



LONGITUDINAL SURVEY ON THE PREVALENCE OF *Escherichia coli* O157:H7 IN BOVINE FAECES AND THE SLAUGHTERED CARCASSES FROM MAJOR ABATTOIRS IN CROSS RIVER STATE, NIGERIA

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ABSTRACT

Escherichia coli O157: H7 is an emerging foodborne pathogen causing life-threatening disease outbreaks with cattle being the main asymptomatic reservoir. This study investigated the prevalence of *E. coli* O157: H7 in bovine faeces and slaughtered carcasses from selected abattoirs in Cross River State, Southern Nigeria. A total of 360 samples each of bovine faeces and carcass were collected from abattoirs and examined for *E. coli* O157: H7 using standard serological and culture methods. The overall prevalence of *E. coli* O157: H7 in bovine faeces and slaughtered carcasses were 19.72% (71) and 29.72% (107). With regards to sampling areas, the northern, central and southern senatorial districts had the prevalence of 13.33%, 25.80% and 20.0% respectively from bovine faeces while 22.50%, 38.33% and 28.33% was recorded from bovine slaughtered carcasses respectively. The monthly prevalence of *E. coli* O157: H7 from bovine faeces was high in January (29.73%), December (25.00%) and October (23.26%) while 43.24%, 37.21% and 35.00% was recorded in January, October and December respectively from slaughtered bovine carcasses. The ratio of faeces/carcass contamination was highest in September (1:2.3), June and August (1:1.6). There was a statistically significant difference ($p < 0.05$) in the occurrence of *E. coli* O157: H7 from bovine faeces and slaughtered carcass samples in the study areas while the faecal/carcass cross contamination ratio among the various sampling areas had no significant difference ($p > 0.05$). The isolation of *E. coli* O157: H7 from bovine faeces and slaughtered carcasses highlights the potential threat of slaughtered meat to public health in the sampled communities. Continuous surveillance of this pathogen in the study area and the implementation of good animal husbandry, pre-skinning decontamination as well as incorporation of hazard analysis and critical control points (HACCP) in the abattoirs are of immense importance.

Key words: *E. coli* O157: H7, faeces, carcass, foodborne, Nigeria

INTRODUCTION

Escherichia coli O157: H7, an enterohemorrhagic *E. coli* (EHEC), is one of the most common causes of foodborne infections in humans. It colonizes the gastrointestinal tract and is associated with a range of symptoms, including watery or bloody diarrhoea, vomiting, haemorrhagic colitis and haemolyticuraemic syndrome, which are characterized by acute renal failure affecting mainly children and the immunocompromised (Griffin and Tauxe 1991).

Cattle are the primary reservoirs of *E. coli* O157:H7 and ground beef and beef products are identified as major sources of foodborne transmission (Ferens and Hovde, 2011; Croxen, 2013). Microbiological contamination of carcasses can occur during processing and manipulation, such as skinning, evisceration, storage and distribution at abattoirs. Faecal matter is a major source of contamination and could reach carcasses through direct deposition, as well as by indirect contact through contaminated equipment, workers, installations and air (Pal, 2007). Cattle slaughtering operations, such as bleeding, dressing and evisceration expose sterile muscle to microbiological contaminants that were present on the skin, the digestive tract and in the environment (Bacon *et al.*, 2000; Abdalla *et al.*, 2009). It has also been further reported that, cross-contamination can occur during further processing of carcasses in the processing plants, during distribution and storage of beef at retail markets (Abdissa *et al.*, 2017).

In the United States, it has been reported that 1 in 4 animals at slaughter shed this pathogen in faeces during the summer months (Elder *et al.*, 2000). Case-control studies of sporadic cases of *E. coli* O157:H7 infections in the United States, Canada and Europe have identified eating undercooked beef, visiting farms and handling animals as principal risk factors for infection (Elder *et al.*, 2000).

There is paucity of information on the prevalence of *E. coli* O157:H7 in animals' faeces and carcasses in Nigeria. Hence, this study sought to determine the prevalence of the pathogen in bovine faeces and slaughtered carcasses from selected abattoirs in Cross River State, Nigeria. Information obtained on this emerging foodborne pathogen of public health importance will be used to create awareness in the public and formulate preventive measures associated with food production, processing, and distribution continuum.

MATERIALS AND METHODS

Collection of Faecal Samples from Slaughtered Cattle
This study was carried out in selected highly populated

communities in Cross River State, Nigeria where cattle rearing is prominent, involving migrants from the northern parts of the country and whose grazing areas are usually unrestricted

The study area was mapped out according to political senatorial districts i.e Northern, Central and Southern Districts. Each senatorial district was further mapped out into two sampling areas each comprising of two most populated Local Government Areas. The choice of the sampling areas was based on the population, presence of abattoirs, cattle pens and livestock activities. The Sampling which was restricted to abattoirs and bi-weekly was performed between June, 2011 to February, 2012

A total of 360 fresh faecal samples (60 from each sampling area) were collected from post-eviscerated cattle at the following selected abattoirs: Obudu/Abuochiche (SA1), Okuku/Igoli (SA2), Ikom/Edor (SA3), Apiapum/Ugep (SA4), Akampka town/Awi (SA5) and Anantigha/IkotOmin/Atimbo (SA6), chosen based on their location and patronage.

Triplicate samples were collected both from the lower ileum and colonic regions of the intestine. About 50g of faeces pat was collected from each cattle using sterilized dissecting knife, spoons, forceps and spatula. Samples were collected in clean, leaked proof, screw-capped, wide-mouthed plastic containers containing Amies transport medium and transported in ice cold box to the Microbiology Laboratory, University of Calabar for analysis within 24 hrs.

Collection of Meat Samples from Slaughtered Cattle

A total of 360 fresh meat samples (60 from each sampling area) were collected from the slaughtered cattle whose faeces were sampled. About 50g portion of each sample was cut off from the pre-retail stock using a sterile knife and forceps, wrapped in aluminium foil and placed in a sterile polyethene bag. All samples were transported in ice cold box (4°C) to the laboratory for microbiological analysis within 24 hrs.

Enrichment of samples

About 1.0ml of each faecal sample suspension from transport medium was introduced into 10ml of buffered peptone water containing cefixime (0.05mg/l) and vancomycin (8.0mg/l) (BPW-CV) and vigorously vortexed for 30s to homogenize the mixture. It was then incubated at 37°C for 24hrs for enrichment. For the bovine meat samples, about 10g of each sample was homogenized in 90ml of phosphate

buffered saline containing cefixime (0.05mg/l) and vancomycin (8.0mg/l) by blending for 1min using Q-link blender (QL-2L40). The resultant slurry was also incubated at 37°C for 24hrs.

Presumptive Identification of E. coli O157 Antigens in Enriched Samples

The Diagnostic Automation Enzyme-linked Immunosorbent Assay (ELISA) technique comprising of anti-*E. coli* O157 antibodies impregnated in the wells was used for the qualitative detection of *E. coli* O157 antigens in all the enriched samples (Milley and Sekla, 1993; Bell *et al.*, 1996).

Isolation of E. coli O157: H7

All enriched samples with positive ELISA results were analysed using the standard *E. coli* O157: H7 culture technique as recommended by Warburton (2006). The samples were serially diluted to 10⁻³ using physiological saline (0.85%w/v NaCl) and spread plated on sorbitol MacConkey agar supplemented with cefixime (0.5mg/l) and potassium tellurite (2.5mg/l) (SMAC-CT) and incubated at 42°C for 24hrs for sorbitol-negative colourless. Growths on 4-Methylumbilliferyl-β-D-Glucuronide (MUG) medium and fluorescence of the broth under UV light at 650nm wavelength was used as a confirmatory test. Also, H7 typing using standard *E. coli* O157: H7 antisera (Difco Laboratories, Detroit, Mich.) and other biochemical tests typical to *E. coli* were used as confirmatory tests. The data were analysed with SPSS version 11.0 software (SPSS Inc, Chicago, IL).

RESULTS

Faecal and carcass samples from slaughtered cattle had the highest percentage occurrence of 19/60 (31.67%) and 27/60 (45.00%) respectively in sampling area 4 (Apiapnum/Ugep Abattoirs). Although significant difference in the percentage occurrence of the pathogen in carcass samples from the various sampling areas was observed (p>0.05), no significant difference was observed among the faecal samples and between the faecal and carcass samples at p >0.05 (Table 1)

Monthly percentage isolation from the colonic content was highest and ranged from 87.50% in September to 100.00% in June while the ilial contents had lower percentage isolation ranging from 0.00% in December to 66.67% in November. Relatively, the monthly percentage occurrence of the pathogen from the ilial and colonic contents and among the iliac content were significantly different at p<0.05 while no significant difference was obtained in the monthly percentage isolation among the colonic content at p>0.05. (Table 2)

Highest faeces/carcass cross contamination ratio of 1:1.9 was obtained in sampling area 2 (Okuku/Igoli Abattoirs) while the least value of 1:1.4 was obtained in sampling areas 4 (Apiapum/Ugep Abattoir) and 6 (Anantigha/IkotOmin/Atimbo Abattoirs). The faecal/carcass cross contamination ratio among the various sampling areas had no significant difference (p>0.05). (Table 3)

Generally, the monthly percentage occurrence of *E. coli* O157: H7 showed that highest prevalence of 29.73% and 43.24% from faeces and carcasses respectively were both obtained in January. Highest cross-contamination ratio of 1:2.3 was obtained in September while the least ratio of 1:1.3 obtained in February and November. There was significant difference (p<0.05) in the monthly occurrence of *E. coli* O157: H7 among the bovine faecal samples and between the bovine faecal and slaughtered carcass samples. However, the monthly occurrence among the slaughtered carcasses and the monthly faeces/carcass contamination ratio had no significant difference (p<0.05). (Table 4)

Table 1: Prevalence of *E. coli* O157: H7 in faeces and slaughtered carcasses of cattle from various sampling areas

Sampling Area	No. of Samples	No of Positives samples (%)	
		BF	SC
SA1 (Obudu/Abuochiche Abattoirs)	60	9(15.00)	14(23.33)
SA2 (Okuku/Igoli Abattoirs)	60	7(11.67)	13(21.67)
SA3 (Ikom/Edor Abattoirs)	60	12(20.00)	19(31.57)
SA4 (Apiapum/Ugep Abattoirs)	60	19(31.67)	27(45.00)
SA5 (Akpampka town/Awi Abattoirs)	60	14(23.33)	20(33.333)
SA6 (Anantigha/IkotOmin/Atimbo Abattoirs)	60	10(16.67)	14(23.33)
Total	360	71(19.72)	107(29.72)

p>0.05 p< 0.05

Table 2: Monthly occurrence of *E. coli* O157: H7 in bovine faeces from ilial and colonic regions of the intestines

Month	No. of Samples	No. of positive samples (%)	
		Ilial Content	Colonic Content
June	38	5	1(20.00) 5(100.00)
July	37	11	5(45.45) 10(90.91)
Aug	43	9	4(44.44) 9(100.00)
Sept.	44	8	3(37.50) 7(87.50)
Oct.	43	10	6(60.00) 10(100.00)
Nov.	38	6	4(66.67) 6(100.00)
Dec.	38	3	0(0.00) 3(100.00)
Jan.	40	10	5(50.00) 9(90.00)
Feb.	39	9	5(55.56) 9(100.00)
Total	360	71	33(46.48) 68(95.77)

p<0.05 p>0.05

Table 3: Relative occurrence of *E. coli* O157: H7 in bovine faeces and slaughtered carcasses from various sampling areas

Sampling Faeces/ Carcass cross	No. of samples		No. of Positive Samples(%)	contamination ratio
	from each source BF	SC		
SA1 60	9(15.00)	14(23.33)		1:1.6
SA2 60	7(11.67)	13(21.67)		1:1.9
SA3 60	12(20.00)	19(31.67)		1:1.6
SA4 60	19(31.67)	27(45.00)		1:1.4
SA5 60	14(23.33)	20(33.33)		1:1.4
SA6 60	10(16.67)	14(23.33)		1:1.4
Total 360	71(19.72)	107(29.72)		1:1.5

p>0.05
BF= Bovine Faeces; SC= Slaughtered Carcass

Table 4: Monthly relative occurrence of *E. coli* O157: H7 in bovine faeces and slaughtered carcasses

Sampling Faeces/ Carcass cross	Month	No. of Samples		No Positive samples (%)	contamination ratio
		BF	SC		
	June	38	5(13.16)	8(21.05)	1:1.6
	July	44	8(18.18)	12(27.27)	1:1.5
	Aug	43	9(20.93)	14(32.56)	1:1.6
	Sept.	38	3(7.89)	7(18.42)	1:2.3
	Oct.	43	10(23.26)	16(37.21)	1:1.6
	Nov.	38	6(15.79)	8(21.05)	1:1.3
	Dec.	40	10(25.00)	14(35.00)	1:1.4
	Jan.	37	11(29.73)	16(43.24)	1:1.5
	Feb.	39	9(23.08)	12(30.77)	1:1.3
	Total	360	71(19.72)	107(29.72)	1:1.5

p<0.05 p>0.05 p>0.05

BF= Bovine Faeces; SC= Slaughtered Carcass

DISCUSSION

Enterohaemorrhagic *E. coli* (EHEC) especially those of the serotype O157:H7 are major human pathogens that cause life threatening sequelae. Transient carriage of this pathogen especially by cattle is asymptomatic which enables the bacteria to circulate easily between humans, animals and the environment (Xia *et al.*, 2010).

The prevalence rate of 19.70% from the bovine faecal samples in our study is higher than the values reported by other studies such as 2.00% by Abdissa *et al.*, (2017), 2.4% by McEvoy *et al.* (2003), 3% by Conedera *et al.* (1997), 7.5% by Van Donkersgoed *et al.* (1999) and 15.7% by Chapman *et al.*, (1997 while it was observed to be lower than the value of 47.8% as reported by Akanbi *et al.*, (2011). However, there was no statistically significant difference (p>0.05) obtained within the various sampling areas. This

may be explained by the fact that migration of herds of cattle between the various senatorial districts is rampant and unrestricted. Numerous studies in several countries have shown that this pathogen is present in the gastrointestinal tract of cattle in varying percentages (Armstrong *et al.*, 1996). They also observed that faecal shedding may not be a reliable index of measuring the prevalence of *E. coli* O157: H7 in cattle as some of the organism may be restricted to the rumen since they are relatively acid tolerant. In this study, faecal samples were collected directly from the iliac and colonic regions of the gastrointestinal tract thereby reducing the risk of not identifying organisms that may be restricted in GIT and not shed. *E. coli* O157: H7 can therefore be said to have an even distribution in its infection in cattle within the study area.

E. coli O157:H7 has also been shown to be more frequently found in cattle faecal samples during the warmer months (Bonardi *et al.*, 2001; Chapman *et al.*, 1997; Chapman *et al.*, 2001; Conedera *et al.*, 2001; Garber *et al.*, 1999; Paiba *et al.*, 2002; Sargeant *et al.*, 2000; Van Donkersgoed *et al.*, 1999).

The monthly prevalence of *E. coli* O157: H7 from bovine faeces had significant difference (p<0.05) with higher prevalence during the dry periods. It is worth noting that the carriage of *E. coli* O157: H7 have been shown to fluctuate significantly over time in USA feedlots (Le Jeune *et al.*, 2004). Similarly, results were also obtained when cattle at slaughter tested for *E. coli* O157: H7 had highest and lowest prevalence rates in the warm and cold months respectively in Finland (Lahti *et al.*, 2001) and UK (Chapman *et al.*, 1997; Paiba *et al.*, 2002). However, Ogden *et al.* (2004) found the prevalence of *E. coli* O157: H7 in Scottish beef cattle at slaughter to be greater during the cooler months (11.2%) than the warmer months (7.50%). Since a large number of variables such as management practices, diets fed, animal factors and isolation methods can influence the prevalence and comparison among studies, these parameters should be carefully evaluated.

In comparison of the monthly isolation of *E. coli* O157: H7 in bovine faeces from iliac and colonic regions of the intestine, the percentage frequency of positive samples was higher (p<0.05) from the colonic region with 100% obtained during most of the warmer periods. Recent research work has attempted to study the gastrointestinal colonization responses by *E. coli* O157: H7 (Nart *et al.*, 2008). The terminal rectum (Recto-anal Junction, RAJ) has been reported to be an area rich in lymphoid follicles (Mahajan *et al.*, 2005) and has been suggested that adherence to these

sites may be as a result of tropism of the pathogen for the bovine terminal rectum though the reason for this tropism is still obscure (Lim *et al.*, 2007). The combine effects of a reduced protective mucous barrier coupled with raised intrarectal pressure during defaecation may facilitate colonization of the colon by the promotion of cell-to-cell contact and induced type III secretion (Nart *et al.*, 2008). This implies positive cultures obtained from the lower ileum may be transient as suggested by reports from Lim *et al.* (2010).

Result of the occurrence of *E. coli* O157: H7 in slaughtered carcasses showed an overall prevalence of 29.72% with significant difference ($p < 0.05$) among the various sampling areas. In contrast, the prevalence of *E. coli* O157:H7 in slaughtered carcass samples in this study was higher than the 13.3% prevalence reported by Tizeta *et al.*, (2014) in Ethiopia, 3.2% by McEvoy *et al.*, (2003) in Ireland, 2.5% by Elder *et al.*, (2000) in the USA, 8.8% by Abong (2008) in South Africa and Hajian *et al.*, (2011) in Iran; and lower than 53% prevalence reported by Dahiru *et al.*, (2008) in fresh beef meat in Kano, Nigeria. The significant difference observed in the prevalence among the various sampling areas may probably be due to the different hygienic standards that prevailed during slaughtering processes at various abattoirs. This is supported by the observation that slaughtered carcasses from abattoirs that supplied meat to larger populations had higher prevalence of the pathogen as was the case in Apiapum/Ugep and Akamkpa/Awi abattoirs each supplying meat to over three Local Government Areas. The slaughtering processes in these abattoirs were generally unhygienic due to the rush in order to meet up with the high demands.

This also explains the significantly higher ($p < 0.05$) prevalence of the pathogen in slaughtered carcasses relative to that obtained for faecal samples. The possibility of faecal cross contamination of carcasses is very certain since in all abattoirs, same equipment were used to slaughter all cattle scheduled for a particular day. Faeces of infected cattle can therefore contaminate slaughtered carcasses of uninfected ones. There was therefore a significant relationship in the monthly *E. coli* O157: H7 prevalence in bovine faeces and faeces/carcass contamination ratio which suggests that the main source of carcass contamination is from bovine faeces. Conversely, Alonso *et al.* (2007) working in a feedlot cattle slaughtered at an abattoir in Northern Italy obtained *E. coli* O157: H7 prevalence of 24% in bovine faeces and 11% in carcass samples. Lower carcass prevalence relative to that from bovine faeces obtained in their study may be due to high hygienic conditions that prevailed in the sampled abattoir that drastically reduced cross contamination to a minimal level. However, they confirmed the possibility of cross contamination through phage typing and PFGE analyses of the positive isolates. Elder *et al.*, (2002) therefore predicted that reduction of carcass prevalence by targeting pre-evisceration and post-processing can be achieved if sanitary procedures are strictly followed.

Escherichia coli O157:H7 was found to be prominent in the State, with highest prevalence in bovine slaughtered carcasses. Bovine faeces serve as the main source of carcass contamination with generally higher prevalence during the warmer months. The findings of this study imply a number of measures need to be taken to lessen the risk of transmission of this pathogen along the meat chain from farm to fork. Best practices as well as incorporation of hazard

analysis and critical control points (HACCP) must be put in place to reduce these risks.

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