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# Assessment of *Escherichia coli* O157:H7 Contamination in Soil and Water Sources Proximal to Abattoirs Within Cross River State, Nigeria

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**Abstract:** Abattoir activities generate numerous waste and microorganisms that have polluting effects on its environment and consequently could have negative impact on the health of its surrounding residents. This study assessed *Escherichia coli* O157:H7 contamination on adjourning soil and water sources proximal to Abattoirs within Cross River State, South-Eastern Nigeria. A total of 288 composite soils were collected into sterile polyethene bags. A total of 144 water samples were collected in triplicates aseptically into sterile 200 ml plastic bottles. Standard microbiological and biochemical tests were carried out to isolate and identify *E. coli* O157:H7 from the samples collected. Results showed that surface and subsurface soil samples closest to abattoir slabs had highest prevalence value (22.92%) which decreased significantly ($p<0.05$) with increase in distance away from the slabs. Spring water sources close to abattoirs had highest prevalence (15.28%) while least value of 3.47% was obtained from boreholes. Prevalence values differed significantly ($p<0.05$) among the different water sources. Effluents generated from the abattoir causes contamination when introduced to the adjoining soil and water bodies, making them unsuitable for domestic use. The isolation of *E. coli* O157:H7 strains further demonstrates the need for adequate waste disposal and treatment.

**Keywords:** *Escherichia coli*, Abattoirs, Effluents, Wastewater, Soil Contamination

## 1. Introduction

An abattoir or slaughterhouse is a facility where animals are slaughtered for consumption as food [1]. The abattoir sector is an essential constituent of the livestock business that typically provides meat supply for over 150 million people in Nigeria [2]. Abattoirs are sources of pollution and effluents from abattoirs are commonly known worldwide to contaminate or degrade their surrounding environments whether directly or indirectly by utilizing huge amount of water and producing enormous amounts of wastewater [3-4]. Abattoir waste consists mainly of bones, undigested ingest and occasionally aborted foetuses (solid waste) while the liquid wastes comprise of blood, urine, water, dissolved solids and gut contents [5].

Environmental pollution is in the increasing trend, thereby confronting its sustainability [6] especially in cities [7] and this is a global concern. Human activities such as animal production and meat processing negatively impact on soil and natural water composition. Abattoirs in Nigeria have been known to dispose their wastes into surface water bodies without any prior treatment of the effluent which often leads to pollution of the soil, water bodies and the environment

directly or indirectly [8-9].

The inappropriate discharge of untreated abattoir wastewater draining into the soil can cause soil oxygen to become less available as an electron acceptor, prompting denitrifying bacteria to reduce available nitrate to gaseous nitrogen which enters the atmosphere with resultant negative effects. Similarly, the anaerobic methanogens may produce excessive methane at a higher rate than aerobic methanotrophs could cope with, thus contributing to greenhouse effect and global warming. Physicochemical properties of the soil may become altered, resulting in the loss of certain soil microbes [10]. Physicochemical and microbiological qualities of soil and water samples contaminated with faecal matter from cattle have been reported to contain high turbidity values and pathogenic bacteria including *E. coli,* respectively which poses a high risk to the environmental health [11].

Pathogens present in animal carcasses or shed in animal wastes may include rotaviruses*, Hepatitis E virus, Salmonella spp., E. coli* O157: H7, *Yersinia enterocolitica*, *Campylobacter* spp., *Cryptosporidium parvum*, and G*iardia lamblia* [12].

Improper disposal and management of these harmful wastes from abattoirs lead to various contamination to the soil, air, and water quality resulting to serious environmental hazards which can lead to broad spectrum of health complications and diseases [13-14]. Furthermore, Ojo and Alamuoye also reported that unsustainable management of slaughterhouse wastes could lead to transmission of zoonotic diseases such as colibacillosis, salmonellosis, brucellosis and helminths in humans [15].

The bacteriological characteristics of abattoir wastewater and its possible effect on receiving water bodies which is likely to cause pollution with intensified environmental and public health hazards has not been documented in Cross River, Nigeria. Therefore, the aim of this study was to assess *E. coli* O157:H7 contamination from discharged abattoir effluents on adjoining soil and water sources proximal to Abattoirs within Cross River State, South-Eastern Nigeria.

# 2. Materials and Methods

### *2.1. Study Area*

This research was carried out in selected highly populated communities in Cross River State. The state is located in the Niger Delta Region, Southern Nigeria and it occupies an area of 299,10km$^2$ and an estimated population of 3,920,208 as at 2006 Nigeria population Census [16]. It is bounded to the North by Benue State, to the West by Enugu and Abia States, to the East by the Republic of Cameroon and to the South by Akwa Ibom State and the Atlantic Ocean.

### *2.2. Sampling Design*

The study area was mapped out according to political demarcations i.e. Northern, Central and Southern Senatorial Districts. Each senatorial district was further mapped out into two sampling areas each comprising of two local Government Areas found in the state. Each sampling area was designated SA.

### *2.3. Sample Collection*

Surface and subsurface soil samples were collected twice over nine months sampling period (from June 2009 to February 2010) from areas where the carcasses were displayed and at various points proximal to the slaughter slabs within the abattoirs environment. Four cardinal directions (North, South, East and West) with reference to each slaughter slab were mapped out and samples collected at points with distances of 0 (samples beside slab site), 10, 30 and 50 m from the carcass site. Samples from points with the same distance to the slab (irrespective of the direction of sampling) were combined to form a composite sample.

A total of 288 composite soil samples (72 from each of the four sampling points) were collected into sterile polyethene bags of about 100g capacity and transported in ice cold box to the Microbiology laboratory, University of Calabar, for analysis within 24 hrs.

Water samples were collected from all available water sources reported to be used for various purposes during each slaughtering process. Sampling of water sources was carried out twice during each sampling period and in all abattoirs selected for this study.

A total of 144 water samples were collected in triplicates using aseptic techniques into sterile 200 ml plastic bottles and transported to the laboratory in ice cold box for analysis within 6-12hrs. A total of 36-60 samples per sampling area were collected during the entire study period. Water sources sampled included boreholes, spring, wells and streams. The samples used as control were collected from an area devoid of butchering activities.

### *2.4. Preparation of Water and Soil Samples*

For each sample, 30 ml of the water source sample was transferred into a sterile 100 ml conical flask and combined with 30ml of double strength (2x concentration) modified buffered peptone water (mBPW, pH7.2$\pm$0.2) to obtain a 1:1 dilution. The mixture was vortexed and incubated overnight at 37°C for 18-24 h [17].

Each soil sample was gently dried in hot air oven at 35°C for 1 h to evaporate the moisture content. The dried sample was crushed and 10 g mixed with 20 ml of sterile normal saline (0.85% w/v NaCl) and homogenized by vigorous shaking for 5 min. The homogenized mixture was subsequently enriched by pipetting 10 ml of mixture into 90 ml of buffered peptone water and incubated at 37°C for 24 h.

### *2.5. Sample Processing*

The diagnostic automation Enzyme-linked immunosorbent assay (ELISA) technique was used for the qualitative detection of *E. coli* O157 antigens in all enriched samples [18].

Two drops of each sample was introduced into separate

wells until all the required number of wells (excluding the control wells) was used depending on the number of enriched samples to be assayed. Two drops of the positive and negative control solutions were also dropped into their respective wells. The contents were incubated at 37°C for 30 min and 2 drops of the enzyme conjugate added to each well. After a period of 30 min incubation, the contents were washed thrice using deionized water and 2 drops of chromogen added to each with gentle shaking. The results were compared to those of the positive and negative control wells.

A significant colour change to yellow indicated the presence of the *E. coli* O157:H7 antigen bound by the anti-*E. coli* O157:H7 antibodies impregnated in the wells. An optical density (OD) reading greater than 0.15 also confirmed a positive result.

All enriched samples with positive ELISA results were analysed using the standard *E. coli* O157:H7 culture technique as recommended by [19].

All enriched ELISA positive meat and faecal samples were serially diluted to $10^{-3}$ using physiological saline (0.85% w/v NaCl). 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). Approximately 0.1ml of enriched soil samples were plated directly on SMAC-CT undiluted. Volumes of 30-50ml (depending on the turbidity) of enriched, ELISA positive water samples were filtered through a 0.45 $\mu m$ millipore filter paper before plating on SMAC-CT. All cultured samples were incubated overnight at 42°C for 24 h. Sorbitol-negative colonies that appeared colourless to grey on SMAC-CT were considered positive for *E. coli* O157:H7. Three randomly selected suspected colonies were isolated on each plate and separately subcultured on nutrient agar slants and stored at 4°C in a refrigerator.

Standard biochemical tests typical for *E coli* such as indole, methyl red, Voges Proskauer, citrate and lysine decarboxylase were also performed on the isolates.

### *2.6. Methylumbilliferyl-β −D-Glucoronide (MUG) Fluorescence Confirmatory Test*

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°C for 18-24 h. 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 (about 92% of *E. coli* other than *E. coli* 0157:H7 produce the enzyme glucoronidase which cleaves MUG to produce a blue fluorescent product). 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.

### *2.7. Confirmation of Escherichia coli O157:H7 by H7 Antigen Typing*

This analysis was performed using standard *E. coli* O157:H7 antisera (Difco Laboratories, Detroit, Mich.) produced from rabbits 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 [20]. A wire loop was used to remove a loopful of the colony and suspended in a drop of normal saline. An equal amount of *E. coli* O157:H7 antiserum was added and mixed by rocking back and forth for 1min.

Colonies that agglutinated rapidly with the *E. coli* O157:H7 antisera were considered as confirmed positive *E. coli* O157:H7 colonies.

### *2.8. Statistical Analysis*

The data were analysed with SPSS version 11.0 software (SPSS Inc, Chicago, IL). Percentages were compared using a Pearson chi square ($x^2$) test for dependent samples or Fisher's extract "t"- test where appropriate. Means were compared using the student "t"-test for dependant samples.

The prevalence of *E. coli* O157:H7 strains from various sources in soil and water samples were analysed by statistical comparison of the survival values (log cfu/ml) of all pairs using one-way analysis of variance (ANOVA: Jump in version 4.0.3, SAS institute Inc, Cary, N. C., USA) to determine significant differences at a probability level of 0.05.

## 3. Results

Soil samples proximal to abattoir slabs were analysed and the highest percentage prevalence of *E. coli* O157:H7 obtained at the following distances: Site 0 (29.17%), 10m (16.67%), 30m (8.33%) and 50m (4.17%) with overall percentage prevalence of 22.92%, 9.72%, 3.47%, and 1.39% respectively. The total percentage prevalence from all samples analysed was 9.38%. The overall percentage prevalence from the various locations were observed to differ significantly at $p<0.05$. Prevalence values of the pathogen at various distances to the slaughter slabs are presented in Table 1.

The effect of seasonal variations on the prevalence of *E. coli* O157:H7 in soil samples at different distances from the slaughter slabs were determined. The monthly prevalence of *E. coli* O157:H7 in soil samples from different locations to the slaughter slabs is shown in Table 2. The highest monthly prevalence from site (0) samples (43.75%) was obtained in the month of January, 10m samples (18.75%) obtained in the month of June and August, 30m samples (6.25%) obtained in the months of July, September, October, January and February and 50m samples (6.25%) obtained in the months of August and September. No significant difference ($p>0.05$) was observed in the monthly prevalence among the site (0) samples and 10m samples. However, the monthly prevalence among the 30m samples and 50m samples differed significantly at $p<0.05$ with highest values obtained during months of heavy rains.

The highest percentage prevalence of *E. coli* O157:H7 was

obtained in spring water while the least value of 8.33% was obtained in borehole water sources. The overall percentage frequency of occurrence obtained were 3.47%, 8.33%, 11.11% and 15.28% for borehole, streams, wells, and spring respectively. Significant difference ($p<0.05$) was observed in the overall percentage frequency of isolation of *E. coli* O157:H7 from various water sources while no significant difference ($p>0.05$) was observed in the total frequency of occurrence from the various sampling areas. Values obtained from the various water sources in the sampling areas are presented in Table 3.

***Table 1.*** *Prevalence of Escherichia coli O157:H7 in soil samples collected at various distances proximal to slaughter slabs in various sampling areas.*

| Sampling area | Distance from slaughter slabs | | | | Total prevalence (%) |
|---|---|---|---|---|---|
| | Site (0) (%) | 10m (%) | 30m (%) | 50m (%) | N= 96 |
| SA1 | 50 (20.83) | 3 (12.50) | 1 (4.17) | 0 (0.00) | 9 (9.38) |
| SA2 | 7 (29.17) | 3 (12.50) | 0 (0.00) | 1 (4.17) | 11 (11.46) |
| SA3 | 3 (12.50) | 1 (4.17) | 1 (4.17) | 0 (0.00) | 5 (5.21) |
| SA4 | 8 (3.33) | 2 (8.33) | 2 (8.33) | 0 (0.00) | 12 (12.50) |
| SA5 | 6 (25.00) | 4 (16.67) | 0 (0.00) | 0 (0.00) | 10 (10.42) |
| SA6 | 4 (16.67) | 1 (4.17) | 1 (4.17) | 1 (4.17) | 7 (7.29) |
| Overall prevalence | 33/144 (22.9) | 14/144 (9.72) | 5/144 (3.47) | 2/144 (1.39) | 54/576 (9.38) |

N=24 for all the distances where soil samples were collected; $p< 0.05$

***Table 2.*** *Monthly prevalence of Escherichia coli O157:H7 in soil samples at various distances to slaughter slabs.*

| Sampling month | Distance from Abattoir | | | | Total frequency (%) |
|---|---|---|---|---|---|
| | Site (0) (%) | 10m (%) | 30m (%) | 50m (%) | N=64 |
| June 2009 | 4 (25.00) | 3 (18.75) | 0 (0.00) | 0 (0.00) | 7 (10.94) |
| July 2009 | 3 (18.75) | 2 (12.50) | 1 (6.25) | 0 (0.00) | 6 (9.38) |
| August 2009 | 2 (12.50) | 3 (18.75) | 1 (6.25) | 1 (6.25) | 6 (9.38) |
| September 2009 | 2 (12.50) | 1 (6.25) | 1 (6.25) | 1 (6.25) | 5 (7.81) |
| October 2009 | 1 (6.25) | 0 (0.00) | 1 (6.25) | 0 (0.00) | 2 (3.13) |
| November 2009 | 4 (25.00) | 1 (6.25) | 0 (0.00) | 0 (0.00) | 5 (7.81) |
| December 2009 | 6 (37.50) | 2 (12.50) | 0 (0.00) | 0 (0.00) | 8 (12.50) |
| January 2010 | 7 (43.75) | 1 (6.25) | 1 (6.25) | 0 (0.00) | 9 (14.06) |
| February 2010 | 4 (25.00) | 1 (6.25) | 1 (6.25) | 0 (0.00) | 6 (9.38) |
| Overall prevalence | 33/144 (22.92) | 14/144 (9.72) | 5/144 (3.47) | 2/144 (1.39) | |

N=16 for total number of soil samples collected at various distances per month; $p<0.05$

***Table 3.*** *Occurrence of Escherichia coli O157:H7 in water sources proximal to some abattoirs located in various sampling areas.*

| Sampling | Borehole | | Wells | | Spring | | Stream | | Total |
|---|---|---|---|---|---|---|---|---|---|
| Area | TS | PS (%) | TS | PS (%) | TS | PS (%) | TS | PS (%) | prevalence (%) |
| SA1 | 24 | 0 (0.00) | - | - | 12 | 2 (16.67) | - | - | 6/2 (5.55) |
| SA2 | 24 | 1 (4.17) | - | - | 12 | 3 (25.00) | - | - | 6/4 (11.11) |
| SA3 | 24 | 1 (4.17) | 12 | 1 (8.33) | - | - | 24 | 1 (4.17) | 0/3 (5.00) |
| SA4 | 24 | 2 (8.33) | 12 | 2 (16.67) | 12 | 2 (16.67) | 12 | 2 (16.67) | 8 (13.33) |
| SA5 | 12 | 0 (0.00) | 12 | 1 (8.33) | 24 | 3 (12.50) | 12 | 1 (8.33) | 0/5 (8.33) |
| SA6 | 36 | 1 (2.78) | - | - | 12 | 1 (8.33) | 12 | 1 (8.33) | 0/3 (5.00) |
| | 144 | 5 (3.47) | 36 | 4 (11.11) | 72 | 11 (15.28) | 60 | 5 (8.33) | |

- Not available, TS = Total No of samples; PS= No of positive samples; $p<0.05$

# 4. Discussion

In this study, *E. coli* O157:H7 contamination of adjourning soils proximal to abattoirs were analysed. High prevalence values were obtained at distances of 0m (22.92%) and 10m (9.72%) from the slaughter slabs. Values were observed to decrease significantly ($p<0.05$) with increasing distances away from the slabs. This observation certainly revealed the role of slaughter effluents from the slabs in the contamination of adjourning soils with greater effects experienced at distances closer to slabs. The whole body of pre-eviscerated cattle and the gastrointestinal contents of post-eviscerated ones were frequently washed on slaughter slabs. The isolation of pathogenic microorganisms including *E. coli* from abattoir effluents has been documented in the South East, Nigeria [21] The findings of this study is supported by Akinyeye et al. who reported that *E. coli* had the highest frequency of occurrence in abattoir effluents contaminated soil in Benin, Edo State, Nigeria [22]. Neboh et al. also demonstrated that the total bacterial population obtained from the contaminated soil was greater than that in the soil without wastewater contamination [23]. Ibekwe *et al.* reported that *E. coli* O157:H7 can survive in soil for more than 90 days which provides a very significant pathway for recontamination of the environment. The possibility of prolonged survival of *E. coli* O157:H7 in soil linked to the intensive agricultural production system pose potential risk of food crop contamination [24].

The role of runoff water from rainfall in the transportation

of organic constituents of improperly disposed wastes is demonstrated through the observation that prevalence values of *E. coli* O157:H7 at far distances (30-50m) to the slabs increased during the rainy periods. The effect of rainfall has also been documented [25]. In order to minimize risks of infection to producers and consumers within the study area, frequent detection o*f E. coli* O157:H7 in abattoir soil is required.

Water-borne *E. coli* O157:H7 transmissions have been attributed to the ingestion of contaminated drinking water, food processed using such contaminated water sources or recreational waters. The present study confirms the presence of *E. coli* O157:H7 in water sources proximal to abattoirs in Cross River State. The results showed that *E. coli* O157:H7 had the highest prevalence of 15.28% from spring water sources and the least value of 3.47% was obtained for borehole water with significant difference $(p<0.05)$ observed in the values obtained from the various water sources. This could suggest that abattoir activities can influence the bacterial community and serve as a source of environmental pollution to water sources proximal to abattoirs. This is in accordance to the findings of Olayinka *et al*. who demonstrated that abattoir effluents have environmental pollution tendency and abattoir effluents on surface water alter the microbial characteristic of the receiving water [26]. The findings of Seiyaboh and Izah also revealed that abattoir effluents had impact on bacteriological quality of tidal creek of Ikoli, Bayelsa State [27]. Receiving water bodies downstream are used for bathing, washing, watering of animals, watering of crops, and other domestic purpose and contamination by abattoir wastewater. This could result in outbreaks of *E. coli* O157:H7 infection through cross contamination by infected crops. One of the critical concerns with food safety is the transfer of pathogens from contaminated soil to edible portions of plants and is a is a cause for concern over threats to public and environmental health. Domestic cattle have been considered as reservoirs of Shiga toxigenic *E. coli* strains for human infections and abattoir effluents serve as a channel for transmission of STEC into the environment. Prevalence of STEC harbouring *stx 1* and *stx 2* in farm cattle have been documented in South Korea [28]. The slaughtering of cattle in the abattoir coupled with the unchecked discharge of abattoir effluent containing wastes and carcasses of these animals portends potential environmental hazards to the receiving bodies and human health [29].

## 5. Conclusion

The study reveals the presence of *E. coli* O157:H7 in soil and water sources proximal to abattoirs in Cross River State. The contamination of surface water and soil as obtained in this study further confirms the dangers associated with discharging untreated wastewater to the soil and surface water, thus the need for adequate treatment to ensure decontamination. Relevant health authorities should enforce compliance with requirements and regulations governing abattoir operations. The treatment of water obtained from surrounding rivers in residential areas before they are used for domestic purposes to prevent occurrence of zoonotic diseases is highly recommended.

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