

Research Article

Epidemiology of Verocytotoxigenic Escherichia Coli (Vtec) O157 Serotype in Cattle in Federal Capital Territory, Abuja, Nigeria

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Abstract

Verocytotoxigenic Escherichia coli (VTEC) are a group of pathogenic E. coli bacteria that are causing major food safety concerns across the globe. Historically, infection has been most commonly associated with VTEC serogroup O157 in most parts of the world. The objective of the study was to establish the occurrence of VTEC O157 serotype in cattle in the Federal Capital Territory (FCT) Abuja, Nigeria. The strength of association of the infection with sex, age and season were also determined. A cross sectional epidemiological design and multi stage sampling technique were used. Samples of freshly voided faeces were collected from both apparently healthy and diarrhoeic cattle in selected abattoirs and cattle herds. Enriched samples were analyzed bacteriologically and biochemically after which they were characterised using commercially procured sorbitol MacConkey and latex agglutination test kits. A total of 718 faecal samples from cattle were analyzed for the presence of VTEC O157. Seventeen (2.4%) were positive. Three hundred and fifty eight were from cattle herds out of which 8 (2.23%) were positive while 360 were from abattoir out of which 9 (2.25%) were positive. There was no strong association ($p > 0.05$) between sex and infection with VTEC O157. A strong association ($P < 0.05$) existed between age and infection with VTEC O157. Calves were more likely to be infected than the adults. There was also a strong association ($P < 0.05$) between season and infection with VTEC O157 and infection was more likely to occur in the dry season than the wet season. As a major food animal in Nigeria, infection in cattle provides an epidemiological causal association to the infections in humans.

ABBREVIATIONS

VTEC: Verocytotoxigenic Escherichia coli

INTRODUCTION

Since 1977, it has been recognised that some diarrhoeagenic strains of Escherichia coli produce toxins that have an irreversible cytopathic effect on cultured vero cells - a line of African green monkey kidney cells [1,2]. Such verocytotoxigenic Escherichia coli (VTEC) have been shown to comprise of over 100 different serotypes of O157 and non O157 VTEC [3,4]. *E. coli* O157 and other VTEC infections cause a spectrum of illness, from mild non bloody diarrhoea to haemorrhagic colitis [5]. Haemolytic uraemic syndrome (HUS) in which renal failure is accompanied by haemolytic anaemia; thrombocytopenia and central nervous

system manifestation complicates 2 - 7% of cases after the onset of diarrhoea [6].

Cattle are believed to be the principal reservoir for the VTEC [7]. and consumption of improperly cooked ground beef contaminated with bovine faeces at slaughter is an important risk factor for human infection [8]. The first reported cases of VTEC infection were linked to the consumption of undercooked minced beef [9]. and since then minced beef has been implicated in numerous outbreaks [10]. Unpasteurised milk, cream and cheese made from unpasteurised milk are other foods of animal origin implicated in VTEC [11].

The number of organisms believed to be sufficient to cause infection is low (as low as 10 cfu) compared to Campylobacter (500 organisms) and Salmonella (100, 000) (CDC, 2008). VTEC

can survive the high acidity of the stomach and with such small numbers capable of causing disease, infection can occur without any growth of the bacteria in food. The incubation period for VTEC infection ranges from 1 – 8 days but is typically between 2 and 4 days [12]. The relatively long incubation period of VTEC has significant implications for investigation. It makes recall bias (inability to remember accurately what foods were eaten or where the patient was during the possible exposure period) more likely [13].

The distribution of *E. coli* O157: H7 and other VTEC is global. Among countries with surveillance systems, the incidences of VTEC infections vary widely, reflecting differences in incidence, diagnostic activity, and reporting. High incidence has been reported from regions of Canada, Scotland, and Argentina [14]. In most European countries and the USA, the annual incidence may range from one to four infections per 100 000 population [14]. Available literature revealed that there is less shedding of the organisms in cattle of slaughter age than in younger cattle [15] reported that excretion was significantly associated with age and was greatest in cattle under 24 months. Most countries record the highest incidence rate of VTEC infection in late summer. Studies have found that peak *E. coli* O157 faecal shedding rates occur during summer and early fall, and they vary from as low of 10% to as high as 61% on some farms [16].

VTEC O157 constitute an emerging enteric zoonotic disease. In Nigeria, the infection has been reported in different parts of the country but no work has been done in FCT. Some of the reported cases include [17,18] in [19] in Plateau state. There is need to investigate any significant differences in sex, age and season with the infection in FCT, Nigeria.

In FCT, Abuja, cattle settlements are scattered in the Area Councils giving rise to close interaction between humans and livestock and their owners. The exposure potentials of the infection to human population are enhanced therefore by the extensive livestock farming practice and the livestock production activity in FCT and environ. The food culture and habits of indigenes and residents of FCT who consume beef and other dairy products purchased from unhygienic retail outlets may also predispose them to the infection.

Food borne spread of VTEC disease usually results from well recognized lapses in food handling, notably failure to achieve adequate cooking temperatures [20,21] or contamination of ready-to-eat products [22] VTEC poses an occupational risk to caterers and others who handle food, mainly because of its low infective dose [12] Withdrawal of feed before slaughter, however, can lead to increased shedding of *E. coli* that presumably can include VTEC [23] These problems may be addressed, in general terms, by the hazard analysis and critical control point (HACCP) approach [24] which makes food businesses responsible for assessing the risks in their food preparation procedures and for instituting appropriate control measures.

The objective of the study is to establish the occurrence and prevalence of VTEC O157 in cattle in the Federal Capital Territory, Nigeria. No work has been done on VTEC O157 in FCT hence the need for this work. Information generated from this study will help in defining the epidemiology of VTEC in Nigeria and in designing effective control measures

MATERIALS AND METHODS

The study was conducted in the Federal Capital Territory (FCT), Abuja consisting of six Area Councils namely; Abuja municipality, Bwari, Gwagwalada, Kuje, Kwali and Abaji. Abuja is located at the centre of the country between latitude 8° and 9° 25' North of the equator and longitude 6°45' and 7°45' East of Greenwich Meridian [25]. The temperature varies in different area councils [26]. The area has a tropical climate marked with two distinct seasons – rainy season (April to October) and dry season (November to March). The study population consisted of selected cattle herds and slaughter cattle from abattoirs and slaughter houses in FCT. A multi-stage sampling technique was used in selecting Area Councils, Cattle herds/abattoir and the animals.

Faecal samples were collected from cattle in both the abattoirs and cattle herds. The samples were collected from freshly voided faeces on the ground. It is thought that isolation rates may be improved by taking faecal samples in preference to rectal swabs, by increasing the sample size, by increasing the number of individual samples and by repeat sampling [27] All the faecal samples collected were transported to the laboratory for analysis under aseptic conditions. Suspected colonies ex-EMB were subjected to biochemical tests [28] for confirmation as typical *E. coli*. These were done before inoculation into sorbitol MacConkey (SMAC) and Cefixime-Tellurite sorbitol MacConkey (CT-SMAC) agar for the identification of VTEC O157 and non O157 serotypes. *E. coli* isolates ex –EMB were subcultured into plates of SMAC and CT-SMAC. The plates were incubated at 37° C for 18-24 hours Non sorbitol fermenting (NSF) isolates that appear as colourless or neutral gray with smokey centre and 1-2mm in diameter on the two plates were presumptive of *Escherichia coli* O157 [29] Sorbitol fermenting isolates that appear pinkish in colour were presumptive of *Escherichia coli* non O157 [29]

Commercially procured latex agglutination test kits from Oxoid Ltd, Hampshire, England were used to further characterise the isolates that were non sorbitol fermenting.

RESULTS

Faecal samples from a total of 718 cattle were analysed for the presence of VTEC O157. Three hundred and fifty eight (358) of the samples were from cattle herds while 360 were from slaughter cattle. Out of the total number screened, 17 (2.4%) were positive for O157. The number of samples positive for O157 for cattle herds were 8 (2.23%) and for slaughter cattle 9 (2.25%) (Table 1).

The chi square analysis of the result showed that there was no significant association between sex and VTEC infection in cattle (Table 2).

The age distribution was carried out for cattle herds only where the age of the animals could be determined (Table 3). The animals were further categorised according to their specific age groupings (Table 4). The strength of association for O157 was determined using the Fisher's exact test and the value 0.006 obtained was less than the p value 0.05. The level of significance for VTEC non O157 tested using chi square analysis was 0.003 which is less than p value 0.05 ($X^2 = 9.029$). This indicated a strong association between age and infection with VTEC in cattle.

Fisher's exact test was also used to check whether there is a significant association between season of the year and prevalence of VTEC infection in cattle (Table 5). The level of significance was 0.000 for VTEC O157 which is less than p value 0.05. Chi square was used to test for non O157 and the value 5.651 is greater than X^2 value 3.841 and the p value was 0.017 which is less than p value 0.05. This indicates a very strong association between season and infection of cattle with VTEC.

DISCUSSION

In recent years, *Escherichia coli* (*E. coli*) O157, has emerged as a pathogen of increasing importance, responsible for numerous outbreaks of gastro-intestinal infection worldwide. The result of

this research indicated that the prevalence rate of VTEC O157 in FCT (2.4%) was within the range of other findings. Prevalence rates for VTEC O157:H7 among cattle vary from less than 1% to 36%, depending on the country, type of herd studied and other [30]. Published research on *E. coli* O157:H7 in cattle has reported prevalence rates of 1.8% [31] 3.4% [32] 7.5% [33] 8% of adult cows [34] and 15.7% [35]. [36] Reported that the proportion of animals infected with verotoxin-producing *E. coli* varied from 0 – 60%. In England and Wales VTEC O157 was recovered from 0.47% of beef carcasses at slaughter and from 0.83% of faeces from live cattle submitted for diagnostic purposes in 1994 and 1995 [37-38] in a research carried out in Ireland between 1998-1999 isolated 6 out of 250 samples of VTEC O157 tested, reporting a prevalence rate of 2.4% in faeces of slaughtered cattle. A United Kingdom-wide survey of beef carcass contamination in 1994/5 recovered VTEC O157 from 0.47% of carcasses and suggested that cross-contamination was probable [15]

There was no significant association ($P > 0.05$) between sex and infection with VTEC O157 in cattle in this study. This agreed with the work carried out by [39] in Danish Dairy farms in which they reported a non significant tendency of bull calves to have a higher prevalence than heifers within the age group of 2-6 months. Although more female cattle were carriers of the organism than the male in this study, the result of the statistical analysis suggested a non significant association. The higher prevalence in females may not be unconnected to the role they play as mother in gathering food for the calves especially during scarcity. [40] studied VTEC prevalence among dairy, feedlot and cow-calf herd in Washington, the prevalence for Stx gene (6%) in samples from feedlots was significantly lower than those from dairy (20%) and range (Stx: 21%) facilities.

The age distribution was carried out only in Cattle herds because most cattle presented for slaughter in the abattoir were adults. There was a strong association ($P < 0.05$) between age and infection with VTEC O157 in cattle. Calves were more associated with infection than the adults. [39]. in their work on Danish Dairy farms reported that a strong effect of age was seen with 2-6 month old calves as the high-risk age group (8.6% positive) in contrast to calves less than 2 months (0.7% positive) and cows (2.4% positive) [41]. reported that in concurrence with previous studies, it appears that cattle and in particular less than 14 week old weanling calves are the primary reservoir for VTEC on the dairy farm. In this work, specific age distribution showed that calves of less than 6 months were more affected with 4 positive compared to those of other ages.

Samples were collected in two seasons (dry and wet) in this study and there was also a strong association ($P < 0.05$) between season and infection with VTEC O157 in cattle. The dry season was more associated than the wet season. Studies have found that *E. coli* O157 faecal shedding rates occur during summer and early fall, and they vary from low of 0% to as high as 61% on some farms [16]. Literature showed that no factors have been identified, other than season that consistently affect the *E. Coli* O157 shedding rates in cattle [16,32]. These data on cattle carriage of O157 correlate with the seasonal variation in the incidence of human disease [42].

Table 1: Prevalence of VTEC O157 in Cattle.

Subject	No tested	positive	% positive
Cattle herds	358	8	2.23
Slaughter cattle	360	9	2.25
Total	718	17	2.4

Table 2: Sex Distribution of VTEC O157 in Cattle.

Sex	Total tested	positive	No negative
Male	332	5	326
Female	386	12	373
Total	718	17	699

($X^2=1.981$; $p=0.159$) for VTEC O157
($X^2=2.336$; $p=0.126$) for VTEC non O157

Table 3: Age Distribution of VTEC O157 in Cattle herds.

Age	Total tested	positive	No negative
Calves	137	7	130
Adults	221	1	220
Total	358	8	350

(Fisher's exact test =0.006) for VTEC O157
($X^2=9.029$; $p=0.003$) for VTEC non O157

Table 4: Age specific distribution of VTEC O157 in cattle herds.

Age group	No tested	VTEC O157
		Positive
<6 months	78	4
7-12 months	59	3
13-24 months	109	1
>25 months	112	-
Total	358	8

Table 5: Seasonal Distribution of VTEC O157 in Cattle.

Season	Total tested	No positive	No negative
Dry	397	14	381
Wet	323	3	320
Total	718	17	701

(Fisher's exact test =0.000) for VTEC O157
($X^2=5.651$; $P=0.017$) for VTEC non O157

CONCLUSION

The study established the occurrence and prevalence of VTEC O157 in cattle in FCT, Abuja, Nigeria. Cattle are major food animals in Nigeria and infection with VTEC portends an epidemiological causal association to the infection in humans. The result showed that warmer seasons (dry season) stimulate the presence of VTEC infection in animals and thus, as a consequence, increases the number of human cases. The prevalence was also higher in younger calves (< 6 months) probably as a result of undeveloped immune system. In Nigeria, many farmers still engage in traditional husbandry systems which predispose the animals to infections (VTEC inclusive). The bio security system and other farm management practices remain very poor and could harbour micro-organisms. Humans also live in close contact with their livestock such as cattle, sheep and goat, pigs and chicken. These food animals interact freely and closely with their owners and handlers and pose a risk in the epidemiology of VTEC. Fulani herdsmen and their women who tend the flock and milk the cows may get infected since cattle remain the major ruminant reservoir of VTEC (CDC, 2008). [43] All stakeholders involved in livestock and food industry in the country must therefore brace up to the challenge of control and eradication of the infection.

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