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Isolation and characterization of shiga toxin-producing *Escherichia coli* O157:H7 from cattle feces within Zaria Metropolis, Nigeria

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ABSTRACT

Background: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a significant zoonotic pathogen implicated in severe human illnesses such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). Cattle are known asymptomatic reservoirs, and their fecal shedding of the pathogen poses a public health risk through contamination of food and water sources. This study aimed to isolate and molecularly characterize STEC O157:H7 from cattle feces within Zaria Metropolis, Nigeria. **Methods**: A cross sectional study was conducted. A total of 328 cattle faecal samples were collected from Eight (8) different Fulani cattle herds. Faecal samples where enriched, selectively cultured, and screened for *E. coli* O157:H7 using Wellcolex rapid latex agglutination kit and multiplex polymerase chain reaction (PCR) to detect *stx1, stx2, eaeA* and *hlyA* genes. **Results:** A total of 26(**7.93%**) isolates were positive for *E. coli* O157:H7 using Wellcolex rapid latex agglutination kit. Out of which, 1(0.31%) isolate was found in bull while 25(7.62%) were in cows, 3(0.91%) isolates were in calves while 23(7.02%) in adult cattle and 4(1.22%) were in Bokoloji while 22(6.71%) were in Bunaji, a total 26 isolates were subjected to multiplex PCR, only 2(7.69%) expressed the shiga toxin genes (*stx1*). **Conclusion:** This study highlights the presence of shiga toxin producing *E. coli* O157:H7 in the cattle population of Zaria and the need for stringent control measures to prevent transmission to humans.

Introduction

Escherichia coli O157:H7 has emerged as a pathogen of public health significance globally. The organism can cause a wide range of diseases in humans, from mild diarrhea to life-threatening conditions such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) [1]. Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a major foodborne pathogen responsible for significant morbidity and mortality worldwide. It produces Shiga toxins (stx1 and stx2) that cause severe gastrointestinal illnesses, such as HC and

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HUS, which can lead to kidney failure, particularly in children and the elderly [2]. Cattle are asymptomatic reservoirs of STEC O157:H7, shedding the bacteria in their feces, which can contaminate water, food, and the environment [3, 4]. This makes cattle an important target for monitoring and control strategies aimed at preventing human infections

Transmission to humans occurs through contaminated food, water, or direct contact with infected animals or their environment [5, 6]. In sub-Saharan Africa, including Nigeria, there is limited data on the prevalence of STEC O157:H7, despite the heavy reliance on cattle for food and agriculture. Zaria metropolis, located in Kaduna State, Nigeria, is a significant cattle-rearing area, where free-range system of rearing cattle is the predominant practice. The risk of zoonotic transmission of STEC O157:H7 to humans in such areas is a growing concern [7].

Despite the importance of the pathogen, limited studies have explored its prevalence and genetic makeup in cattle from this region. This study aimed to determine the prevalence of *E. coli* O157:H7 in cattle feces and to characterize its virulence factors through molecular techniques.

Materials and Methods

Study Area

The study was conducted in Zaria metropolis (11°3'N; 7°42'E) comprising of Zaria and Sabon Gari Local Government Areas of Kaduna State, Nigeria (Figure 1), a region known for its high livestock density and commercial slaughterhouses [8].

Study Design

A cross sectional study was conducted. A total of 328 faecal samples were randomly collected from apparently healthy cattle different Fulani cattle herds within Zaria metropolis between April and July 2023.

Sample Collection, Transportation and Storage

The faecal samples were aseptically collected from the rectum of cattle using sterile obstetrical gloves into labeled sterile containers. The samples were transported under refrigeration to the Bacteriology laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for laboratory analyses. All samples were processed immediately on arrival to the laboratory.

Selective Enrichment and Isolation of *E.coli*

One gram of each faecal sample was enriched in 5ml of Tryptone soya broth and incubated at 37ºC for 24 hours [9]. A loopful of the enriched samples were inoculated on Eosin methylene-blue (EMB) agar, and then incubated aerobically at 37ºC for 24 hours. The colonies with a greenish metallic sheen on EMB agar which is a typical feature of *E. coli* were suspected to be *E. coli*.

Phenotypic Identification of *Escherichia coli* Isolated from Cattle Faeces

The suspected *E. coli* isolates were biochemically characterized according to the method described by [16]. The isolates were subjected to different biochemical tests which include; Triple sugar iron, MR-VP (Methyl Red and Voges Proskauer), Simmons citrate, SIM (Sulphide, Indole, Motility) and Urease tests. Isolates that were positive for indole and methyl red tests but negative for Voges Proskauer, urease and Citrate utilization tests were identified as *E. coli* [10].

The *E. coli* isolates were transferred to the Sorbitol MacConkey agar to check for the presence of sorbitol and non-sorbitol fermenters. Colourless colonies on Sorbitol MacConkey agar (non-sorbitol fermenters) were suspected to be *E. coli* O157:H7. The suspected *E. coli* O157 isolates were transferred to nutrient agar to be stored for additional tests. Pinkish colonies on Sorbitol MacConkey agar (sorbitol fermenters) were suspected to be *E. coli* non O157 and were selected and used for further analysis.

Serological Screening of *E. coli* O157:H7

The non-sorbitol fermenting *E. coli* colonies were serologically tested by using Wellcolex rapid latex agglutination kit to confirm and identify as O157:H7 (Oxoid Ltd., Basingstoke, UK). Identification of STEC was carried out following the Manufacturer's instructions for the presence of O157 antigens. The suspected *E. coli* O157:H7 isolates were subcultured on EMB and incubated aerobically at 37ºC for 24 hours. The culture was further enriched in tryptone soya broth at 37ºC for 24 hours. Fifty microlitre of the enriched culture was dropped onto a slide, and one drop of Wellcolex rapid latex agglutination kit for O157:H7 was added and mixed on each circle of the Reaction Card using sterile application sticks, the mixtures were rocked for one minute, and samples that agglutinated were identified as *E. coli* O157:H7 [11].

Molecular Detection of stx1, stx2, eaeA and hlyA Genes from *E. coli* O157:H7

1) **DNA extraction**: DNA was extracted from the 26 serologically identified *E. coli* O157:H7, using the Quick-DNA™ Miniprep Plus Kit (Zymo Research, California U.S). Briefly, 200 µl sample was added to a microcentrifuge tube and 200 µl BioFluid & Cell Buffer (Red) 20 µl Proteinase K were added. The tube was mixed thoroughly and then incubated at 55ºC for 10 minutes. 1 volume Genomic Binding Buffer was added to the digested sample and mixed thoroughly. The mixture was transferred to a Zymo-Spin™ IIC-XLR Column in a Collection Tube. It was then centrifuged (at 12,000 x g) for 1 minute. The Collection Tube with the flow through was discarded. 400 µl DNA Pre-Wash Buffer was added to the column in a new Collection Tube and then centrifuged for 1 minute. The Collection Tube was emptied. 700 µl g-DNA Wash Buffer was added and centrifuged for 1 minute. The Collection Tube was emptied. 200 µl g-DNA Wash Buffer was added and centrifuged for 1 minute. The Collection Tube with the flow through was discarded. The DNA was transferred to a clean microcentrifuge tube. 50 µl DNA Elution Buffer was added, incubated for 5 minutes, and then centrifuged for 1 minute. The pellets were discarded while the supernatant containing the DNA templates was used for polymerase chain reaction.
2) **Multiplex PCR for *stx1, stx2, eaeA* and *hlyA:*** Detection of stx1, stx2, eaeA and hly (Table 1) were performed using 2xEasyTaq^{R} PCR Super Mix for PAGE (TRANS^{R}). 25µl reactions containing 12.5µl of 2xEasyTaq^{R} PCR Super Mix, 1µl of each primer of 20 pmol concentrations, 0.5µl of DNase-free water, and 4µl of DNA template were used. Samples were subjected to 35 PCR cycles, each consisting of 1min of denaturation at 95ºC, 2 min of annealing at 65ºC for the first 10 cycles, decrementing to 60ºC by cycle 15 and 1.5min of elongation at 72ºC, incrementing to 2.5min from cycles 25 to 35.
3) **Gel Electrophoresis:** A 1.5% agarose gel was prepared by suspending 1.5 grams of agarose powder in 100mL of 1x Tris-Borate-EDTA (TBE) buffer and heated on a hot plate until completely dissolved. Ethidium bromide (2.5μl) was added to the liquid agarose before pouring into a gel caster set and allowed to solidify at room temperature. The caster was rightly placed into an electrophoresis tank and flooded with 1x TBE buffer to the maximum level before carefully removing the comb. The PCR products (5μl each) were then loaded into the wells using a 10μl Eppendorf pipette. Four microliters (4μl) of 100bp ladder (BioLabs Inc. New England) mixed with 2μl Gel Loading Dye Blue 6x (BioLabs Inc. New England) was also loaded into one of the wells before connecting the tank to a power pack and plugging it to the mains supply. The products were electrophoresed at 85 volts for 50mins. Immediately after electrophoresis, the agarose gel was viewed using a GelDoc^{TM} XR + (BioRad). The gel image was captured and labelled accordingly.

Statistical Analysis

Data obtained were expressed in percentages and presented in tables, chats and plates. Values obtained were analysed using statistical package for social science (SPSS) (Version 21.0). Chi-square was used to determine the difference in isolation rate between different cattle population. P values <0.05 was considered significant for the study.

Results

A total of 26(7.93%) phenotypically identified E. coli were positive for E. coli O157:H7 (Plate 1, Table 2). Out of which, 1(0.31%) isolate was found in bull while 25(7.62%) were in cows (Table 4); Based on age, 3(0.91%) isolates were in calves while 23(7.02%) in adult cattle (Table 5) and based on breeds sampled 4(1.22%) were in Bokoloji while 22(6.71%) were in Bunaji (Table 3).

Only 2(7.69%) out of the 26 E. coli O157:H7 isolates expressed the shiga toxin genes. This gives a detection rate of 7.69%. All the two genes that expressed the shiga toxin genes harbor only stx1 gene. None of stx2, eaeA and hlyA gene were expressed (Table 6, Figure 2).

Table 1. Shiga toxin primer sequences and expected sizes of PCR amplicons products.

Target gene	Direction	Primer sequence (5´3´)	Amplified segment (bp)	Reference
stx$_1$	Forward	ATAAATCGCCATTCGTTGACTAC	180	
	Reverse	AGAACGCCCACTGAGATCATC		[12].
stx$_2$	Forward	GGCACTGTCTGAAACTGCTCC	255	
	Reverse	TCGCCAGTTATCTGACATTCTG		[12].
EaeA	Forward Reverse	GACCCGGACAAGCATAAGC CCACCTGCAGCAACAAGAGG	384	[12].
hlyA	Forward Reverse	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAAGCT	534	[12].

Table 2. Prevalence of *E. coli* O157: H7 from different locations in Zaria metropolis, Nigeria.

Herd	Location	Number of samples collected	*E. coli* O157:H7 Positive	Prevalence Rate (%)
Herd A	Nagoyi	41	1	**2.44**
Herd B	Kofar kona	41	3	**7.32**
Herd C	Dambo	41	2	**4.88**
Herd D	Bizara	41	7	**17.07**
Herd E	Bassawa	41	4	**9.76**
Herd F	Dogarawa	41	5	**12.20**
Herd G	Bomo	41	3	**7.32**
Herd H	Hanwa	41	1	**2.44**
Total		**328**	**26**	**7.93**

Chi square = 7.853, df = 7, p value = 0.346

Table 3. Breed-specific prevalence of *E.coli* O157:H7 in cattle within Zaria metropolis, Nigeria.

Breed	Number of samples collected	*Number of E. coli* O57:H7 Positive	Prevalence rate in *E. coli* O157: H7(%)
Bunaji	246	22	6.71
Bokoloji	82	4	1.22
TOTAL	328	26	7.93

O157: H7; Odd ratio = 1.437, chi square= 0.501, p= 0.479.

Table 4. Sex-specific prevalence of *E.coli* O157:H7 in cattle within Zaria metropolis, Nigeria.

Sex	Number of samples collected	*Number of E. coli* O157:H7 Positive	Prevalence in *E. coli* O157: H7(%)
Bull	48	1	0.31
Cow	280	25	7.62
TOTAL	328	26	7.93

O157: H7; Odd ratio = 1.102, chi square= 4.841, p= 0.028.

Table 5. Age-specific prevalence of *E.coli* O157:H7 in cattle within Zaria metropolis, Nigeria.

AGE	Number of samples collected	*Number of E.coli* O157:H7 Positive	Prevalence rate in *E.coli* O157: H7(%)
Calves	22	3	0.91
Adult	306	23	7.02
TOTAL	328	26	7.93

O157: H7; Odd ratio = O.515, chi square= 1.053, p= 0.305.
Calves: Pre-weaned calves (suckling calves) were considered as calves.
Adult Cattle: Weaned cattle were considered as adults.

Table 6. Detection of *stx1, stx2 eaeA and hlyA* in *E.coli* O157:H7 from cattle feaces within Zaria metropolis, Nigeria.

Identity no	Location	Serotypes	*Stx1(%)*	*Stx2(%)*	*EaeA(%)*	*HlyA(%)*
SFAA16	Herd A	O157	**1(3.85)**	**0(0.00)**	**0(0.00)**	**0(0.00)**
SFAG11	Herd G	O157	**1(3.85)**	**0(0.00)**	**0(0.00)**	**0(0.00)**
Prevalence			**7.69**	**0**	**0**	**0**

Figure 1. Map of Zaria and its environment showing study area. Source satellite image 2019.

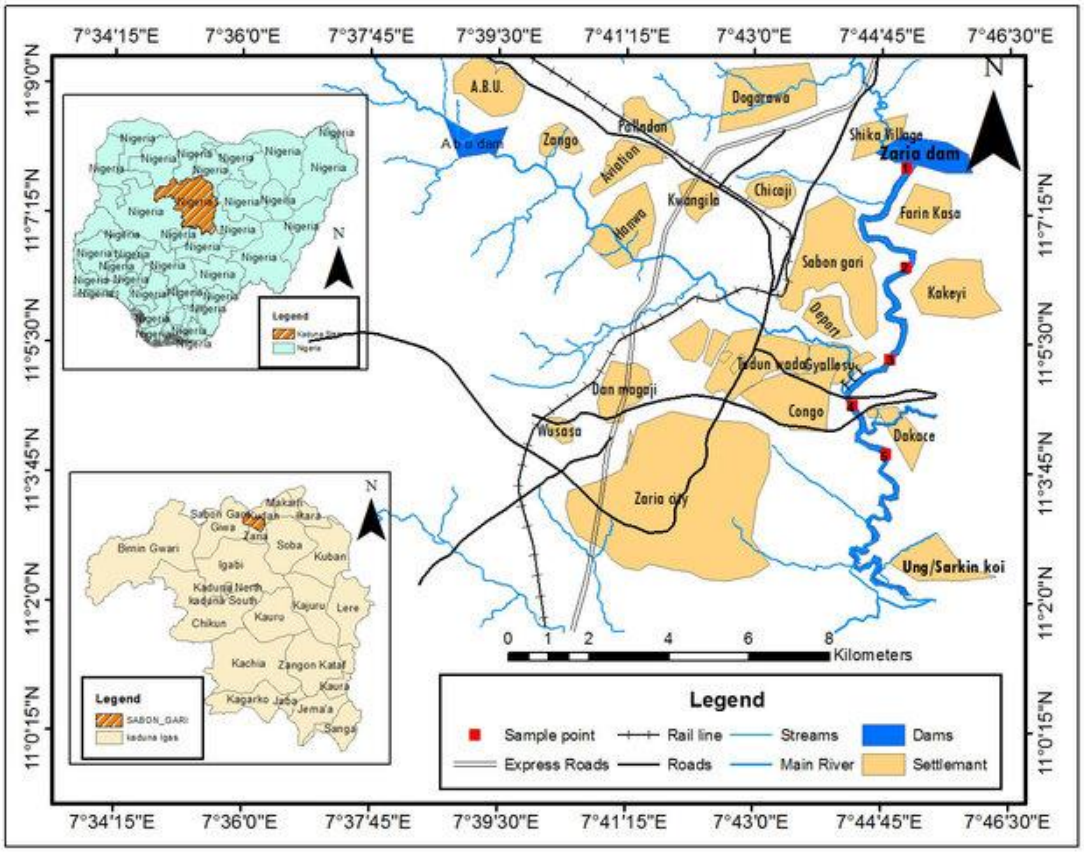


Figure 2. Agarose gel electrophoresis result for Multiplex PCR detection of *Stx1, stx2, eaea and hly* gene in *Escherichia coli O157:H7*. Lane M: 100bp ladder (BioLabs), lanes 2 and 4 are positive isolates; lanes 1, 3 and 5 are negative results and lanes 6 and 7 negative control.

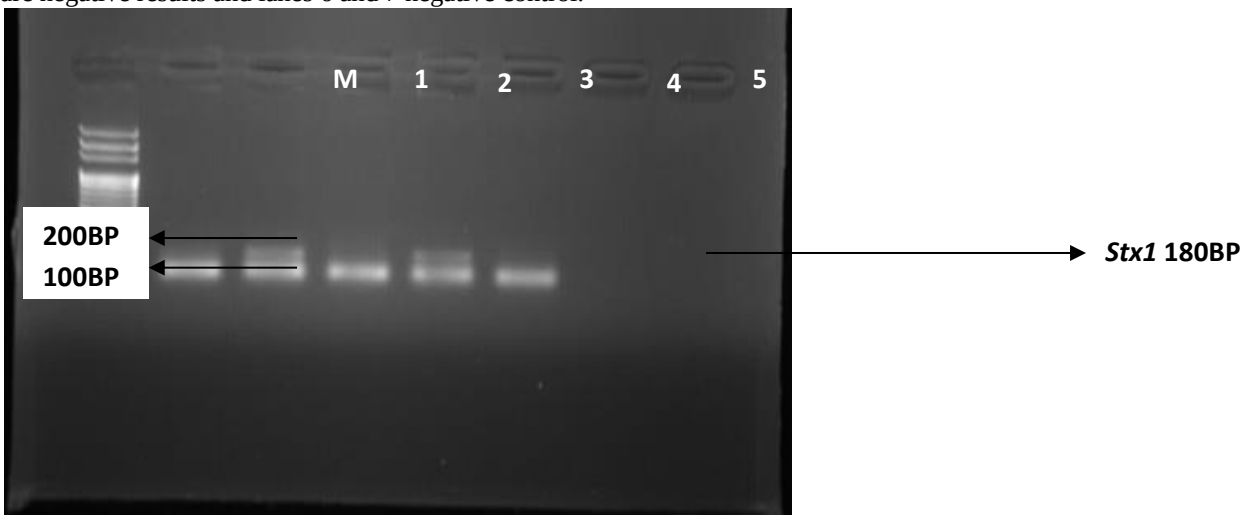
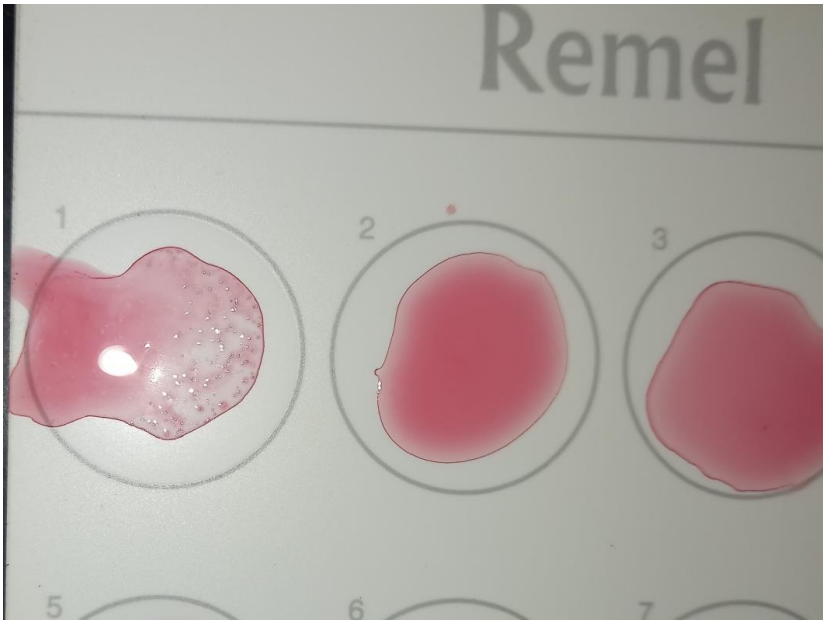


Plate I. Positive agglutination result from sample SFAA16 using Wellcolex rapid latex agglutination kit for confirmation and identification as *E. coli* O157:H7.



Discussion

The 7.93% prevalence of *E. coli* O157:H7 observed in this study is consistent with other reports from African countries, where prevalence rates typically range from 5% to 10% in cattle populations [3]. Several other authors have also reported isolation rates of less than10% of STEC O157 in ruminant feces in tropical countries Manna *et al.*,and Leomil *et al.*, [13,14]. A lower prevalence of 4.5% was reported in apparently healthy cattle in Borno and Adamawa States Moses *et al.*, [15], 2 % from apparently healthy cattle in Lagos Aibinu *et al.*, [16].

Higher prevalence of 12.4% was obtained from the work conducted by Gonzalez *et al.*, [17] where *E. coli* O157:H7 was the most prevalent serotype. Also, a prevalence of 51.4% from the cattle faeces in England and Wales was obtained Paiba *et al.*, [18].

The higher prevalence of *E. coli* O157:H7 in cows 25(7.62%) compared to bull 1(0.31%) may be due to several factors (Table 4). Cows, particularly lactating cows, have different physiological and management conditions compared to bull. For instance, lactating cows often undergo hormonal and metabolic changes that may impact gut flora and immune function, potentially increasing their susceptibility to colonization by pathogens such as *E. coli* O157:H7 [19]. Moreover, management practices such as the stress associated with calving may further predispose cows to bacterial shedding [20].

The significantly higher prevalence of *E. coli* O157:H7 in adult cattle 23(7.02%) compared to calves 3(0.91%) (Table 5) is consistent with the finding of Smith *et al.*, [21] which have shown that adult cattle tend to harbor STEC more frequently due to longer exposure to environmental and dietary factors that promote the colonization and shedding of these pathogens.

The breed-related differences observed in this study (Table 3), with Bunaji 22(6.71%) cattle showing a higher prevalence of *Escherichia coli* O157:H7 than the Bokoloji breed 4(1.22%), suggest that breed-specific factors may influence susceptibility to colonization by this pathogen. Cattle breeds often have different genetic traits that may affect their immune responses to pathogens. Bunaji cattle, which are indigenous to Nigeria and widely kept in Zaria Metropolis, may possess genetic traits that make them more susceptible to *E. coli* O157:H7 colonization. Studies have shown that certain cattle breeds have gut environments that are more conducive to the persistence of *E. coli* O157:H7, potentially due to genetic factors influencing the expression of receptors that bacteria use to adhere to host cells [22].

A detection rate of 7.69% was obtained from *Stx 1* (Table 6). This is in agreement with the research conducted by lawan *et al.*, [23], where he

got a prevalence of 8% STEC O157:H7. Similarly, lawan *et al.,* [23] got prevalence of 0% for *eaeA* gene.

Asanthi *et al.*, [24] in Malaysia, got a lower prevalence of 4% STEC O157:H7 from ruminant feces samples using multiplex PCR. Also a lower prevalence of 4.69% *E. coli* O157:H7 of was recorded from ruminants faeces by Yakubu *et al.*, [25]. These lower prevalences could be attributed to the fact that cattle are the main reservoirs of STEC.

The presence of Shiga toxin genes, particularly *stx1*, in 2 of the isolates emphasizes the zoonotic risk posed by cattle as reservoirs of STEC.

Interestingly, none of the isolates were positive for the *stx2* or *eaeA* genes, which are often associated with more severe disease outcomes, such as HUS [21]. The absence of these genes in all of the isolates might suggest a lower pathogenic potential, although it is well established that even strains harboring only *stx1* can cause serious illness.

The finding that 2 of the 26 isolates carried the stx1 gene highlights the potential for transmission to humans, particularly in areas where cattle feces can contaminate food or water sources. This underscores the need for improved hygiene practices in cattle handling and slaughtering processes within Zaria Metropolis to mitigate the risk of zoonotic transmission.

Conclusion

This study confirms the presence of *Escherichia coli* O157:H7 in cattle feces within Zaria Metropolis, with (7.93%) of the fecal samples testing positive for the pathogen. Molecular characterization revealed that two isolates harbored the stx1 gene, indicating the presence of potentially pathogenic strains.

Recommendations

Personal and environmental hygiene measures should be practiced when handling cattle and its products. Free range rearing system should be controlled and monitored. Good manure treatment strategy should be adopted. Continuous surveillance, along with public health interventions aimed at reducing the contamination of food and water sources by cattle feces, is essential to prevent potential outbreaks in the human population. Finally, set educational programs to publish awareness about STEC safety issues among cattle herders, crop and vegetable farmers, milk and milk products handlers as well as meat and meat product consumers.

Conflict of interest

None

Financial disclosure

None

References

1- Pruimboom-Brees I, Rowland AL, Ahrens S. Global epidemiology and trends of E. coli O157:H7 infections: Implications for livestock and public health. J Food Prot 2023; 86(5), 679-687.

2- Nataro JP, Kaper JB. Diarrheagenic Escherichia coli infections: An update on pathogenesis and treatment. Clin Microbiol Rev 2018; 31(1):e00011-17. Doi:10.1128/CMR.00011-17.

3- Abdullahi AM, Barde AA, Yahaya H. Prevalence and molecular characterization of Escherichia coli O157:H7 from cattle in Northern Nigeria. Afr J Microbiol Res 2022; 16(3), 175-182.

4- Abubakar A, Usman YA, Suleiman S. Prevalence and antimicrobial susceptibility profile of shiga-toxin producing Escherichia coli in abattoirs of Northwestern Nigeria. BMC Vet Res 2022; 18(1), 58.

5- Lim JY, Yoon JW, Hovde CJ. A brief overview of Escherichia coli O157:H7 and its plasmid O157. J Microbiol Biotech 2020; 30(4):477-491. Doi:10.4014/jmb.2001.01029.

6- Ojo EF, Akinola O, Oranusi S. Molecular detection of shiga-toxin genes in E. Coli from bovine sources in Southern Nigeria. J Pathogens 2021; 689432.

7- Dapjala D, Elisha I, Odiko S, Olatunji V. Prevalence and molecular characterization of Shiga toxin-producing Escherichia coli O157:H7 from animal feces in abattoirs and cattle farms in Northern Nigeria. Internat J Infect Dis 2020; 95:387-392. Doi:10.1016/j.ijid.2020.02.054.

8- National Bureau of Statistics (NBS). Agricultural production survey: Livestock farming in Kaduna State. Abuja, Nigeria (2020).
9- Akanbi O, Aremu S, Ayansina B. Molecular detection of Shiga toxin genes in Escherichia coli O157:H7 isolates from cattle in Nigeria. J Infect Dev Countr 2022; 16(4):659-666. Doi:10.3855/jidc.15562.
10- Salim W, Ugochukwu E, Adamu S. Isolation and molecular characterization of Shiga toxin-producing Escherichia coli from animal feces in Nigeria. A J Microbiol Res. 2019; 13(8):148-154. Doi:10.5897/AJMR2019.8961.
11- Fagan KP, McGrath N, Traber KE. Comparative analysis of virulence factors in E. coli O157:H7 isolates from cattle and humans. Vet Microbiol 2021; 260:109171. Doi:10.1016/j.vetmic.2021.109171.
12- Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. Clin Microbiol Rev 2020; 33(3):e00031-19. Doi:10.1128/CMR.00031-19.
13- Manna SK, Brahmane MP, Manna C, Batabyal K, Das R. "Occurrence, virulence characteristics and antimicrobial resistance of Escherichia coli O157 in slaughtered cattle and diarrhoeic calves in West Bengal, India," Let App Microbiol 2006; 43(4):405–409.
14- Leomil L, Aidar-Ugrinovich L, Guthetal BEC. "Frequency of Shiga toxin-producing Escherichia coli (STEC) isolates among diarrheic and non-diarrheic calves in Brazil. Vet Microbiol 2003; 97(2):103–109.
15- Moses AE, Ameh JA, Egwu GO. Prevalence of Escherichia coli serogroup O157, O118, O111, O26 in cattle faeces, dairy products and water samples in Borno and Adamawa States. Proceedings of the 42nd Congress of Nigerian Veterinary Medical Association 2005; 162-168.
16- Aibinu IE, Peters FR, Amisu KO, Adesida SA, Ojo MO. Multi-drug resistance in Escherichia coli O157 strains and the public health implication. J Amer Sci 2007; 3(3): 22-33.
17- Gonzalez AG, Cerqueira AM, Guth BE, Coutinho CA, Liberal MH. Souza RM, et al. Serotypes, virulence markers and cell invasion ability of Shigatoxin-producing Escherichia coli strains isolated from healthy dairy cattle. J Appl Microbiol 2016; 121, 1130–1143.
18- Paiba GA, Wilesmith JW, Evans SJ, Pascoe SJ, Smith RP. Prevalence of faecal excretion of verocyto-toxigenic Escherichia coli O157 in cattle in England and Wales. Vet Rec 2003; 153(12): 347-353.30.
19- Omotayo OG, Ogunleye TE, Adesokan HK. Influence of physiological and environmental factors on the shedding of Escherichia coli O157:H7 in cattle. J Vet Med 2022; 19(3), 145-154.
20- Oluwaseun A, Ibrahim SY, Okeke RA. Antibiotic resistance patterns of shiga toxin-producing Escherichia coli isolated from livestock in Nigeria. Infect Dis Rep 2022; 14(4), 812-818.
21- Smith DR, Johnson EA, McAllister TA. Cattle as reservoirs of Shiga toxin-producing Escherichia coli: Mechanisms of persistence and control strategies. J Anim Sci 2023; 102(5), 2512–2525.
22- Adeleye AO, Lawal SO, Adewale AB. The role of genetic factors in susceptibility to bacterial infections in livestock: A focus on Escherichia coli O157:H7. J Gen Anim Sci 2021; 9(2), 23-30.
23- Lawan MK, Bello M, Lawan J, Grande L, Morabito S. Detection of pathogenic

Escherichia coli in samples collected at an abattoir in Zaria, Nigeria and at different points in the surrounding environment. Internat J Environ Res 2015; 12:679-691 doi:10.3390/ijerph12010067 9

24- Asanthi P, Charles MC, Gary AD, Narelle F. Characterization of shiga toxigenic Escherichia coli O157 and Non-O157 isolates from ruminant feces in Malaysia. BioMed Res Internat 2014; 382403 http://dx.doi.org/10.1155/2015/38240.

25- Yukubu RO, Lawan MK, Kwaga JK, Kabir J. Isolation, molecular detection and antimicrobial susceptibility profile of Escherichia coli O157:H7 in household - reared small ruminants in Zaria metropolis, Kaduna state, Nigeria. Sahel J Vet Sci 2020; 17(4):16-23.

Lawal A, Kabir L, Balarabe F, Abubakar Y, Usman M, Lawal F, Umar Y. Isolation and characterization of shiga toxin-producing Escherichia coli O157:H7 from cattle feces within Zaria Metropolis, Nigeria. Microbes Infect Dis 2025; 6(3): 5020-5028.