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
Lalaahan Nuclear Research Institute in Animal Health Lalahan, Ankara-TURKEY

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Abstract: Faecal specimens from 172 diarrhoeic and 130 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

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). Cryptosporidiosis is an intestinal protozoan 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
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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 available 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 μg), 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.

**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 frequency, 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 probably 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 _Cryptosporidium_ 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

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<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. positive samples/No.materials examined</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium spp.</td>
<td>109/172</td>
<td>p≥0.05 NS</td>
</tr>
<tr>
<td>Escherichia coli K99</td>
<td>35/109</td>
<td>p≥0.05 NS</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Prevalence of enteropathogens in diarrhoeic and non-diarrhoeic cattle
only sensitive to Nalidixic acid in both diarrhoeic and healthy animals. This finding may be attributable to the common practice of using antibiotics indiscriminately 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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### References


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