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Epidemiology of Verocytotoxigenic *Escherichia Coli* (Vtec) Non O157 Serotypes in Cattle in Abuja, Nigeria

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Abstract: ***The most frequently implicated E. coli serotype causing haemorrhagic colitis and haemorrhagicuraemic syndrome (HUS) is VTEC 0157. However, non – O157 VTEC is now known to be as prevalent as VETC O157 infection (or even more) in most parts of the world. The objective of the study was to establish the occurrence of non O157 VTEC serotypes in cattle in the Federal Capital Territory (FCT) Abuja, Nigeria. The level of significance of the infection with sex, age and season were also tested. The research work took place at Abuja, the capital of Nigeria. Abuja lies between 6^{o} 45'and 7^{o} 45'East of the Greenwich meridian and 8^{o} and 9$^{o.}$ 25'North of the Equator The cross sectional epidemiological method and multi staged sampling technique were used in this study. Samples were collected from the freshly voided faeces of 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 prepared latex agglutination test kits. A total of 718 faecal samples from cattle were analyzed for the presence of VTEC non O157. Thirty eight (5.23%) were positive for non O157. No significant difference (p > 0.05) existed between O157 VTEC infection and sex of the cattle. A significant difference was found between age and infection with non O157 cattle. More calves were associated than the adult cattle. There was also a significant association (P < 0.05) between season and infection with non - O157VTEC in cattle. Infection occurred more in the dry season. The study established the occurrence and prevalence of non O157VTEC in cattle in FCT, Abuja, Nigeria. As a major food animal in Nigeria, infection in cattle provides 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.***

Keywords: Cattle, Distribution, Prevalence, Verocytotoxigenic Escherichia coli (VTEC) non O157 serotypes.

1. Introduction

Verocytotoxigenic *Escherichia coli* (VTEC) were first recognized as a cause of serious human illness in 1982 following two outbreaks of gastro intestinal illness in the United States among 47 customers of a fast food restaurants chain [1]. The report described a clinically distinctive gastrointestinal illness associated with *E. coli* O157: H7 apparently transmitted by under cooked meat. Since then, VTEC have been recognized worldwide and have caused numerous large outbreaks by a variety of transmission vehicles. Other serogroups of VTEC are sometimes called non – O157 VTEC [2]. *E. coli* O157:H7 is far exceeded by the non – O157 VTEC serotypes, several of which have been associated with human illness [3]. Some non – O157 serotypes including O26; O103, O111 and O145 have been associated with occasional outbreaks of human disease and others still with sporadic cases [4].

The predominant VTEC serotype associated with outbreaks and sporadic cases of serious VTEC illnesses is VTEC O157[5] and despite early recognition of non O157 VTEC strains as human pathogens [4], VTEC O157 remains the major focus of clinical and food diagnostic laboratories in many jurisdictions. However, over 380 other serotypes of VTEC has been isolated in humans [5] and increasing awareness of non O157 VTEC as causes of human illness has prompted expanded clinical diagnosis, investigation and surveillance of these organisms[6].

Non O157 VTEC serotypes are seriously emerging as an enteric zoonotic disease. In Nigeria,[7] reported isolation of VTEC serotypes in feacal and milk samples from adult cattle and calves; however, none of the isolates belonged to VTEC O157. Unamba-Opara et al. [8], at Nsukka, Nigeria, stated that of the 50 strains of randomly selected sorbitol positive samples tested, 4% was positive for VTEC group.

VTEC represent the only pathogenic group of *E. coli* that has a definite zoonotic origin, although not all the VTEC strains have been demonstrated to cause disease in humans. Six non – O157 groups have been identified by the Centre for Disease Prevention and Control [2] as being responsible for over 70% of non – O157 STEC – associated illness (O26, O45, O103, O111, O121 and O145) [9]. Both the European Food Safety Authority (EFSA) and the U.S. Department of Agriculture (USDA) have issued recommendations for laboratory testing for these pathogens [10], [11]. There are considerable differences in the ratio between cases of VTEC O157 and VTEC non – O157 infection. These differences are likely to reflect that in some countries surveillance is mainly based on laboratory methods specific for VTEC O157 only [12].

The prevalence of non – O157 VTEC in Canadian beef appears to be at least equal to and is possibly greater than that of O157: H7 VTEC. A 1990 study found that of 225 samples of ground beef from Ontarian meat processing plants, 82 (36.4%) were positive for verotoxin production by verocell assay. Verocytotoxigenic*E. coli* were isolated from

10.4% of the positive samples and none of the isolates was of the O157 serotype [13]. Testing of 400 ground beef samples from Calgary and Winnipeg in 1989 – 1990 recovered 8 O157:H7 isolates and 18 non – O157 isolates [14].

In Abuja 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 [3].

There is no published record of any study on non – O157 VTEC in Abuja, FCT, Nigeria hence the need for this research. The main objective of the study is to establish the prevalence and distribution of non O157 VTEC in FCT, Nigeria.

2. Materials and Methods

The study was carried out in the Federal Capital Territory (FCT) Abuja which is located between latitude 8^{o} and 90 25` North of the equator and longitude 6$^{o}$ 45` and 7^{o}45` East of the Greenwich meridian [15]. The study population included cattle herds and slaughtered cattle at randomly selected abattoirs. The study was cross sectional and a multi-staged sampling technique was used in selecting the area councils, sites and animals. Three area councils out of six in FCT were selected randomly in this study by balloting namely: Gwagwalada, Kuje and Abuja Municipality.

Faecal samples from seven hundred and eighteen (718) cattle were analyzed for the presence of non – O157 VTEC. Three hundred and fifty eight (358) of these samples were from cattle herds while 360 were from slaughtered cattle. Samples were collected from both apparently healthy and diarrheic cattle in selected abattoirs and cattle herds. Precautions were taken to avoid cross-contamination of samples in transit and at the laboratory. An enrichment media of buffered peptone water (BPW) supplemented with 8mg/litre vancomycin, 10mg/litre cefsulodin and 0.05g/litre cifixine (BPW-VCC) was used to suppress the growth of gram positive organisms, *Aeromonas*and *Proteus*spp. [16]. About 0.5g of faecal sample was inoculated into 5ml of the prepared BPW-VCC and incubated at 37% for 6-8hours[16].

E. coli samples were identified by first culturing them in plain McConkey agar then subcultured into eosin methylene blue (EMB) agar incubated for 37^{o}C for 18-24 hours. Isolates exhibiting the typical greenish sheen coloration were subjected to biochemical tests for further confirmation. The isolates ex- EMB were subcultured into plates of sorbitol McConkey (SMAC) and cifixine – tellurite sorbitol McConkey (CT-SMAC) agar. The sorbitol fermenters (SF) stored in nutrient agar slants were further characterized using latex agglutination test kits (sero screen and polyvalent serocheck) commercially procured from Oxoid ltd, Hampshire, England and used according to the manufacturer's specifications.

3. Result

The prevalence of VTEC non – O157 in cattle was 5.23%. The prevalence for cattle herds was 5.85% while that for slaughtered cattle was 5.0% (Table I). The prevalence of the specific non – O157 serotypes in cattle in FCT was determined (Table II and Fig.1). Ten (10) samples (1.31%) were positive for O26 (6 in cattle herds and 4 in slaughter cattle). Seven (7) samples (0.97%) were positive for O103 (3 in cattle herds and 4 in slaughter called). Seven (7) samples (0.97%) were also positive for O145 (4 in cattle herds and 3 in slaughter cattle). Three (3) samples (0.42%) respectively were positive for O111 (2 in cattle herd and 1 in slaughter cattle) and O91 (none for cattle herd and 3 for slaughter cattle). Eight other non – O157 VTEC serotypes not specific to the kits ordered from Oxoid, England were considered untyped (5 for cattle herds and 3 for slaughter cattle).

There is no association between sex and VTEC infection in cattle. The age distribution was carried out for cattle herds only (Table IV). There was a strong association between age and infection with VTEC in cattle (Table V). Chi square was used to test whether there is a significant association between season of the year and prevalence of non O157 (Table VI). A strong association existed between season and infection of cattle with non O157 VTEC.

4. Discussion

Verocytotoxin producing *E. coli* (VTEC) have emerged as significant pathogens causing a range of severe and potentially fatal illnesses. It is now apparent however that *E. coli*serogroups other than O157 (non - O157 VTEC) also make a significant contribution to human diarrhea disease and in many European countries, are isolated more frequently than VTEC O157[17]. This study was carried out to establish the occurrence of non - O157 VTEC in cattle in the Federal Capital Territory. Faecal samples from a total of 718 cattle were analyzed and 38 (5.23%) were positive.

Global testing of beef cattle faeces revealed prevalence rates range for non – O157 VTEC of 2.1 to 70.1% [18] while faecal testing of dairy cattle worldwide showed prevalence rates for non – O157 VTEC from 0.4 – 74.0%[19]. The prevalence rate of VTEC non – O157 in cattle in FCT, Nigeria fell within the range of this and other published research work. The testing of 400 ground beef samples from Calgary and Winnipeg in 1989-1990 showed a prevalence rate of 0.45% for non – O157 VTEC [14]. The value of 5.25% recorded in this work may be due to the fact that in infected herd; carrier animals may be shedding the organism in low numbers, thus making isolation difficult. Even as that, shedding is also intermittent in the faeces and recovery of the organism may prove rather difficult. This is collaborated by [20] which reported that shedding is influenced by the age of the animals, diet, stress, population density, geographical location and season.

Our result may also have been influenced by the random sampling selection method which may not have favoured the

sampling of some animals known as 'super shedders'; which are thought to contribute disproportionately to transmission of infection [21].

In this research work, 026 with prevalence of 1.31% (10) was highest followed by O103 and O145 with prevalence of 0.97% (7 each) then O111 and O91 with prevalence of 0.42% (3) respectively. According to EFSA [11] report, a restricted range of serotypes (i.e. O26, O103, O91, O145 and O111) are associated with public health risks. In USA, a twenty year study that confirmed the importance of non O157 VTEC strain in human infections, pointed out that the most common were O26 (22%), O111(16%), O103 (12%), O121(8%), O45(7%) and O145(5%) [6]. As described by [22], 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 from sick and healthy people (ratio 76:3). The O26 VTEC serogroup probably occurs exclusively in cattle, their foodstuffs and humans [22]

The picture of the dominance of the O157:H7 serogroup as a cause of VTEC illness in North America has been challenged by the findings of studies indicating that non – O157 may be the cause of 30% to 50% of VTEC illness [23]. Reports from other countries including those that export beef to Canada and the USA, indicated that non – O157 VTEC are of primary importance in those countries [22].

There was no significant association ($P > 0.05$) between sex and infection with non - O157 in cattle in this study. This is in agreement with the work carried out by [24] 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. There was a strong association ($P < 0.05$) between age and infection with non - O157 in cattle. Calves were more likely to be infected than the adults. Nielson et al.[24] in their work on Danish Dairy farms reported that a strong effect of age was seen, with 2-6 month old calves being the high-risk age group (8.6% positive) in contrast to calves less than 2 months (0.7% positive) and cows (2.4% positive). Age specific distribution in this study showed that calves less than 6 months were more affected, with 7 positive for non O157. A strong difference ($P < 0.05$) existed between season and infection with non - O157 in cattle. The dry season was more associated than the wet season. Griffin [25], reported seasonal variation in the cattle carriage of VTEC and correlated it with the seasonal variation in the incidence of human disease.

5. Conclusion/Recommendation

The findings in this work show that non – O157 VTEC are widely distributed in cattle in FCT, Nigeria. The non – O157 VTEC serotypes found in FCT is similar to the ones obtained in other parts of the world. The study revealed that non – O157 VTEC is prevalent in cattle, a major food animal thus suggesting an epidemiological causal association to the infection in humans. The presence of these pathogens on bovine farms and abattoirs in Abuja, Nigeria is of serious public health implication; emphasis should be focused on the consequences of infecting consumers through the consumption of contaminated beef and beef products.

A clean carcass initiative should be adopted at all abattoirs, applying a policy of zero tolerance to visible contamination on carcass and red offal. Pre and post meat inspection operations should be appropriately carried out. Routine extensive screening of herds, abattoirs for VTEC should be an ongoing policy. Campaigns should be undertaken at the farm level to raise farmers' awareness on the serious illnesses caused by VTEC and their role in its control. The results of this work and the recommendations have been communicated to relevant ministries and agencies.

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Table 1: Prevalence of VTEC Non – O157in Cattle

Subject	No tested	No positive	% Positive
Cattle Herd	358	20	5.58
Cattle Slaughter	360	18	5.0
Total	718	38	5.23

Table 2: Distribution of VTEC Non – O157 In Cattle

Subject	No positive	O26	O103	O145	O111	O91	Others Untyped
Cattle Herd	20	6	3	4	2	-	5
Cattle Slaughter	18	4	4	3	1	3	3
Total	38	10	7	7	3	3	8
Prevalence %	5.25	1.31	0.97	0.97	0.42	0.42	1.11

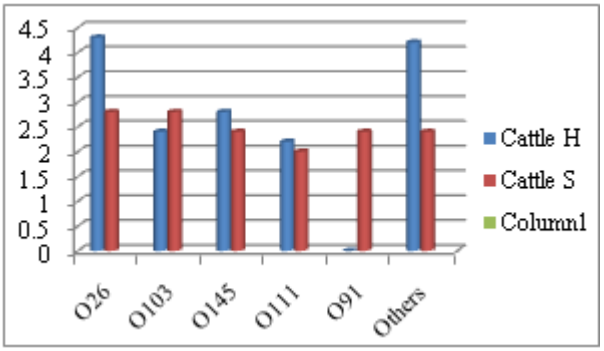

Figure 1: Distribution of VTEC non – O157 in Cattle

Table 3: Sex Distribution of Non – O157 VTEC in Cattle

Sex	Total tested	No positive	Non negative
Male	332	13	319
Female	386	25	386
Total	718	38	680

($p>0.05$)

Table 4: Age Distribution of Non – O157 VTEC In Cattle Herds

Age	Total tested	No Positive	No negative
Calves	137	14	123
Adults	221	6	215
Total	358	20	338

($p<0.05$)

Table 5: Specific Age Distribution in Cattle Herds

Age group	No tested	No positive
< 6 months	78	7
7 – 12 months	59	3
13 – 24 months	109	3
>25 months	112	1
	358	14

Table 5: Seasonal Distribution of Non O157VTEC In Cattle

Season	Total tested	Total positive	Total negative
Dry	397	28	367
Wet	323	10	313
Total	718	38	680

($p<0.05$)