Original Article

Isolation and Molecular Detection of Shiga Toxin-producing *Escherichia coli* (STEC) Pathotypes in Cattle Slaughtered at Gusau Modern Abattoir, Zamfara State, Nigeria

Mukhtar Mamuda[1], Kabiru Haliru Ahmad[2], Mohammed Kabiru Lawan[3], Aminu Shittu[4]

[1]Department of Veterinary Public Health, Ministry of Agriculture and Natural Resources, Zamfara, [2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, [3]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, [4]Quantitative Epidemiology and Animal Health Research Group, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria

Abstract

Background: Cattle have remained a primary reservoir for Shiga toxin-producing *Escherichia coli (STEC)*. This zoonotic pathogen causes serious diarrhea in humans and animals by producing toxins that can severely damage the lining of the intestines and kidneys. The prevalence of these diarrhoeagenic agents in cattle has not been fully ascertained in most States in Northwestern Nigeria. Therefore, this study aimed to determine the prevalence of STEC and other pathotypes of *E. coli* from cattle slaughtered at Gusau Modern Abattoir, Zamfara State, Nigeria. **Materials and Methods:** A total of 120 faecal samples were collected from mid-September to mid-October 2021. The faecal samples were collected from inside and around the rectum using a gloved hand and transported in an icebox container to the bacterial zoonoses laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, for processing. The samples were processed according to the standard bacteriological procedure for the isolation and identification of *E. coli* on Eosin Methylene blue and Cefixime- Tellurite Sorbitol MacConkey (CT-SMAC) agars to *E. coli* O157:H7 and non-O157 serogroups. The serogroups were confirmed using commercially available antisera (wallcolex and prolix agglutination kits) obtained from SSI Institute, Denmark. **Results:** The results revealed an isolation rate of 35 (29.2%) for *E. coli* isolates. All 35 *E. coli* isolates were positive on CT-SMAC agar, in which the colourless colonies were presumed to be *E. coli* O157:H7; in contrast, pinkish colonies were presumed to be *E. coli* non-O157. Serotyping was performed, where 12(34.3%) were confirmed to be O157:H7, while 15(42.9%) were confirmed to be non-O157 serogroups (O26, O45, O101, O103, O111, and O145). It was observed that out of 5 (five) *E. coli* isolates subjected to multiplex PCR, only 1(20%) with the sample I.D number 42WFAM harbored the *stx2* gene. **Conclusion:** This study established the presence of *E. coli* O157:H7 and non-O157 serogroups in cattle slaughtered at the Gusau modern abattoir, indicating a high public health risk to people living in the study area.

Keywords: Abattoir, *Escherichia coli*, molecular detection, serotypes, Shiga toxin-producing *Escherichia coli*, Zamfara State

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) is a significant foodborne pathogen responsible for severe gastrointestinal diseases in humans, including haemorrhagic colitis and haemolytic uremic syndrome. Cattle are recognised as the principal reservoir of STEC, with both O157 and non-O157 serotypes implicated in zoonotic transmission through contaminated meat and other food products (1-5). The asymptomatic carriage and faecal shedding of STEC by cattle pose a persistent public health risk, particularly in regions where abattoir hygiene and meat processing practices may facilitate cross-contamination (3,4,6,7).

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The global burden of STEC-related illnesses underscores the importance of surveillance and molecular characterisation of these pathogens in the beef production chain. Studies from various countries have demonstrated considerable genetic diversity amongst STEC isolates, with multiple serotypes

Address for correspondence: Dr. Mukhtar Mamuda, Department of Public Health, Ministry of Agriculture, Gusau, Zamfara, Nigeria.
E-mail: mamood42@yahoo.com

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and virulence gene profiles detected in cattle and beef products (1,2,8-11). Molecular techniques, such as polymerase chain reaction (PCR) and whole-genome sequencing, have enabled the identification of key virulence factors, including *stx 1*, *stx 2*, *eae* and *hlyA* genes, and the assessment of antimicrobial resistance patterns, which are critical for risk assessment and outbreak prevention (1-3,5,6,9,10).

Despite increasing awareness, data on the prevalence and molecular characteristics of STEC in Nigerian abattoirs remain limited. Recent studies in Nigeria and other African countries have highlighted the presence of both O157 and non-O157 STEC in cattle, with notable variation in serogroup distribution, virulence gene carriage and antimicrobial resistance (4,12,13). The role of abattoir hygiene and worker practices has also been identified as a key determinant in the spread and persistence of STEC contamination (4,6).

Given the public health implications and the economic importance of cattle production in Nigeria, there is a pressing need for comprehensive studies focusing on the isolation and molecular characterisation of STEC in local abattoirs. This research aims to address this gap by investigating the prevalence, serotype diversity and virulence gene profiles of STEC *E. coli* pathotypes in cattle slaughtered at Gusau Modern Abattoir, thereby contributing to improved food safety and public health interventions in the region (4-6,12,13).

Materials and Methods

Study area

The study was carried out at the Gusau Modern Abattoir, located in Gusau, the capital city of Zamfara State, Northwestern Nigeria. Gusau lies approximately between latitude 12°10′N and longitude 6°40′E. The area is characterised by a Sudan savanna vegetation zone, with a semi-arid climate marked by distinct wet and dry seasons. The wet season typically spans May to October, while the dry season lasts from November to April. Average annual rainfall ranges between 600 and 1000 mm, and temperatures can vary from 15°C during the Harmattan to over 38°C in the dry season.

Gusau serves as a major commercial hub in Zamfara State, with livestock farming being a significant economic activity. The Gusau Modern Abattoir is strategically situated to cater to the high demand for meat processing in the region. It receives animals from surrounding rural communities and neighbouring states, making it an important point for meat supply and distribution. The facility operates daily, handling cattle, sheep and goats and plays a vital role in food security and public health within the state [Figure 1] (14).

Sample size determination

The sample size was calculated using the formula by Thrusfield (15) at 95% Confidence level (CL) and a previous prevalence of 7.5% (16).

$$n = \frac{Z^2 \times P(1-p)}{d^2} = n = (1.962 \times P_{exp}\,(1 - P_{exp})/d^2;$$

n = Sample size

z = Appropriate value for standard normal deviation for the desired 95% CL (confidence level) = 1.96

p = Previous prevalence = 7.5% at 95% CL (16)

q = Probability of failure = $(1 - p)$

d = Error margin or desired absolute precision/confidence interval of 5%

$$n = \frac{(1.96)^2 \times 0.075 \times (1-0.075)}{(0.05)^2} = 106.$$

13.5% was added to increase the precision.

n = 106 + 13.5% =120.

Study design

A cross-sectional study was conducted between September and October 2021 at the Gusau Modern Abattoir, Zamfara State, Nigeria. Faecal samples were systematically collected from slaughtered cattle during routine operations at the abattoir.

Sample collection, transportation and storage

Faecal samples were collected, mainly from cattle designated for slaughter at the lairage or immediately after being slaughtered at the abattoir. Samples were placed on sterile bottles and maintained cool on an ice-packed cold box and transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, for analysis. Samples not immediately processed were stored at 4°C until needed for analysis.

Ethical approval

The study was reviewed and approved by the Animal Use and Ethics Committee at Ahmadu Bello University, Zaria, with permission No. ABUCAUC/2021/100.

Escherichia coli isolation and identification

The procedure was in accordance with the International Standard Organization – Reference (ISO 16654) method for the isolation of *E. coli.*

The collected samples were pre-enriched in nutrient broth and incubated for 24 h at 37°C. After the appearance of confirmed growth, a loopful was streaked onto MacConkey agar (MAC) medium plates and incubated at 37°C for 24 h. The pink lactose-fermenting colonies were picked up with a sterile loop and transferred onto eosin–methylene blue (EMB) agar, then incubated for 24 h at 37°C. Discrete colonies with a metallic green sheen typical of *E. coli* were inoculated into the semi-solid medium by stabbing technique, incubated for 24 h at 37°C and kept at 4°C. The suspected colonies were used for further morphological investigation, such as biochemical and molecular characterisation. The isolated *E. coli* strains

Figure 1: Map of Zamfara State showing the study area (14)

upon specific media were confirmed at the species level using different biochemical tests such as sulphur reduction, iMviC and urease test (17,18). The *Staphylococcus aureus* ATCC 29737 was used as a negative control and *E. coli* STEC (*stx* 1, *stx 2*, *eaeA* and *hlyA*).

Biochemical test for the identification of *Escherichia coli*

The presumptive *E. coli* (both O157 and non-O157) were subcultured from nutrient agar onto EMB agar, incubated at 37°C for 24 h and biochemically characterised using methods described by Khandaghi *et al.* (19). The isolates were subjected to eight different biochemical tests, which include: Triple Sugar Iron, methyl red and Voges–Proskauer, Simmons citrate, sulphide indole motility medium, urea agar test, sucrose and sorbitol.

Rapid latex agglutination test for *Escherichia coli* O157:H7

A commercially available latex agglutination kit (OK O Pool) from SSI Diagnostica, Denmark, was used for *E. coli* O157 and H7 antisera, which were used to confirm *E. coli* following conventional biochemical test reagents. The kit contains two test reagents: an O157 test reagent and an H7 test reagent. The O157 test reagent consists of a red latex particle coated with antibodies specific for *E. coli* O157, and the H7 test reagent consists of a blue latex particle coated with antibodies specific for *E. coli* H7 antigen. The principle of the test reaction is that when a drop of the reagent is mixed on a card with the suspension of an *E. coli* O157 organism, rapid agglutination occurs through the interaction of specific antigen O157 organisms with the O157 antibodies in the test reagent.

Test procedure for O157 (somatic antigen)

Confirmed *E. coli* isolates after conventional biochemical testing were subcultured on freshly prepared cefixime–tellurite Sorbitol MacConkey agar and incubated at 37°C for 18–24 h. With the use of a mixing stick, a sufficient amount of growth (non-sorbitol fermenting cultures) was picked, just enough to cover the blunt end of the stick. Forty microliters of normal saline (0.85% NaCl w/v) were placed in two circles on the reaction card and mixed thoroughly.

Serotyping was performed on the isolates to determine the O antigen according to Kok *et al*. (20) using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co.).

For each test sample, one drop of O157 test latex and one drop of O157 control latex were applied and mixed by rocking the container and carefully spreading the latex over the entire

area of the circle. Rapid agglutination was used to confirm the presence of the O157 antigen.

Test procedure for H7 (flagella antigen)

Cultures positive on the latex test for O157 antigen were grown for 18–24 h at 37°C in Tryptone-soya broth. Forty microliters of the broth were placed in two circles on the reaction card. The H7 test latex and H7 control latex were applied separately on the circle of the card and mixed by carefully spreading the latex over the entire area of the circle. The reaction was allowed for 1 min, and then, observed for the presence of agglutination. Positive results showed agglutination with clear clumping of the latex particle. The rate of agglutination appearance is dependent on the quality and quantity of the cultured antigen. In case of a negative result, the latex does not agglutinate, and the appearance of the suspension remains substantially unchanged throughout the reaction; the results are shown in Table 1.

Virulence genes primer sequences

Table 2 presents the primer sequences used for PCR amplification of key virulence genes (*stx 1*, *stx 2*, *eaeA* and *hlyA*) in this study. It includes the forward and reverse primer sequences in 5′–3′ orientation and the expected amplicon sizes in base pairs for each target gene.

Results

Out of one hundred and twenty faecal samples analysed on EMB for the presence of *E. coli*, 75 (62.5%) produced bacteria growth morphologically typical of *E. coli.* Out of these 75 (62.5%) isolates, 35 (29.2%) showed biochemical characteristics of *E. coli.*

Table 1: Seroprevalence of *Escherichia coli* isolated from cattle faeces at Gusau Modern Abattoir, Zamfara State, using latex agglutination kits (*n*=35)

Breed	O157:H7	Non-O157	Overall prevalence
White Fulani	4	9	13
Sokoto Gudali	5	4	9
Red Bororo	3	1	4
Adamawa Gudali	0	1	1
Total, *n* (%)	12 (34.3)	15 (42.9)	27 (77)

Out of the 35 faecal samples examined for the presence of *E. coli* O157:H7 and non-O157 serotypes, 27 (77.1%) were positive for *E. coli* [Table 1]. The overall prevalence of O157:H7 was 12 (34.3%), while Non-O157 isolates accounted for 15 (42.9%). Amongst the breeds, the White Fulani showed the highest prevalence, at 13 (37.1%), followed by the Sokoto Gudali with 9 (25.7%), then Red Bororo with 4 (11.4%) and the Adamawa Gudali with 1 (2.9%) [Figure 2].

Breed-wise distribution revealed the highest prevalence amongst White Fulani cattle (13 isolates), followed by Sokoto Gudali (9 isolates), Red Bororo (4 isolates) and Adamawa Gudali (1 isolate). Both O157:H7 and non-O157 serotypes were detected across most breeds, except Adamawa Gudali, where only non-O157 *E. coli* was identified.

Five latex agglutination-positive isolates were further analysed using multiplex PCR for the detection of virulence genes (*stx 1, stx 2, eaeA* and *hly*). Only one non-O157 *E. coli* isolate (42WFAM) was positive for the *stx 2* gene, as demonstrated by agarose gel electrophoresis [Plate 1]. None of the isolates harboured *stx 1, eaeA* or *hly* genes.

Molecular detection of virulence genes

E. coli isolates were tested for the presence of the virulence genes stx_1, stx_2, *eaeA* and *ehlyA* in PCR assays. These genes code for Shiga toxin 1, Shiga toxin 2, intimin and enterohaemolysin, respectively. All reactions were conducted in a 25-µl reaction mixture containing 2 µl of template DNA, 10 mM Tris-HCl (pH 8.4), 50 mM KCl buffer, 2.5 mM $MgCl_2$, 200 µM concentrations of each deoxynucleoside triphosphate (Promega, Madison, WI) and 1.526 U of Taq DNA polymerase (Promega). Oligonucleotide primers [Table 2] were used at concentrations of 0.5 µM. Samples were amplified in a thermocycler (Mastercycler, Eppendorf, Hamburg, Germany) using the conditions described by Sipos *et al*. (22) [Plate 1].

Discussion

The high overall prevalence of *E. coli* (77.0%) observed in this study confirms the widespread occurrence of this organism in cattle faeces, particularly in abattoir settings. Similar high carriage rates of *E. coli* amongst slaughtered cattle have been reported in Nigeria and other developing countries,

Table 2: Primer sequences and size of polymerase chain reaction amplification products

Target gene	Direction	Primer sequence (5'-3')	Amplified segment (bp)	Reference
stx1	Forward	ACACTGGATGATCTCAGTGG	614	(21)
	Reverse	CTGAATCCCCCTCCATTATG		
stx2	Forward	CCATGACAACGGACAGCAGTT	779	(21)
	Reverse	CCTGTCAACTGAGCAGCACTTTG		
eaeA	Forward	GTGGCGAATACTGGCGAGACT	890	(21)
hlyA	Reverse	CCCCATTCTTTTTCACCGTCG	165	(21)
	Forward	ACGATGTGGTTTATTCTGGA		
	Reverse	CTTCACGTGACCATACATAT		

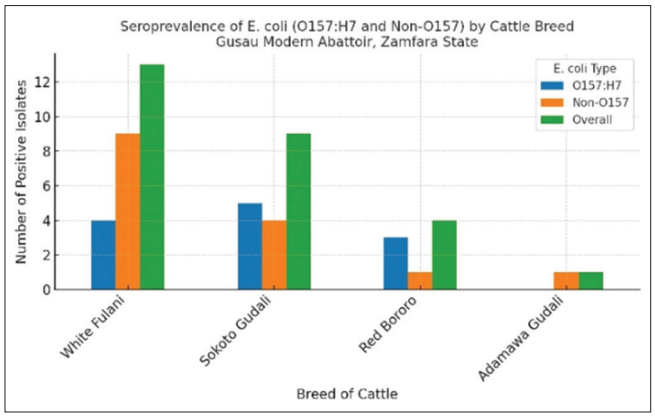

Figure 2: Distribution of *Escherichia coli* O157:H7 and non-O157 isolates across cattle breeds in Gusau Modern Abattoir, Zamfara State. *E. coli: Escherichia coli*

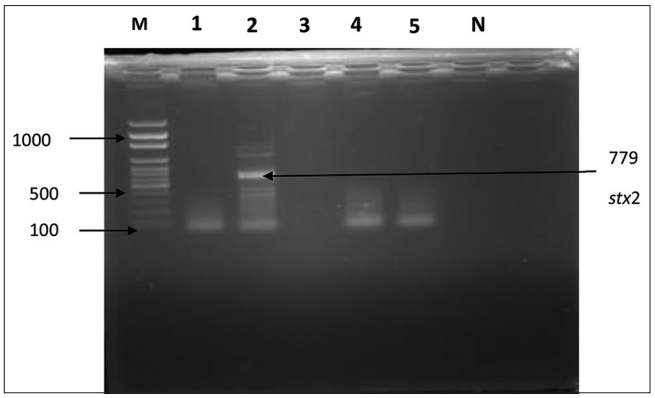

Plate 1: Multiplex polymerase chain reaction amplification of *stx 1*, *stx 2*, *eaeA* and *hly* genes (lanes 1–5 were *Escherichia coli* isolates, lane M – 100 bp molecular ladder, lane N – negative control and lane 2 – positive *stx 2* gene)

highlighting cattle as important reservoirs of potentially pathogenic strains (23,24).

The prevalence of *E. coli* O157:H7 (34.3%) recorded in this study is higher than reports from Bauchi State and other regions of Nigeria, where lower prevalence rates have been documented (23) Differences in geographical location, animal management practices, hygiene conditions at slaughter facilities and sampling methodologies may account for this variation. The relatively high detection rate in the present study raises concerns regarding abattoir hygiene and meat safety in the study area.

Non-O157 *E. coli* isolates were more prevalent than O157:H7, which is consistent with previous studies indicating that non-O157 strains are more commonly carried by cattle and are increasingly implicated in foodborne infections (25,26). Although often overlooked, non-O157 *E. coli* strains may possess significant virulence attributes and should be included in routine surveillance programmes.

The detection of the *stx 2* gene in one non-O157 isolate aligns with earlier findings reporting low but significant occurrence of Shiga toxin genes in cattle-derived *E. coli* isolates (19). Importantly, *stx 2* is strongly associated with severe human illnesses such as haemorrhagic colitis and haemolytic uremic syndrome (27). The absence of *stx 1, eaeA* and *hly* genes in most isolates suggests a lower overall virulence potential; however, even a small proportion of toxigenic strains poses a significant public health risk.

Breed-related differences observed in this study, particularly the higher prevalence amongst White Fulani and Sokoto Gudali cattle, may be influenced by management systems, movement patterns and environmental exposure, as previously reported by Ahmad *et al.* These findings emphasise the need for targeted interventions at the abattoir and farm levels.

Public health implications

The presence of *E. coli* O157:H7 and non-O157 strains, including a Shiga toxin-producing isolate, in cattle slaughtered for human consumption represents a potential public health hazard. Inadequate hygiene during slaughter and meat processing may facilitate contamination of carcasses, abattoir environments and water sources, thereby increasing the risk of foodborne transmission to humans.

Given the association of *stx 2*-positive *E. coli* with severe disease outcomes, continuous monitoring of both O157 and non-O157 *E. coli* in cattle is essential. Strengthening abattoir sanitation, enforcing meat inspection standards and promoting good hygienic practices amongst meat handlers are critical control measures. Furthermore, incorporating molecular surveillance of virulence genes into routine monitoring programmes will enhance early detection and prevention of zoonotic transmission, in line with the One Health approach advocated by the World Health Organization (27).

Conclusion

This study demonstrated a high prevalence of *E. coli*, including O157:H7 and non-O157 serotypes, in cattle slaughtered at the Gusau Modern Abattoir, Zamfara State. The detection of these pathogenic strains highlights a potential public health risk associated with beef production and handling in the study area. The confirmation of only one isolate by PCR suggests the need for more sensitive and comprehensive molecular diagnostic approaches. Strengthening abattoir hygiene practices, routine monitoring and public health awareness are recommended to reduce the risk of foodborne transmission.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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