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Shiga Toxin Producing Strains of *Escherichia coli* (STEC) Associated with Beef Products and Its Potential Pathogenic Effect

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Authors' contributions

This work was carried out in collaboration between all authors. Author IMO designed the study and wrote the manuscript. Authors EU and EO carried out the animal study while Author GM screened meat products for STEC. All authors read and approved the final manuscript.

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ABSTRACT

Background: Contaminated beef products sold in Nigeria are widely consumed by all with little or no knowledge of the presence or occurrence of possible pathogen.
Methods: The current study investigated the presence of shiga toxin producing strains of *E. coli* (STEC) in beef products using selective and chromogenic media. Furthermore, the pathogenicity of STEC was determined *in vivo* using established methods.
Results: *E. coli* was isolated from all beef samples while 30 % (mean) were reported to be STEC. There was a dose-dependent increase in the levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase observed in the serum of all mice exposed to STEC. This was also confirmed by an obvious damage in the livers and kidneys of the affected mice.
Conclusion: By and large, the result of this study shows that beef products sold in Benin City, Edo State, Nigeria are loaded with STEC, which could pose grave problem for consumers. Hence, effort should be made to minimize their presence in beef, and to prevent possible outbreaks.

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1. INTRODUCTION

Escherichia coli are Gram negative, lactose fermenting, facultative aerobic rods. There are over 170 known serogroups of *E. coli,* distinguished by the expression of O (somatic), H (flagella) and/or K (capsular) antigens. Over the past decade, several groups of pathogenic *E. coli* have been identified, with shiga toxin-producing *Escherichia coli* (STEC) or enterohemorrhagic *E. coli* (EHEC) being the most notorious, based on its threat to public health. STEC produces two phage-encoded cytotoxins called Shiga toxins (Stx1 and Stx2), and have been implicated as the causative agent in several human diseases including mild non bloody or severe bloody diarrhea (haemorrhagic colitis), haemolytic uremic syndrome (HUS) and renal failure [1,2]. Cattle are considered to be the principal natural reservoir of this pathogen [3], but strains of this pathogen are also prevalent in the gastrointestinal tracts of other domestic animals, particularly ruminants [4].

STEC comprises of serologically different strains, with *E. coli* O157:H7 being the serotype that has been linked to most of the outbreaks of food-borne diseases and has led to the largest number of haemolytic uremic syndrome (HUS) cases in humans [5]. In most human infections, transmission occurs primarily by ingestion of contaminated food: most often inadequately cooked meat, especially minced beef, raw milk and milk products as well as ready to eat (RTE) products such as fresh fruits and vegetables [6].

During the processing of beef carcass, the presence of *E. coli* is regarded as an indicator of faecal contamination. Their occurrence is influenced by factors such as the level of faecal contamination of live cattle, efficiency of evisceration and hygienic practices in the abattoir.

Also during processing, faecal contamination or transfer of bacteria from the animal's hide to the carcass often facilitates the transmission of pathogenic *E. coli* during the food chain [7]. Although reported outbreaks of *E. coli* O157 in Africa are few to date, available information indicates that the pathogen has wide geographic distribution. Since the 1992 outbreak, culture-proven *E. coli* O157 diarrheal illness has been reported from multiple locations in Africa, particularly in Kenya, Nigeria, Côte d'Ivoire, and Central Africa Republic [8,9]. In Egypt, 6 (5%) of 125 meat samples obtained from slaughter houses yielded *E. coli* O157 [10]. Also, because *E. coli* O157 is not detected by the usual conventional methods used to isolate and identify the traditional enteric bacterial pathogens, coupled with the fact that microbiology laboratories in most developing countries especially in Africa do not routinely test for this pathogen, *E. coli* O157 infections may go unrecognized for years [11]. Reports on African dysentery outbreaks attributed to *Shigella* (with pathogenesis similar to that of *E. coli* 0157) sometimes indicate that samples were not tested until several months into an outbreak [12,13]. This is unfortunate because the spectrum of clinical illness resulting from *Shigella* infection overlaps considerably with that of *E. coli* O157 and mixed outbreaks have been reported [14].

STEC, like other *E. coli* survive in the intestine of various animal reservoirs, including ruminants and monogastric animals [15,16,17,18] and are easily attached to the gastrointestinal tract where the shiga toxins are elaborated to cause a number of negative effects.

This study therefore firstly aimed at investigating the potential occurrence of STEC in beef samples and secondly determining their potential pathogenic effects *in vivo.*

2. MATERIALS AND METHODS

2.1 Materials and Sample Collection

The Nutrient agar, Eosin Methyl Blue (EMB) agar, Muller Hinton agar and MacConkey agar used in this study were obtained from Titan Biotech Ltd (Rajasthan, India), while the CHROMagar STEC base was obtained from CHROMagar (Paris, France). A total of 60 different samples were obtained from two major markets in Benin City, Edo State, Nigeria, between January and April, 2016. All experiments were done in the central laboratory of Benson Idahosa University, Benin City, Edo State, Nigeria.

2.2 Microbiological Analysis

The mean coliform and mean enterobacteriaceae counts were determined as previously reported [19]. Briefly, 25 g of beef samples were placed in

225 ml of peptone water, following which serial dilutions were made. 1 mL each of the serial dilutions (4^{th} and 5^{th}) was transferred to already prepare EMB, MacConkey and Nutrient agar plates in triplicates. The respective agar plates were then incubated at 37°C for 24 hours.

2.3 Identification of Bacterial Isolates

Clearly distinct colonies were identified based on their cultural, morphological and biochemical characteristics, principally characteristic of the Enterobacteriaceae family. Pure cultures of all colonies exhibiting typically dark colonies with green metallic sheen on EMB agar and pink colonies on MacConkey agar were sub-cultured and re-plated on CHROMagar STEC base. *E. coli* strains harbouring the shiga-toxin gene were noted for the mauve colouration while other enterobacteriaceae were either inhibited or were indicated with colourless to bluish colouration.

STEC used for animal studies were cultured on trypton soy broth for 12 to 18 hr, until the isolate reached its exponential growth phase.

2.4 Animal Studies

Healthy mice weighing between 20 to 25 g were obtained and divided into four groups (A, B, C and D), with each groups containing at least, 3 mice. Group A served as the control group, group B were administered shiga toxin (oral gavage) daily for 1 week, group C were administered shiga toxin 3 times during the study period (1 week), while group D were administered a single dose of shiga toxin. Each dose was equal to 2 ml per kg of body weight, with each ml containing approximately 200 cfu/ml of the isolates at OD_{600}. The control mice were also administered 2ml of trypton soy broth void of STEC. All animal experiment was carried out in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines.

2.4.1 Serum indicator enzyme test

Upon the last administration of mice to STEC, they were allowed to fast for approximately 10 to 12hours prior to being sacrificed. The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP) were measured as previously described [20].

2.5 Histopathology

Mice were sacrificed by cervical vertebra dislocation, the kidney and liver were removed and fixed in 4% formalin overnight at 4°C. Following overnight treatment, the organs (kidney and liver) were run through alcohol-xylene for dehydration and clearing, and further embedded in paraffin. A thin section of the organs were cut, deparaffinized and hydrated before being stained with hematoxylin-eosin (HE) for microscopic observation. Slides were prepared and examined under a microscope.

3. RESULTS AND DISCUSSION

The results of the current study shows that beef products sold in Benin City, Edo State, Nigeria are sources of human exposure to pathogenic *E. coli*. A total of 24 different species of *E. coli* were obtained from all beef samples, out of which 7(30%) were further confirmed to be shiga-toxin producing strains of *E. coli* (data not shown).

Table 1 shows the ALT, AST and ALP activities of the mice serum orally administered STEC at different doses following a short-time exposure (7 days). There was generally a dose dependent increase in the activities of the liver indicator enzymes. For example, the activity of ALP was twice as high as that of the control following the single exposure while it was about three times as high following the triple exposure. Meanwhile, with daily treatment, the ALP level was over 40-fold higher (Table 1). This was also the case for ALT and AST (Table 1), although the activity difference vs. control at the highest doses (daily exposure) was 6- and 9-fold for ALT and AST, respectively.

The release of ALT, AST and ALP into the serum is attributed to cellular damage [21]. The results of the histopathological study tend to favour the above claim, as clear damage was observed with both the liver and kidney of the studied mice [at all doses] (Plate 1 and 2).

Meat is often reported as a reservoir of a number of pathogenic microorganisms, and their contamination is mainly due to poor sanitary conditions maintained during slaughtering, transportation and unhygienic environment under which they are processed. STEC or EHEC remain a serious threat to public health. In the United States for example, EHEC remains among the most common organism isolated from patients with bloody stool [22]. Unfortunately, in most developing countries where *E. coli* remains

the common cause of diarrhea in children, isolating this pathogen and differentiating it from other non-pathogenic *E. coli* remain a serious challenge. Also, little is known about its occurrence, distribution, potential source(s) of infection/contamination as well as its potential health effect in most developing countries, including Nigeria. The current study was therefore aimed at investigating the occurrence of STEC in beef samples, and to determine its potential toxicity *in vivo,* following short term exposure.

The presence of faecal coliform and STEC in beef samples from different markets in Benin City, Edo state, Nigeria is an indication of the poor sanitary environment under which they are processed. This assertion has previously been reported by Omoruyi et al. [19]. The sources of these coliform could vary. For example, animals in abattoirs located in Benin City are slaughtered on the floor with little or no proper disinfection, nor are good sanitary conditions maintained by butchers, abattoir personnel, beef sellers and transportation personnel during the processing of beef. This is worrisome, owing to the fact that abattoirs in most developing countries are generally less developed [23]. In other cases, they could be modern or very simple but many of them irrespective of the type may constitute a threat to public health because of unsanitary conditions [24]. Also, transportation vehicles have been implicated as a possible source of STEC in beef carcass [25]. In that study, Mark [25] reported 2% of sampled vehicles used in the transportation of cattle to contain STEC.

Shiga toxin producing *Escherichia coli* (STEC) have been implicated as the causative agent in several human diseases including mild non-bloody or severe bloody diarrhoea (haemorrhagic colitis), haemolytic uremic syndrome (HUS) and renal failure [1,2]. Approximately 6.3% of STEC infected individuals develop HUS, with a fatality rate of 4.6%. Children and immunocompromised individuals are more susceptible, with 15.3% of children under five years of age developing HUS following STEC infection [26].

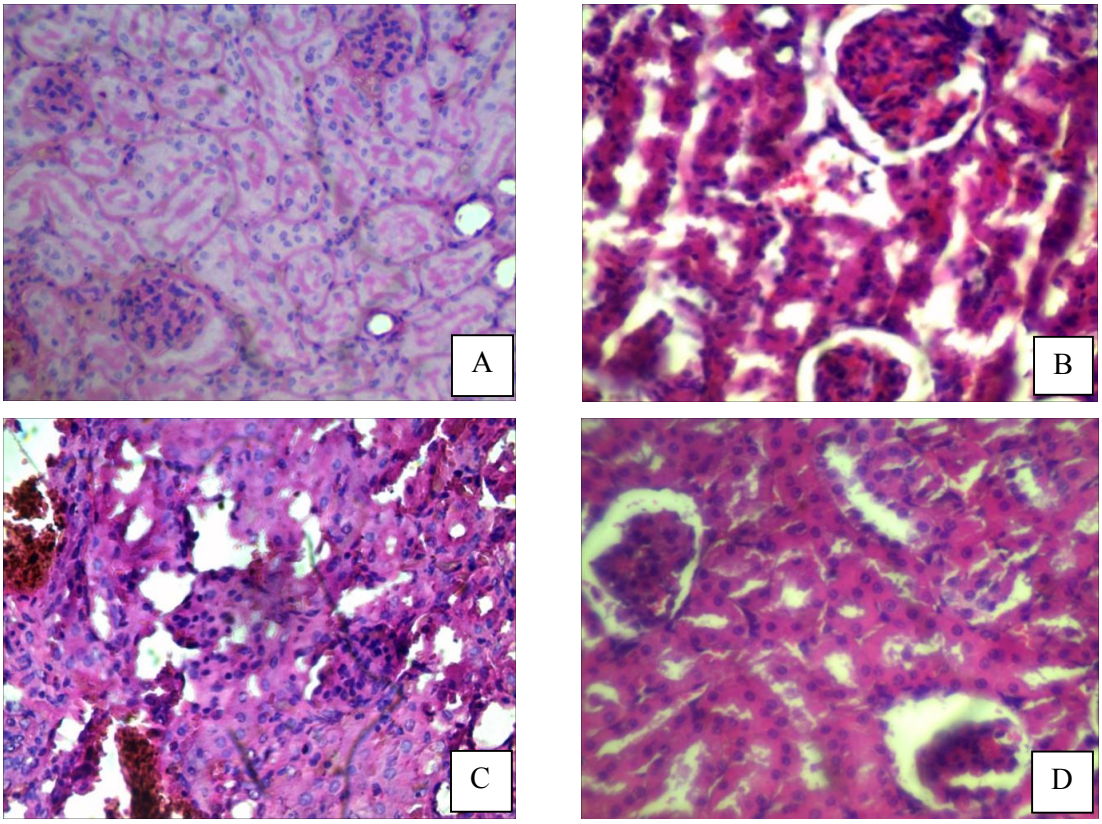

Plate 1. Histopathology of mice kidney following short term exposure to STEC; A: Control; B: Daily exposure; C: Triple exposure; D: Single exposure

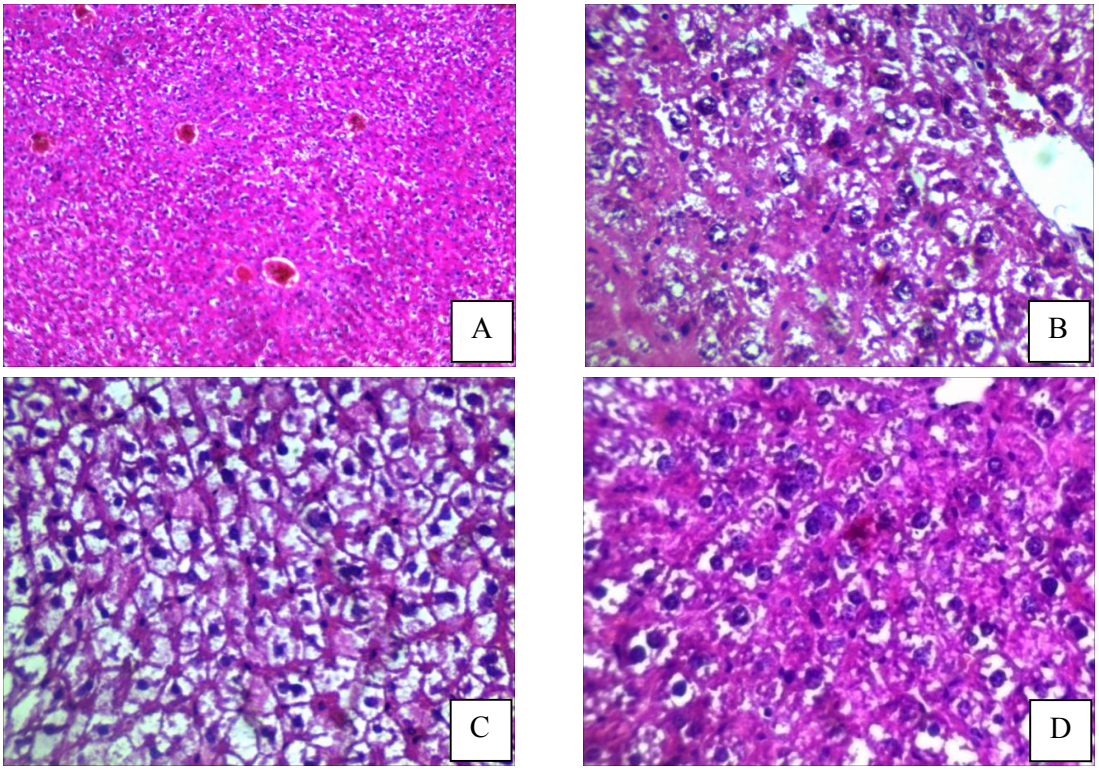

Plate 2. Histopathology of mice liver following short term exposure to STEC; A: Control; B: Daily exposure; C: Triple exposure; D: Single exposure

Table 1. The levels of ALT, AST and ALP in the serum of mice exposed to different concentrations of STEC

	Parameters (µ/L)		
	ALT	**AST**	**ALP**
Control	35.3 ± 4.8	14.0 ± 2.6	3.5 ± 0.4
Group A	239.0 ± 2.61	130.0 ± 5.40	147.3 ± 10.90
Group B	95.0 ± 4.53	84.0 ± 3.35	11.0 ± 4.78
Group C	60.7 ± 15.00	69.0 ± 1.41	8.28 ± 7.30

Key: Group A: Daily dose; Group B: Triple dose; Group C: Single dose

The results of this study (30% STEC occurrence) are in keeping with previous studies both in Nigeria and other countries. In a recent study, Tafida et al. [27] reported STEC (2.2%) from a number of retailed food samples in Zaria metropolis, Nigeria. Adwan and Adwan [28] also reported 14.7% of STEC from beef samples sold in Palestine. This is also in agreement with a similar study by Hussein [29], who reported *E. coli* 0157:H7 prevalence rates in the range of 3 to 19.7%, and that of Magwira et al. [30] in Botswana, who reported a prevalence rate of 2.3%. In that study, they also reported a prevalence of 5.2% in meat cubes and 3.8% from raw ground beef. A prevalence of 2% in sausages was also reported in Egypt [31]. Interestingly, the presence of *E. coli* O157 in all these studies were mostly attributed to faecal contamination from infected animals as well as the unsatisfactory hygienic conditions maintained during processing.

Measurement of the plasma activities of the enzymes AST, ALT and ALP is a common way of detecting liver damage in humans and animals. They are intracellular enzymes that are released when liver cell death occurs or when the structural integrity of the liver is damaged [29]. Also, high levels of the enzymes have been attributed to infection of the heart [32] and are generally associated with tissue necrosis, cardiovascular disease and cellular damage. The current increase observed with this study especially following short term exposure implies

that a lot need to be done on making the Nigerian citizens aware of the importance of maintaining a healthy lifestyle and environment during processing of beef.

4. CONCLUSION

In conclusion, the isolation of STEC from beef products sold in Benin City, Edo State, Nigeria is an indication of the poor sanitary conditions and unhygienic environment under which beef are processed and sold. The high levels of liver enzymes recorded and the damage on the liver and kidney following short term exposure further calls for serious concern. Furthermore, the presence of STEC may lead to possible outbreak, and this may pose a grave problem for the citizenry. It is therefore recommended that efforts should be made by both government, patrons and proprietors of abattoirs, beef sellers and the general public to ensure that only healthy beef are slaughtered and that such beef are processed and sold under hygienic conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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