JJBS

Volume 6, Number 1, March .2013
ISSN 1995-6673
Pages 73 - 76

Jordan Journal of Biological Sciences

Short Communication

A Novel Report on the Prevalence of Enterohaemorrhagic *Escherichia coli* non-O157 Isolated from Cattle in Kaduna State, Nigeria

Jasini A. Musa[1], Mashood A. Raji[1], Haruna M. Kazeem[1]and Nicodemus M. Useh[1, 2,*]

[1]*Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria*

[2]*Laboratory of Molecular Biology of Infectious Diseases, Department of Population Medicine & Diagnostic Sciences, College of Veterinary Medicine, Cornell University, 14853 Ithaca, New York, United States of America*

Received: September 22, 2012; Accepted: November 24, 2012

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 rearers 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.

* Corresponding author. e-mail: nicodemus.useh@fulbrightmail.org.

2. Materials and Methods

2.1. Study Area

The study area was Kaduna State, which is located between latitude 10 ^{0} and 11 ^{0}N and longitude 7 ^{0} and 8 ^{0}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 ^{o}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 ^{o}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

Farm	Positive *E. coli* serogroups			
	Specific Prevalence (%)	O111	O118	O126
Farm A	0.0	0 (0.0)	0 (0.0)	0 (0.0)
Farm B	0.0	0 (0.0)	0 (0.0)	0 (0.0)
Farm C	3.0	0 (0.0)	0 (0.0)	1 (3.0)
Farm D	3.1	0 (0.0)	0 (0.0)	1 (3.0)
Farm E	17.4	2 (8.7)	2 (8.7)	0 (0.0)
Farm F	13.0	0 (0.0)	1 (4.4)	2 (8.7)
Farm G	0.0	0 (0.0)	0 (0.0)	0 (0.0)
Farm H	6.1	1 (3.0)	0 (0.0)	1 (3.0)
Total	4.5	3 (1.2)	3 (1.2)	5 (2.1)
$\alpha^2 = 0.04$, 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).

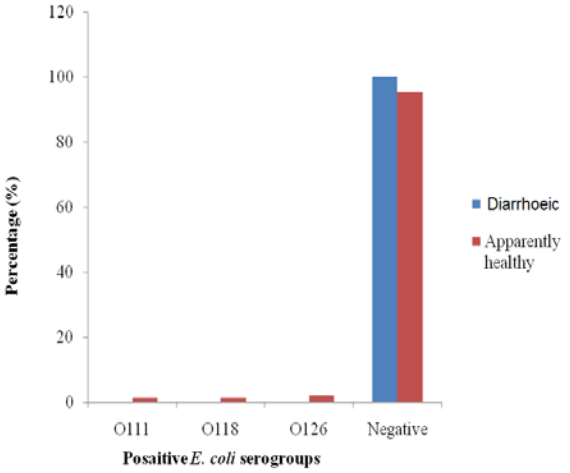

Figure 1. Relationship between health status (%) and *E. coli* serogroups isolated from commercial cattle farms in Kaduna State, Nigeria

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.

Acknowledgements

E. coli monospecific antiserum was generously donated by David Bukbuk, Head, Department of Veterinary Microbiology & Parasitology, University of Maiduguri, Nigeria. Manuscript was prepared using a facility provided by Professor Dr. Heinrich Neubauer, Director, Friedrich-Loeffler-Institute of Bacterial Infections and Zoonoses (*Clostridium* National Reference Laboratory), Federal Research Institute for Animal Health, Jena, Germany via a German Research Foundation (DFG) fellowship to NM Useh for which the authors are most grateful.

References

Barrow G I and Feltham R K A. 1993. **Cowan and Steel's Manual for the Identification of Medical Bacteria**, 3rd Edition. Cambridge University Press.

Bettelheim K A. 2000. Role of non-O157 verocytotoxin-producing *Escherichia coli* (VTEC). *J Applied Microbiol*., **88**: 38-50.

Bettelheim KA.2003. Non-O157 verotoxin-producing *Escherichia coli*: A problem, paradox, and paradigm. *Experimental Biol Med*., **228**: 333-344.

Blanco M, Blanco J E, Mora A, González E A, Blanco J. 2000. Serotypes and virulence genes of verocytotoxigenic *E. coli* (VTEC) isolated from cattle in Spain. In: Duffy, G., Garvey, D., Coia, P.J., Wasteson, Y. and McDowell D.A. (Eds),**Verocytotoxigenic *E. coli* in Europe, 3. Pathogenicity and virulence of verocytotoxigenic *E. coli***. Teagasc. The National Food Centre, Dublin.

Blanco JA. 2006. A manual: Kit for *E. coli* serotyping, including O2, O26, O78, O86 and O141 sera. *Laboratorio de Referencia de E. coli (LREC)*, Espana, pp. 1-7.

Blanco M, Blanco J E, Bahbi C A, Mora A, Alonso M P, Varela G, Gadea M P, Schelotto F, Gonzalez E A and Blanco J. 2006. Typing of intimin (eae) genes from enteropathogenic *Escherichia coli* (EPEC) isolated from children with diarrhoea in Montevideo, Uruguay: Identification of two novel intimin variants (μB and §R/β2B). *J Med Microbiol*., **55**: 1165-1174.

Buchanan R L, Doyle M P. 1997. Food borne disease significance of *Escherichia coli* O157:H7 and other enterohaemorrhagic *E. coli*. *Food Technol*., **51:** 69-76.

Cheesbrough M. 2000. **District Laboratory Practice in Tropical Countries.** Lower price editions, part 2, Cambridge University Press.

Clarke S C. 2001. Diarrhoeagenic *Escherichia coli*–an emerging problem? *Diag Microbiol Infect Dis*., **41**: 93-98.

Djordjevic S P, Hornitzky M A, Barley G, Gill P, Vanselow B, Walker K and K. A. Bettelheim. 2001. Virulence properties and serotypes of shiga toxin-producing *Escherichia coli* from healthy Australian slaughter-age sheep. *J Clin Microbiol*., **39**: 2017- 2021.

Eklund M, Scheutze F and Siitonen A. 2001. Clinical isolates of non-O157 shiga toxin-producing *Escherichia coli*: serotypes, virulence, characteristics and molecular profiles of strains of the same serotypes. *J Clin Microbiol*., **39**: 2829-2834.

Field A and Graham J H. 2003. **How To Design Experiments**. Sage publications Ltd.

Montenegro M A, Bulte M, Trumpf T, Aleksic S, Reuter G, Bulling E and Helmuth R. 1990. Detection and characterization of faecal verotoxin-producing *Escherichia coli* from healthy cattle. *J Clin Microbiol*., **25**: 1417-1421.

Nataro J P and Kaper J B. 1998. Diarrhoeagenic *Escherichia coli*. *Clin Microbiol Rev*., **11**: 142-201.

Pearce M C, Evans J, McKendrick I J, Smith A W, Knight H I, Mellor D J, Woolhouse M. E J, Gunn G J and Low J C. 2006. Prevalence and virulence factors of *Escherichia coli* serogroups O26, O103, O111, and O145 shed by cattle in Scotland. *Applied Environ Microbiol*., **7**: 653 659.

World Health Organization (WHO). 1998. Zoonotic non-O157 shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO scientific group meeting, Berlin, Germany. Department of Communicable Disease Surveillance and Response. June, 23-26, pp.1-38.