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Incidence of Shiga Toxin-Producing *Escherichia coli* (STEC) in Camels, Cattle and Humans in North-Eastern Nigeria

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Abstract: *This study was designed to determine the incidence of Shiga Toxin-producing Escherichia coli (STEC) in camel, cattle and humans in North-Eastern Nigeria. Sorbitol Macconkey agar was used for primary isolation after pre-enrichment in tryptone soya broth supplemented with novobiocin. The results showed overall total of 4.8% STEC isolates recovered from slaughtered camels, cattle as well as diarrheic and non-diarrheic stool of human patients with gastroenteritis in Mubi The highest incidence rate of 9.3% was recorded from cattle followed by camels (3.8%) and then humans (1.2%). There was significant (P < 0.05) seasonal trend in the prevalence of STEC in cattle and camels which were more in the wet season. The study did not show any significant influence of sex in incidence of STEC from the various sources. But there was significant (P < 0.05) influence of age in humans with children below the age of 4 years most hit. The presence of STEC in faeces of these animals and that of humans in Mubi has sent a strong signal on the poor nature of hygiene and food safety packages in Nigeria. It is therefore, recommended that there should be proper maintenance of slaughter houses and regular microbiological monitoring of carcasses to minimize the risk of zoonotic diseases spreading across all the nook and crannies of the country.*

Keyword: *Incidence, Shiga Toxin, Escherichia Coli, Livestock, Humans, Nigeria.*

INTRODUCTION

The occurrence of *E. coli* O157 has rarely been reported in a study on feacal samples of camel from the United Arab Emirates (Moore and Mc Calmon, 2002). Studies in five east African countries on feacal and serum samples from 400 camels failed to detect STEC or anti-*Stx* antibodies (El-Sayed *et al.,* 2008). However, Rahimi *et al.* (2012) reported a low prevalence of 2% in camel carcass in Iran.

According to Hussein and Bollinger (2005a) the prevalence of *E. coli O*157 ranged between 0.3 and 19.7% in feedlot cattle, 0.7 and 27.3% in cattle on irrigated pasture, and 0.9 and 6.9% in cattle grazing rangeland forages. In the global scene, the prevalence rates of non-O157 STEC

ranged between 4.6 and 55.9% in feedlot cattle, 4.7 and 44.8% in pasture grazing cattle. The prevalence rates varied widely because of variations in environmental conditions and management practices. Cattle hides have been identified as an important source of microbial contamination of carcasses (Ridell and Korkeala, 1993; Bell, 1997). Estimation of the incidence of carriage of STEC is complicated by the fact that feacal shedding may be transient and is almost certainly influenced by a range of factors including diet, stress, population density, geographical region, and season (Synge *et al.,* 1994 and Clarke *et al.,* 1994).

A study done to examine the incidence of STEC in seven domestic animals observed that sheep, goats, deer and cattle were the common domestic animal reservoirs of STEC (Beutin *et al*, 1993). Other domestic animals such as dogs, pigs and cats showed low prevalence of STEC, while chickens have been found to be negative for STEC.

Due to the occurrence of STEC in meat and its impact on public health and food safety, USDA, 2012 Inspection Service (FSIS) investigated ground beef for *E. coli* O26, O45, O103, O111, O121, O157 and O145. Since cattle are the major reservoir of STEC, studies have shown an association between cattle population and the incidence of STEC in humans. The incidence of STEC in humans has been found to be higher in areas with high cattle population and where manure has used for agricultural practices (Frank *et al.,* 2008). Similarly, other studies showed that the incidence has been higher in rural areas where people have frequent contact with cattle (Michel *et al.,* 1999). A study done in Nebraska also showed that 1.2% of stool samples collected from patients with gastroenteritis were positive for STEC (Fey *et al.,* 2000). The objective of the study was to determine the incidence of STEC in camels, cattle as well as humans with gastroenteritis in North-Eastern 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 38,741km². 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 km² 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.

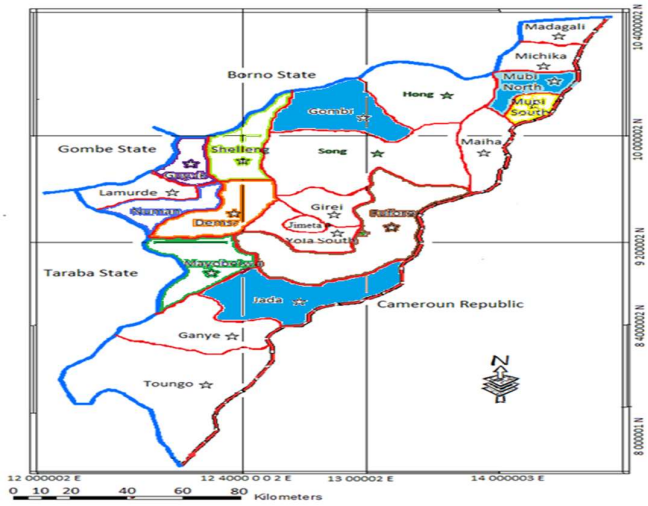

Figure 2: Map of Adamawa State Showing Study Site

Camel/ Cattle: Six hundred faecal samples each were collected from Camel and Cattle of both sexes slaughtered at the Mubi main abattoir. The average number of camel / cattle slaughtered daily according to the abattoir records were 50 and 60 during the dry season respectively. The figures for camels dropped sharply to an average of 15 camels per day in the wet season. The sources of camels and cattle are usually local markets, Chad, Niger, Cameroon and Central Africa. The common breed of camels found in Mubi and its environs is the single- humped breed (*Camelusdromedarius*) while red Bororo and Kuri breeds of cattle are dominant in the study area. 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. The two seasons comprise: wet (June to October) and dry season (November to May).

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 capital. The three hospitals are general hospital Mubi. Lokuwa medical center and Lamorde clinic.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 patients the 3 hospitals in Mubi.. Faecal samples were also collected from camels and 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.

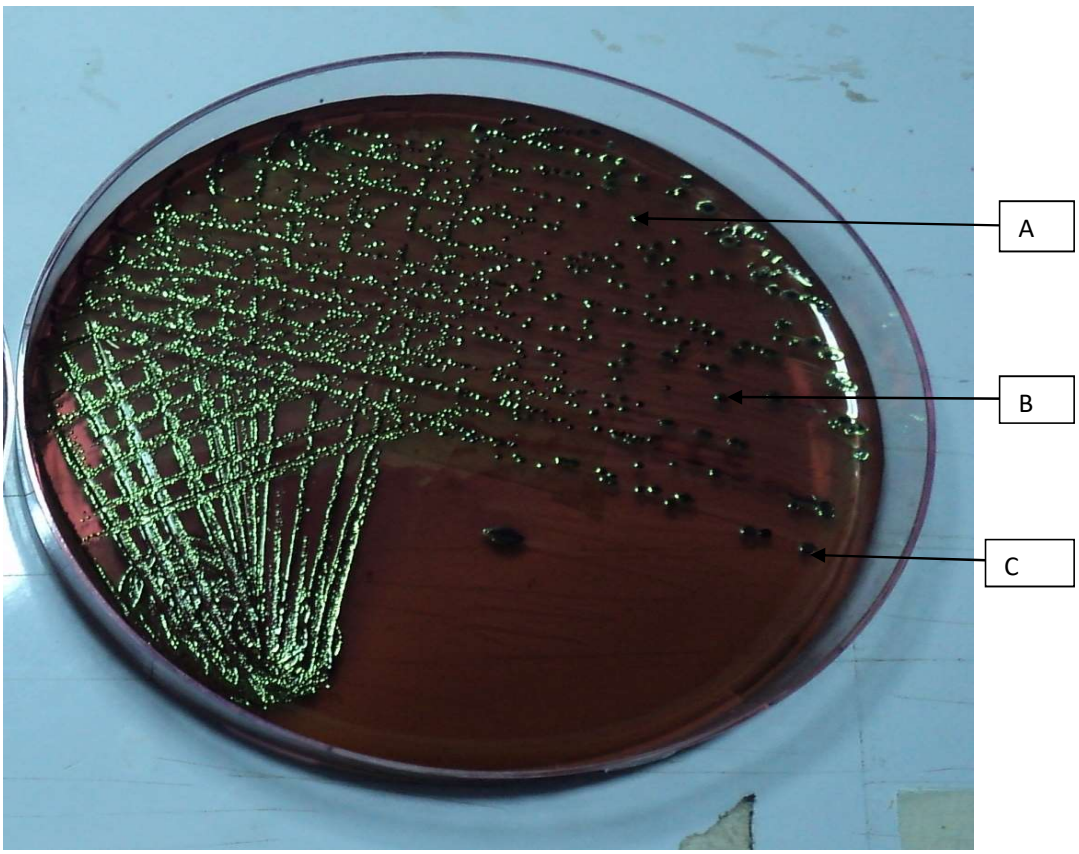

Plate 1: Eosin Methylene Blue agar (EMB) plate showing greenish metallic sheen colonies (presumptive for *Escherichia coli* with discrete colonies A, B and C.)

Data analysis

Data were subjected to descriptive statistics such as frequencies, percentages and means to know the prevalence of *Escherichia coli* in cattle, camels and humans in Mubi, Nigeria. A

student t-test using SAS (2000) software package 8.1 version was also used to analyze the differences in incidence among the various sexes, ages, and seasons.

RESULTS AND DISCUSSION

incidence of STEC in camels

The results show that, 3.8% of camels investigated were positive for STEC, and the highest (10.0%) incidence rate was recorded in the month of September, followed by 8.0% in June and 6.0% each in July, December and January respectively as shown in Table 1. No STEC was isolated during the months of August, February and March. The reason for that was not clear enough. There was significant ($P < 0.05$) seasonal trend in the isolation of STEC from camels (Table 2). The highest isolation rate (6.9%) was recorded in wet season. This was significantly higher than the rate (1.7%) obtained in the dry season. The incidence of STEC in male and female camels were 4.7% and 3.3% respectively (Table 2). The results showed no significant ($P < 0.05$) difference in the incidence of STEC in male and female camels tested.

The results suggested that camels faeces are good sources of STEC O157 and non- O157 in Mubi abattoir. This is because in the developing countries, high level of carcass contamination with faeces from gastrointestinal tracts of food animals is common in abattoirs and slaughtering slabs especially in wet season characterized with high rate of humidity related diseases. This is specifically true in Nigeria, where butchering of meat is done in open concrete floors under unhygienic slaughtering and meat processing conditions with inadequate slaughtering facilities such as potable water (Olatoye, 2010).

The values obtained in the present study are comparable with the figures reported by Rahimi *et al.* (2012) from Iran. However, this finding is at variance with previous reports on camel feacal samples from the United Arab Emirates and East African countries (El-Sayed *et al.,* 2008), where *E. coli* O157:H7 was not identified (Moore and Mc Calmon, 2002). The differences observed may be due to differences in geographical locations and laboratory techniques used for the detection. However, those studies targeted only serogroup O157 and one of the reasons advanced for failure to detect STEC in camels around the desert could be that, the free life pattern of camels in the desert minimizes the contact of camels with other animal species and therefore the transmission of STEC (El-Sayed *et al.,* 2008).

Table 1: incidence of STEC isolated from camels slaughtered in Mubi

Month	**Year**	**Num tested**	**Num STEC +ve**	**percentage +ve**
June	2010	50	4	8.0%
July	2010	50	3	6.0%
August	2010	50	0	0%
September	2010	50	5	10.0%
October	2010	50	2	4.0%
November	2010	50	3	6.0%
December	2010	50	3	6.0%
January	2011	50	0	0%
February	2011	50	0	0%
March	2011	50	0	0%
April	2011	50	2	4.0%
May	2011	50	1	2.0%
Total		600	23	3.8%

Table 2: Influence of sex and season on incidence of STEC isolated from camels in Mubi

Season	**Sex**				**Total (%)**	**Means**
	Female		Male			
	Num tested	NumSTEC +ve(%)	Num tested	NumSTEC +ve(%)		
Wet	149	10(6.7%)	98	7(6.5%)	17(6.9%)	4.0[A]
Dry	219	2(0.9%)	134	4(2.3%)	6(1.7)	1.5 [B]
Total	368	12(3.3%)	232	11(4.7)	23(3.8%)	
Means		5.2[A]		4.0 [A]		

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

incidence of STEC in cattle

The results indicated that, 9.3% of cattle examined were positive for STEC (Table 3). The highest, (24.0%) incidence 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 incidence 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 incidence 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 incidence of STEC with higher rate recorded in the wet than in the dry season. These different incidence rates could be explained by sampling time and seasons.

Table 3: incidence of STEC isolated from cattle slaughtered in Mubi

Month	**Year**	**Num tested**	**Num STEC +ve**	**Percentage +ve**
June	2010	50	7	14%
July	2010	50	6	12%
August	2010	50	2	4%
September	2010	50	12	24%
October	2010	50	11	22%
Novembe	2010	50	4	8%
December	2010	50	6	12%
January	2011	50	0	0%
February	2011	50	1	2%
March	2011	50	0	0%
April	2011	50	3	6%
May	2011	50	3	6%
Total		600	56	9.3%

Table 4: Influence of sex and season on incidence 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%)	17.1[A]
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

Incidence of STEC in Human patients with gastroenteritis

The incidence of STEC isolated from human patients with gastroenteritis receiving medical attention in 3 hospitals in Mubi was 1.2% (Table 5). 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 6). There was no clear seasonal variation observed in the distribution of STEC in the human stool (Table 7). But there was significant ($P < 0.05$) variation in STEC incidence 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 6).

In humans however, the results of this study revealed no clear seasonal variation in the incidence 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 incidence 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 incidence 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 5: incidence of STEC isolated from human patients with gastroenteritis receiving medical attention in 3 hospitals in Mubi

Month	Year	Num tested	Num STEC +ve	Percentage +ve
June	2010	50	1	2%
July	2010	50	0	0%
August	2010	50	1	2%
September	2010	50	1	2%
October	2010	50	1	2%
November	2010	50	0	0%
December	2010	50	2	4%
January	2011	50	0	0%
February	2011	50	0	0%
March	2011	50	1	2%
April	2011	50	0	0%
May	2011	50	0	0%
Total		600	7	1.2%

Table 6: Distribution of STEC isolates according to age among the diarrheic and non diarrheic human stool (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)	278	2 (0.7%)	322 (53.7%)	5 (1.6%)	7 (1.2%)	
Mean	(46.3%)	0.73 [A]		1.66 [A]		

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

Table 7: Influence of season on incidence of STEC isolated from stool of human patients with gastroenteritis receiving medical attention in 3 hospitals in Mubi

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

Overall incidence of STEC isolated from camels, cattle and humans in Mubi

The results showed that, 4.8% of the animals and humans were positive for STEC (Table 8). Comparatively, the results indicated that cattle (9.3%) had the highest incidence followed by camels (3.8%), while humans (1.2%) recorded the least isolates. This study further revealed that, the incidence of STEC was significantly higher (P < 0.05) in the month of September than any other month (Fig. II). The prevalence of STEC in the months of June (8%), July (6%) and August (2%) were not significantly different from those of the months of October (9.3%), November (4.7%), December (7.3%), March (0.7%), April (3.3%) and May (2.7%).

Table 8: Overall incidence of STEC isolated from camels, cattle and humans in Mubi

Month/ Year	Number of STEC +ve (%)			Overall	Means
	Camel	Cattle	Human		
Jun 2010	4(8%)	7(14%)	1(2%)	12(8.0%)	4.0 ^{AB}
Jul 2010	3(6%)	6(12%)	0(0%)	9(6.0%)	3.0 ^{AB}
Aug.2010	0(0%)	2(4%)	1(2%)	3(2.0%)	1.0 ^{AB}
Sep 2010	5(10%)	12(24%)	1(2%)	18(12.0%)	6.0 ^{A}
Oct 2010	2(4%)	11(22%)	1(2%)	14(9.3%)	4.7 ^{AB}
Nov 2010	3(6%)	4(8%)	0(0%)	7(4.7%)	2.3 ^{AB}
Dec 2010	3(6%)	6(12%)	2(4%)	11(7.3%)	3.6 ^{AB}
Jan 2011	0(0%)	0(0%)	0(0%)	0(0%)	0.0^{B}
Feb 2011	0(0%)	1(2%)	0(0%)	1(0.7%)	0.0 ^{B}
Mar 2011	0(0%)	0(0%)	1(2%)	1(0.7%)	0.3 ^{AB}
Apr 2011	2(4%)	3(6%)	0(0%)	5(3.3%)	1.6^{AB}
May 2011	1(2%)	3(6%)	0(0%)	4(2.7%)	1.3 ^{AB}
Total	23(3.8%)	56(9.3%)	7(1.2%)	86(4.8%)	
Means	1.9 ^{B}	4.5^{A}	0.6^{B}		

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

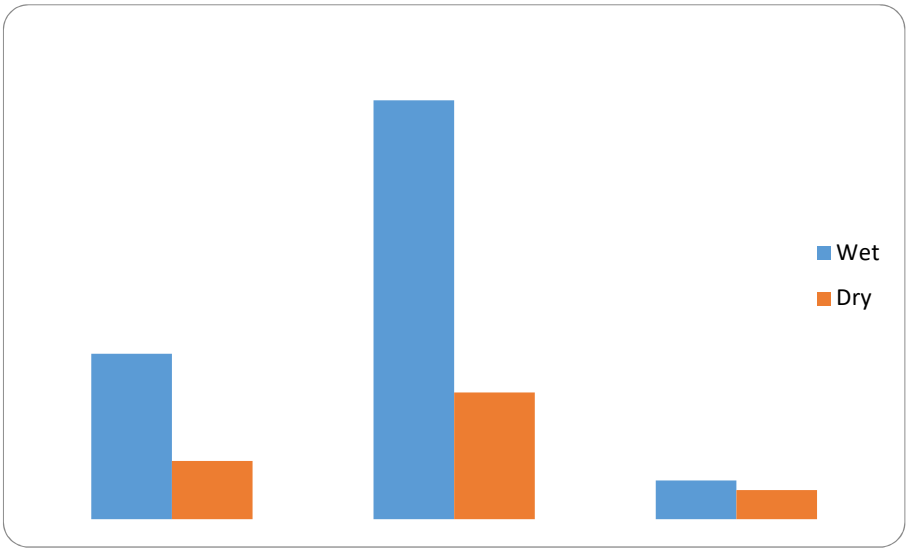

Figure II: Histogram showing influence of season on incidence of STEC isolated from camels, Cattle and Human in Mubi

CONCLUSSION AND RECOMMENDATIONS

It is therefore concluded that, the overall total of 4.8% STEC recorded in Mubi alone is high enough to pose a threat to public health in Nigeria. Importantly, more STEC isolates came from cattle which is the main source of animal protein consumed by humans in the country. The significant ($P < 0.05$) seasonal incidence recorded during the wet season, calls for more veterinary attention in our slaughter slabs nationwide if the public safety is anything to go by. The prevalent serogroups recorded in this study were O157 and non – O157 which signal a strong warning on food processing hygiene and safety packages in Nigeria. It is therefore recommended that, there should be improvement in sanitary conditions in our slaughter houses to minimize the risk of human infections by the bacteria. Further systematic research be conducted on the meat of these animals to evaluate the level of contamination by STEC.

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