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The Prevalence of *Cryptosporidium parvum* in Lambs around Konya

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Abstract: This study was conducted between January and May 2003 in small-holder sheep farms in Konya. A total of 471 faecal specimens obtained from 1-60 – day-old lambs were examined for the oocysts and coproantigens of *Cryptosporidium parvum* by Modified Ziehl Neelsen (MZN) staining technique and ELISA. The oocysts and coproantigens of *C. parvum* were identified in 14 (2.97%) and 43 (9.13%) of the 471 lambs, respectively. The infected lambs examined in the study were asymptotically infected with *C. parvum*.

Key Words: *Cryptosporidium parvum*, lambs, prevalence, Konya

Konya Yöresindeki Kuzularda *Cryptosporidium parvum*'un Yaygınlığı

Özet: Bu çalışma, 2003 yılı Ocak-Mayıs ayları arasında, Konya merkez ve köylerindeki kuzularda *Cryptosporidium parvum* enfeksiyonunun yaygınlığını belirlemek amacıyla yapılmıştır. Bu amaçla, 1-60 günlük toplam 471 kuzu dışkıları Modifiye Ziehl Neelsen boyama metodu (MZN) ve ELISA tekniği kullanılarak *Cryptosporidium parvum* oocistleri ve coproantijenleri yönünden incelendi. MZN boyama metodu ile, 14 (% 2,97) dışkı örneğinde *C. parvum* oocistleri tespit edilirken, ELISA tekniği ile 43 (% 9,13) kuzu dışkısı *C. parvum* coproantijenleri yönünden pozitif bulundu. Bu çalışmada pozitif olarak tespit edilen kuzuların klinik ve mikroskopik muayeneleri, enfeksiyonun subklinik olduğunu göstermiştir.

Anahtar Sözcükler: *Cryptosporidium parvum*, kuzu, prevalans, Konya

Introduction

Cryptosporidium parvum is a protozoan parasite which in particular causes severe diarrhoea in neonatal ruminants, and is a primary aetiologic agent of neonatal diarrhoea syndrome (1-5). The most prominent clinical sign of ovine cryptosporidiosis is diarrhoea lasting 2 to 12 days and this is sometimes accompanied by anorexia, dehydration, reduced milk intake, growth retardation, stiffness, hyperpnoea, slow gait and depression (6-9). Concurrent infections with *Salmonella* sp., *Clostridium perfringens*, enterotoxigenic *Escherichia coli*, rotavirus, coronavirus and bovine diarrhoea virus may rarely occur, but it was stated that *Cryptosporidium* sp. was the primary pathogen (9). Cryptosporidiosis is of great importance because it causes high morbidity and mortality in animals. Human infections have been associated with exposure to infected animals, particularly calves and lambs (2,7,10-12).

The majority of the studies about cryptosporidiosis are related to human and calves. It is known that domestic livestock play an important role in environmental contamination by producing *C. parvum* oocysts. *Cryptosporidium* oocysts spread to animals through contaminated feed and water, with the faeces of clinically ill animals and latently infected animals serving as reservoirs of infection (11).

Cryptosporidiosis is usually diagnosed by microscopic examination of sporulated oocysts in faeces. The oocysts are bright and much smaller than coccidia and helminth eggs. Special stains are needed for *C. parvum* oocysts so that the protozoa can be distinguished from yeasts. A large number of staining techniques have been used to detect *Cryptosporidium* oocysts. The most widely used have been the modified acid-fast procedures, which differentiate red-stained oocysts from similarly sized and shaped green-stained yeast forms (10). 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 and needs an experienced microscopist, along with difficulty in the detection of oocysts in subclinical infections (13-18).

Humoral antibodies to *Cryptosporidium* sp. have been detected by indirect fluorescent antibody and enzyme-linked immunosorbent assay (ELISA) techniques. However, the non-specificity of antibody-based methods due to cross-reactivity with other microorganisms can be problematic (14). Direct fluorescent (DF) antibody tests with a specific antibody reagent have been used for identification of the organism in faeces. An ELISA test based on the detection of specific antigens in faecal specimens for diagnosis of cryptosporidiosis has the advantage of being more sensitive and less user-dependent than routine acid fast staining (13-18). A polymerase chain reaction technique has been developed recently, but to date it has not been tested in large clinical trials (16).

Cryptosporidium sp. was first reported in 1- to 3-week-old diarrhoeic lambs in Australia (19). Since then, it was reported that cryptosporidiosis has been associated with high morbidity and mortality in lambs in several countries (4,5,20-23). However, there are limited data on its prevalence rate in sheep. Ovine cryptosporidiosis was first detected by microscopic examination as 12% in diarrhoeic lambs in the vicinity of Elazığ in Turkey in 1989 (24). Erman et al. (25) detected *Cryptosporidium* spp. oocysts in 35 of 150 (23.3%) diarrhoeic lambs, and in 1 of 50 (2%) non-diarrhoeic lambs by carbol-fuchsin staining. No other reports have been published to date. The aim of this study was to determine the prevalence of *C. parvum* in lambs using the Modified Ziehl Neelsen (MZN) staining method and ELISA around Konya.

Materials and Methods

This study was conducted from January to May 2003.

Samples: A total of 471 faecal specimens were obtained from 1-60 - day-old lambs. Initially, faecal smears were prepared for microscopic examination, and stained with MZN staining for the diagnosis of *C. parvum* oocysts. Faecal specimens were stored in sodium-acetate-acetic-acid-formaldehyde (SAF) filled plastic bags at 2-6 °C until used for ELISA.

Microscopic examination: The microscopic examination of *C. parvum* was performed with MZN (26). For preparation, thin faecal smears were dried at room temperature and fixed in methanol for 2 min and then flooded with basic fuchsin solution for 5 min and rinsed in 50% ethanol. Then the slides were decolourised in 1% sulphuric acid for 2 min. Following washing, the slides were pooled in methylene blue solution for 1 min, washed and air dried, and examined under a 100 X objective.

The intensity of infection was assessed semiquantitatively by counting the numbers of oocysts in 20 randomly selected microscope fields under the immersion objective. The scores are categorised as negative (no oocysts), slight (1-5 oocysts), moderate (6-10 oocysts) and severe (>10 oocysts).

Enzyme immunoassay: *C. parvum* coproantigens were detected by a commercial ELISA kit (BioX diagnostics). Stool in SAF was processed according to the manufacturer's recommendations. Briefly, approximately 2 g faecal samples were diluted in buffer. The diluted samples were added to the wells of the microplate, which was coated with specific antibody of *C. parvum*. The plate was incubated at room temperature for 1 h, and rinsed with the washing solution. Then the diluted conjugate was 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 + substrate) 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 were read using a plate reader with a 450 nm filter (Anthos htll). 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.

Statistical analysis: The results were analysed by chi-square test (27).

Results

C. parvum was detected in 43 (9.13%) and 14 (2.97%) of 471 faecal samples by ELISA and MZN staining, respectively. MZN-positive specimens were also positive with ELISA. Twenty-nine specimens (6.35%) which were negative with MZN staining were positive with ELISA (Table 1).

Table 1. The prevalence of *Cryptosporidium parvum* infection in lambs.

MZN staining	ELISA		Total (%)
	Positive	Negative	
Positive	14	-	14 (2.97)
Negative	29	428	457 (97.03)
Total (%)	43 (9.13)	428 (90.87)	471

The prevalence of the infection according to the age groups was as follows: 4 (2.35%), 7 (4.73%), 2 (3.33%) and 1 (1.07%) by MZN, and 12 (7.06%), 19 (12.84%), 5 (8.33%) and 7 (7.53%) by ELISA, in the age groups of 1-7, 8-14, 15-30, and 31-60 days, respectively. The highest rate of *C. parvum* infection was in lambs 8-14 days old (Table 2).

None of the positive animals had diarrhoea. The low numbers of oocysts in the microscopic examinations indicated that the intensity of infection was slight.

Discussion

Clinical cryptosporidiosis has been reported mainly in lambs younger than 1 month of age (1). Diarrhoea occurring in clinical infections causes high mortality and morbidity in neonates (3,5-8). *Cryptosporidium* oocysts have been observed in clinically healthy animals as well as in infected animals (2,10).

In this study, *C. parvum* coproantigens and oocysts were detected in 43 (9.13%) and 14 (2.97%) of 471

faecal samples by ELISA and MZN staining, respectively. *Cryptosporidium* infection rates of lambs were low in the present study compared with previous studies conducted in other countries (5,8,20,21,27-29). The difference is thought to be potentially related to the non-diarrhoeic lambs in this study.

In the present study, subclinical *Cryptosporidium* infection was detected in 12 (7.06%), 19 (12.84%), 5 (8.33%) and 7 (7.53%) of lambs in the age groups of 1-7, 8-14, 15-30 and 31-60 days, respectively. *C. parvum* infection was most commonly detected in the group of lambs 8-14 days old whilst the infection rate was 9.52% (36 of 378) and 7.53% (7 of 93) in lambs 1-30 and 31-60 days old, respectively. No statistically significant differences were detected between the groups ($P > 0.05$).

The ingestion of low numbers of viable oocysts can initiate infection in susceptible lambs (11,22,30,31). In this study, the observation of the low numbers of oocysts by microscopical examination may indicate that the intensity of the infection was slight. Therefore, environmental contamination with small numbers of infective oocysts can be dangerous for livestock and public health.

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Table 2. The prevalence of *Cryptosporidium parvum* infection in different age groups of lambs.

Age groups (days)	Number of examined lambs	Number of positive lambs (%)	
		MZN	ELISA
1 - 7	170	4 (2.35)	12 (7.06)
8 - 14	148	7 (4.73)	19 (12.84)
15 - 30	60	2 (3.33)	5 (8.33)
31 - 60	93	1 (1.07)	7 (7.53)
Total	471	14 (2.97)	43 (9.13)
		$\chi^2 = 2.99$	$\chi^2 = 3.48$
		$P = 0.392$	$P = 0.323$

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