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PREVALENCE AND SERO-GROUPING OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) IN CATTLE AND HUMANS IN MUBI ADAMAWA, NORTH-EASTERN NIGERIA

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ABSTRACT

The objectives of the study were to determine the prevalence and sero-group of Shiga Toxin-producing *Escherichia coli* (STEC) in cattle and humans in Adamawa, North-Eastern Nigeria. A commercially prepared ***E. coli*** **Non-O157 Identification Kit, Prolex™ was used to sero-group the isolates.** Presumptive *E. coli* colonies on nutrient agar (CM3, Oxoid) slants were picked and streaked on to sorbitol McConkey agar cultures (SMAC) (CM813, Oxoid) and then incubated for 24 hrs at 37^0C. The Prevalence of STEC in Adamawa is found to be 9.3% and 1.2% among cattle and humans respectively with overall prevalence of 5.25%. The results of STEC sero-grouping in this study revealed that all the STEC isolates were within the six known STEC sero-groups reported in literature. It is worth noting to have 56 STEC isolates from cattle of which all tested positive for at least one of the

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Introduction:

There are over 200 STEC serotypes identified, but many have not been implicated in livestock and human illness (Bonnet *et al.,* 1998; Paton and Paton 1998; Karmali *et al.,* 2010). A restricted range of serotypes such as O157, O26, O103, O91, O45 and O111 are associated with public health risks, and these serotypes are most

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six STEC serotypes except serotype O45. Out of the overall 63 STEC isolates sero-grouped, 8 (12.69%) of the isolates were O157 and 55 (87.31%) were non – O157seroypes. The non – O157serogroups were O26, 25 (39.68%), O91, 18(28.57%), O103, 7(11.1%) and O111, 5 (7.9%). The presence of STEC sero-types in faeces of these animals coupled human detection of same strains indicate the level of poor hygiene and danger associated to food safety. It is therefore, recommended that, there should be improvement in slaughter house hygiene and meat handling to minimize the risk of human infections.

Keywords: Sero-Grouping, *Escherichia Coli*, Livestock, Humans, Nigeria.

frequently isolated from food animals (Willshaw *et al.,* 2001; Bennett and Bettelheim, 2002). However, other serotypes are becoming a cause of serious public health concern in the world. For instance, serotype O157 have been extensively studied and shown to be involved in many cases and outbreaks of human diseases *(*Bell *et al.,* 1994; Conedera *et al.,* 1997; *Chapman, 1997; Elder et al.,* 1997; Tarr *et al.,* 2005*). In addition, infections caused by non-O157 serotypes have also been frequently associated with severe illness in humans* (Griffin *et al.,* 1991; Pradel *et al.,* 2000; Moses, 2005).

In Nigeria, studies conducted at the south-western part reported the isolation of *E. coli* 0157:H7 and other pathogenic *E. coli* strains from human patients with diarrhoea (Olorunshola *et al.,* 2000; Okeke *et al.,* 2000a and 2003b). Similarly, the isolation of non-O157 STEC and some EPEC serotypes was reported from feaces of diarrheic calves collected from various farms in Zaria, North-central Nigeria (Tekdek *et al.,* 1995). Also in North-Eastern Nigeria, Moses, (2005) isolated STEC O157 from HIV infected patients and non-O157 from human and cattle faeces. The incidence of STEC in camel calves diarrhoea, although not studied extensively, has been reported in Eastern Sudan (Mohammed *et al.,* 2000) and recently, Rahimi *et al.* (2010) reported the prevalence of STEC O157:H7 in camel carcass during processing in Iran. The prevalence and distribution of STEC sero-groups O157, O26, O45, O91, O103 and O111 which are associated with public health risks are unknown in cattle and humans around Adamawa in spite of the poor public health situations. It is against this background that this study was conceived to determine the prevalence and sero-

group of Shiga toxin-producing *E. coli* (STEC) isolated from cattle and humans in Mubi-Adamawa State, Nigeria.

MATERIALS AND METHOD

The Study Area

Adamawa State is located at the area where the River Benue enters Nigeria from Cameroon Republic and is one of the six states in the North-East geopolitical zone of Nigeria. It lays between latitudes 7^0 and 11^0 North of the Equator and between longitudes 11^0 and 14^0 East of the Greenwich Meridian (Mohammed, 1999). It shares an international boundary with the Republic of Cameroon to the East and interstate boundaries with Borno to the North, Gombe to the North-West and Taraba to the South-West (Adebayo, 1999; ASMLS, 2010), as shown in Figure 1.

Figure 1: Map of Nigeria Showing Adamawa State

According to Adebayo and Tukur (1997), Adamawa State covers an area of land mass of about 38,741km^2. The state is divided into three Senatorial Zones

(Northern, Central and Southern) which translates to three agricultural zones as defined by INEC (1996), which are further divided into 21 Local Government Areas (LGAs) for administrative convenience.

The major occupation of Adamawa people is farming. The mineral resources found in the state include iron, lead, zinc and limestone (Adebayo & Tukur, 1997).

The state has minimum and maximum rainfall of 750 and 1050 mm per annum and an average minimum and maximum temperature of 15^0C and 32^0C, respectively. The relative humidity ranges between 20 and 30% with four distinct seasons that include early dry season (EDS, October – December); late dry season (LDS, January – March); early rainy season, (ERS, April – June) and late rainy season (LRS, July – September), according to Adebayo (1999). The vegetation type is best referred to as guinea savannah (Areola, 1983; Adebayo & Tukur, 1997). The vegetation is made up of mainly grasses, aquatic weeds along river valleys and dry land weeds inter-spersed with shrubs and woody plants. Plant heights ranges from few centimeters (Short grasses) to about one meter tall (tall grasses), which form the bulk of animal feeds.

Cash crops grown in the state include cotton and groundnuts, sugarcane, cowpea, benniseed, bambara nuts and tiger nuts, while food crops include maize, yam, cassava, sweet potatoes, guinea corn, millet and rice. The communities living on the banks of rivers engage in fishing, while the Fulani and other tribes who are not resident close to rivers are pastoralists who rear livestock such as cattle, sheep, goats, donkeys, few camels, horses and poultry for subsistence (Adebayo & Tukur, 1997; Adebayo, 1999).

The Study Site

The study was conducted in Mubi region, located at the northern part of old Sardauna Province, which now forms Adamawa North Senatorial District as defined by INEC (1996). The region lies between latitude 9^0 30´´ and 11^0 North of the Equator and Longitude 13^0 and 13^0 45´´ East of Green witch Meridian. Mubi region is bordered in the North by Borno State, in the West by Hong and Song LGAs and in the South and East by the Republic of Cameroon. It has a land area of about 4,728.77 km^2 and human population of about 759,045 going by NPC (1991) census projected figure as shown in figure 2. It has an international cattle market linking neghbouring countries to southern Nigeria where cattle are consumed.

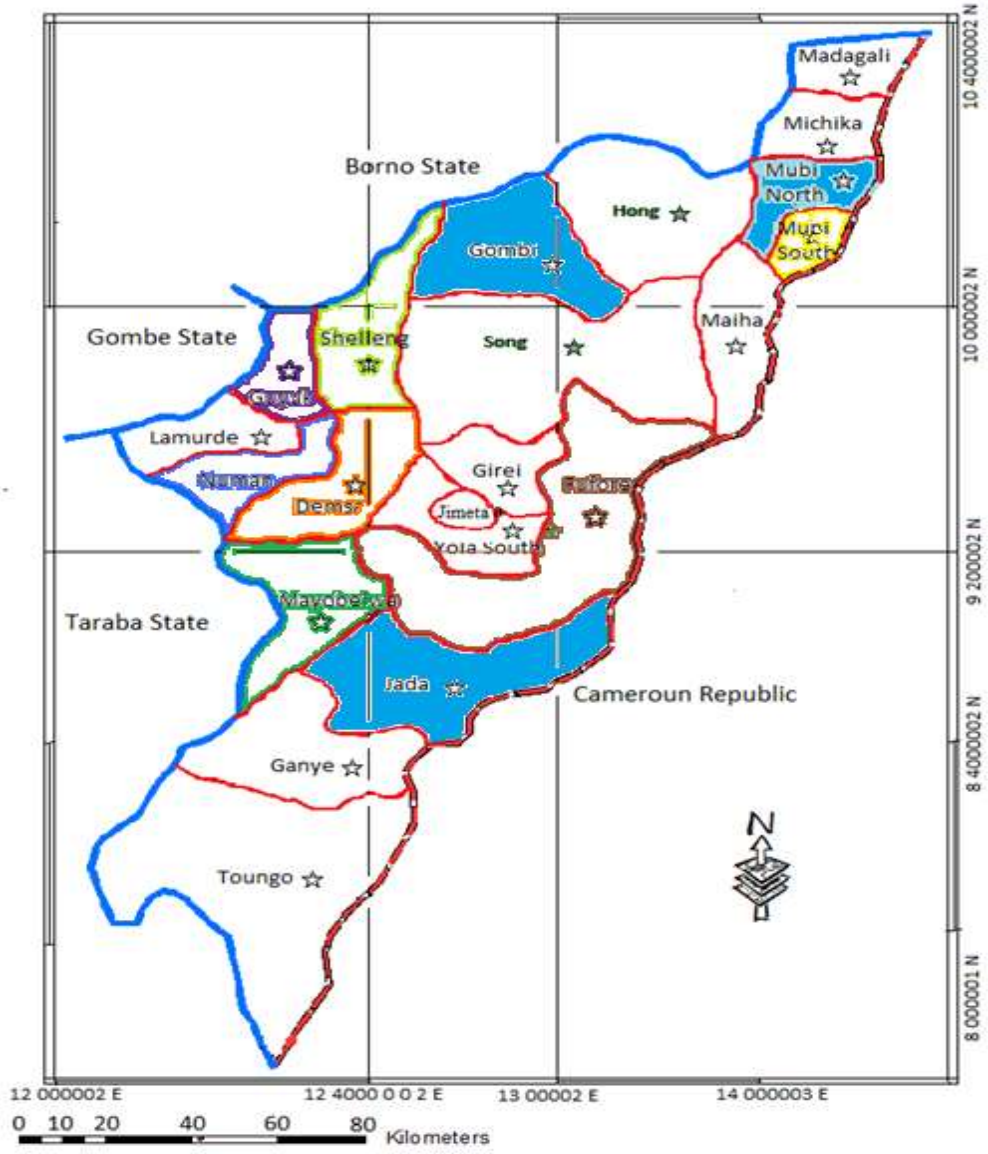

Figure 2: Map of Adamawa State Showing Study Site

Source of Samples (Camel, Cattle and Human)

Cattle: Six hundred faecal samples were collected from Cattle of both sexes slaughtered at the Mubi abattoir. The average cattle slaughtered daily according to the abattoir records were 75 during the dry season. The figure may drop to an average of 40 cattle per day in the wet season. The sources of cattle to the abattoir are usually local markets around and imports from Chad, Niger, Cameroon and

Central Africa. The common breed of cattle found in Mubi and its environs are white Fulani, red Bororo, sokoto Gudali and Kuri breeds. The sampling was carried out for a period of one year (June, 2009 to May, 2010) according to the two climatic seasons of the study area.

Humans: Six hundred (600) stool samples were collected from human patients of both gender and all ages receiving medical attention in three major hospitals in Mubi, Adamawa state. The three hospitals are Mubi general Hospital, which is the biggest state owned hospital; Federal Polytechnic Mubi medical center and Newlife medical center (A private owned hospital). The ages of sampling group were 0 – 4, 5 – 14, 15 – 24, 25 – 39 and above 40 years old. Stool types considered were both diarrheic and non-diarrheic.

Isolation and identification of STEC

Culture: - Diarrhoeic and non-diarrhoeic stool samples were randomly collected from out and inpatients of the three hospitals. Faecal samples were also collected from cattle slaughtered at Mubi abattoir. All samples were collected in sterile well labeled containers and were transported to the Federal Polytechnic Mubi microbiology laboratory in ice pack to avoid deterioration prior to analysis. At the laboratory, the samples were enriched in modified tryptone soya broth (mTSB) (CM0989, Oxoid) supplemented with novobiocin (SR0181, Oxoid) using the ratio of 1:9 (1g of faeces in 9ml of mTSB) and incubated at 37°C for an initial period of 6 hours and then for a further period of 12 to 18 hours. A loopful of the broth culture was streaked on to Mac Conkey agar plates using a sterile wire loop. The plates were then incubated for 24 hrs at 37^0C and then observed for growth. Pinkish to red colonies (Lactose fermenting colonies) were picked and then streaked on Eosin Methylene Blue (EMB) agar plates. Typical *E. coli* colonies (greenish metallic sheen on EMB agar) were picked as presumptive isolates (plate. I). Plates with mixed cultures were sub cultured to obtain a pure culture of *E. coli.*

Nutrient agar (CM3, Oxoid) slants were prepared in sterile bijou bottles and the presumptive isolates were inoculated onto them and incubated at 37^0C for 24 - 48hrs. After growth was observed, the slants were then stored in the refrigerator for further tests.

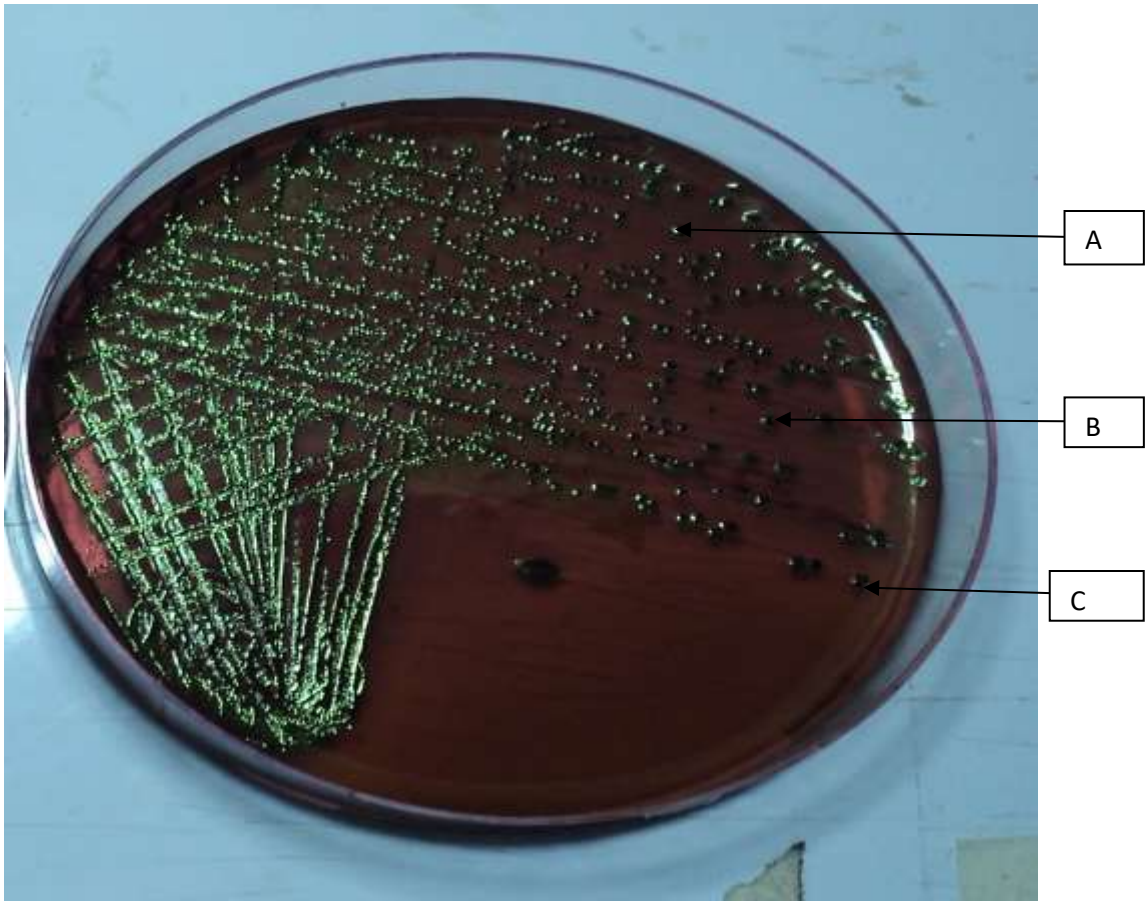

Plate 1: Eosin Methylene Blue agar (EMB) plate showing greenish metallic sheen colonies (presumptive for *Escherichia coli* with discrete colonies A, B and C.)

Identification of *E. coli* O157: Presumptive *E. coli* colonies on nutrient agar (CM3, Oxoid) slants were picked and streaked on tosorbitol McConkey agar cultures (SMAC) (CM813, Oxoid) and then incubated for 24 hrs at 37^0C. *Escherichia coli* O157 generally produced colourless colonies (non-sorbitol fermenting colonies) when cultured on this media, thus distinguishing it from other Shiga Toxin-Producing *Escherichia coli* (STEC) sero-groups and other micro-flora. Although sorbitol fermenting *E. coli* O157 stains (non motile) have emerged.

Detection of *E. coli* non-O157 sero-groups (*E. coli* O26,O103,O111,O91 and O45) A commercially prepared ***E. coli* Non-O157 Identification Kit, Prolex™ (PL 1070, Pro-Lab Diagnostics** Round Rock, Texas, USA)**was used to sero-group the isolates.**

Data analysis

Data were subjected to descriptive statistics such as frequencies, percentages and means to know the prevalence of *Escherichia coli* in cattle and humans in Mubi Adamawa, Nigeria. A student t-test using SAS (2000) soft ware package 8.1 version was also used to analyze the differences in prevalence among the various sexes, ages, and seasons.

RESULTS AND DISCUSSION

Prevalence of STEC in cattle

The results indicated that, 9.3% of cattle examined were positive for STEC (Table 1). The highest, (24.0%) prevalence was recorded in the month of September, this was followed by (22.0%) and (14.0%) for the months of October and June respectively. The months of July, December and January had (12.0%) each, while no STEC was isolated during the months of January and March. There was significant ($P < 0.05$) seasonal influence on prevalence of STEC in the cattle tested (Table 4). The highest isolation rates (20.0%) and (12.8%) were recorded in wet season for female and male cattle respectively. The results agree with some earlier studies that ruminants remained the natural reservoir for human STEC infections (Caprioli *et al.*, 2005; Hussein 2007). However, the figure obtained in this study was rather low compared to previous reports in India, Bangladesh and Nigeria (Islam *et al.*, 2008).This discrepancy in prevalence might be due to the regional differences and management systems. Similarly, Caprioli *et al*. (2005) reported that the use of specific immuno-concentration procedures for STEC O157 as used by researchers strongly enhanced the sensitivity of the isolation methods and higher rates of recovery. The results of this study again showed a significant seasonal prevalence of STEC with higher rate recorded in the wet than in the dry season. These different prevalence rates could be explained by sampling time and seasons.

Table 1: Prevalence of STEC isolated from cattle and humans in Mubi

Month/Year	Number of STEC +ve (%)		Overall	Means
	Cattle	Human		
Jun 2010	7(14%)	1(2%)	8(8.0%)	4.0 AB
Jul 2010	6(12%)	0(0%)	6(6.0%)	3.0 AB
Aug.2010	2(4%)	1(2%)	3(2.0%)	1.0 AB

Sep 2010	12(24%)	1(2%)	13(13.0%)	6.0 A
Oct 2010	11(22%)	1(2%)	12(12%)	4.7 AB
Nov 2010	4(8%)	0(0%)	4(4%)	2.3 AB
Dec 2010	6(12%)	2(4%)	8(8%)	3.6 AB
Jan 2011	0(0%)	0(0%)	0(0%)	0.0B
Feb 2011	1(2%)	0(0%)	1(1%)	0.0 B
Mar 2011	0(0%)	1(2%)	1(1%)	0.3 AB
Apr 2011	3(6%)	0(0%)	3(3%)	1.6AB
May 2011	3(6%)	0(0%)	3(3%)	1.3 AB
Total Means	56(9.3%) 4.5A	7(1.2%) 0.6B	63(5.25%)	

Means with the same letter were not significantly different ($P < 0.05$)

Table 2: Influence of sex and season on prevalence of STEC isolated from cattle in Mubi

Seasons	Sex				Total (%)	Means
	Female		Male			
	Num tested	Num STEC +ve (%)	Num tested	Num STEC +ve (%)		
Wet	138	29(20.0%)	109	14(12.8%)	43(17.4%)	17.1A
Dry	221	8(3.6%)	132	5(3.8%)	9(2.6%)	5.9 B
Total	359	37(10.3%)	231	21(9.1%)	56 (9.3%)	
Means		12.4 A		8.4 A		

Means with the same letter are not significantly different ($P < 0.05$

Prevalence of STEC in Human patients attending hospitals in Mubi

The prevalence of STEC isolated from human patients with gastroenteritis receiving medical attention in 3 hospitals in Mubi was 1.2% (Table 1). Only 2 patients out of the 7 STEC positive isolates had diarrhea and no significant association was found between STEC positive samples and diarrheal disease (Table 3). There was no clear seasonal variation observed in the distribution of STEC in the human stool (Table 3). But there was significant ($P < 0.05$) variation in STEC prevalence among the age groups. Four of the 7 (57.1%) human patients that were positive for STEC were within the age group of less than 4 years old

which was significantly ($P < 0.05$) higher than the other age groups (5 -14 years, 15 – 24 years, 25 – 39 years and above 40 years old) with 14.3% each (Table 3). In humans however, the results of this study revealed no clear seasonal variation in the prevalence of STEC. It is important to note that quantitative feacal shedding of STEC is considered a more important factor than prevalence in influencing the risk of human exposure and infection with STEC. Interestingly, the high prevalence recorded in camels and cattle during the wet season in this study corresponded with the human incidences recorded in the same season. This agrees with the reports of Ogden *et al.*(2004) that, the prevalence of *E. coli*O157 in beef cattle at slaughter was found to be greater ($P < 0.05$) during the cooler months (11.2%) than during the warmer months (7.5%) which explain increased human infections at that time. This was the reverse of the known seasonality of human infections with STEC (WHO, 1998).

Table 3: Distribution of STEC isolates according to age among the human Patients (n=600).

Age group (yrs)	Diarrheic stool		Non- diarrheic stool		Total STEC +ve	Age group means
	Num tested	NumSTEC +ve	Num tested	Num STEC +ve		
≤ 4 (n=112)	52	1	60	3	4(57.1%)	3.46 [A]
5 – 14(n=128)	63	0	65	1	1 (14.3%)	0.88 [B]
15 – 24 (n=131)	57	1	74	0	1 (14.3)	0.86 [B]
25 – 39 (n=109)	51	0	58	1	1 (14.3)	0.77[B]
≥ 40 (n=120)	55	0	65	0	0 (0%)	0.00[C]
Total (n=600) Mean	278 (46.3%)	2 (0.7%) 0.73[A]	322 (53.7%)	5 (1.6%) 1.66 [A]	7 (1.2%)	

Means with the same letter were not significantly different ($P < 0.05$)

Table 4: Influence of season on prevalence of STEC isolated from stool of human patients attending hospitals in Mubi

Seasons	Sex				Total (%)	Means
	Female		Male			
	Num tested	Num STEC +ve (%)	Num tested	Num STEC +ve (%)		

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Wet	151	2(1.3%)	147	2(1.4%)	4(1.3%)	1.3 ^{A}
Dry	147	2(1.4%)	155	1(0.6%)	3(1.0%)	1.4 ^{A}
Total	298	4(1.3%)	302	3(0.9%)	7 (3.8%)	
Means		1.3^{A}		1.3 ^{A}		

Means with the same letter were not significantly different ($P < 0.05$)

Sero-grouping of STEC isolates from cattle and humans

The results indicated that, 56 isolates of STEC belong to 5 different sero-groups whereas 12.5% were O157 and 87.5% were non-O157 sero-group. Sero-group O26 accounted for 22 (39.3%) of the isolates, which was the most frequently significantly ($P < 0.05$) detected sero-group among the non – O157 (Table 5). There was no significant differences between the detection rates of sero-groups O103 (10.7%) and O157 (12.5%). However, sero-group O91 accounted for 32.1% of the STEC isolates from cattle. There were significant differences ($P < 0.05$) between the rates of detecting the four sero-groups encountered among the six sero-groups tested from human STEC isolates (Table 5). Only 14.3% of O157 sero-group was isolated and was significantly lower than 85.7% non- O157 sero-groups obtained from the study. The non- O157 sero-groups included O26 (42.8%), O111 (28.6%) and O103 (14.3%) in descending order. However, using descriptive statistics, sero-group O26 was the most frequently encountered among the sero-groups. None of the isolates belong to sero-groups O45 and O91. The overall results of sero-grouping are shown in figure II. The results showed that out of the six sero-groups tested; only one was not detected, that was sero-group O45. A total of 63 isolates belonging to five sero-groups O157 comprising of 14.0% isolates and 86.0% non – O157 comprising of O26 (40.7), O91 (23.2%), O103 (12.8%) and O111 (9.3%). In addition to sero-group O45, sero-group O91 were also not found among STEC isolates from human. STEC were found in 7 out of 600 (1.2%) stool specimens from randomly selected human patients suffering from gastroenteritis, of which there was no significant association between prevalence of STEC and diarrheal disease. However, it is also possible to speculate that the STEC organisms present in the human patients in this study were not responsible for diarrheal disease as STEC was present in only two of the patients with diarrhea. In comparison with the present work (Fey *et al.,* 2000)

reported that 1.2% of stool samples in Nebraska from patients with gastroenteritis were positive for STEC. Moreover, according to CDC food borne outbreak online database, there were four confirmed STEC outbreaks in North Dakota from 1998 to 2009. Three of the outbreaks were caused by *E. coli*O157 while one was caused by *E. coli*O111 (CDC, 2012e) which lends support to this study.

Table 5: Distribution of STEC isolates from cattle and humans to various sero-groups.

Cattle		Humans		
Sero-group	No of isolates (n=56)	Sero-group	No of isolates (n=7)	Sero-group Total (n=63)
O157	7 (12.5%)	**O157**	1 (14.3%)	8 (12.69%)
O26	22 (39.3%)	**O26**	3 (42.8%)	25 (39.68%)
O45	0 (0%)	**O45**	0 (0%)	0 (0%)
O91	18 (32.1%)	**O91**	0 (0%)	18 (28.57%)
O103	6 (10.7%)	**O103**	1 (14.3%)	7 (11.1%)
O111	3 (5.4%)	**O111**	2 (28.6%)	5 (7.9%)
Total	56 (100%)	**Total**	7 (100%)	63 (100%%)

Results of the bacteriological studies showed that, out of the 1200 faecal/stool samples collected from cattle and humans (n = 600 each) 36.6% and 28.5% samples respectively showed typical growths of *Eschrichia coli* as shown in figure II. The results indicated that, out of the 540 biochemically presumptive *E. coli* isolates, 25.2% were non- sorbitol fermenters, with 33.8% and 8.8% from cattle and human respectively. Motility test showed that 25.7% of the confirmed non- sorbitol fermenting *E. coli* isolates were motile (Table 6)

Table 6: Biochemical and morphological characteristics of STEC isolated from cattle and humans in Mubi

Sample source	*E. coli* +ve (%)	Indole +ve (%)	NSF (%)	Motility (%)
Cattle (n = 600)	220(36.6%)	216 (98.1%)	73 (33.8%)	21 (28.8%)
Human (n = 600)	171(28.5%)	164 (95.9%)	12 (7.3%)	2 (16.6%)
Total (n = 1200)	391(32.58%)	380 (97.2%)	85(21.7%)	23(25.7%)

NSF: non sorbitol fermenters

CONCLUSION AND RECOMMENDATIONS

The results of STEC sero-grouping in this study revealed that all the STEC isolates were within the six known STEC sero-groups reported in literature. It is not surprising to have 56 STEC isolates from cattle of which all tested positive for at least one of the six STEC serotypes. The sero-groups recorded in this study were O157, non – O157 such as O26, O91, O103 and O111 with 27.0%, 36.6% and 28.5% samples showing typical growths of *Eschrichia coli*. The presence of STEC sero-types in faeces of the animals indicate the level of poor hygiene and danger associated to food safety. It is therefore, recommended that, there should be improvement in slaughter house hygiene to minimize the risk of human infections.

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