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Monitoring Enterohaemorrhagic *Escherichia coli* O157:H7 in the Vegetable Food Chain in Ghana

[1]Eric S. Donkor, [2]Richard Lanyo, [3]Moses L. Akyeh,
[4]Boniface B. Kayang and [4]Jonathan Quaye
[1]Department of Microbiology,
University of Ghana Medical School, P.O. Box 4236, Accra, Ghana
[2]Department of Environmental Science, University of Ghana, Accra, Ghana
[3]Noguchi Memorial Institute for Medical Research, P.O. Box LG 581, Accra, Ghana
[4]Department of Animal Science, University of Ghana, Accra, Ghana

Abstract: The study was carried out to assess the occurrence of *Escherichia coli* O157:H7 in the vegetable food chain in Accra, the capital city of Ghana. A total of 272 samples of various types of vegetables were screened for *Escherichia coli* and *Escherichia coli* O157:H7 using standard microbiological methods. In addition, 80 samples of water used for irrigation, 40 manure soil samples and 250 stool samples of various livestock were also screened. Overall, a total of 243 (37.9%) *Escherichia coli* isolates were obtained from all the specimens screened. The overall prevalence rates of *Escherichia coli* for vegetables were 35.3 and 29.4% for external and internal vegetable parts, respectively. The prevalence rates of *Escherichia coli* for irrigation water, manured soils and livestock faeces were 26.3, 52.5 and 24%, respectively. Overall, only one *Escherichia coli* isolate from irrigation water was detected to be *Escherichia coli* O157:H7 which translates to an overall prevalence rate of 0.4% among the *Escherichia coli* population. The study shows that *Escherichia coli* O157:H7 is present in the vegetable food chain in Accra but is relatively uncommon. Despite the low prevalence of the organism and its isolation from only irrigation water, the findings of the study call for public health attention owing to the very low infectious dose of *Escherichia coli* O157:H7 and the common practice of vegetable irrigation.

Key words: *Escherichia coli* O157:H7, Accra, irrigation, manure, livestock

INTRODUCTION

Escherichia coli are bacteria that normally inhabit the intestines of humans and animals. Most strains are known to be harmless, but several of them can cause mild to serious disease. One strain in particular, *Escherichia coli* O157:H7 is considered as an emerging pathogen of immense public health significance and is associated with severe clinical conditions including, hemorrhagic colitis leading to bloody diarrhea, hemolytic uraemic syndrome and kidney damage (Riley *et al*., 1983). According to Petridis *et al*. (2002) three unique characteristics of *Escherichia coli* O157:H7 that distinguish it from other *Escherichia coli* strains are:

- Its dangerous health implications especially with the possibility of kidney damage.
- Its unusual persistence in the environment: *Escherichia coli* O157:H7 survives at low temperatures and under acidic conditions.
- Its very small infective dose: a few bacteria, from 10 to 100 cells, are sufficient to cause disease.

Corresponding Author: Eric S. Donkor, Department of Microbiology, University of Ghana Medical School, P.O. Box 4236, Accra, Ghana

Table 1: Largest *Escherichia coli* O157:H7 outbreaks

Year	Place	No. of people sick	Contamination source
1989	Montana, USA	243	Undercooked ground beef
1996	Sakai, Japan	5,727	Poorly washed white radish sprouts
1996	Scotland, UK	496	Undercooked ground beef
2000	Walkerton, Canada	>2,000	Contaminated drinking water
2002	Pennsylvania, USA	51	Petting infected dairy animals

Petridis *et al.* (2002)

Since the first reported foodborne illness associated with *Escherichia coli* O157:H7 in 1982 in Michigan and Oregon in USA, it has been isolated from a variety of foods and livestock animals and accumulated research data has led to its recognition as an important foodborne and zoonotic pathogen (Doyle and Schoeni, 1984; Doyle, 1991; Riley *et al*., 1983). Though outbreaks of the organism have occurred worldwide, most of them were in Canada, United Kingdom, United States of America, with the largest outbreak occurring in Japan in 1996 (Table 1).

Cattle appear to be the main reservoir of *Escherichia coli* O157:H7 although this pathogen has been isolated from other animals including pigs and poultry (Petridis *et al*., 2002; CDC, 2006). The organism resides in the intestinal tract of live animals and is shed in the faeces that may contaminate food, water and the environment. The association of *Escherichia coli* and *Escherichia coli* O157:H7 with vegetables is usually related to farming practices involving the use of contaminated irrigation water or manured soils (Bryan, 1982; Wachtel *et al*., 2002a, b; Cote and Quessy, 2005; Mukhejee *et al*., 2007). Apart from vegetables, human infection is associated with consumption of a number of contaminated foods including meat, especially undercooked beef, raw milk, yoghurt, salamis, cheese and unpasteurised apple cider (Doyle and Schoeni, 1984; Doyle, 1991; Riley *et al*., 1983).

Most of the information on *Escherichia coli* O157:H7 emanated from developed countries and there appears to be very little information on the organism in the developing world. In Ghana, with the exception of raw milk which is hardly consumed by Ghanaians, *Escherichia coli* O157:H7 has hardly been isolated and therefore there is little information on it (Aning *et al*., 2002). Vegetable farming is an important occupation in Ghana and its cultivation involves high usage of animal manure and polluted water for irrigation. As these practices are major risk factors of *Escherichia coli* O157:H7 and vegetables are largely consumed by Ghanaians on a regular basis, the study examined the occurrence of *Escherichia coli* O157:H7 in the vegetable food chain.

MATERIALS AND METHODS

The study was carried out in Accra from September 2006 to August, 2007. Sampling was done along the vegetable food chain which involved collection of samples of vegetables, irrigation water, manured soils and livestock faeces. Overall a total of 272 vegetable samples, comprising 68 each of pepper, tomatoes, cabbage and lettuce were collected at the market level. A total of 80 samples of water used for irrigation, comprising streams (30), wells (20) and pipe borne (30) were collected. Forty manure soil samples and 250 stool samples of livestock animals were also collected. All the samples were initially screened for *Escherichia coli* and then for *Escherichia coli* O157:H7 using standard microbiological methods (Marshall, 1992).

Isolation of *Escherichia coli* from Vegetables

Bacteriological examinations to isolate *Escherichia coli* were carried out on internal and external parts of the vegetable samples. For examination of the external parts, whole vegetable samples were weighed and washed with saline of equivalent volume. After washing, the resultant saline was used to prepare a dilution range of 10^{-1}-10^{-6}. For examination of the internal parts, whole pepper samples were opened aseptically after sanitizing with chlorine (100 ppm) and 1 g of the inner tissues

transferred into MacContey bottles containing 1 mL saline solution. The mixture was macerated and 1 mL used to prepare a ten fold serial to obtain a dilution range of 10^{-1}-10^{-6} for each sample. One milliliter of the diluents of each sample was used to inoculate eosin methylene blue agar and incubated at a temperature of 44°C for 18-24 h. Suspected *Escherichia coli* (blue to black metallic sheen appearance) were confirmed by biochemical tests (Baron *et al*., 1994).

Isolation of *Escherichia coli* from Irrigation Water, Manure Soil and Livestock Faeces

One millilitre of the water sample was diluted in 9 mL of the standard saline solution and subsequently used to prepare a ten fold serial dilutions of 10^{-1}-10^{-6}. A soil sample was analysed by weighing 20 g (dry weight) into 180 mL standard saline solution and, subsequently used to prepare a ten fold serial dilutions of 10^{-1}-10^{-6}. One milliliter of the diluents of each sample was used to inoculate eosin methylene blue agar and incubated at a temperature of 44°C for 18-24 h. Livestock faecal specimens were inoculated directly onto agar plates and incubated. Suspected *Escherichia coli* were confirmed by biochemical tests (Baron e*t al*., 1994).

Isolation of *Escherichia coli* O157:H7

Escherichia coli isolates were purified and cultured on sorbitol macConkey agar at 37°C for 18-24 h. Suspected *Escherichia coli* O157:H7 which appeared as colourless colonies were purified and confirmed serologically by latex agglutination using a serotyping kit of the organism (Baron *et al*., 1994).

RESULTS

Overall, a total of 243 *Escherichia coli* were isolated from manured soils, livestock stool, irrigation water and vegetables. This translates to an overall prevalence rate of 37.9%. The overall prevalence rates of *Escherichia coli* for vegetables were 35.3 and 29.4% for external and internal vegetable parts, respectively (Table 2). Among the various vegetables studied, the highest prevalence of *Escherichia coli* externally was associated with lettuce (47.1%); the highest prevalence internally was associated with cabbage and lettuce (39.7%); tomatoes had the lowest prevalence both externally (22.1%) and internally (11.7%). The prevalence rates of *Escherichia coli* for irrigation water, manured soils and livestock faeces were 26.3, 52.5 and 24%, respectively (Table 3). Among the various types of water used for irrigation, the prevalence rates of *Escherichia coli* were, stream water (50%), Well water (30%) and pipe borne water (0%) (Table 3).

With the exception of irrigation water, *Escherichia coli* isolates from vegetables, manured soils and livestock faeces did not test positive for *Escherichia coli* O157:H7. One *Escherichia coli* isolate from irrigation water (stream) was detected to be *Escherichia coli* O157:H7. This translates

Table 2: *Escherichia coli* isolated from vegetable samples from the market

Vegetables	Vegetable part	N	(%)
Lettuce	External part	32	47.1
	Internal part	27	39.7
Cabbage	External part	20	29.4
	Internal part	18	26.4
Tomatoes	External part	15	22.1
	Internal part	8	11.7
Pepper	External part	29	42.6
	Internal part	27	39.7
Total	External part	96	35.3
	Internal part	80	29.4

N = No. of samples contaminated with *E. coli*, % = Proportion of samples contaminated with *E. coli*

Table 3: *Escherichia coli* isolated from irrigation water, manure soil and livestock stools

Type of sample	N	(%)
Stream water	15	50.0
Well water	6	30.0
Pipe borne water	0	0.0
Manure soil	21	52.5
Livestock stool	60	24.0

N = No. of samples testing positive for *E. coli*, % = Proportion of samples testing positive for *E. coli*, Overall proportion of water samples testing positive for *E. coli* was 26.3% (N = 21)

Table 4: Occurrence of *Escherichia coli* O157:H7 among *Escherichia coli* isolates

Samples	No. of *E. coli* isolated	No. of *E. coli* O157:H7 isolated
Vegetables	80	0
Stream water	39	1(3.3%)
Shallow wells	24	0
Pipe borne water	0	0
Manure soils	40	0
Livestock stools	60	0
Total	243	1 (0.4%)

to an isolation rate of 3.3% of stream water and an overall prevalence rate of 0.4% among the *Escherichia coli* population screened (Table 4). Thus 99.6% of the all the *Escherichia coli* isolated from the various samples sources were not of the O157:H7 serotype.

DISCUSSION

Escherichia coli was commonly isolated from the specimen screened, especially irrigation water (streams) and manure soils. This has also been reported by other studies (Wachtel *et al*., 2002b; Rai and Tripathi, 2007). *Escherichia coli* is carried as normal intestinal flora of humans and animals and also occurs in the soil. From these sources the organisms can contaminate the environment and occur in water and food items such as vegetables (Badrakh *et al*., 2008). The presence of *Escherichia coli* in food and water is therefore indicative of probable faecal contamination and the organism is referred to as a faecal coliform.

Despite the high prevalence rate of *Escherichia coli* (37.9%), the prevalence of *Escherichia coli* O157:H7 among *Escherichia coli* isolates in the study was low (0.4%). Similar findings have been reported and the organism appears to be a rare serotype of *Escherichia coli* (Aning *et al*., 2002). Despite the low prevalence of *Escherichia coli* O157:H7 observed, its isolation from the vegetable food chain is of public health significance due to its very low infectious dose and the fact the organism causes very severe clinical conditions including kidney damage.

Studies have shown that *Escherichia coli* O157:H7 can get internalised in vegetables through contaminated irrigation water and soil (Solomon *et al*., 2002). For this reason it is important to take a closer look at irrigation water which was the only source of *Escherichia coli* O157:H7 in the study. Though *Escherichia coli* O157:H7 was not isolated from vegetables, internalisation of *Escherichia coli* occurred in the various vegetables at an overall rate of 29.4%. The internalisation of pathogens within growing vegetables which are eaten raw is of primary concern since those bacteria are protected against removal by washing. From this study, given the occurrence of *Escherichia coli* O157:H7 in irrigation water and the tendency of internalisation of *Escherichia coli* in about 30% of vegetables, serious attention should be paid to the safety of irrigation water. It is interesting to note that while *Escherichia coli* does not occur naturally in water, some irrigation water (streams and wells) sampled showed similar prevalence rates to livestock stool or soil where the organism occurs as normal flora. This indicates a high faecal pollution rate of these irrigation water, probably from both animal and human faeces (Badrakh *et al*., 2008). It is also worth noting that pipe borne water used for irrigation

was completely free of *Escherichia coli*. This type of water which is treated and safe for human consumption appears to be the best choice for irrigation. However, in Accra where the study was carried out, it is less frequently used by farmers due to cost and availability. Well water and streams which may have suffered faecal pollution according to this study, are more available and usually without cost and are thus used by a large majority of farmers.

Comparing outbreaks of *Escherichia coli* O157:H7 in the developed and developing worlds, epidemiological information have shown that outbreaks are more common in the developed world and scarce in the developing (Petridis *et al*., 2002). Some outbreaks in the developed world have been linked to irrigation water and contaminated irrigation water is recognised as an important vehicle for transmission of *Escherichia coli* O157:H7 (Petridis *et al*., 2002). In many developing countries, disposal of animal and human excreta is still a major problem, resulting in gross contamination of environmental water such as streams, as observed in this study. Though outbreaks of *Escherichia coli* O157:H7 are still rare in the developing world, the ineffective disposal of excreta in many of these countries could be a major risk factor for future outbreaks. In Ghana, this study is one of the few documentations of the organism in addition to previous documentation in raw milk (Aning *et al*., 2002). Available information shows that, though isolation rate of *Escherichia coli* O157:H7 is very low, the organism appears to be widespread in the country and there exist the potential of an outbreak unless risk factors are dealt with.

The study concludes that *Escherichia coli* O157:H7 is present in the vegetable food chain in Accra but is relatively uncommon. A major intervention to improving the safety of vegetables in Ghana as far as *Escherichia coli* O157:H7 is concerned is related to the safety of irrigation water. There is the need therefore to use safe water for irrigation in vegetable farming and also to heat vegetables sufficiently before consumption.

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