2023 MULTIDISCIPLINARY CONFERENCE ON SUSTAINABLE DEVELOPMENT
Timisoara, 25-26 May

OCCURRENCE OF VERO-TOXIGENIC PRODUCING *E. COLI* (VTEC) IN SOME COMMERCIAL LIVESTOCK FARMS IN KANO STATE, NIGERIA

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Abstract: *Vero-toxin-producing E. coli (VTEC) have gained increasing global concern as food-borne pathogens and are the only diarrhoeagenic E. coli pathogroup with an ascertained zoonotic origin, with ruminants being regarded as the main animal reservoir. This study aimed at determining the occurrence of VTEC in some commercial livestock farms in Kano State, Nigeria. A total of 240 samples were collected from the three Agro-climatic zones in Kano State with 80 samples per zone. The samples were processed in accordance with the International Standards Organisation reference method (ISO 16654) for the isolation of E. coli ,195 (81.3%) samples were suggestive of E. coli on Eosin Methylene Blue Agar (EMB). The Isolates were further screened biochemically [42 (17.5%) isolates are positive] and on CT Smac agar for selection of E. coli 0157 where 30 (12.5%) isolates were suggestive of E. coli 0157. These isolates were further screened using Latex Agglutination Test, where 24 (10%) isolates were confirmed to be E coli 0157. This study showed that Cattle from commercial livestock farms shed E. coli 0157 in their feaces. E. coli 0157 is widely distributed across commercial livestock farms.*

• Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC) are members of a set of pathogenic *Escherichia coli* strains and are significant food borne pathogen associated with serious disease outbreak globally (7, 13). Verocytotoxin-producing *Escherichia coli* are the major zoonotic food-borne organisms causing several illnesses in both humans and animals (8).
VTEC can be divided into sub-groups based on the presence of biochemicals called O antigens on the cell surface and further sub-divided into serotypes (also called serovars) based on the presence of different H flagella and K capsular antigens (19). The bacterium has the ability to produce a potent cytotoxins called Shiga toxins (*Stx*), which are the most virulence factor during pathogenesis of diseases caused by the VTEC (12). The *stx2* is more significant than *stx1* in terms of causing severe diseases in human and importantly linked with increased risk of haemolytic uremic syndrome in Verocytotoxin-producing *E. coli* infection (17).
Ruminants are the major source of VTEC with cattle as the principal reservoir (10, 12). Enterohaemorrhagic *Escherichia coli* (EHEC) is the most important subset of verotoxin producing *E. coli* (VTEC) and can cause a broad spectrum of clinical manifestations in humans ranging from asymptomatic infections to mild diarrhoea or haemorrhagic colitis which occasionally progresses to haemolytic uraemic syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults (14). People are vulnerable to HUS after direct contact with faeces or consumption of animal products that are contaminated such as meat, milk and milk products (10).
The study determined the occurrence of Vero-toxin producing *E. coli* (VTEC) in some commercial livestock farms in Kano State through cultural isolation, biochemical characterization and latex agglutination test from feacal sample. It also determined the occurrence of E. coli 0157 between different breeds and sex of animals in commercial livestock farms in Kano State.

• Material and method

Study area
All samples were collected from within Kano state. Kano State is the commercial center of Northern Nigeria and is the second largest city in Nigeria after Lagos. According to the 2006 census, Kano is the most populous state in Nigeria, with about 9,383,682 million people. There are two distinct seasons; wet season (May - September) and dry season (October-April). The temperature ranges between 21- 39°C (22).
It is situated in Sudan savanna zone within latitude 13053'N and 10025'N and longitude70 0'E and 10053'E. Farming is the main occupation of its people, who are predominantly Hausa/Fulani engaged in production of crops like millet, sorghum, maize, cowpea, groundnut, pepper, onion, etc., and rearing animals like cattle, sheep, goat, poultry, etc. (9). KNARDA has divided Kano State into three administrative division called Agricultural Development Programme (ADP) zones namely, zone I, zone II and zone III.

Study Design
A cross sectional Study was utilized to collect feacal samples from some livestock farms within the State. The farms were selected using simple random sampling method from the three agricultural zones in the State namely Zone I (Rano Zone), Zone II (Danbatta Zone) & Zone III (Gaya Zone). A total of 240 samples were collected from the three Agro-climatic zones in Kano State with 80 samples per zone. Systematic random method was used to obtain the samples from the animals on the farm with every fourth animal being selected. The faecal samples were taken from each of the sampling animals via rectal scooping of the faeces using a sterile polythene bag, and were transported to the Department of Veterinary Public Health and Preventive Medicine Laboratory, Ahmadu Bello University Zaria inside ice packed cool box for analysis.

Isolation and identification of *Escherichia coli*
The procedure was in accordance with the International Standards Organisation reference method (20) for isolation of *E. coli*.
Primary culture – 10g of the sample was suspended on to 90mls of 0.1% of peptone water and then homogenise. Then 10mls of the homogenised sample was inoculated on 90mls of tryptone soy broth then incubated at 37°c for 24hrs for enrichment. A loopful of the overnight culture was streaked on Eosin Methylene Blue (Oxoid, U.K.) then incubated at 37°c for 24hrs for the detection of *E. coli* through production of greenish metallic sheen colonies.
Secondary culture –The well- separated colonies from above were picked up and inoculated on nutrient agar (Oxoid, U.K.) slants and incubated at 37°C then stored at 4°Cfor further identification.

Biochemical test
Colonies growing on nutrient agar slants were subjected to further biochemical tests namely; Simmon citrate, Urea, Triple Iron Sugar (TSI), sulfate, Indole, Motility (SIM), Methyl Red (MR), Vogesproskeur (VP). Various reactions of the tests such as color change, motility and gas formation were used to interpret results as either positive or negative after 24 hours incubation. These tests were carried out as described in the methodology of (6).
Positive isolates were also be further characterized using sugars; Glucose, Xylose, Lactose, Mannitol, Sorbitol and Sucrose.
Isolates that were identified biochemically as *E. coli* were further screened on Cefixime Tellurite Sorbitol MacConkay agar (CT-SMAC, Oxoid Basingstoke, UK) by incubating at 37° C for 24 hours.

Latex agglutination test
Colonies that appeared colorless on CT-SMAC (nonsorbitol fermenters) were presumed to be *E. coli* O157 and were preserved on Nutrient agar slants for further confirmation using Latex agglutination test (pro-Lab Diagnostics, Richmond Hill, Canada).

Data Analysis
Data were presented in forms of charts and tables. SPSS version 20.0 was used where analysis such as chi-square (χ^2) was used to show association between categorical variables.

• Results and discussions

Out of the 240 samples ,195 (81.3%) of the isolates were suggestive of *E. coli on Eosine Methylene Blue (EMB) agar*. On further biochemical test, 42 (17.5%) were suggestive of *E. coli*. Thirty isolates (12.5%) were suggestive of *E. coli* 0157 on CT smac. These isolates were further screened using Latex Agglutination Test where 24 (10%) isolates were confirmed to be *E coli* 0157: H7 (Table 1). These showed high occurrence of *E.coli* O157: H7 (10% of isolates). High occurrence of *E.coli* O157: H7 was also reported by (11) of 8.7% from healthy cattle in Spain. Similarly, In Egypt, (16) reported prevalence 6.7% from raw milk, rectal swabs from apparently healthy and diarrhoeic calves and stool samples of children. Also researchers such as, (4, 5) reported occurrence of 33.9% and 36.7% of VTEC respectively. VTEC shed in faeces of ruminants can contaminate the environment, water sources, and cause diarrhoeal related infections when this contaminated water is used as drinking water without treatment (15). Evidence has shown that contact with animal faeces is a risk factor for sporadic *E. coli* O157:H7 infection (9).

Table 1
Isolation of *E. coli* O157: H7

Test	Total number Sampled	Number Positive	%
EMB	240	195	81.3
Biochemical	240	42	17.5
CT Smac	240	30	12.5
Latex Agglutination	240	24	10.0

Table 2
Distribution of *E. coli* O157: H7 across the Agro-climatic Zones

Zones	Total Sampled	Number Positive (%)
Zone I	80	9 (11.3%)
Zone II	80	9 (11.3%)
Zone III	80	6 (7.5%)
Total	240	24 (10%)

$\chi^2 = 0.833$, df =2, p value =0.6590

The prevalence of *E. coli* O157: H7 across the Agro climatic zones was 11.3% for Zone I & II, and 7.5% for Zone III. The relationship between the prevalence and location of the Zones o is not statistically significant (Table2). Occurrence of VTEC was higher in Zone I and Zone II. These are areas with more intensification of livestock farming, intervention programs and size capacity of than those of Zone III. This may pose hazard and increased risk of exposure to the human beings residing in the areas investigated.
The occurrence of *E. coli* O157: H7 among the various breeds was; White Fulani 10%, Bokolo 13.3%, Crosses 5.7% and Friesians 13.3%. But the relationship observed in this study was not statistically significant (Table 3). The occurrence was higher in Bokolo and Friesians than that of crosses and white Fulani. The Bokolo and Friesians are mostly kept in conventional and high capacity farms. By implication, there might be more chances of spread of the organism among the breeds on these farms.
The sex distribution of *E. coli* O157: H7 was observed as Males 7.9% and Females 25.7% and the differences between is statistically significant (Fig. 1). The occurrence is higher in female animals than male animals. Since traditionally female animals are kept for longer periods than male animals, there will be prolonged period of shading of this organism in the environment .

Table 3
Breed Distribution of *E. coli* O15: H7

Breed	Total Sampled	Number Positive (%)
White Fulani	80	8 (10%)
Bokolo	60	8 (13.3%)
Crosses	70	4 (5.7%)
Friesians	30	4 (13.3%)
Total	240	24 (10.0%)

$\chi^2 = 2.540$, df =3, p value =0.4680

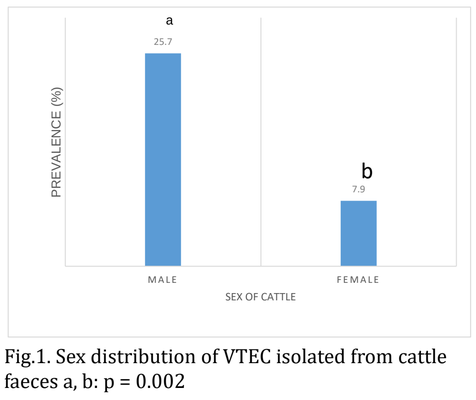

Fig.1. Sex distribution of VTEC isolated from cattle faeces a, b: p = 0.002

• Conclusions

This study has established that Cattle from the commercial livestock farms in Kano state shed *E. coli* O157: H7 in their feces, *E. coli* O157: H7 is widely distributed across commercial livestock farm. There's is need for public enlightenment about preventive and control measures of spread of Pathogenic *E. coli* in farm and environment; Further studies are required to ascertain the genes of *E. coli* O157: H7 circulating on these farms.