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Abstract

This study was carried out to investigate the presence of enterohaemorrhagic Escherichia coli (EHEC) of cattle that may pose a risk to human beings. Two hundred and forty (240) faecal samples were obtained from 8 randomly selected commercial cattle farms in Kaduna state, Nigeria. E. coli colonies from 76 (31.2 %) faecal samples were confirmed by Gram staining reactions and biochemically using indole, methyl red, Voges Proskauer and citrate (IMViC), triple sugar iron and motility tests respectively. Characterization of the isolates revealed three heterogeneous serogroups (O111, O118 and O126) from apparently healthy cattle, while no E. coli serogroup was isolated from diarrhoeic cattle. The prevalence of non-O157 isolates was 4.5 %. Association between the serogroups and source of samples (farms) was significant (P<0.05). The O126 serogroup isolated from apparently healthy cattle occurred more frequently, followed by O118 and O111 respectively. Although it is not known whether the presence of EHEC subgroups in apparently healthy cattle in the study areas may pose a health threat, it is safe to assume that the human population in these areas, including cattle rears and veterinarians, is at risk of exposure to the EHEC subgroups reported in the study. Data from the study possibly suggest cattle as important source of enterohaemorrhagic E. coli in Kaduna State, Nigeria.

Key Words: Cattle, enterohaemorrhagic Escherichia coli, serogroups, Nigeria.

1. Introduction

The term 'enterohaemorrhagic Escherichia coli ' (EHEC) was originally used to describe strains that cause haemorrhagic colitis (HC) and haemolytic-uraemic syndrome (HUS) (Nataro and Kaper, 1998), express shiga toxins (stx), cause attaching and effacing (A/E) lesions on epithelial cells and possess large plasmid. In accordance with the latest nomenclature, these strains are called shiga toxin-producing E. coli (formerly shiga-like toxin-producing E. coli) (WHO, 1998). Cattle appear to be the main reservoir of EHEC from which the organisms have been isolated (Clarke, 2001; Djordjevic et al., 2001). E. coli O111 is the most frequently implicated non-O157 strain causing gastroenteritis with HUS, particularly in the United States of America and Europe (Bettelheim, 2000; Pearce et al., 2006). Most studies indicated that majority of O111 serogroups were recovered from individuals with HC and HUS than from cattle (Bettelheim, 2003). Cattle and human O118 serogroups represent the same clones and are similar in virulence attributes. Evidence for zoonotic transmission of E. coli O118 serogroups have been documented (Buchanan and Doyle, 1997).

E. coli O126 has been reportedly isolated from the faecal samples of cattle and human beings. The serogroup O126 has not been implicated in cases of haemolytic uraemic syndrome (Buchanan and Doyle, 1997; Bettelheim, 2000). Some other non-O157 serogroups of EHEC have been implicated in diarrhoea, HC and HUS in humans (Eklund et al., 2001; Bettelheim, 2003). In the present study, we report the prevalence of enterohaemorrhagic E. coli from the faeces of cattle in Kaduna State, Nigeria for the first time.

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2. Materials and Methods

2.1. Study Area

The study area was Kaduna State, which is located between latitude 10°N and 11°N and longitude 7°E and 8°E, North-Western Nigeria. Eight commercial cattle farms were randomly selected from five different local government areas of Kaduna State, Nigeria.

2.2. Sample Collection

A total of two hundred and forty (240) faecal samples from apparently healthy (233) and diarrhoeic (7) cattle were collected from 8 randomly selected commercial farms using stratified sampling technique (Field and Graham, 2003). Faecal material (1-2 g) was aseptically collected from the rectum of each animal using clean disposable hand gloves. The samples were placed in separate sterile bottles containing 9-10 mL of tryptone soya broth (TSB) and kept in a cold box at 4 °C, and transported to the Bacteriology Diagnostic Laboratory, Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria and processed immediately.

2.3. Isolation and Identification of Suspected Colonies

Bacterial isolation, identification and biochemical tests were carried out using standard procedures described elsewhere (Barrow and Feltham, 1993; Cheesbrough, 2000). Briefly, samples were streaked on sorbitol macConkey agar and suspected positive colonies were confirmed using biochemical tests.

2.4. Biochemical Characterization

Colonies growing on sorbitol macConkey agar (SMAC) suspected to be E. coli were subjected to biochemical tests (indole, methyl red, Voges-Proskauer, citrate (IMViC), triple sugar iron, TSI and motility) (Cheesbrough, 2000).

2.5. Serogrouping of Somatic ‘O’ Isolates

All confirmed E. coli isolates were sub-cultured onto nutrient agar slants and stored at 4 °C for serogrouping (Blanco et al., 2006). Somatic ‘O’ isolates of enterohaemorrhagic Escherichia coli O111, O118 and O126 were identified using monospecific E. coli antisera (SIFIN Berlin, Germany) (Blanco, 2006).

2.6. Statistical Analysis

Data obtained from the apparently healthy and diarrhoeic cattle were analyzed using Student’s t-test and values of P<0.05 were significant.

3. Results

3.1. Spatial Distribution of Enterohaemorrhagic E. coli

Out of the 240 faecal samples collected from 8 randomly selected commercial cattle farms, the specific prevalence rate for each farm ranged between 0.0 % (Farm A, FA; Farm B, FB; Farm G, FG) and 17.4 % (Farm E, FE) respectively. A total of 11 (4.5 %) E. coli serogroups from apparently healthy cattle were found, of which 2 (8.7 %) isolated from Farm E (FE) and 1 (3.0 %) from FH were O111, 2 (8.7 %) from FE and 1 (4.4 %) from Farm F (FF) were O118, 1 (3.0 %) each from Farms C (FC), D (FD) and H (FH), and 2 (8.7 %) from farm F (FF) were O126 serogroups respectively. E. coli serogroup O126 occurred more frequently, followed by O111 and O118 respectively. All the farms had one or more serogroups, except FA, FB and FG where no E. coli serogroup was isolated. A prevalence rate of 2.1 % was recorded for E. coli O126 and 1.2 % each for O111 and O118 respectively. The prevalence rate of non-O157 which was 4.5 % was statistically significant (P<0.05) (Table 1).

Table 1. Distribution of E. coli serogroups among commercial cattle farms in Kaduna State, Nigeria

<table>
<thead>
<tr>
<th>Farm</th>
<th>Specific Prevalence (%)</th>
<th>O111</th>
<th>O118</th>
<th>O126</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Farm B</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Farm C</td>
<td>3.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Farm D</td>
<td>3.1</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Farm E</td>
<td>17.4</td>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Farm F</td>
<td>13.0</td>
<td>0.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Farm G</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Farm H</td>
<td>6.1</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>4.5</td>
<td>3.0</td>
<td>3.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

\[ \alpha = 0.04,\]

\[ P<0.05 \]

3.2. Distribution of Enterohaemorrhagic E. coli in Relation to Age

The age distribution of serogroups isolated from commercial cattle farms identified 1 (3.2 %) E. coli serogroup as O111, isolated from the young (0-1 year). E. coli O111, O118 and O126 were isolated from adults where O126 serogroup had the highest prevalence (7.4 %) rate. However, the specific prevalence varied among the adult species of cattle ranging between 4.5 % (in cattle older than 3 years) and 7.4 % (for those older than 1-2 years). The relationship observed between age and E. coli serogroups was not statistically significant (P>0.05) in this study.

3.3. Distribution of Enterohaemorrhagic E. coli in Relation to Breed

The specific prevalence rate ranged between 0.0 % (Holstein and Simmentals) and 8.1 % (Friesian) in the exotic breeds of cattle and 6.9% in locals (Rahaji). One (2.7 %) O111 and 2 (5.4 %) O126 serogroups were isolated from Friesian breed of cattle. At least one non-O157 serogroup was isolated from different types of local breeds. The relationship between breed and serogroups was not statistically significant (P>0.05).
3.4. Distribution of Enterohaemorrhagic E. coli in Relation to Sex

The relationship between sex and E. coli serogroups showed that E. coli serogroups were distributed according to the sex of cattle. A total of 1 (2.1 %) each for O111 and O118 serogroups and 2 (4.2 %) for O126 were isolated from males, while 2 (1 %) each for O111, O118 and 3 (1.5 %) for O126 were isolated in females. Overall, 11 (4.5 %) with one or more serogroups were identified. The relationship between sex and E. coli serogroups was not statistically significant (P>0.05).

3.5. Distribution of Enterohaemorrhagic E. coli in Relation to Health Status

The relationship between health status and E. coli serogroups plummeted (0 %) in diarrhoeic and increased (4.5 %) in apparently healthy cattle respectively. Thus, the relationship between health status and E. coli serogroups from commercial cattle farms was not significant (P>0.05) (Fig. 1).

![Figure 1](image.png)

Figure 1. Relationship between health status (%) and E. coli serogroups isolated from commercial cattle farms in Kaduna State, Nigeria

4. Discussion

The prevalence of E. coli non-O157 isolated from commercial cattle farms in Kaduna State, Nigeria, was 4.5 %. The authors found individual rates of 2.1 % in males and 1 % in females each for O111 and O118 serogroups respectively. This result agreed with the findings of Bettelheim (2003) and Pearce et al. (2006) who reported prevalence rate of 1-2 % for E. coli O111 and O118. Serogroup O126 is not frequently associated with disease in humans. Thus, the most common serogroups associated with disease in humans, which were also isolated from apparently healthy cattle in this study were E. coli O111 and O118. The prevalence of 1.7 % for O111 serogroup recorded in young animals agreed with the work of Blanco et al. (2000), who reported that calves are important reservoirs of E. coli non-O157. No E. coli serogroup was isolated from diarrhoeic cattle in this study, further supporting our suspicion that cattle may be reservoirs of colibacillosis in the area investigated.

The relationship between sex and the E. coli serogroups revealed that males (8.4 %) recorded higher number of E. coli non-O157 serogroups as compared to females (3.5 %). Montenegro et al. (1990) reported 11.6 % and 3.0 % prevalence rates in cows and bulls using DNA hybridization technique, but the serogroups, except E. coli O126 were different. In addition, differences in the areas of study and the changing dynamics of disease may have contributed to this disparity. It is not known whether the presence of EHEC subgroups in apparently healthy cattle, reported in the current study may pose a significant health hazard to human beings residing in the areas investigated. However, it is safe to assume that the human population in these areas, including cattle rearers and veterinarians, is at risk of exposure to the EHEC subgroups reported in the study. Thus, there is the need for veterinary and human public health officials to educate the communities on the public health hazards of colibacillosis. In conclusion, this study revealed that cattle are important reservoir of EHEC in Kaduna state, Nigeria and research should be carried out to establish the extent to which human beings are at risk of being exposed, especially in tropical Nigeria, where cattle owners maintain close contact with animals in the residential areas.

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References


