

# Prevalence of *Escherichia coli* O157 in Fruits, Vegetables and Animal Faecal Waste Used As Manure in Farms of Some Communities of Akwa Ibom State-Nigeria

A. E. Moses<sup>1, \*</sup>, R. A. James<sup>2</sup>, U. S. Ekanem<sup>3</sup>

<sup>1</sup>Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, University of Uyo, Uyo, Nigeria

<sup>2</sup>Department of Environmental Health Management, Centre for Wetlands Studies, University of Uyo, Uyo, Nigeria

<sup>3</sup>Department of Community Health, Faculty of Clinical Sciences, College of Health Sciences, University of Uyo, Uyo, Nigeria

## Email address

amoses264@gmail.com (A. E. Moses), abasiekongr@yahoo.com (R. A. James), uwemedimbukekanem@yahoo.com (U. S. Ekanem)

\*Corresponding author

## To cite this article:

A. E. Moses, R. A. James, U. S. Ekanem. Prevalence of *Escherichia coli* O157 in Fruits, Vegetables and Animal Faecal Waste Used As Manure in Farms of Some Communities of Akwa Ibom State-Nigeria. *Central African Journal of Public Health*.

Vol. 2, No. 1, 2016, pp. 22-27. doi: 10.11648/j.cajph.20160201.14

**Received:** August 12, 2016; **Accepted:** August 24, 2016; **Published:** September 30, 2016

**Abstract:** Fresh fruits and vegetables have been implicated as vehicles for the transmission of microbial food-borne diseases worldwide. This research aimed to assess the prevalence of *E. coli* O157 serogroup in fruits, vegetables and animal faecal manures used in some farms in Uyo and Ikot Ekpene communities of Akwa Ibom State, Nigeria. A total of 250 ready-to-eat fruits such as egg plant, carrot and cucumber and vegetables including fluted pumpkin, water leaf and okra were randomly collected from farms, markets, vendors and sale outlets in the study areas. Animal (cow, goat, poultry, swine) fecal waste samples used as manure (n=100) were collected from various farms in the area. The study employed standard microbiological analysis of these samples. Out of a total of 250 fruits and vegetable samples tested, 39 (15.6%) *E. coli* were isolated from fruits and vegetables, of which 30 (22.1%) were from washed and 9 (7.9%) from unwashed samples. None of the *E. coli* isolates belonged to the *E. coli* O157 serogroup. Out of the 100 samples of animal dung tested, 45% yielded *E. coli* and *E. coli* O157 serogroup accounted for 3% of the isolates. Cow (n=2) and goat (n=1) fecal samples were associated with *E. coli* O157. Although none of the fruits and vegetables tested yielded *E. coli* O157 serogroup, the results of this study have identified cow and goat dung as potential sources of *E. coli* O157 contamination of farm produce if used as manure. The detection of *E. coli* in fruits and vegetables, and *E. coli* O157 in cattle and goat droppings in particular, is a clear indication of food safety risk. Consumers of farm products should wash them thoroughly with potable water before consumption to drastically reduce the risk of infection with enteropathogens.

**Keywords:** *E. coli* O157, Fruits, Vegetables, Animal Faecal Manure, Nigeria

## 1. Introduction

Fresh fruits and vegetables are vital to the health and well-being of humans because they provide essential vitamins, minerals and fiber. Families either grow fruits and vegetables for their own use or purchase from local farmers or retail outlets. Fruits and vegetables are important component of a healthy diet and there is a global move to increase their consumption [1].

Fresh fruits and vegetables have been implicated as

vehicles for the transmission of microbial food-borne diseases worldwide [2]. Advances in agronomic practices, processing, preservation, distribution and marketing have enabled the fresh fruits and vegetables industries to supply high quality fresh fruits and vegetables to many consumers all year round. However, some of these practices are responsible for the incidence and spread of human illnesses associated with an increasing number of pathogenic bacteria, viral, and parasitic microorganisms [3].

*Escherichia coli* is a common inhabitant of the gastrointestinal tract of animals and man. It has the ability to

survive for extended periods in water and soil, under frozen and refrigerated temperatures, and in dry conditions and can only be destroyed by thorough cooking or pasteurization. Most strains are harmless, while some have caused severe food and water-borne disease outbreaks worldwide [4]. *E. coli* is a significant cause of diarrhea especially in young children and adults in developing countries and localities of poor sanitation, and *Escherichia coli* O157 is a highly pathogenic strain of *Escherichia coli* [5].

According to Davis and Kendall [6], *E. coli* O157 is of particular public health concern because of its severe consequences of infection. Reports of sporadic outbreaks of disease caused by *Escherichia coli* O157 serogroup have been on the increase since it was first identified in the early 1980s as a new group of pathogens implicated in human mortality and morbidity. Human illness typically follows consumption of food or water that has been contaminated with microscopic amount of cattle faeces, and cattle is regarded as a primary reservoir of *E. coli* O157 [5]. The illness it causes is often a severe bloody diarrhea, associated with painful abdominal cramps, but without much fever [5]. In 3% - 5% of cases, a complication called Haemolytic Uraemic Syndrome (HUS) can occur several weeks after the initial symptoms. Other complications are anaemia, profuse bleeding and kidney failure [7].

Root crops and leafy vegetables have the greatest risk of infection from manure application to soil or in direct (or indirect) contact with cattle, deer and sheep [6]. Other carriers include birds, insects and squirrels [6]. The animal carriers do not appear sick (asymptomatic) but they carry and shed the bacteria in their faeces. They contaminate fresh fruits and vegetables through contact with faeces, sewage and untreated irrigation water or surface water. Other sources include poor harvesting and handling, including at point of preparation by street vendors, in food service establishments and in the home [6].

Numerous outbreaks of gastrointestinal disease have been linked to consumption of fresh fruits and vegetables; and the source of contamination can occur at various stages of production through actual sale of the final product [8]. The proportion of total outbreaks of food-borne disease attributed to fresh fruits and vegetables varies between countries. A total of 70% of the total food-borne illness in the USA [9] and 75% of the total fresh produce outbreaks in Brazil were attributed to fruits and vegetables [2]. Between 1995 and 2006, 22 produce outbreaks were documented in the United States, with nearly half traced to lettuce or spinach grown in California river [10]. An outbreak associated with a bagged mixed-produce product, including lettuce, occurred in Minnesota in September 2005 [11] and a large multi-state outbreak involving bagged spinach occurred in August/September, 2006 [12].

Problems linked with pathogens like *E. coli* in fresh farm produce and the associated public health issues have been reported in a number of countries worldwide [2, 10, 12]. *Escherichia coli* is a significant cause of diarrhoea in developing countries and localities of poor sanitation.

Several outbreaks of gastroenteritis have been linked to the consumption of *E. coli* contaminated fresh fruits and vegetables. Fresh or minimally processed fruits and vegetables are potential vehicle for transmission of pathogenic microorganisms to man, hence the consumption of fruits and vegetables contaminated with pathogenic *E. coli* strains are known to cause human disease such as gastroenteritis. Outbreaks of *E. coli* serotype O157 infection have been associated with consumption of contaminated apple cider, lettuce, radish, alfalfa sprouts, and other mixed salads [13]. The *E. coli* O157 low infectious dose, survival under adverse conditions, potential for extreme disease severity and reports of outbreaks compels this study. Therefore, this study was aimed to investigate the presence of *E. coli* O157 in fruits and vegetables, and animal faecal manures used in some farms in Uyo and Ikot Ekpene areas of Akwa Ibom State, Nigeria.

## 2. Materials and Methods

### 2.1. Study Site

Uyo and Ikot Ekpene Local Government Areas of Akwa Ibom State were considered as the study site in this study. The two areas were selected because they represent the major towns in Akwa Ibom State with a large concentration of producers, distributors (traders) and consumers of fruits and vegetables, and farmers commonly use animal manure in farms.

### 2.2. Study Design

This was a cross sectional descriptive study involving collection of fresh fruits and vegetables from various farms, local markets, vendors and sales outlets in Uyo and Ikot Ekpene communities as well as animal faecal manure from these farms.

### 2.3. Sample Collection

A total of 250 assorted fruits and vegetables were randomly collected from various farms, local markets, vendors and sales outlets in Uyo and Ikot Ekpene; and placed in sterile container. They were ready-to-eat fruits such as eggplant, carrot and cucumber; and vegetables including fluted pumpkin, water leaf and okra. They were grouped into distilled water washed (n=54) and unwashed fruits (n=56), and washed (n=60) and unwashed (n=80) vegetables. A total of 100 animal faecal waste samples were randomly collected from cattle, poultry dropping, swine dung and goat droppings from farm houses and placed in universal bottles containing transport medium. All faecal samples were transferred to the laboratory for analysis within 3 hours of collection.

### 2.4. Laboratory Analysis

Fruit and vegetable samples were ground to paste in a sterile mortar and pestle. One gram (1g) of the homogenized sample was suspended in nine milliliters (9mls) of tryptone

soy broth (Oxoid Hamsphire, England) and incubated at 37°C for 18-24 hours. One milliliter (1ml) of the tryptone soy broth suspension was plated on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 18-24 hours.

All animal faecal samples were cultured overnight in Brain Heart Infusion (BHI) broth before inoculated onto Eosin Methylene Blue (EMB) agar and incubated at 37°C for 18-24 hours.

Pure culture of all colonies on EMB agar that exhibited typical dark purple red colour with metallic sheen which characterized *E. coli* were subcultured on MacConkey agar for identification of lactose fermenting colonies. Biochemical tests such as indole, citrate, carbohydrate fermentation tests and MR-VP test were done on all lactose fermenting colonies to confirm *E. coli* [14].

#### 2.4.1. Phenotypic Identification of *E. Coli* O157

Pure cultures of all positive *E. coli* were putatively identified as *E. coli* O157 following growth on cefixime-tellurite sorbitol-MacConkey (CT-SMac) agar as described by Vernozy-Rozand [15]. After 18-24hr incubation at 37°C, non-sorbitol fermenting (NSF) colonies on the CT-SMac agar were screened for the *E. coli* O157 using the dry-spot *E. coli* O157 agglutination test kit as described by the manufacturers (Oxoid, UK).

#### 2.5. Data Analysis

Frequency tables were used to show prevalence rates of *E.*

**Table 1.** Distribution of *Escherichia coli* isolates in washed and unwashed fruits and vegetable samples in study areas.

Sample	No. Tested	Unwashed Sample	<i>E. coli</i> No.(%)	Washed Sample	<i>E. coli</i> No.(%)	Total <i>E. coli</i> (%)	<i>E. coli</i> O157 (%)
Ready-to-eat fruits	40	20	2 (10 )	20	0	2 (5)	0
Egg plant	30	16	5 (31.3 )	14	0	5 (15 )	0
Carrot	40	20	6 (30)	20	2 (10)	8 (20)	0
Sub-Total	110	56	13 (23.2 )	54	2 (3.7)	15 (13.6)	0
Vegetables	50	30	8 (27)	20	2 (10)	10 (20)	0
Fluted pumpkin	50	30	3 (10)	20	0	3 (6)	0
Water leaf	40	20	9 (45)	20	3 (15)	12 (30)	0
Sub-Total	140	80	20 (25)	60	5 (8.3)	25 (17.9)	0
Grand Total	250	136	30 (22.1)	114	9 (7.9)	39 (15.6)	0

Table 2 indicates the frequency of isolation of *E. coli* and *E. coli* O157 serogroup from animal waste manure. Out of 100 animal faecal samples tested, 45 (45.0%) yielded *E. coli*, of which 3 (3.0%) were of *E. coli* O157 serogroup. Piggery

*coli* and *E. coli* O157 strains obtained from the various samples tested in this study.

### 3. Results

The results obtained from the analysis of two hundred and fifty (250) samples of fruits and vegetables in Uyo and Ikot Ekpene communities are presented in table 1. Of the 250 assorted fruits and vegetables tested, *E. coli* was isolated in 39 (15.6%) of the samples, of which 15 (13.6%) were from fruits and 25 (17.9%) were from vegetables. Unwashed fruits and vegetables had 13 (23.2%) and 20 (25%) *E. coli*, respectively, while 2 (3.7%) and 5 (8.3%) *E. coli* were isolated from washed fruits and vegetables, respectively.

In the overall ready-to-eat fruits (washed and unwashed) cucumber had the highest *E. coli* isolates, 8 (20.0%), while eggplant had the least 2 (5.0%). The unwashed carrot had most of the *E. coli* isolates, 5 (31.3%) and least in unwashed egg plant, 2 (10.0%). The washed cucumber alone had *E. coli*, 2 (10.0%) and none in washed eggplant and carrot.

In the overall vegetables (washed and unwashed), *E. coli* was mostly isolated in okra, 12 (30.0%) and least in waterleaf, 3 (6.0%). In addition, okra had the highest *E. coli* isolates in both unwashed and unwashed samples, 9 (45%) and 3 (15%), respectively. Unwashed waterleaf had the least *E. coli* isolate, 3 (10%) and none in washed samples. None of the *E. coli* isolates were of O157 serogroup.

dung had the highest yield of *E. coli*, 6 (60.0%) followed by cow dung, 23 (46.0%). Only, goat dung and cow dung that harboured *E. coli* O157 serogroup, 1 (10.0%) and 2 (4.0%), respectively.

**Table 2.** Distribution of *E. coli* and *E. coli* O157 serogroup in animal manure obtained from the study areas.

Animal faecal waste (manure)	No. Tested	<i>E. coli</i> (%)	<i>E. coli</i> O157 (%)
Poultry dropping	30	12 (40.0)	0
Piggery dung	10	6 (60.0)	0
Goat dung	10	4 (40.0)	1 (10.0)
Cow dung	50	23 (46.0)	2 (4.0)
Total	100	45 (45.0)	3 (3.0)

## 4. Discussion

According to the report by FAO and WHO [1], *E. coli* is a more specific indicator of faecal contamination of food. The presence of *Escherichia coli* in fruits (13.6%) and vegetables (17.9%) in this study could be a direct reflection of the sanitary quality during the process of cultivation, watering, harvesting, transportation, storage, or packing of the produce as suggested by Beuchat [13].

In this study, the overall prevalence of *E. coli* was 39 (15.6%) detected in fruits and vegetables obtained from some communities (Ikot Ekpene and Uyo) in Akwa Ibom State, Nigeria. This indicates the level of faecal contamination that must have occurred during handling or processing of these products. The frequency of *E. coli* isolates in this study is higher than that reported by Eni *et al.* [16] in Sango Ota, Ogun State, Nigeria (4.2%) and elsewhere in Jaipur city, India [17], where 11.8% *E. coli* were isolated from fruit and vegetable salad. The frequency of *E. coli* isolated in vegetables alone in this study was higher than that isolated in fruits (17.9% versus 15.6%). Also, the rate of vegetable contamination by *E. coli* in this study was higher compared to that reported by Mukherjee *et al.* [18] in leafy vegetables (10.7%).

It was observed in this study that washed fruits and vegetables had low levels of *E. coli* contamination (7.9%) compared to the unwashed samples (22.1%). This is consistent with FAO and WHO's [9, 10] report that washing and rinsing fruits and vegetables in potable water would aid in removing micro-organisms like *E. coli* from the produce. A 3-fold reduction in *E. coli* was observed in fruits and vegetables that were washed with clean water in this study. However, it has been documented that 10 to 100-fold reductions can sometimes be achieved if the produce are further treated with chemical disinfectants [9]. The result of this study therefore shows that adequate washing with clean water can go a long way in reducing bacterial load on the farm produce even in the absence of disinfectants as earlier documented [19]. Although washing of produce with plain clean water can reduce the number of pathogens, it cannot be relied upon to totally eliminate enteropathogens such as *E. coli* O157:H7 as earlier documented [6, 20].

When all the *E. coli* isolated from fruits and vegetables in this study were subjected to serological test to detect *E. coli* O157 serogroup, the results revealed that none of the isolates tested positive for the O157 serogroup. A similar result has been documented by Hosein *et al.* [21]. Also, Johnston *et al.* [21] did not detect any *E. coli* O157:H7 in the 175 samples of various leafy vegetables obtained from the USA and Mexico.

Indiscriminate waste disposal, transportation and storage facilities can increase the risk of *E. coli* contamination of fruits and vegetables [10]. With increasing urbanization and scarcity of land for agricultural practices in Akwa Ibom State, fresh vegetables are often seen grown indiscriminately in areas close to livestock farms. Moreover, animals

normally stray into crop farms and defecate, thereby contaminating the soil. This may lead to food safety risks arising from direct or indirect exposure of crops to animals' wastes [22, 23, 24].

In this study, among the ready-to-eat fruits examined, *E. coli* was most frequently isolated in cucumber (20.0%) followed by carrot (15.0%). Incidentally, these are among the most consumed ready-to-eat fresh fruits in our locality. It has been observed that fruits and vegetables that get in contact with the soil have a higher risk of being contaminated, especially if the soil is contaminated with animal/human waste or manure that is not well-composted [19]. It is instructive to note that fruits and vegetables comprise a diversified range of plant parts (leaves, roots, tubers, fruits, and flowers) and any of these parts are either consumed directly, partially cooked or otherwise. Production practices, growth conditions and the location of the edible part during growth (soil, soil surface, and aerial part) will in combination with intrinsic, extrinsic, harvesting and processing factors affect their microbial status at the time of consumption [25]. Hence, any part that hosts microbial pathogens becomes a risk to humans especially the ready-to-eat ones such as cucumber, carrot, and others that may require no boiling process before being consumed. For example, those used for salad preparation.

The total *E. coli* isolates from vegetables in this study was higher in Okra (30.0%), followed by fluted pumpkin (20.0%). This observation is consistent with the report that vegetables with rough outer skin are at higher risk of harboring micro-organisms like *E. coli* [25]. On the other hand, it was generally observed that eggplants (fruit) and waterleaf (vegetable) with smooth surfaces had few or none detected with *E. coli*. This is likely because plant produce with smooth surfaces are said to be at less risk of microbial contamination as well as those that grow above soil surface and are usually plucked or harvested without contacting the soil [25].

Among the animal wastes examined in this study, piggery dung harbored the most *E. coli* isolates (60%) followed by cow dung (4%). Two of the *E. coli* isolates from cow dung were identified as *E. coli* O157 serogroup (4.0%) while one of the isolates from goat dung (10%) was of *E. coli* O157 serogroup. Ruminant animals have been reported to be the most common reservoirs for *E. coli* pathogens, with cattle being considered the primary maintenance reservoir host [26, 27]. Renter and Sargent [28] reported that the prevalence of *E. coli* O157 in cattle may vary from 0 to over 50% depending on location and season. Doane *et al.* [29] had reported that ungulates like deer, sheep, goats and numerous other domestic and wild animals including pigs and chickens can harbour *E. coli* O157. In addition, Jay *et al.* [30] reported 23% of faecal sample of pigs positive for *E. coli* O157 in California, USA whereas in this study none of the pigs faeces harboured *E. coli* O157.

Contamination of fruits and vegetables with enterohaemorrhagic *E. coli* O157:H7 may occur when cattle

and perhaps other ruminants inadvertently enter the farms, or when improperly composted animal manure has been applied as fertilizer [19, 31, 32]. To reduce the risk of contamination of produce through the use of animal composted manure, certified organic methods require that these manure applied to fields must be properly composted before application. Also, harvesting of the farm produce for human consumption should be delayed for at least 90 days after the application, for edible portions of the crop not in direct contact with the soil and 120 days for edible portions in direct contact with the soil [9]. Leafy crops fertilized with inadequately composted manure and those fertilized with animal manure were found to have a higher risk of *E. coli* contamination [18, 33]. Also, because contaminated manure may create airborne dust particles, it is possible that fruits on trees and vines may become contaminated. Apart from animal manure, workers on farms and packinghouses may also be a source of *E. coli* O157 contamination as suggested by Beuchat [19].

## 5. Limitations

Authors could not carry out molecular characterization of the isolates due to limited funding for the study.

## 6. Conclusion

In conclusion, although none of the fruits and vegetables tested yielded *E. coli* O157 serogroup, the results of this study have identified cow and goat dung as potential sources of *E. coli* O157 contamination of farm produce if used as manure. The detection of *E. coli* in fruits and vegetables, and *E. coli* O157 in cattle and goat droppings in particular, is a clear indication of food safety risk in these communities. It is common to observe grazing animals, particularly cow, littered the environment with faeces that may become a source of these enteropathogens to farms. At the same time, the use of not well-composted animal wastes in the fertilization of fresh fruits and vegetable farms in our locality is as common practice. The outcome of this study has shown that these practices can significantly increase the risk of *E. coli* contamination in fresh produce grown in such farms. Consumers of farm products, especially the ready-to-eat ones, are advised to wash them thoroughly with potable water before consumption to drastically reduce the risk of infection with enteropathogens in contaminated farm produce.

## Competing Interest

Authors of this article declared existence of no competing interest in this research.

## Authors' Contributions

AEM conceptualize the research and wrote part of the manuscript, RAJ carried out the sample collection and laboratory analysis, USE conducted the literature search and

also wrote part of the manuscript. All authors read and agreed on the conclusions of this research.

## Acknowledgements

We wish to acknowledge the cooperation of all fruit and vegetable sellers and farm owners in Ikot Ekpene and Uyo used in the course of this study. The technical assistance offered by Mr. Anietie Samuel, Department of Medical Microbiology, University of Uyo Teaching Hospital, Uyo is hereby appreciated.

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