purpose of this study was to document the presence of *Cryptosporidium* spp. in cattle in Ankara and its environs in order to provide information on the prevalence, intensity and age distribution of the infection in these herds, and to elucidate the occurrence of cryptospordiosis in combination with other infectious agents such as *E. coli* K 99 and rotavirus.

Materials and Methods

Faecal Samples

For a period of one year, fresh faecel samples were collected from 172 diarrhoeic and 130 non-diarrhoeic calves and cattle in small-holder farms in Ankara. Each animal was sampled once, and the age and clinical status of the animal, as well as the date and place of collection were recorded.

Faecal Examinatoin

Faecal smears were prepared on glass slides, using a wooden, cotton tipped swab, and were air dried. Pasty faecal material was diluted 1:1 in tap water to make thin

smears. In all samples, oocysts were identified by the Safranin-Methylene Blue (S-MB) technique (22), in which oocysts appear as bright orange round bodies, usually within a clear halo, against a blue background. Sheather's sugar flotation method (23, 24) was used for confirmation when the number of oocysts in faecal material was fairly low. Microscopical examination was performed with magnifications of 400x and 1000x. In each smear 20 fields were searched and the results were graduated as follows (at 1000 x magnification):

- \- No oocyst found per 20 fields
- \+ 1-5 oocysts found per 20 fields
- ++ 6-20 oocysts found per 20 fields
- +++ > 20 oocysts found per 20 fields

Microbiological Examination

Cryptosporidium-positive faecal samples were subjected to microbiological examination for the presence of *E. coli* K99 (25) and rotavirus. Isolates of *E. coli* were obtained after a primary plating of the faecal specimens on EMB, blood agar and Mc Conkey agar, and then isolated coliforms were subcultured on Minca-Isovitalex agar. After overnight incubation at 37°C, the growth of each colony was tested for the presence of K99 antigen by slide agglutination using K99-specific antisera, kindly provided by Dr. M. Contrepois, INRA, Clermont-Ferrand, France. For the detection of rotavirus in faeces, a commercially available Enzyme Immunoassay kit was used (Rotascreen EIA, Mercia Diagnostics, UK).

Results

The results of the survey are shown in Table 1. *Cryptosporidium* spp. were found in 109 (63.3%) of 172 diarrhoeic animals and 90 (69.2%) of 130 non-diarrhoeic animals. The prevalence of the infection peaked in cattle over three years old in both the diarrhoeic and non-diarrhoeic groups (71% and 79.4%, respectively). An oocyst shedding level of (+) was the most common in all age groups, with the exception of non-diarrhoeic animals from 20 days to 5 months of age in which (+) and (++) oocyst shedding levels were identical. The percentage distribution of positive samples, with reference to age groups of diarrhoeic and non-diarrhoeic animals is illustrated in Fig 1.

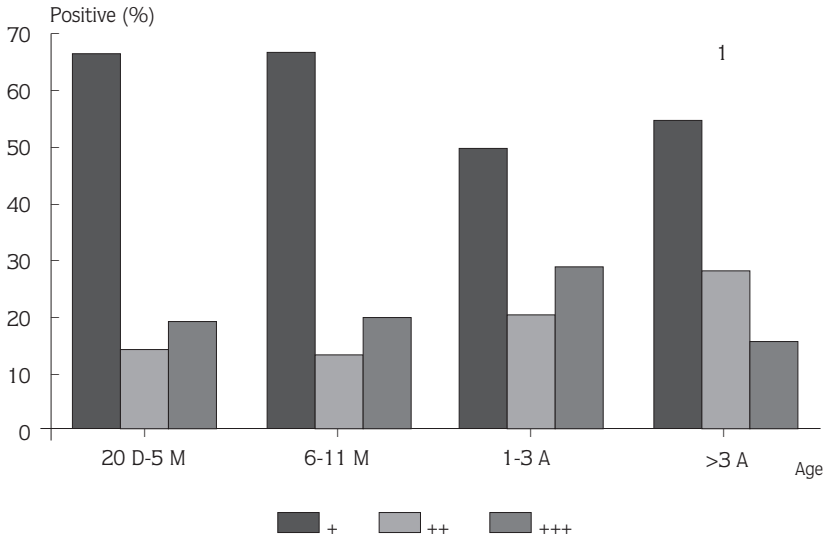
One hundred nine diarrhoeic and 90 non-diarrhoeic *Cryptosporidium*-positive faecal samples were examined for the presence of *E. coli* K 99 and rotavirus. *E. coli* K99 was isolated from 35 (32.1%) of the diarrhoeic and 23 (25.5%) of the non-diarrhoeic animals. Rotavirus was not detected in any of the *Cryptosporidium*-positive faecal specimens (Table 2).

Table 1. The Prevalence of *Cryptosporidium* spp. infection in diarrhoeic and non-diarrhoeic cattle in Ankara, Turkey

Locality	No. animals examined	No. (%) positive	Diarrhoeic		Non-diarrhoeic	
			No. examined	No. (%) positive	No. examined	No. (%) positive
BALA	37	28(75.6)	23	21(91.3)	14	7(50.0)
ELMADAĞ	32	23(71.8)	15	7(46.6)	17	16(94.1)
İMRAHOR	57	37(64.9)	25	17(68.0)	32	20(62.5)
HASANOĞLAN	61	45(73.7)	25	18(72.0)	36	27(75.0)
SİNCAN	30	20(66.6)	23	13(56.5)	7	7(100.0)
PURSAKLAR	16	12(75.0)	11	8(72.7)	5	4(80.0)
SARAY	26	12(46.1)	19	9(47.3)	7	3(42.8)
ÇUBUK	17	7(41.1)	10	4(40.0)	7	3(42.8)
DIFFERENT	26	15(57.6)	21	12(57.1)	5	3(60.0)
TOTAL	302	199(65.8)	172	109(63.3)	130	90(69.2)

Table 2. Prevalence of enteropathogens in diarrhoeic and non-diarrhoeic animals.

Pathogen	No. positive samples/No.materials examined		Significance
	Diarrhoeic	Non-diarrhoeic	x^2 analysis
Cryptosporidium spp.	109/172	90/130	p≥0.05 NS
Escherichia coli K 99	35/109	23/90	p≥0.05 NS
Rotavirus	-	-	-



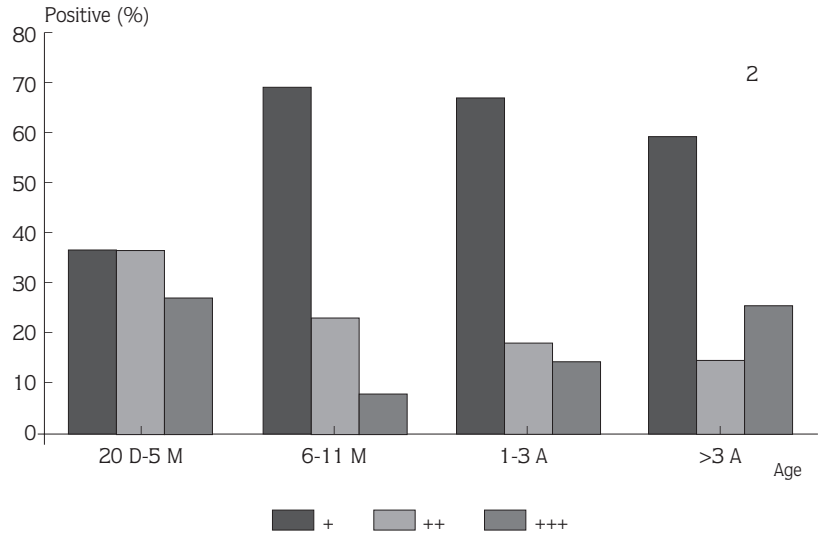


Figure 1. Distribution of Cryptosporidium-positive specimens with reference to age-groups of hosts 1. Diarrhoeic 2. Non-diarrhoeic

Discussion

The overall prevalence of *Cryptosporidium* spp. infection (65.8%) in animals of a wide range of ages is hardly comparable to the prevalences reported from other countries, where such investigations have been almost exclusively comprised of 1-30-day-old (13, 14, 26). The infection was detected in 63.3% of the diarrhoeic animals and 69.2% of the non-diarrhoeic animals; however, this difference was not statistically significant ($p \geq 0.05$). The prevalence of infection and the intensity of oocysts were highest in cattle over three years of age in both the diarrhoeic and non-diarrhoeic groups. Henriksen and Krogh (9) found an infection rate of 5% in animals over two years of age at a (+) oocyst shedding level. In the present study, the infection rate was 57.8% in both diarrhoeic and non-diarrhoeic animals over three years of age at an oocyst shedding level of (+). The intensity of oocysts did not vary significantly in the faecal samples of diarrhoeic and non-diarrhoeic animals at

three levels. The present observations indicate that bovine cryptosporidiosis can be observed at any age. Since *Cryptosporidium* spp. infection was detected in both diarrhoeic and non-diarrhoeic animals with the same frequency and was not found to be associated with any severe or prolonged clinical manifestations, this agent is considered not to be a significant pathogen. Our observations, thus, support the suggestions that cryptosporidiosis is common in both healthy and diarrhoeic animals. In the survey region, the animals are kept in the same barn as the infected animals, which probably leads to reinfections. However, the infections were selflimited and did not seem to be of a severe clinical nature, similar to cases reported by Anderson (23, 27), who revealed that although at later ages calves appear to be more heavily infected, they do not show severe clinical symptoms. This may also be due to acquired immunity.

The present study demonstrates *E. coli* K 99 organisms to be distributed extensively not only in diarrhoeic animals but also in healthy ones. *E. coli* K 99 occurred in 32.1% of the diarrhoeic and in 25.5% of the non-diarrhoeic animals concurrently with cryptosporidiosis. Nevertheless, typical diarrhoea problems were not seen on these farms, nor was the pathogenic significance of the mix infection clear. These results are in accordance with previous studies in which *E. coli* K99 was reported in healthy calves intermixed with diarrhoeal calves (10, 28).

In this survey, rotavirus was not detected in any of the *Cryptosporidium*-positive animals. While other surveys have shown rotavirus to be of major importance as a non-bacterial agent of diarrhoea (13, 29), our observations show rotavirus to be uncommon, in fact non-existent, even in diarrhoeic animals. This finding is similar to those of Bulgin et al. (30).

Because the prevalence of the infection and the intensity of oocyst shedding were identical in both diarrhoeic and non-diarrhoeic animals, and because mix infections of *Cryptosporidium, E. coli* K 99 and rotavirus were not found to be associated with any severe clinical symptoms, this study provides no evidence of an association between diarrhoea and infection with *Cryptosporidium* spp., *E. coli* K99 or rotavirus in the animals examined. Cryptosporidiosis, as demonstated in the present study, is not only seen in young calves but also in older cattle, and can be a problem at all ages.

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