Open Journal of Veterinary Medicine, 2016, 6, 89-94
Published Online June 2016 in SciRes. http://www.scirp.org/journal/ojvm
http://dx.doi.org/10.4236/ojvm.2016.66011

A Cross Sectional Sero-Study of Verocytotoxigenic *Escherichia coli* (VTEC) Serotypes in Apparently Healthy and Diarrhoeic Cattle in Abuja, Federal Capital Territory (FCT), Nigeria

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Received 21 May 2016; accepted 18 June 2016; published 21 June 2016

Abstract

It is reckoned worldwide that verocytotoxigenic *Escherichia coli* (VTEC) serotypes are important food borne pathogens causing severe health problems in humans. A cross sectional epidemiological study was carried out to determine the prevalence of VTEC serotypes (O157 and non O157) in both apparently healthy and diarrhoeic cattle in Abuja, FCT. A total of 718 faecal samples collected from abattoirs and cattle herds from Abuja, FCT representing 381 from apparently healthy and 337 from diarrhoeic cattle were analyzed. Primary isolation of typical *E. coli* was done using Eosin Methylene Blue (EMB) agar and performing biochemical tests. Samples were further analyzed using Cefixime, Tellurite-Sorbitol McConkey (CT-SMAC) agar to identify sorbitol and non sorbitol fermenting *E. coli*. Further characterization of both the sorbitol fermenting and non sorbitol fermenting *E. coli* was done using commercially procured latex agglutination test kits from Oxoid, United Kingdom. The prevalence of VTEC O157 in apparently healthy cattle was 1.84% and 2.96% for diarrhoeic cattle while the prevalence of non O157 VTEC was 3.67% and 7.12% for apparently healthy and diarrhoeic cattle respectively. There was no strong association ($p > 0.05$) between faecal consistency and infection with VTEC O157. A strong association ($p < 0.05$) however existed between faecal consistency and infection with non-O157 VTEC. Diarrhoeic cattle appear likely to be more affected. The implication of the study is that individuals in contact with cattle such as veterinarians, abattoir workers and cattle herdsmen are at risk of exposure to VTEC and proper hygienic control measures should be adopted.

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How to cite this paper: Enem, S.I., Oboegbulem, S.I., Okoli, C.E. and Godwin, E.E. (2016) A Cross Sectional Sero-Study of Verocytotoxigenic *Escherichia coli* (VTEC) Serotypes in Apparently Healthy and Diarrhoeic Cattle in Abuja, Federal Capital Territory (FCT), Nigeria. *Open Journal of Veterinary Medicine*, **6**, 89-94. http://dx.doi.org/10.4236/ojvm.2016.66011

Keywords

Sero-Study, VTEC Serotypes, Apparently Healthy, Diarrhoeic, Cattle

1. Introduction

Verocytotoxigenic *Escherichia coli* are highly significant zoonotic threat to public health globally. The serogroup O157 is particularly important and recently non O157 serogroups namely, O26, O111, O103 and O45 have emerged and been associated with severe food borne illness in humans [1]. A study in Irish cattle showed that while VTEC O157 are being carried by cattle presented for slaughter in Ireland, a number of other verotoxin producing strains such as O26, O111, O103 and O145 are beginning to emerge [2].

Food borne spread of VTEC infection usually results from well recognized lapses in food handling, notably failure to achieve adequate cooking temperatures [3] [4] or contamination of ready-to-eat products [5]. Their low infective dose combined with the severity of symptoms associated with the infection make them a significant concern [1], poses an occupational risk to caterers and others who handle food, mainly because of its low infective dose [6].

VTEC rarely cause disease in animals and ruminants are recognized as their main natural reservouir [7] [8]. Cattle are considered to be the major animal source of VTEC that are virulent to humans and the ecology of this microorganism in cattle farming has been extensively studied [9]. Harbouring of *E. coli* O157 in cattle is a significant concern for public health because of their transmitting capability to humans through contaminated foods and water with faeces from cattle [10] [11]. Monitoring of ruminants is essential to evaluate risk factors associated with VTEC infection in humans. Faeces, hides, and pre-chilled carcasses are the best samples to monitor VTEC at slaughter and to compare data among countries [12].

Although, *E. coli* O157 is the most renowned VTEC, other serogroups, including O26, O111, O103, O145 and O121 have the potential to cause serious human illness [13] [14]. Six non-O157 groups have been identified by the Centre for Disease Prevention and Control [15] as being responsible for over 70% of non-O157 VTEC—associated illness (O26, O45, O103, O111, O121 and O145) [16]. A prevalence of VTEC O157, O26, O111 and "O not determined (OND)" as 6.3, 3.8, 0.6 and 2.5 percent respectively was also reported [17]. Unlike O157 VTEC, some non O157 VTEC such as O5, O26, O111 and O118 can be isolated from calves with diarrhea [18] [19].

The aim of this study was to assess the prevalence of VTEC O157 and non-O157 serotypes in apparently healthy and diarrhoeic cattle in Abuja, FCT with the view to raising awareness amongst population at risk to fashion out creative hygienic means of controlling the infection.

2. Materials and Methods

A cross sectional epidemiological study was used in this research which was carried out between May, 2011 and April, 2012. Faecal samples were collected from 718 cattle in selected cattle herds (5) and slaughter houses (3) in Abuja, FCT. Of that number, 381 were collected from apparently healthy cattle while 337 faecal samples were from diarrhoeic cattle. Among the cattle herds selected, 137 were calves (less than one year old) while 221 were adults. All the samples collected from abattoir were from adults as calves were not routinely slaughtered in abattoirs. Faecal samples were collected from freshly voided faeces to identify and differentiate the diarrhoic from the apparently healthy. It is reported that isolation rates may be improved by taking voided faecal samples in preference to rectal swabs [20].

Samples (about 0.5 g in each case) were first inoculated into 5 ml of an enriched media (Buffered Peptone Water supplemented with 8 mg/l vancomycin, 10 mg/l cefsulodin and 0.05 mg/l cefixine (BPW-VCC) and incubated for 37˚C for 6 - 8 hrs [21] to suppress the growth of gram positive organisms. Confirmed *E. coli* samples showing typical greenish sheen colouration when cultured into eosin methylene blue (EMB) agar were subcultured into cifixine—tellurite sorbitol McConkey (CT—SMAC) agar. The non sorbitol fermenters (NSF) and the sorbitol fermenters (SF) were further characterized using latex agglutination test kits obtained from Oxoid ltd, Hampshire, UK. The test kits were used according to the specifications of the manufacturers.

3. Results

Faecal samples were collected from both cattle herds and slaughter houses. Of the 358 samples analyzed from cattle herds, 207 were from apparently healthy while 151 were from diarrhoeic cattle. Of the 360 samples from slaughter cattle, 174 were from apparently healthy while 186 were from diarrhoeic cattle (**Table 1**). The prevalence of VTEC O157 is 1.84 in and 2.96 in apparently healthy and diarrhoeic cattle respectively (**Table 2**) while the prevalence of VTEC non O157 was 3.67% and 7.12% in apparently healthy and diarrhoeic cattle respectively (**Table 3**). The specific prevalence for the non O157 VTEC isolated from cattle in FCT was determined and the prevalence for O26 was highest (**Table 4**). The "O" not determined ("O"ND) represents the serotypes that were not typed in this research due to unavailability of the serocheck agglutination test kits specific to them.

Pearson's chi square was used to analyze the significance of the faecal consistency to the infection with both VTEC O157 and non-O157 VTEC. There was no strong association ($p > 0.05$) between faecal consistency and infection with VTEC O157. A strong association ($p < 0.05$) however existed between faecal consistency and infection with non-O157 VTEC. Diarrhoeic cattle appear likely to be more affected.

4. Discussion

Verocytotoxigenic *Escherichia coli* (VTEC) have become a very important and world-wide reported food-borne pathogen. In this study, an assessment of the prevalence of VTEC O157 and non O157 serotypes were carried out and the result indicated prevalence for VTEC O157 of 1.84% and 2.96% for apparently healthy and diarrhoeic cattle respectively. The prevalence for non O157 VTEC was 3.67% and 7.12% for apparently healthy and diarrhoeic cattle respectively. The result of the finding is closely related to other findings reported in published literatures [22]-[24].

Table 1. Sample collection analysis.

Subject	No collected	Apparently healthy	Diarrhoeic
Cattle herds	358	207	151
Slaughter cattle	360	174	186
Total	718	381	337

Table 2. Prevalence of VTEC O157 in Apparently Healthy and Diarrhoreic cattle.

Type cattle	No tested	No positive	% positive
Apparently Healthy	381	7	1.84
Diarrhoeic cattle	337	10	2

($p > 0.05$).

Table 3. Prevalence of VTEC non O157 in Apparently Healthy and Diarrhoreic cattle.

Type cattle	No tested	No positive	% positive
Apparently Healthy	381	14	3.67
Diarrhoeic cattle	337	24	7.12

($p < 0.05$).

Table 4. Specific prevalence of non O157 VTEC in apparently healthy and diarrhoeic cattle.

Subject	No collected	% positive	O26	O10	O145	O111	O91	"O"ND
Apparently Healthy	381	14	3	2	3	2	1	3
Diaorrhoeic	337	24	7	5	4	1	2	5
Total	718	38	10	7	7	3	3	8
Prevalence %		5.25	1.31	0.97	0.97	0.42	0.42	1.11

A wide range of prevalence estimates ranging from 0.1% to 62% of E. coli O157 in cattle was reported worldwide [10] [11] [25]. Bonardi [17] reported a prevalence of VTEC O157, O26, O111 and "O" not determined as 6.3%, 3.8%, 0.6% and 2.5% respectively. A study in Irish cattle showed that while VTEC O157 are being carried by cattle presented for slaughter in Ireland, a number of other verotoxin producing strains such as O26, O111, O103, O145 are beginning to emerge [2]. Roopnarine [26] detected by dry spot test E. coli isolates with prevalence of 2.2%, 2.2%, 4.4% and 6.7% belonging to non O157 strains O91, O111, O103 and O157 respectively.

In this study, the specific non O157 VTEC isolated were O26 (1.31%), O'ND (1.11%), O103 (0.97%), O145 (0.97%), O111 (0.42%) and O91 (0.42%) in the descending order of number of isolation. Brooks [27] in a twenty year study in USA, confirmed the importance of non O157 VTEC strain in human infection pointing out that the most common were O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%) and O145 (5%). EFSA, [12] reported that a restricted range of serotypes (*i.e*. O26, O103, O91, O145 and O111) are associated with public health. Bettelheim, [28] stated that O26 VTEC should be considered as pathogen for both cattle and humans being isolated from sick and healthy cattle (ratio 4:3) as well as sick and healthy people (ratio 76:3).

The percentage of positive isolates in diarrhoeic cattle exceeded that of apparently healthy in this study. For VTEC O157, diarrhoeic was 2.97% as against the 1.84% for apparently healthy. Also, for non O157 VTEC, diarrhoeic was 7.12% as against the 3.67% for apparently healthy. Sanz [29] recovered VTEC strains from 10 (23%) of 43 calves with diarrhea, from 24 (29%) of 83 healthy calves, from 40 (44%) of 91 healthy cows waiting at the slaughter house and from 6 (22%) of healthy grazing cattle. Mohammad [30] reported the isolation from cattle and buffalo calf diarrhea, serotypes of verocytotoxigenic *E. coli* strains.

5. Conclusion

The assessment of the prevalence of VTEC O157 and non O157 serotypes in cattle herds and abattoirs in Abuja, FCT showed that both apparently healthy and diarrhoeic cattle carry the organism in certain degrees. Population at risk (abattoir workers, butchers, cattle herdsmen, veterinarians) should adopt proper hygienic and safety measures to tackle the problem of VTEC infection in cattle in the capital territory. Diorrhoeic cattle should be treated before presenting them for slaughter to prevent spread of VTEC and other infections. Farmers' awareness campaign should be raised on the public health implication of VTEC infection.

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