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Research Paper

Occurrence and phenotypic characterization of *Escherichia coli* O157:H7 in cattle population under traditional-herding system of management in Sokoto State, Nigeria

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To investigate the occurrence and determine the prevalence of Escherichia coli O157 in Traditional cattle herds in Sokoto State Nigeria, rectal swab samples were collected from 402 cattle in 36 randomly selected herds, from the period May 2013 to April 2014. The faecal samples were subjected to selective-culture on SMAC and E. coli O157:H7 was confirmed using E. coli O157 and H7 antisera respectively. The E. coli O157 isolates were assessed for phenotypic expression of enterohemolysis and verotoxin-production as virulence properties, by standard methods (what is the name of the standard method). Of the herds surveyed, 10 (27.8%) were found to have at least one positive isolation of E. coli O157:H7 in feacal samples examined. Within-herd prevalence ranged from 6.7 to 20.0%, with an average of 12.9 +/- 5% SD, and the individual-animal prevalence was 4.0% of the 402 rectal swab samples examined. The prevalence was highest among female (4.5%) than male (3.2%) cattle respectively, and also among weaned calves (7.0%) and yearling heifers. Eleven (68.8%) of the E. coli O157:H7 isolates were positive for enterohemolysis, while verotoxin assay showed 4 (25%) as verotoxin-producers. No significant association was observed between verotoxin-production and enterohemolysin expression among the isolates tested ($p > 0.05$). This study confirmed the presence of E. coli O157:H7, including the potential pathogenic strains in cattle managed under traditional-herding system in Sokoto state. This is potential public health threat for human infection in the state, which needs to be controlled.

Key Words: *Escherichia coli* O157:H7, characterization, cattle herds

INTRODUCTION

Verotoxin-producing *Escherichia coli* (VTEC) have emerged as important enteric foodborne pathogens of considerable public health significance (Beutin *et al*., 2004; Brooks *et al*., 2005) and have been linked to range of disorders such as; hemorrhagic colitis (HC) or the hemolytic-uremic syndrome (HUS), which is the main cause of acute renal failure in children and the elderly (Gyles, 2007; Grant *et al*., 2011). The bacteria elaborate one or both shiga-toxins (stx1 and stx2/ vt1 and vt2), locus of enterocyte effacement (eae) and EHEC-hemolysin (enterohemolysin) as important parameters for pathogenicity of the STEC strains (Fairbrother and Nadeou 2006; Magwedere *et al*., 2013). While sero-grouping is important, the presence of these virulence

attributes is essential for determining the potential pathogenic E. coli strains.

Domestic ruminants, mainly cattle, sheep and goats have been established as major natural reservoirs for STEC and play a significant role in the epidemiology of human infections (Griffin and Tauxe, 1991).

Consequently, E. coli strains, recovered from animal reservoirs and harboring stx, eae, and/or ehxA genes, are thought to represent a subpopulation of STEC strains that may pose a higher risk to human health (Caprioli *et al*., 2005).

The organism can be transmitted to humans through person-to-person contact, ingestion of food or water contaminated with animal faeces, and by direct contact with infected animals (Caprioli et al., 2005; Smith *et al*., 2014). Escherichia coli O157 infection in cattle and sheep is invariably subclinical; and thereby acting as asymptomatic carriers for human infection (Dean-Nystrom *et al*., 1998; Mainil and Daube, 2005). Several outbreaks of human E. coli O157 infection were found to originate from foods of bovine origin such as beef and undercooked meat- products, unpasteurized raw-milk and milk products (Karmali, 2004; Gyles, 2007). Epidemiologic surveys of fecal samples from cattle populations have shown E. coli O157 prevalence that varied from 0 - 28% at animal level (Hancock *et al.,* 1997; Dunn *et al.,* 2004), herd- level prevalence of 1.5-6% and within herds prevalence of 0 - 4% respectively (Omisakin *et al.,* 2003; Fairbrother and Nadeou, 2006). The prevalence was also found to be higher among weaned calves and heifers than in adult cattle (Zhao *et al.,* 1995; Elder *et al* 2000, Neilsen *et al.,* 2002; Urumova *et al.,* 2015). A survey of cattle and beef meat in a northern province of South Africa reported E. coli O157:H7 prevalence of27.7% (Ateba and Mbewe, 2011).The risk of meat contamination was found to dependon the *E. coli* O157 carriage status of the food-animals (McEvoy *et al.,* 2003). Therefore, assessment of the carriage status of *E. coli* O157 in animal population is essential in determining the risk of human exposure.

Studies in different parts of Nigeria have also reported varying prevalence of E. coli O157 infection among cattle populations in herds and farm environment (Ameh *et al.,* 2002; Smith *et al.,* 2003; Moses *et al.,* 2005; Oluyege and Famurewa, 2015; Enem and Oboegbulem, 2015). E. coli O157 prevalence of 11.2% and 1.5 % had been recorded from surveys of cattle farms and herds in Borno state of Nigeria (Ameh *et al.,* 2002; Moses *et al.,* 2005). Sokoto state, located in the north-western part of Nigeria has abundant ruminant resources concentrated mainly in rural and semi-urban cattle herdsdistributed within the 23 Local Government Areas (MAHF 2013). The state is the second largest ruminant producing state in the country, after Borno (RIM 1991), and a large number of ruminant species of animals also enter the state from the neighboring Niger and Benin Republic. The cattle from these traditional herds account for approximately 67.5% of cattle slaughtered and beef consumed in the State. Despite the position of Sokoto state being a large area of ruminant production in Nigeria, little is known on the occurrence and distribution of E. coli O157:H7 infection among the cattle population from traditional herds. The only available reports were a survey on exotic dairy-breeds of cattle by Luga *et al.,* (2007), who reported 0.9% animal-level carriage prevalence of E. coli O157. In another survey that involved traditional cattle herds, E. coli O157 herd- and individual animal- prevalence of 21.8% and 5.9% were respectively recorded (Abubakar, 2010), including enterohemolytic and antibiotic-resistant strains. The purpose of this study was to establish the occurrence, and determine the prevalence of E. coli 0157 strains among cattle managed under traditional herding system in Sokoto state. The aims were to; define the proportion of cattle herds and individual cattle infected with E. coli 0157:H7 and assess the isolates for expression of enterohemolysis and verotoxin-production, as epidemiologic virulence properties.

MATERIALS AND METHODS

The study area

The study was conducted in 8 randomly selected Local Government Areas (2 LGAs each) from the 4 Agricultural Zones designed by Sokoto Agricultural and Rural Development Agency (SARDA). Sokoto State of Nigeria is a semi-arid region, located to the extreme north-western Nigeria (between longitudes 4° 8´E and 6° 54´E and latitudes 12°N and 13° 58´N) and covered a total land area of about 32,000 square km. The state has an estimated human population of about 3.2 million (NPC 2006), and the estimated of animal population of 1.8 million cattle, 2.6 million sheep, 2.9 million goats, 48,000 camels and variable species of poultry (RIMS, 1991; MAHF 2012). The state housed numerous small-holding- and a small number of large-holding cattle herds distributed with in the 23 Local Government Areas (LGA). The cattle kept are mostly indigenous and /or mixed breeds, in average herd-size of 8- 30 animals.

Selection of Herds and Animals

The target population for this study was cattle raised by rural and semi-urban farmers in Sokoto state of Nigeria. For the purpose of this study, the number of herds and animals were selected using a two-stage sampling techniquewith list of cattle herds (obtained from Divisional Veterinary Offices) as primary sampling frame and the individual cattle as secondary sampling unit (Elder *et al*., 2000).Sample size for the herd's and animal-level survey was calculated by the method of Van Donkersgoed *et al.* (1999), using a herd prevalence of 1.7% (Moses *et al.,*

2005) and animal-level prevalence of 11.2% from previous studies (Ameh *et al.,* 2002). The cattle sampled were divided into four age-groups according to NAERLS 2012, Urumova *et al.,* 2015) as; Pre-weaned calves (day1 to 6 months), Weaned calves (6 - 11 months), Yearling Heifers (12 – 24 months) and Adult cattle (above 24 months).

Sample Collection and Microbiological Analysis for E. coli O157:H7

Fecal sample from each selected animal was collected by rectal swabbing using a sterile cotton-tipped swab, and the swab was then placed in a tube containing 3 ml of Buffered peptone water supplemented with 50 µg/L cefixime and 4 mg/L vancomycin. The tubes collected per day were transported in ice-cooled box to the veterinary microbiology laboratory of Usmanu Danfodiyo University Sokoto, for bacteriological analysis. The laboratory examination for E.coli was initiated within 24 h of the collection of the samples. Each rectal swab suspension in Buffered Peptone Water supplemented with vancomycin and Cefsoludin (BPW-VC) was pre-enriched at 37°C for 12 h without agitation. This was followed by sub-culturing aerobically onto Sorbitol-MacConkey agar plate (SMAC: CM-0813, Oxoid, Basingstoke, UK) at 37°C overnight. Non-sorbitol fermenting (colorless) colonies with the morphology and biochemical characteristics of E. coli was presumed to be E. coli O157 isolates (March and Ratnam 1989; McFaddin 2000; Brenjchi, 2011). Three discrete sorbitol negative colonies were tested for O157 and H7 antigens, using a commercial E. coli O157:H7 latex agglutination test kit (DR 620: OXOID; Basingstoke, UK) and H7 antisera; based on manufacturers' instructions.

Enterohemolysin Detection

The E. coli O157 isolates were sub-cultured onto blood agar plates containing 5% washed sheep erythrocytes and 10 mM $Cacl_2$ (1.1gm/L) (Beutin *et al.,* 1989, Bettelheim *et al.,* 1995). After incubation at 35-37°C for 24 h, the plates were examined for enterohemolysis expression after 4 and 24 h of incubation based on the interpretation of Bettelheim *et al.* (1995).

Verotoxin production Assay

Detection of verotoxin-producing E. coli O157 strain was done using the Ridascreen ELISA® kit (R-Biopham AG, Darmstadt, Germany) using the method adopted from Bonardi *et al.,* (2000), and in accordance with the recommendations by the manufacturer's. The E. coli O157 colonies were transferred to nutrient broth/ plates and studied after 24 h at 37°C. The cut-off value was determined by adding 0.1 to the absorbance value of the negative control. Any strains with results over this value were considered verotoxin producing. Culti-Loop® E. coli O157:H7 (Oxoid, Basingstoke, UK), and E. coli O157:H7 beef isolate (courtesy of Dr Abdullahi D.M., ABU Zaria) were used as positive control.

Data analysis

All the data collected were entered into Microsoft Excel spread sheet (Microsoft Corp, WA, USA). The prevalence of E. coli O157:H7 and the frequencies of enterohemolytic and verotoxin-producing phenotypes among the isolates was determined by dividing the number of positive samples by the total number of samples examined.The significance of association between E. coli O157:H7 carriage and age and sex of the animals, and between the virulence properties was assessed by the χ^2 -test with yates correction, using Graphad® statistical software. Statistical significance was regarded at a p-value of less than 0.05.

RESULTS

During the one year period for survey of the 36 randomly selected cattle-herds, rectal swab samples were obtained from a total of 402 animals and examined for the carriage of E. coli O157:H7. The distribution of herds and cattle sampled is presented in (Table 1). The animals comprises of 155 (38.6%) male and 247 (61.4%) female cattle respectively, and age categorization of the sampled animals was; 48 suckling-calves, 71 weaned-calves, 68 yearling-Heifers and 215 adult cattle.

Prevalence of E. coli O157:H7 among cattle in the Selected Herds of Sokoto State

Of the 36 cattle herds surveyed, 10 were found to carry at least one E. coli O157-infected animal, revealing a herd-level prevalence of 27.8%. The proportion of infected animals within the E. coli O157-positive herds (With-in Herd Prevalence) ranges between 6.7% to 20.0%, with an average of 12.9% +/- 5% SD (Table 2). The prevalence of infection based on herd-size was 4.3% from herds with animal population of less than 20 and 7.0% for those with 20 animals and above. The prevalence of infection showed no significant association with the herd- size ($p > 0.05$).

E. coli O157:H7 was isolated from faecal samples of 16 out of the 402 cattle examined, revealing an overall animal-level prevalence of 4.0%. Of the positive cattle, 11 (4.5%) and 5 (3.2%) were from female and male

Table 1. Distribution of Herds and Cattle Sampled by Local Government Areas of Sokoto State.

LGA	No. Herds		No. Animals Present	No. Animals Selected
	Recorded	Selected		
WAMAKKO	12	3	119	38
BINJI	12	3	99	35
SOKOTO METROP	36	9	268	95
GORONYO	12	3	91	29
TAMBUWAL	16	4	153	45
SABON-BIRNI	20	5	161	56
ILELLA	28	7	239	76
SHAGARI	8	2	88	28
Total	144	36	1218	402

Table 2. Proportional Distribution and With-In Herds Prevalence of E. coli O157:H7in cattle herds.

	NUMBER HERDS				NUMBER ANIMALS		
Local Government	Tested	Positive	%	Herd ID	Tested	Positive	%
BINJI	3	0	0.0	Hd/BNJ01	8	0	0.0
				Hd/BNJ02	16	0	0.0
				Hd/BNJ03	11	0	0.0
WAMAKKO	3	1	33.3	HD/WMK01	10	0	0.0
				HD/WMK02	13	2	15.4
				HD/WMK03	15	0	0.0
ILELLA	7	2	28.6	HD/ LLA01	10	0	0.0
				HD/ LLA02	12	2	16.7
				HD/ LLA03	15	1	6.7
				HD/ LLA04	11	0	0.0
				HD/ LLA05	10	0	0.0
				HD/ LLA06	10	0	0.0
				HD/ LLA07	8	0	0.0
WURNO	3	0	0.0	WNO H- 01 A	8	0	0.0
				WNO H-02 A	11	0	0.0
				WNO H-01 B	10	0	0.0
SABON BIRNI	5	3	60.0	SBN01 A	9	0	0.0
				SBN02 A	15	1	6.7
				SBN03 A	8	1	12.5
				SBN01 B	13	0	0.0
				SBN02 B	11	2	18.2
SOKOTO METRO	9	3	33.3	SKK HS01	14	1	7.1
				SKK HS02	8	0	0.0
				SKK HS03	19	3	15.8
				SKK HS04	9	0	0.0
				SSK HS05	11	0	0.0
				SSK HS06	10	1	10.0
				SSK HS07	7	0	0.0
				SSK HS08	9	0	0.0
				SSK HS09	8	0	0.0
TAMBUWAL	4	1	25.0	TBW Hd-01 A	15	0	0.0
				TBW Hd-02 A	10	2	20.0
				TBW Hd-03 B	11	0	0.0
				TBW Hd-04 B	9	0	0.0
SHAGARI	2	0	0.0	SGR Hd-0A	12	0	0.0
				SGR Hd-0B	16	0	0.0
TOTAL	36	10	27.8		402	16	4.0

animals respectively. Although the rate of E. coli 0157 infection was higher among female cattle, no statistical significance was found on the prevalence in relation to sex of the sampled animals ($p > 0.05$). Of the 48 pre-weaned calves investigated for E. coli O157:H7 infection; only one (2.1%) was positive, 7.0% of the weaned-calves, 3 (4.4%) of the yearling-heifers and 7(3.3%) of the adult cattle were found positive for E. coli O157:H7 (Table 3). The prevalence was significantly higher among weaned calves and yearling-heifers ($p > 0.05$: Figure 1).

Table 3. Age related prevalence of E. coli O157:H7 among cattle population in Sokoto State.

Age Category	No. Cattle		
	Tested	Positive	% (Prevalence)
Pre-Weaned	48	1	2.1
Weaned calves	71	5	7.0
Yearling Heifers	68	3	4.4
Adults	215	7	3.3
Total	402	16	4.0

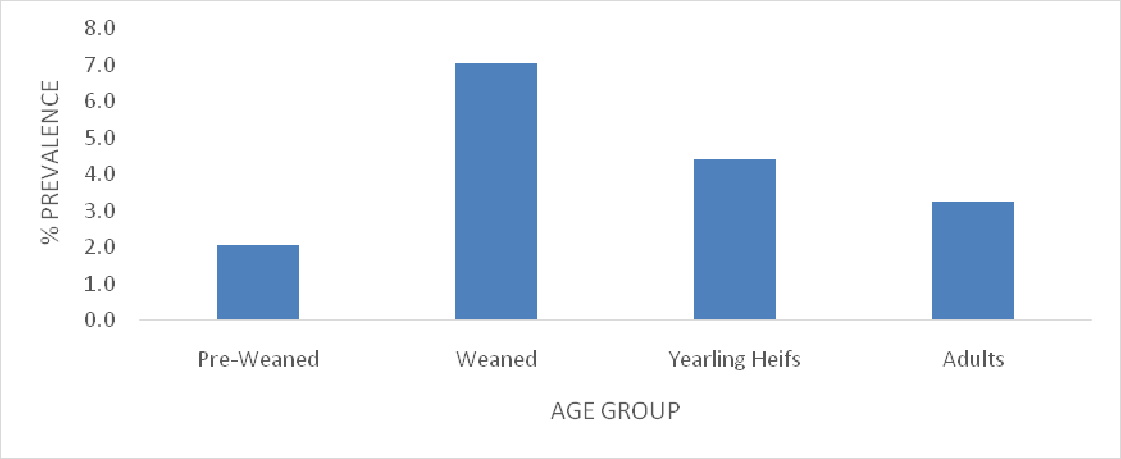

Figure 1: Prevalence of E. coli O157:H7 in cattle by age groups.

Table 4. Frequency of Enterohemolysin and Verotoxin Detection Among E. coli O157:H7 Isolated From Cattle in Sokoto State.

Enterohemolysis Status	No. Tested	Verotoxin positive	%	Number isolate per Verotoxin Type		
				vt1	vt2	vt1&2
Enterohemolysis +ve	11	3	27.3	2	0	1
Non-Enterohemolytic	5	1	20.0	0	0	1
TOTAL	16	4	25.0	2	0	2

Detection of Enterohemolysin and Verotoxin-producing E. coli O157 Phenotypes Isolated from Cattle

Sub-culturing of the 16 E. coli O157:H7 isolates on WSBA+cacl$_2$ revealed 11 (68.75%) enterohemolytic and 5 (31.25%) as non-enterohemolytic strains respectively (Table 4). Verotoxin assay detected a total of 4 (25.0%) isolates that expressed verotoxin-activity, of which 2 (12.5%) each were detected as vtx1 and vtx1&2 producers respectively. No isolate of E. coli O157:H7 expressed vt2-production. The verotoxin-positive isolates were detected among 3 (27.3%) of the 11 enterohemolytic and 1 (20.0%) of 5 non-enterohemolytic strains (Table 4). Statistically significant association between enterohemolysis and verotoxin production was observed ($p<0.05$).

DISCUSSION

For over a decade, verotoxin-producing Escherichia coli (VTEC) especially E. coli O157:H7/NM have emerged as significant pathogens of clinical and epidemiological importance to public health. Cattle and sheep are the primary reservoirs (Fairbrother and Nadeou, 2006; Gyles 2007), but infection in these animal species is sub-clinical. Prevalence data generated through examining animal fecal-samples from herds has indicated that, up to 30% of the general cattle population may shed the

pathogen in their faeces, accounting for a remarkable amount of zoonotic infection among human population (Berrend, 2008). In the present study, E. coli O157 herd-level prevalence of 27.8% was obtained, with within-herd prevalence that ranges between 6.7% and 20.0%. This result is comparable to findings from other countries (Hancock et al., 1994: Lahti et al. 2001; Synge *et al.,* 2003). As observed in the present study, herd-prevalence of E. coli O157 was often found to be higher than the prevalence at individual animal-level (Lahti *et al.,* 2001, Omisakin *et al*., 2003, Alam *et al.,* 2006). The overall animal-level prevalence of 4.0% among cattle in traditional herds obtained in the present study is similar to what has been reported by Abubakar *et al.* (2010) in the same study area, and in east-china by Wang *et al.,* (2014). Our prevalence data was significantly higher ($P > 0.05$) than 1.5% encountered among breeding cattle from Adamawa and Borno States of Nigeria (Moses *et al*., 2005), an environment similar to Sokoto State. The animal-level prevalence in this study is also similar to what has been reported from certain developing countries (Fairbrother and Nadeou 2006; Gyles, 2007; Wang *et al.,* 2014), but lower than 8-12.4% reported from developed countries; including United Kingdom and certain states of the United States of America (Chapman *et al*., 1993; Hancock *et al*., 1994). This prevalence however, was in contrast with 17.5% reported among apparently healthy cattle in Lagos Nigeria (Smith *et al*., 2003). The difference between the methods of isolation and geographical variations may be the cause of the discrepancies observed. Relatively, recent studies have examined and reported varying prevalence of E. coli O157:H7 in cattle from various locations in Nigeria (Oluyege and Famurewa, 2015; Enem and Oboegbulem, 2015). The age-related prevalence in the present study is in agreement this previous report (Zhao *et al.,* 1995; Neilsen *et al.,* 2002), that E. coli O157:H7 is more frequently carried by weaned-calves and heifers than by pre-weaned calves and adult cattle. Our result is also in concert with the reports that, higher prevalence of E. coli O157 was observed in female than male cattle (Van-Donkersgoed *et al*., 1999; Nielsen *et al*., 2002; Enem and Oboegbulem, 2015).

In our study, 68.8% of the E. coli O157:H7 isolated from cattle were positive for enterohemolysin, among which 3(27.3%) were also Verotoxin producing strains. Similar finding of 57.8% enterohemolytic EHEC has been reported in a preliminary study of cattle herds in the same area (Abubakar, 2010). Our result is higher than what was reported for Serbia by Cobeljic *et al.,* (2005), where 21.5% of VTEC expressed enterohemolytic activity. Enterohemolytic strains of E. coli O157:H7 isolated from cattle and environmental sources have been found to be verotoxin-producers, and are potential human pathogens (Beutin *et al*., 1989; Siegfried *et al*., 2004). The prevalence of verotoxin- producing E. coli O157 detected and the toxin-type distribution in the present study was similar to that obtained by Raji, (2013)from beef isolates in Tanzania, but it was significantly lower than what has been reported among E. coli O157 isolates obtained from beef cattle herds in one study (Alam *et al.,* 2006).

Conclusion

The present study highlights the occurrence of Escherichia coli O157:H7 with a relative high prevalence among cattle (27.8% at herd-level and 4.0% for individual cattle), including those that are traditionally managed in Sokoto State.The occurrence of enterohemolytic and verotoxin-producing phenotypes is of public health concern, as they can be transmitted from animals to humans from the cattle faeces and the herd's environment respectively. Considering the clinical implication of E. coliO157 in human health and veterinary public health, continuous surveillance of E. coli O157 among cattle and other ruminant specie both at herd and abattoir level is recommend. A comprehensive Herd-Health program that can facilitate the control of E. coli O157 at herd/farm-level needs to be design for states and local governments in Nigeria.

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Authors` declaration

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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