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Prevalence of mix infections of *Cryptosporidium spp., Escherichia coli* K99 and Rotavirus in the faeces of diarrhoeic and healthy cattle in Ankara, Turkey and in vitro resistance of *Escherichia coli* K99 to antimicrobial agents

Zişan EMRE, Hatice FİDANCI
Turkish Atomic Energy Authority
Lalahan Nuclear Research Institute in Animal Health Lalahan, Ankara-TURKEY

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Abstract: Faecal specimens from 172 diarrhoeic and130 healthy cattle were examined for the presence of *Cryptosporidium spp., Escherichia coli* K99 and *Rotavirus* in Ankara, Turkey. The prevalence of the *Cryptosporidium spp.* infection in diarrhoeic and non-diarrhoeic cattle was 63.3 % and 69.2 %, respectively. *Cryptosporidium* positive faecal specimens were examined for the presence of *E. coli* K99 and Rotavirus. *E. coli* K99 was isolated from the faeces of 35 diarrhoeic (32.1 %) and 23 healthy (25.5 %) animals. *Rotavirus* was not detected in any of the faecal samples. In vitro drug sensitivities indicated that *E. coli* K99 was sensitive to Nalidixic acid in both diarrhoeic group (91.2 %) and healthy group (87.5 %) of cattle. Virtually all *E. coli* K99 strains were resistant to Colistin sulphate, Ampicilline, Tetracycline and Neomycine in both groups.

Key Words: *Cryptosporidium, Escherichia coli* K99, *Rotavirus,* cattle, antibiotic susceptibility

Ankara ve yöresinde, sağlıklı ve ishalli sığır dışkılarında *Cryptosporidium spp., Escherichia coli* K99 ve *Rotavirus* mix enfeksiyonunun prevalansı ve *Escherichia coli* K99 suşlarının antimikrobiyel ajanlara in vitro direnci

Özet: Ankara ve çevresinden toplanan 172 ishalli ve 130 normal sığır dışkısında *Cryptosporidium spp., Escherichia coli* K99 ve *Rotavirus* karma enfeksiyonunun prevalansı araştırıldı. *Cryptosporidium spp.,* ishalli ve normal sığırlarda sırasıyla % 63.3 ve % 69.2 oranlarında görüldü. *Cryptosporidium spp.,* pozitif örneklerin *E. coli* K99 ile komplikasyon oranları ishalli dışkılarda % 32.1, normal dışkılarda % 25.5 olarak belirlendi. Dışkı örneklerinde *Rotavirus*'a rastlanmadı. İshalli 35 ve sağlıklı 23 sığırdan izole edilen *E. coli* K99 suşunun nalidiksik asit'e duyarlılıkları sırasıyla % 91.2 ve % 87.5 olarak bulundu. İzole ve identifiye edilen tüm *E. coli* K99 suşlarının kolistin sülfat, ampisilin, tetrasiklin ve neomisin'e çoklu direnç gösterdikleri saptandı.

Anahtar Sözcükler: Cryptosporidium, Escherichia coli K99, Rotavirus, sığır, antibiyotik duyarlılığı

Introduction

A variety of infectious agents including *Cryptosporidium spp., E. coli* K99 and *Rotavirus* have been considered as important causes of diarrhoeal disease of calves (1-5). These microorganisms are also present in the intestinal tract of clinically normal calves (1, 4, 6-8). *Cryptosporidium* is an intestinal protozoon parasite belonging to the same family as Isospora and Toxoplasma. It is an established cause of diarrhoea in many animal species (9, 10). Clinically normal animals may also be infected with *Cryptosporidium* (6, 9). Cryptosporidiosis has been recognized alone or in combination with other agents such as *E. coli* K99 and *Rotavirus* as contributing to diarrhoea (4-6, 10). *E. coli* K99 is a well-known enteropathogen of calves (1-4). This microorganism has been found to be distributed widely not only among diarrhoeal but also healthy cattle kept in the same barns (3, 8). In vitro drug sensitivities have indicated that a high percentage of *E. coli* has been resistant to drugs, especially to the drugs commonly used to treat calf diarrhoea (3, 5). The most common enteropathogen in diarrhoeic calves has shown to be *Rotavirus* (1, 2, 4).

The aim of this study was to investigate the frequency of occurrence of *Cryptosporidium, E. coli* K99 and *Rotavirus* in healthy and affected animals in Ankara and its environs, and to show in vitro sensitivity of *E. coli* K99 isolated from diarrhoeal and normal cattle to antimicrobials presently used.

Materials and Methods

Sample collection

For a period of one year, fresh faecal samples were collected from 172 diarrhoeic and 130 non-diarrhoeic

cattle in small-holder farms in Ankara. Each animal was sampled once and the clinical status of the animals was recorded.

Faecal examination

Cryptosporidium oocysts were identified by using Safranin-Methylene blue technique (11). *Cryptosporidium* positive faecal samples were subjected to microbial examination for the presence of *E. coli* K99 and *Rotavirus.* Isolates of *E. coli* were obtained after a primary plating of the faecal specimens on EMB, blood agar and Mc Conkey agar, then isolated coliforms were subcultured on Minco-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 specific antisera (12). The specific K99 antisera was kindly provided by Dr. M. Contrepois, INRA, Clermont-Ferrand, France. For the detection of *Rotavirus* in *Cryptosporidium* positive faeces, a commercially avaliable Enzyme Immunoassay kit was used (Rotascreen EIA, Mercia Diagnostics, Surrey, UK)

Antibiotic susceptibility testing

In vitro antibiotic susceptibility testing was done by the disc diffusion method of Kirby-Bauer (13). Antibiotics tested were Colistin sulphate (10 μg), Gentamicin (10 μg), Streptomycin (10 μg), Ampicillin (10 g), Trimethoprim-sulfamethoxozole (1.25 g-23.75 μg), Tetracycline (30 μg), Nalidixic acid (30 μg) and Neomycin (30 μg). Antibiotic sensitivities were assessed from the diameter of the zone of inhibition of growth around the disc. Isolates with reduced or no zone of inhibition were considered to be resistant in the test.

The relationship between diarrhoea and infection with each of the organisms was tested using a chi-square statistic appropriate for matched data (14).

Results

Cryptosporidium spp., were found in 109 (63.3 %) of 172 diarrhoeic animals and 90 (69.2 %) of 130 non-diarrhoeic animals. *Cryptosporidium* positive 109 diarrhoeic and 90 non-diarrhoeic faecal samples were examined for the presence of *E. coli* K99 and *Rotavirus. E. coli* K99 was isolated from 35 (32.1 %) of diarrhoeic and 23 (25.5 %) of non-diarrhoeic animals. *Rotavirus* was not detected in any of the *Cryptosporidium* positive faecal specimens (Table 1). Resistance of *E. coli* K99 strains from diarrhoeic and normal faecal samples to eight antibiotics is given at Table 2.

Discussion

Since *Cryptosporidium spp.,* infection was detected in both diarrhoeic and healthy animals in the same frequence, and was not found to be associated with any severe or prolonged clinical manifestations, this agent was not considered a significant pathogen. Our observations, thus, support the suggestions that cryptosporidiosis is common in both healthy and diarrhoeic animals (1, 4, 8). In the survey region, the healthy animals are kept in the same barn with the infected animals and this pro- bably causes natural immunity and latent infection. However, the infections were self-limited and did not seem to be a cause of a severe clinical manner; similar to cases reported by Anderson (15, 16).

The present study has shown that *E. coli* K99 organisms were distributed extensively not only diarrhoeal but also in healthy animals. *E. coli* K99 occurred in 32.1 % of diarrhoeic and in 25.5 % of non-diarrhoeic animals at the same time with cryptosporidiosis. Nevertheless, neither the typical diarrhoea problem were seen on these farms nor the pathogenic significance of the mix infection was clear. These results are in accordance with the previous studies in which *E. coli* K99 was reported for healthy calves intermixed with diarrhoeal calves (4, 6, 8).

In this survey, *Rotavirus* was not detected in any of the *Cryptospiridium* positive animals. While other surveys have shown the role of *Rotavirus* as the non-bacterial agent of diarrhoea to be of major importance (1, 3, 17). Our observations have shown that *Rotavirus* was uncommon, in fact non-existent, even in diarrhoeic animals. This finding resembled to that observed by Bulgin et al (5).

The comparison of 35 diarrhoeic and 23 non-diarrhoeic *E. coli* K99 isolates for their resistance to seven antibiotics showed that, a higher proportion of isolates in both groups were widely resistant to the antibiotics tested. Similar resistance patterns were observed by Bulgin et al. (5) and Coates and Hoopes (18). Gentamicin (3, 5, 18) and Colistin were previously found to be effective inhibitors of *E. coli* from domestic animals, however, in the present study *E. coli* K99 isolates were

Table 1. Prevalence of enteropathogens in diarrhoeic and non-diarrhoeic cattle

	No. positive samples/No.materials examined		
Organisms	Diarrhoeic	Non-diarrhoeic	Significance
Cryptosporidium spp.	109/172	90/130	p≥0.05 NS
Escherichia coli K99	35/109	23/90	p≥0.05 NS
Rotavirus	-	-	-

Table 2. Antibiotic resistance of E. coli K99 isolated from the faecal specimens

Antibiotics	Percentage of strains demonstrating resistance	
	Diarrhoeic (35 isolates)	Non-diarrhoeic (23 isolates)
Colistin sulphate	100	100
Gentamicin	76.4	79.1
Streptomycin	94.1	100
Trimethoprim-sulfamethoxozole	94.1	100
Ampicillin	100	100
Tetracycline	100	100
Nalidixic acid	8.8	12.5
Neomycin	100	100

only sensitive to Nalidixic acid in both diarrhoeic and healthy animals. This finding may be attributable to the common practice of using antibiotics indiscriminantly both prophylactically and therapeutically for diarrhoea. Drugs, such as Neomycin and Tetracycline, used extensively in milk replacers are becoming less effective for the control of enteritis because of the increasing resistance of enteric pathogens and such use may even be harmful.

Because of the prevalence of cryptosporidiosis was identical in both diarrhoeic and non-diarrhoeic animals, and because mix infections of *Cryptosporidium*, *E. coli* K99 and *Rotavirus* were not found to be associated with any severe clinical symptoms, it is possible to say that this study has provided no evidence of an association between diarrhoea and an infection with either *Cryptosporidium spp., E. coli* K99 or *Rotavirus* in the animals examined. The present study also supports the suspicion that prophylactic use of some drugs may be detrimental.

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