Original Research Article

Faecal Carriage Of *Escherichia coli* 0157:H7 Serotype By Free-Ranged And Confined Small Domestic Ruminants Within Cross River State, Nigeria

Abstract

Background: The type of animal grazing method to be promulgated into the Federal Legal System has been an issue of controversy following incessant Farmers/Herdsmen clashes in Nigeria. The method to be adopted has to ensure a drastic reduction of common pathogens associated with the consumption of meat and its products notably *Escherichia coli* O157:H7, a human enterohaemorrhagic pathogen. This pathogen has regularly been isolated from cattle by researchers while scanty information exists implicating small domestic ruminants especially in Cross River State, Niger Delta Region of Nigeria where ruminant meat is highly consumed. Similar studies on this pathogen associated with small domestic ruminants do not take into consideration all the different grazing methods practiced.

Aim: This study was aimed at investigating the effects of two commonly used grazing methods (free-ranging and confined grazing) on the potentials of fecal carriage of *Escherichia coli* 0157:H7 by sheep and goats in different locations within Cross River State, Nigeria.

Methodology: A total of 360 fresh recto-anal faecal swap samples each were collected from both confined (penned) and free- ranging goats and sheep within a 5-months sampling duration. Combined culture and serological methods such as growth on sorbitol macconkey agar supplemented with cefixime and tellurite (SMAC-CT), nonfluorescence of 4-Methylumbilliferyl-β —D-Glucoronide (*E. coli*-MUG) cultures under uv light at 650*nm* wavelength, serological identification using ELISA technique and *H* typing with standard *E. coli* H7 rabbit antisera were used in the isolation and identification of the pathogen.

Results: Among the various groups of domestic ruminants, free-ranged and confined sheep had highest overall prevalence of 31/180 (17.22%) and 12/180 (6.67%) respectively compared to free-ranged and confined goats with overall values of 19/180 (10.50%) and 9/180 (5.00%) respectively. Prevalence rates showed no significant difference (P = .05) between confined goats and sheep while significant difference (P =.05) was observed between the free-ranged groups. Also, the monthly values differed significantly at P = .05 between the free-ranged and confined groups.

Conclusion: Confined grazing of ruminants in pens (ranching) significantly reduced the transmission of *E. coli* O157:H7 by goats and sheep which were highly implicated as possible vehicles within Cross River State, Nigeria.

Keywords: Grazing methods; *Escherichia coli* O157:H7*;* Small domestic ruminants*;* Faeces; Nigeria

1. INTRODUCTION

The grazing of small domestic ruminants such as goats and sheep constitute a major aspect of livestock farming in most parts of Nigeria especially in Cross River State where they are used as main source of meat during routine meals, festivals and traditional rites. Free-ranging (open grazing) and confined (penned) methods are presently being used by the herdsmen with the former being more frequent. This practice constantly exposes cultivated crops to destruction by these animals resulting to frequent clashes between the herdsmen and farmers.

In addition to the effects on crops, livestock (including small ruminants) are at risk of pathogens exposure from livestock and wild animal faeces during grazing. Livestock exposure to pathogens will therefore be dependent on the behavioral contact processes between the grazing livestock and the immediate environment hence the grazing method practiced becomes very significant[1, 2]

Escherichia coli O157:H7 is an emerging pathogen with significant public health concern[3]

This pathogen is one of the most important food-borne pathogens, causing diarrhoea, hemorrhagic colitis and haemolytic-uremic syndrome in humans worldwide with potentially life-threatening consequences as a result of systemic Shiga toxin *(stx*) activity [3, 4, 5, 6, 7]

Large ruminants particularly cattle, are considered the primary reservoir for *E. coli* O157:H7, where the organism typically colonizes the lower gastrointestinal tract and is shed in the feces[2,8]. Numerous studies have demonstrated the shedding of *E. coli* O157:H7 from cattle in feces [2, 9, 10, 11, 12, 13, 14] and have reported the bovine gastrointestinal system to be a reservoir for the pathogen. *Escherichia coli* O157:H7 has also been isolated from the carcasses and hides of cattle which suggests a possible cross contamination by faeces harbouring the pathogen during slaughtering and meat handling [9, 10,11,12]

Shedding of *E. coli* O15:H7 by small ruminants is multifactorial and has not been extensively investigated especially in developing countries [15, 16]. Although colonization and persistence factors exhibited by this pathogen to its bovine host has been extensively analysed [8, 14, 16], this has not been

the case with small ruminants. Despite many similarities to the bovine host, the pathology of *E coli* O157:H7 in small domestic ruminants appears to differ significantly to that described in cattle [17].

More recently, feces of small domestic ruminants such as sheep and goats have emerged as important sources of *E. coli* O157:H7 implicated in human infections particularly with the widespread of petting farms and the increasing use of sheep and goats as major sources of meat [18, 19, 20, 21, 22,23]. However, information on this pathogen associated with small domestic ruminants in Nigeria still remain scanty and most often conducted in the Northern part of the country [21, 24, 25]. Also, similar researches conducted by Jacob *et. al.* [18], Kudva *et. al*. [19], and Dahiru *et al*. [24] do not take into consideration the various grazing methods.

It is therefore necessary to establish the dynamics of faecal carriage of this pathogen by small domestic ruminants in relation to their grazing methods in order to adopt a measure that will effectively reduce pathogen carriage and shedding in the region.

This study was therefore carried out to determine the potentials of fecal carriage of *Escherichia coli* O157:H7 by free ranging and confined goats and sheep in different locations within Cross River State, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study area

This research was carried out in selected highly populated communities in Cross River State, Nigeria, whose population is basically comprised of (small-scale) farmers with few traders and civil servants. The main occupation of the inhabitants is therefore subsistence farming and trading while most households keep domestic animals such as chickens, goats, sheep, and pigs whose grazing areas are usually free-ranged though confined in few cases.

2.2 Sampling design

The study area was mapped out according to political demarcations i.e. Northern, Central and Southern Senatorial Districts. Each District was further mapped out into two sampling areas each

comprising of communities from two Local Government Areas. Communities sampled within each selected area were based on the population and livestock activities.

2.3 Sampling procedures

Six (6) sampling areas were mapped out to cover all geographical regions of the State namely Obudu/Abuochiche (SA1), Okuku/Igoli (SA2), Ikom/Edor (SA3), Apiapum/Ugep (SA4), Akampka/Awi (SA5) and Anantigha, Ikot Omin/Atimbo (SA6).

Sampling was restricted to communities within a particular sampling area during each sampling period. A total sampling duration of five (5) months (between September 2017 and January, 2018) was used and monthly sampling performed in each sampling area.

2.4 Sample collection

A total of 360 fresh faecal samples each from goats and sheep (both confined and free ranged) were collected throughout this study (60 from each sampling area) after having obtained approval from the producers.

Samples were obtained from each animal by digital rectal extraction using sterilized gloves to avoid cross contamination. Each sample was immediately placed in a sterile wide-mouth screw capped container containing 15 ml of Amies transport medium (Difco). The samples were transported in a flask with ice packs to the Microbiology laboratory, University of Calabar, for analysis within 6 hrs of rectal retrieval.

2.5 Selective enrichment

About 1.0 ml of each faecal samples suspension from transport medium was introduced in to 10ml of buffered peptone water containing cefixime (0.05mg/l) and vancomycin (8.0 mg/l) (BPW-CV) and vigorously vortexed for 30 seconds to homogenize the mixture. It was then incubated at 37^{o}C for 24hrs for enrichment. Faecal samples from goats and sheep were diluted at a ratio of 1:20 with the enrichment medium (BPW-CV) before incubation [8]

2.6 Presumptive identification of *Escherichia coli* 0157

The diagnostic automation Enzyme-linked immunosorbent assay (ELISA) technique was used for the qualitative detection of *E. coli* 0157 antigens in all enriched samples [22]

2.7 Isolation and identification

All enriched samples with positive ELISA results were analyzed using the standard *E. coli* 0157:H7 culture technique as recommended by Fujisawa *et al*. [23]

The enriched samples were serially diluted to 10^{-3} using physiological saline (0.85%w/v NaCl) and approximately 0.1ml of 10^{-2} and 10^{-3} dilutions were spread plated on sorbitol MacConkey agar supplemented with cefixime (0.5mg/l) and potassium tellurite (2.5mg/l) (SMAC-CT). All cultured samples were incubated overnight at 42^{o}C for 24 hrs. Sorbitol-negative colonies that appeared colourless to grey on SMAC-CT were considered positive for *E. coli* 0157:H7. Three randomly selected suspected colonies were isolated on each plate and separately subcultured on nutrient agar slants and stored at 4^{o}C.

2.8 Growth on 4-Methylumbilliferyl-β −D-Glucoronide (MUG) medium

All positive colonies isolated on nutrient agar slants were further inoculated into test tubes containing *E. coli* with MUG (*E. coli*-MUG) medium and incubated at 42^{o}C for 18-24 h as recommended by Thompson *et al*. [29]. The broth cultures were then observed under ultraviolet (uv) light of long wavelength (650nm) to detect the inability of *E. coli* 0157:H7 to cleave MUG. Positive isolates were considered as those that fermented lactose (yellow broth), produced gas (collected at the tip of the immersed durham tubes) and did not produce any fluorescence.

Other confirmatory biochemical tests typical to *E coli* such as indole, methyl red, voges proskauer, citrate and lysine decarboxylase were also performed on the isolates.

2.9 Serotyping with standard *E. coli* 0157:H7 rabbit antisera

This analysis was performed using standard *E. coli* 0157:H7 rabbit antisera (Difco Laboratories, Detroit, Mich.) and preserved with glycerol using 1:2 dilution.

Slide agglutination technique was used to test resuscitated colonies directly from sorbitol MacConkey agar (SMAC) as recommended by Newell and Ragione [30]. A wireloop was used to remove a loopful of the colony and suspended in a drop of normal saline. An equal amount of *E. coli* 0157:H7 antiserum was added and mixed by rocking back and forth for 1min. Colonies that agglutinated rapidly with the *E. coli* 0157:H7 antisera were considered as confirmed positive *E. coli* 0157:H7 colonies.

Statistical analyses

Pearson's chi-square test was used to analyze the results obtained. Each test was conducted at 95% confidence interval (p=0.05) at the appropriate degrees of freedom. The data were analyzed with SPSS version 21.0 software (SPSS Inc, Chicago, IL).

3.0 RESULTS

The prevalence of E. coli O157:H7 from faeces of free-ranged goats had highest value of 13.33% in SA1 and lowest value of 3.33% in SA3.The highest value of 10.00% for confined goats was obtained in SA4 while the least value of 3.33% was obtained in SA1, SA3, SA5, and SA6. The prevalence values among the free-ranged goats and between the free-ranged and confined goats differed significantly at $p<0.05$ while no significant difference was observed among the confined goats ($p>0.05$).The prevalence values obtained from the various sampling areas are shown in fig 1.0

The percentage prevalence of *E. coli* 0157:H7 in confined and free-ranged sheep faeces from the various sampling areas are presented in fig 2. Free-ranged sheep had highest prevalence of 23.33% in SA1 compared to the highest value of 10.00% obtained in SA2 and SA5 for confined sheep. However, least value of 10.00% was obtained in SA6 for free-ranged sheep while 3.33% was obtained in SA1 and SA3 as least value for confined sheep. Though no significant difference at $p>0.05$ was observed in the prevalence of the pathogen among the free-ranged and confined sheep faeces, the values between the two groups differed significantly at $p<0.05$.

The monthly prevalence of *E. coli* 0157:H7 from confined and free-ranged goats and sheep faeces are presented in table 1. Highest value of 13.87% was obtained in November and December for free-

ranged goats while 8.33% was obtained in January as highest value for confined goats. Free-ranged sheep had 22.22% in December as highest value while 11.11% was obtained in January as highest value for confined sheep. No significant difference was observed in the monthly prevalence within each of the free-ranged and confined ruminant groups ($p>0.05$) though the monthly values differed significantly at $p<0.05$ between the free-ranged and confined groups.

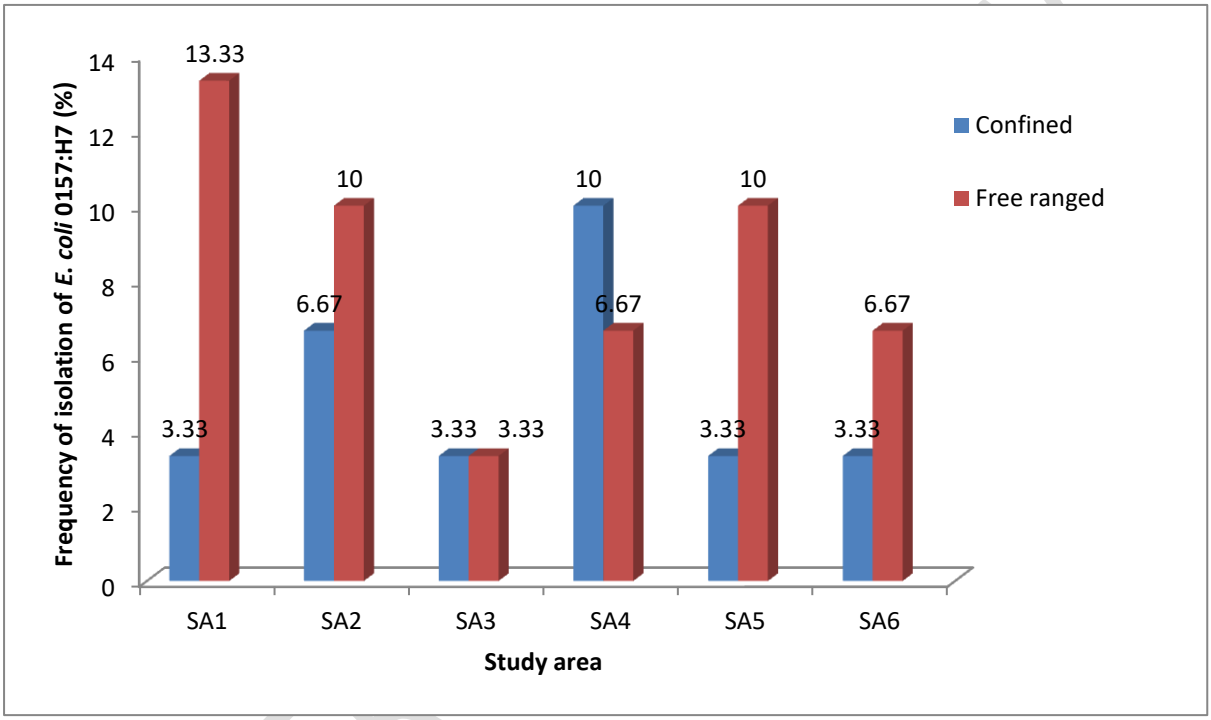

Fig 1. Prevalence of *E.coli* 0157:H7 in faeces from confined and free- ranged goats in various sampling areas. SA1(Obudu/Abuochiche); SA2 (Okuku/Igoli); SA3 (Ikom/Edor); SA4 (Apiapum/Ugep); SA5 (Akampka/Awi); SA6 (Anantigha/ Ikot Omin/Atimbo)

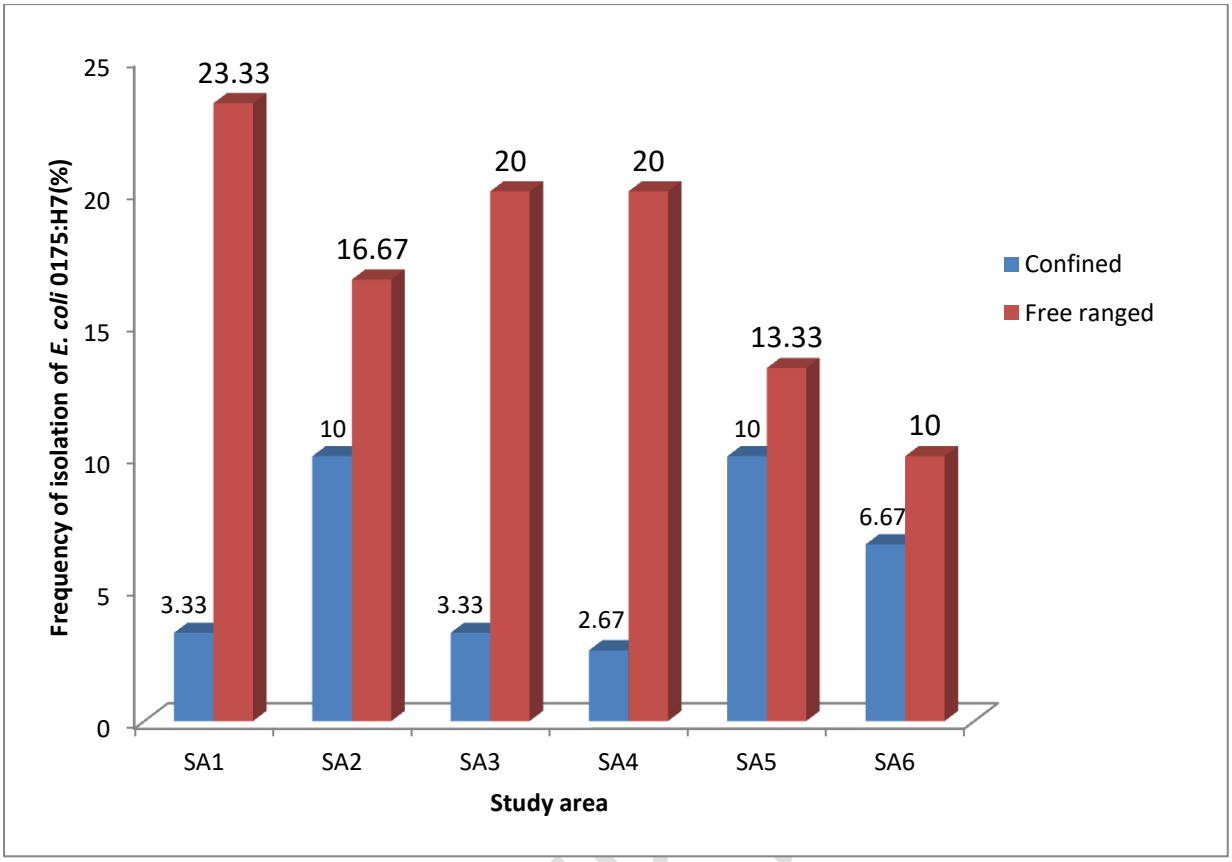

Fig 2 Prevalence of *Escherichia coli* 0157:H7 in faeces from confined and free-ranged sheep in various sampling areas. SA1(Obudu/Abuochiche); SA2 (Okuku/Igoli);SA3 (Ikom/Edor); SA4 (Apiapum/Ugep); SA5 (Akampka/Awi); (SA6) Anantigha/ Ikot Omin/Atimbo

Table 1: Monthly prevalence of *Escherichia coli* 0157:H7 in faeces from confined and free-ranged goats and sheep in various sampling areas

Sampling	Goats				Sheep			
Month	Confined		Free-ranged		Confined		Free-ranged	
	N	n(%)	N	n (%)	N	n(%)	N	n (%)
Sept	36	1(2.78)	36	4(11.11)	36	3(8.33)	36	5(13.89)
Oct	36	2(5.56)	36	2(5.56)	36	1(2.78)	36	7(19.44)
Nov	36	2(5.56)	36	5(13.89)	36	3(8.33)	36	6(16.67)
Dec	36	1(2.78)	36	3(8.33)	36	1(8.33)	36	8(22.22)
Jan	36	3(8.33)	36	5(13.89)	36	4(11,11)	36	5(13.89)
Overall prevalence	**180**	**9(5.00)** *P*=.05	**180**	**19(10.56)** *P* =.05	**180**	**12(6.67)** *P* =.05	**180**	**31(17.22).**

N- total number of animals sampled; n- number of animals shedding the pathogen.

4.0 DISCUSSION

The effect of ruminant grazing methods to the faecal carriage of *Escherichia coli* O157:H7 by small domestic ruminants in Cross River State, Nigeria which hitherto was very elusive has been extensively evaluated in this study.

Though little information on the prevalence of *E. coli* 0157:H7 in goats is available [12], this study obtained overall prevalence from free-ranged and confined goats to be 10.56% and 5.00% respectively with significant difference (*P=.05*) in the prevalence between the two groups. This difference in prevalence may be due to high exposure of the free-ranging group to contaminated environments. Smith *et al*.[1] observed that livestock are at risk of pathogen exposure from the grazing livestock and wild animal faeces as well as the immediate environment. This result is similar to that conducted by Akanbi *et al*.[31] on fecal samples obtained from 40 goats at Kuje and Gwagwalada Abattoirs, North Central Nigeria where 2 fecal samples were positive for *E. coli* O157:H7 with a prevalence rate of 5%. Also, research by Joseph *et al.*[23] showed that the number of goats positive for *E. coli* 0157:H7 ranged from 14-85%. In a similar study carried out by Sima *et al*.[20], 9(75%) of the 12 goat herds and 30(44%) of the 68 faecal samples collected in North Central Florida were positive for *E. coli* 0157:H7. Detection of the pathogen on herd basis ranged from 14-100%. Similarly, Jacob *et al*.[18], working on U.S. meat goats at slaughter obtained *E. coli* O157:H7 prevalence of 11.13%, 2.7% and 2.7% from faeces, hide and carcasses respectively. Conversely, Joseph *et al*. [23], working in Iran sampled 60 goat faecal samples and none was positive for *E. coli* 0157:H7. However, none of the studies above provided comprehensive information on the different grazing methods of the animals. The findings of this research therefore suggest that the prevalence obtained from goats faeces may be sufficient to cause potential food-borne illness in exposed humans if contaminated goat meat, goat milk or dairy products are consumed.

Sheep were also identified as reservoirs of *E. coli* 0157:H7 in the study area with overall percentage prevalence of confined and free-ranged sheep obtained as 6.67% and 17.22% respectively. These

values were observed to differ significantly (*P*=.05) between the two groups and also between the free-ranging goats and sheep. *Escherichia coli* 0157:H7 have been shown to colonize the gastrointestinal tract of sheep than are other pathotypes of *E. coli* [17, 27, 32] . Similar result of 4.69% was obtained by Yakubu *et al*.[21] working on household-reared small ruminants in Zaria, Nigeria. The value for free-ranging sheep was observed to be lower than 31% obtained by Kudva *et al*. [19] among microflora of sheep in June. Mohammed *et al*. [15] sampled 150 apparently healthy farmed sheep and wandering flocks fed on city wastes for *E. coli* 0157:H7. Out of the 150 animals, 13 were *E. coli* O157 shedders with a prevalence of 8.7%. The result of this research finding is comparatively higher with the 2.4% prevalence reported by Joseph *et al*. [23], 2.2% prevalence in confined sheep faeces reported by Paiba *et al.* [16] and the 1.7% described by Sima *et al*.[20]. Differences in geographical locations and identification methods may account for the discrepancies. Most of the identification procedures used by the researchers did not incorporate the rapid biochemical MUG test which eliminates false positive results since *E. coli* O157 antigens have been shown to be identical with those of *Salmonella urbana* and *Citrobacter freundii* [29]. Akanbi *et al*. [31] working on hides and faeces of ruminants at slaughter in major abattoirs in Nigeria obtained *E. coli* O157:H7 prevalence values of 6.3% and 2.5% for sheep and goats respectively. Their study however did not clearly define the grazing method of the ruminants at slaughter since it is a common practice in Nigeria that some free-ranging ruminants in rural communities are not taken to abattoirs but slaughtered privately at various locations. Abattoir environments have been reported as important reservoirs of extended spectrum beta-lactamase (ESBL) Escherichia coli [28]. Sapountzis *et al*.[2] also observed that animal husbandry practices have a profound effect on STEC prevalence in cattle. Generally, free-ranging animals tend to be more exposed to environmental pathogens than their confined counterparts [21]. This may likely explain why the free-ranged groups had significantly higher prevalence than the confined groups. Contaminated soil and water has been shown by Nfongeh *et al*.[33] to serve as possible vehicles for the transmission of *Escherichia coli* O157:H7 in the study area. Commercial feedlots were also identified by Stephens *et al*. [34] as a possible source of *E. coli* O157:H7 transmission to livestock.

Monthly prevalence value in goats had highest in January (8.33%) in the confined group while the free-ranged group had 13.89% during the same month. Also, monthly prevalence value was highest in January (11.11%) in confined sheep while the free-ranged group had highest value of 22.22% in December. Generally, monthly values differed significantly ($p<0.05$) between the confined and free-ranged groups of both ruminants while no significant difference was observed within each group at $p>0.05$. These results are not consistent with the findings of Kudva *et al*. [19] who obtained a prevalence value of 31% in June and 0% in December working on the microflora of sheep. Paiba *et al*. [16] also revealed that faecal shedding of *E. coli* 0157:H7 in sheep faeces was transient and seasonal with values between 31% and 5.7% of sheep positive during certain periods of the rainy season and none during parts of the dry season. The possible reasons for the difference in the monthly prevalence from those of the other researchers may be due to differences in the grazing method, geographical locations, climate and other confounding variables. However, the seasonality influence in this study cannot be fully established due to limitations in sampling duration.

5.0 CONCLUSION

This study provides an initial baseline data on the relationship between grazing methods and the occurrence of *Escherichia coli* 0157:H7 in small domestic ruminant animals in Cross River State, Niger Delta Region of Nigeria. The results reveal that small domestic ruminants are significant sources of *E. coli* O157:H7 for human infection. Confined ruminants have a lower prevalence of *E. coli* O157:H7 than the free-ranged group. Therefore, confined grazing (formation of ranches) practices are highly recommended as against free ranging.

6.0 CONFLICT OF INTEREST

All items used in this research were obtained locally and mainly used in our area of research. There is therefore no conflict of interest between the authors and producers of such items. This research was also completely funded by the authors without assistance from any institution or organization.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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