A probabilistic assessment of the contribution of wastewater-irrigated lettuce to *Escherichia coli* O157:H7 infection risk and disease burden in Kumasi, Ghana

Razak Seidu, Amina Abubakari, Isaac Amoah Dennis, Arve Heistad, Thor Axel Stenstrom, John A. Larbi and Robert C. Abaidoo

ABSTRACT

Wastewater use for vegetable production is widespread across the cities of many developing countries. Studies on the microbial health risks associated with the practice have largely depended on faecal indicator organisms with potential underestimation or overestimation of the microbial health risks and disease burdens. This study assessed the *Escherichia coli* O157:H7 infection risk and diarrhoeal disease burden measured in disability-adjusted life years (DALYs) associated with the consumption of wastewater-irrigated lettuce in Kumasi, Ghana using data on *E. coli* O157:H7 in ready-to-harvest, wastewater-irrigated lettuce. Two exposure scenarios – best case and worst case – associated with a single consumption of wastewater-irrigated lettuce were assessed. The assessment revealed wastewater-irrigated lettuce is contributing to the transmission of *E. coli* O157:H7 in Kumasi, Ghana. The mean *E. coli* O157:H7 infection risk and DALYs in the wet and dry seasons, irrespective of the exposure scenario, were above the World Health Organization tolerable daily infection risk of 2.7×10^{-7} per person per day and 10^{-6} DALYs per person per year. It is recommended that legislation with clear monitoring indicators and penalties is implemented to ensure that farmers and food sellers fully implement risk mitigating measures.

Key words | diarrhoeal disease burden, *E. coli* O157:H7, Ghana, infection risk, lettuce, wastewater irrigation

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INTRODUCTION

Globally, diarrhoeal disease and its complications account for billions of illnesses and millions of deaths annually. Developing countries, particularly those in Africa, have the highest incidence as well as burden of diarrhoeal disease (WHO 2013). In Africa, diarrhoeal disease is the third ranked cause of mortality after HIV/AIDS and lower respiratory tract infections, and led to 800,000 deaths and 55 million lost life years (expressed as diarrhoeal disease burden and measured in disability-adjusted life years (DALYs)) on the continent in 2011 (WHO 2013). A plethora of risk factors have been implicated for the occurrence of diarrhoeal disease in developing countries. These risk factors include, but are not limited to, unimproved water and sanitation facilities, poor hygiene practices and unhygienic food handling. In many cities in developing countries with rapidly expanding populations, wastewater use in vegetable farming is contributing to the mix of diarrhoeal disease risk factors. In these cities, a significant proportion of the vegetables consumed are irrigated with raw or partially diluted wastewater. Farmers using wastewater for irrigation rarely adopt risk-mitigating measures and thus expose themselves and their families, as well as consumers of the wastewater-irrigated vegetables, to pathogens causing diarrhoeal disease (Seidu & Drechsel 2010). However, in most developing countries

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the link between wastewater irrigation and the transmission of diarrhoea-causing pathogenic organisms is poorly understood. This is partly due to inadequate resources to collect and analyse wastewater and wastewater-irrigated vegetables for pathogenic organisms and a general weakness in existing epidemiological surveillance systems to assess and relate the cause(s) of disease outbreaks. Consequently, the actual contribution of diarrhoeal disease attributed to wastewater irrigation is less well characterized.

In urban Ghana, a significant proportion of vegetable salad served by restaurants and street-food sellers originates from wastewater-fed fields (Seidu & Drechsel 2010). It is estimated that daily, about 800,000 people across the cities of the country consume wastewater-irrigated lettuce salad (Seidu & Drechsel 2010). This number is likely to increase in synch with rapid urbanization, which is now 3% in Ghana (Ghana Statistical Service 2010). Studies on potential disease-causing organisms in wastewater and wastewater-irrigated vegetables in Ghana have focused mainly on indicator organisms, particularly faecal coliforms (Keraita *et al.* 2007; Amoah *et al.* 2007), and have not addressed the pathogens directly. These faecal indicator organisms have formed the basis in the construction of models to assess the diarrhoeal disease risk and burden associated with wastewater irrigation (Seidu *et al.* 2008; Seidu & Drechsel 2010). The use of faecal indicator organisms in risk assessment can lead to an underestimation or overestimation of diarrhoeal disease risk and burdens in the absence of data on pathogenic organisms.

In 2011, the Research Council of Norway (RCN) funded a study to, among other things, assess the diarrhoeal disease risk and burden associated with wastewater use in urban Ghana. As part of the RCN study, wastewater-irrigated vegetables were analyzed for *Escherichia coli* O157:H7 in wastewater-irrigated vegetables. *E. coli* O157:H7 diarrhoeal disease outbreaks have been associated with the consumption of vegetables (Jay *et al.* 2004). Following the outbreak of *E. coli* O157:H7 in the United States in 1982 from fast-food chains (Riley *et al.* 1983), additional outbreaks have been related to contaminated vegetables (Michino *et al.* 1999; Hilborn *et al.* 1999). From 1996 to 1999, an estimated 22 outbreaks of *E. coli* O157:H7 reported in California were attributed to the contamination of lettuce and spinach in watersheds due to heavy and increased river flow rates (Cooley *et al.* 2007). Outbreaks associated with the consumption of *E. coli* O157:H7-contaminated vegetables have also been reported in Japan (Michino *et al.* 1999) and Sweden (Söderström *et al.* 2008). In the United States, *E. coli* O157:H7 causes about 73,000 infections and 60 deaths annually (Mead *et al.* 1999), and increased morbidity and mortality has raised public concern due to the fact that infection develops into haemorrhagic colitis and haemolytic uremic syndrome (HUS) which is more pronounced in children, the elderly and the immunocompromised (Abu-Ali *et al.* 2010). *E. coli* O157:H7 diarrhoeal disease outbreaks have also been reported in African countries such as Kenya (Sang *et al.* 1996), Cameroon (Germani *et al.* 1998), Ivory Coast (Dadie *et al.* 2000) and Nigeria (Olorunshola *et al.* 2000). The objective of this study was to assess the *E. coli* O157:H7 infection risk and disease burden associated with the consumption of wastewater-irrigated lettuce in Kumasi, Ghana, where vegetable farmers mainly depend on diluted wastewater from storm water drains and streams as a source of irrigation water (Seidu *et al.* 2013).

METHODS

Farm sites, sampling and analysis of *E. coli* O157:H7

Kumasi is the second largest city in Ghana with a population of over two million and an annual growth rate of 2.7% (Ghana Statistical Service 2010). The city has a number of hotels, restaurants and street-food vendors that serve salad prepared with wastewater-fed vegetables as a side-dish to consumers. Within the city two cross-sectional surveys were undertaken in all the diluted wastewater-irrigated farms ($n = 8$) in the wet (August–November, 2012) and dry (December 2012–May 2013) seasons to assess the level of *E. coli* O157:H7 in ready-to-harvest lettuce.

In each survey, lettuce heads were aseptically collected from beds in the farms with sterile knives into sterile plastic bags (Stomacher® laboratory system, Seward, UK) and sealed. From each bed, the lettuces were collected from the far ends and in the middle to represent triplicate samples. The samples were transported in cooling boxes to the laboratory for analysis. A total of 107 and 89 ready-to-harvest triplicate lettuce heads were collected from the farms in the wet and dry seasons, respectively.

Lettuce samples were weighed, aseptically cut and homogenized with a blender. One millilitre of the blended lettuce was transferred into 9 mL of 0.85% isotonic saline and poured onto Eosine Methylene Blue agar Petri dish plates and incubated at 44 °C for 24 h. Plates showing growth of *E. coli* colonies (blue black with metallic sheen appearance) were sub-cultured onto Sorbitol MacConkey agar plates and incubated at 44 °C for 24 h. Colonies that demonstrated a colourless appearance were isolated and re-cultured for pure colonies (Baron *et al.* 1994). Pure colonies were then serologically confirmed with *E. coli* prolex™ latex agglutination test kit (Oxoid, UK). One drop of *E. coli* O157:H7 antisera was dispensed onto a reaction card and a loop full of the pure colony of the test organism was added. This was then carefully emulsified into a smooth suspension. The card was carefully rocked in a circular motion. If agglutination occurred within 1 minute then the test organisms were considered positive for *E. coli* O157:H7. A similar reaction was carried out as a control using normal saline instead of the *E. coli* O157:H7 antisera. Quantifications were based on colony forming units (CFU) expressed per 100 g of lettuce.

Assessment of *E. coli* O157:H7 infection risk and DALYs

An integrated quantitative microbial risk assessment and DALYs approach was used to assess the *E. coli* O157:H7 infection risk, diarrhoeal disease morbidity and mortality and burden associated with the consumption of wastewater-irrigated lettuce salad. This was based on the following steps.

Hazard identification

The main hazard was *E. coli* O157:H7 in the ready-to-harvest, wastewater-irrigated lettuce. The *E. coli* serotype O157:H7 is regarded as a sub-group of enterohaemorrhagic verocytotoxin-producing *E. coli* associated with bloody diarrhoea (haemorrhagic colitis) and HUS (Su & Brandt 1995).

Exposure assessment

Occurrence of E. coli O157:H7 in ready-to-harvest lettuce: To account for variability and uncertainty in the level of *E. coli* O157:H7 in the lettuce, probability distribution functions (PDFs) were fitted to the wet and dry seasons' *E. coli* O157:H7 data. The PDFs were fitted using the maximum likelihood estimation (MLE) method (Haas *et al.* 1999). The best fit PDF was obtained based on Akaike Information Criteria (AIC). Bootstrapping methods were used to obtain the uncertainties around the parameters of the PDFs.

Amount of lettuce consumed and exposure scenarios: The amount of lettuce consumed per single serving was obtained from a survey involving 270 street-food sellers and restaurants in Kumasi. Lettuce in ready-to-eat salad was weighed (Mettler balance, P-1200 (No. 903316)), PDFs were fitted to the weighed lettuce data, and the best fit PDF and bootstrapped uncertainty parameters were obtained using the MLE method (Haas *et al.* 1999). Two main exposure scenarios were considered in the risk assessment: (i) a worst-case scenario where there is no reduction in the level of *E. coli* O157:H7 in the wastewater-irrigated lettuce salad prior to consumption; and (ii) a best-case scenario where there is 0.5–2 log reduction of *E. coli* O157:H7 in wastewater-irrigated lettuce prior to consumption. The first scenario reflects the situation for most of the street foods where poor food safety practices prevail. The latter scenario is representative for high-class restaurants in the city where treatment of wastewater-irrigated lettuce using disinfectants (e.g., chlorine, salt solution, vinegar) is undertaken during salad preparation (Amoah *et al.* 2007).

The dose (D) of *E. coli* O157:H7 was obtained from the equation

$$D = C_{\text{season}} \times 10^{-r} \times A \tag{1}$$

where C is the concentration of *E. coli* O157:H7 in wastewater-irrigated lettuce salad in a given season (wet or dry); r is the reduction of *E. coli* O157:H7 on the salad (i.e., 0 for the worst-case scenario and log 0.5–2 for the best-case scenario); and A is the amount of lettuce salad consumed.

Dose–response (DR) assessment

It has been shown from outbreak studies and feeding trials that the beta-Poisson DR model best describes the dose(s) of *E. coli* O157:H7 ingested and the probability of infection

(Haas *et al.* 2000; Teunis *et al.* 2008). The approximate form of the beta-Poisson DR model is given as (Furumoto & Mickey 1967)

$$P_{\text{inf}} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \tag{2}$$

where P_{inf} is the probability of infection; D is the dose of *E. coli* O157:H7 ingested via lettuce salad; and β and α are the DR parameters. In this study, the beta-Poisson DR model parameters derived for *E. coli* O157:H7 by Teunis *et al.* (2008) were used. To account for variability and uncertainty, a triangular probability distribution function was fitted to the α and β parameters of the Teunis *et al.* (2008) DR model.

Risk characterization

The *E. coli* O157:H7 infection resulting from a single consumption event of wastewater-irrigated lettuce was assessed. To account for variability and uncertainty separately in the risk estimates, a second-order Monte Carlo simulation was implemented. A description of the model parameters in the second-order Monte Carlo Simulation are presented in Table 1. The risk estimates accounted for both the wet and dry seasons expressed as the probability of infection per consumer per single consumption of lettuce salad. The second-order Monte Carlo risk models were constructed and run in R statistical software version 2.15.0 (www.r-project.org).

Table 1 | Summary of the parameters used in the risk assessment and DALYs estimations

Model parameter	Unit	Probability distribution or assumption	Description
Dose of *E. coli* O157:H7 ingested (*D*)	CFU/g	$C_{\text{wet season}}$ = gamma (shape = 0.052; rate = 0.00019)[1]	U & V
E. coli O157:H7 in irrigated lettuce (*C*)	Log g	$C_{\text{dry season}}$ = gamma (shape = 0.013; rate = 0.00025)[1]	V
Reduction in *E. coli* O157:H7 (*r*)		Uniform (0.5, 2)[2]	U & V
Amount of lettuce consumed per serving (*A*)		Lognormal (3.67; 0.19)[1]	
E. coli O157:H7 DR model		Parameters for the beta-Poisson model [3]:	U & V
		α = Triang (2.62×10^{-4}; 0.373; 398.9)	
		β = Triang (0.056; 39.71; 3.96×10^{4})	
Probability of infection (P_{inf})		Beta-Poisson DR model	U & V
Probability of illness (P_{ill}) with a certain symptom		$P_{\text{ill}} = P_{\text{inf}} \times 0.69$*	C
		$P_{\text{waterdiarr}} = P_{\text{ill}} \times 0.53$*	
		$P_{\text{blooddiarr}} = P_{\text{ill}} \times 0.47$*	
		$P_{\text{HUS}} = P_{\text{blooddiarr}} \times 0.07$*	
		$P_{\text{ESRD}} = P_{\text{HUS}} \times 0.1$#	
Probability of fatality (P_{fatal})		$P_{\text{fatal}} = P_{\text{blooddiarr}} \times 0.0003$#	C
		$P_{\text{fatal}} = P_{\text{HUS}} \times 0.17$#	
Severity weights for illness and fatality		$P_{\text{waterdiarr}} = 0.067$#	C
		$P_{\text{blooddiarr}} = 0.39$#	
		$P_{\text{HUS}} = 0.93$#	
		$P_{\text{fatal}} = 1$#	
Duration of illness	Days	$P_{\text{waterdiarr}}$ = 7 days*	C
		$P_{\text{blooddiarr}}$ = 12.2 days *	
		$P_{\text{HUS}} = 21$#	

U, uncertainty; V, variability; C, calculated; gamma, gamma probability distribution function; Uniform, uniform probability distribution function; Lognormal, probability distribution function.
Source: [1]This study; [2]Amoah *et al.* (2007); [3]Teunis *et al.* (2008); *Su & Brandt (1995); #Havelaar & Melse (2003).

Diarrhoeal disease burden

Diarrhoeal illness and mortality: Infections with *E. coli* O157:H7 can result in a wide range of symptoms and secondary complications. Given a probability of *E. coli* O157:H7 infection (P_{inf}), the probability of illness (morbidity) can be derived as

$$P_{ill}(P_{\text{symp}}|D, \alpha, \beta) = \left(1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}\right) x\, (P_{\text{symp}}) \tag{3}$$

where the first part of the equation is P_{inf} and the second P_{symp} is the probability of an illness with a certain symptom or secondary complication resulting from the infection given a dose *(D)* of *E. coli* O157:H7 with DR parameters α and β.

Equation 3 can be expanded to obtain the probability of mortality (P_{mort}) as follows:

$$P_{\text{mort}}(P_{\text{fatal}}|P_{\text{ill}}) = (P_{\text{ill}})\ x\ (P_{\text{fatal}}) \tag{4}$$

where P_{fatal} is the probability of fatality given the occurrence of illness (P_{ill}) with a given symptom resulting from the consumption of a dose *(D)* of *E. coli* O157:H7.

To assess the illness (P_{ill}) and mortality (P_{mort}) associated with the consumption of *E. coli* O157:H7-contaminated lettuce salad, the *E. coli* O157:H7 disease transition model proposed by Havelaar & Melse (2003), with modifications, was followed. It was assumed that 31% of the infected consumers were asymptomatic (Su & Brandt 1995) while the rest were symptomatic. Of the symptomatic cases, 53% had watery diarrhoea ($P_{\text{waterdiarr}}$) and 47% bloody diarrhoea (haemorrhagic colitis) ($P_{\text{blooddiarr}}$). A case–fatality ratio of 0.03% was applied to cases with bloody diarrhoea (Havelaar & Melse 2003) while those cases with mild watery diarrhoea were expected to fully recover. Among the bloody diarrhoea cases, 7% later had HUS (Su & Brandt 1995) and 10% of the HUS cases developed an end-stage renal disease (ESRD) (Havelaar & Melse 2003). The mortality associated with the acute phase of HUS was assumed to be approximately 17% (Fitzpatrick *et al.* 1991; Havelaar & Melse 2003). The use of this high mortality rate was to account for the relatively poor health services in Kumasi.

Disability-adjusted life years: DALYs estimates were made to assess the burden of *E. coli* O157:H7 related diarrhoeal disease and mortality estimates derived from the previous section. DALYs were estimated using the equation of Murray (1994) as follows:

$$\text{DALYs} = \text{YLLs} + \text{YLDs} \tag{5}$$

where YLL is years of life lost due to mortality and YLD is years lived with a disability with weighing factor between 0 and 1 for the severity of the disability or disease. To derive the YLLs and YLDs for the *E. coli* O157:H7-related diarrhoeal disease and mortality, the following equations were used:

$$YLLs[r, K, \beta] = \frac{KCe^{ra}}{(r+\beta)^2} \left\{e^{-(r+\beta)(L+a)}[-(r+\beta)(L+a)-1] - e^{-(r+\beta)a}[-(r+\beta)a-1]\right\} + \frac{1-K}{r}(1-e^{-rL}) \tag{6}$$

$$YLDs[r, K, \beta] = D\left\{\frac{KCe^{ra}}{(r+\beta)^2} \left\{e^{-(r+\beta)(L+a)}[-(r+\beta)(L+a)-1] - e^{-(r+\beta)a}[-(r+\beta)a-1]\right\} + \frac{1-K}{r}(1-e^{-rL})\right\} \tag{7}$$

where K = age weighting modulation factor; C = constant; r = discount rate; a = age of death; ß = parameter from the age weighting function; L = standard expectation of life at age a. In this study, the age cohort 25–29 was used in the DALYs estimation. This cohort represents the highest proportion of consumers of wastewater-fed vegetables in urban Ghana (Seidu & Drechsel 2010). The severity weights for watery and bloody diarrhoea were taken as 0.067 and 0.39, respectively; and that of HUS was 0.93 (Havelaar & Melse 2003). Deaths resulting from diarrhoea, HUS and ESRD have a severity index of 1. A standard life expectancy of 63 years was used (Ghana Statistical Service 2010). Irrespective of the age group, mild diarrhoea and bloody diarrhoea were assumed to last 7 days and 12.2 days, respectively (Su & Brandt 1995). The HUS cases were estimated to last 21 days (Havelaar & Melse 2003). The DALY models were constructed and run in Microsoft Excel®.

RESULTS

Occurrence of *E. coli* O157:H7 in wastewater-fed lettuce and amount of lettuce salad consumed

Figure 1 shows the frequency distribution of *E. coli* O157:H7 in the wet and dry seasons. The occurrence of *E. coli* O157:H7 was low in the wastewater-fed lettuce, particularly in the dry season. The likelihood of a lettuce sample being positive for *E. coli* O15:H7 was 28% (30 out of 107 samples) and 5% (4 out of 89 samples) in the wet and dry seasons, respectively.

Among the PDFs fitted to the *E. coli* O157:H7 occurrence data, the gamma PDF was the best fit for both the wet season (shape = 0.052; rate = 0.00019; AIC = 404.67) and dry season (shape = 0.013; rate = 0.00025; AIC = 363). Figure 2 shows the fitted PDFs and the bootstrapped parameters of the gamma PDF for the wet season (shape = 0.052 (95% CI: 2.9×10^{-2}–0.128); rate = 0.00019 (95% CI: 8.76×10^{-5}–8.9×10^{-4})) and dry season (shape = 0.013 (95% CI: 2.98×10^{-2}–1.3×10^{-1}); rate = 0.00025 (95% CI: 9.1×10^{-5}–8.9×10^{-4})).

Figure 3 shows the amount of lettuce salad consumed per serving as fitted with the lognormal PDF (Inμ = 3.67; Inσ = 0.19) and bootstrapped parameters (Inμ = 3.67 (95% CI: 3.64–3.69); Inσ = 0.19 (95% CI: 0.18–0.22)) of the PDF.

E. coli O157:H7 infection risk, diarrhoeal disease and DALYs

Table 2 presents a summary of the estimated *E. coli* O157:H7 infection risk, diarrhoeal disease morbidity and mortality and disability adjusted life years (DALYs) associated with the consumption of wastewater-fed lettuce in Kumasi, Ghana. Consumers of wastewater-irrigated lettuce salad were more likely to be infected with *E. coli* O157:H7 in the wet season than in the dry season. In the wet season, the one-time *E. coli* O157:H7 infection risk was 1.7×10^{-1} (95% CI: 0.02–0.39) for the worst-case scenario and 4.2×10^{-2} (95% CI: 0.0013–0.177) for the best-case scenario. In the dry season, the *E. coli* O157:H7 infection risks were 1.8×10^{-2} (95% CI: 0.0002–0.0810) for the worst-case scenario and 2.05×10^{-3} (95% CI: 7.63×10^{-6}–1.36×10^{-2}) for the best-case scenario. The variability and uncertainty of the risk estimates derived from the second-order Monte Carlo simulation under the different seasons and scenarios are presented in Figure 4.

E. coli O157:H7-related diarrhoeal morbidity and mortality

In the wet season, the diarrhoeal disease morbidity per consumer per single serving was 0.12 (95% CI: 0.014–0.269) for the worst-case scenario and 0.028 (95% CI: 0.00089–0.12213) for the best-case scenario (Table 2). The estimated mortalities resulting from the morbidity cases per serving were 8.25×10^{-5} (95% CI: 9.6×10^{-6}–1.9×10^{-4}) and 2×10^{-5} (6.28×10^{-7}–8.6×10^{-5}) for the worst-case and best-case scenarios, respectively, in the wet season. In the dry season, the estimated morbidities per consumer per single serving for the worst-case and best-case scenarios were

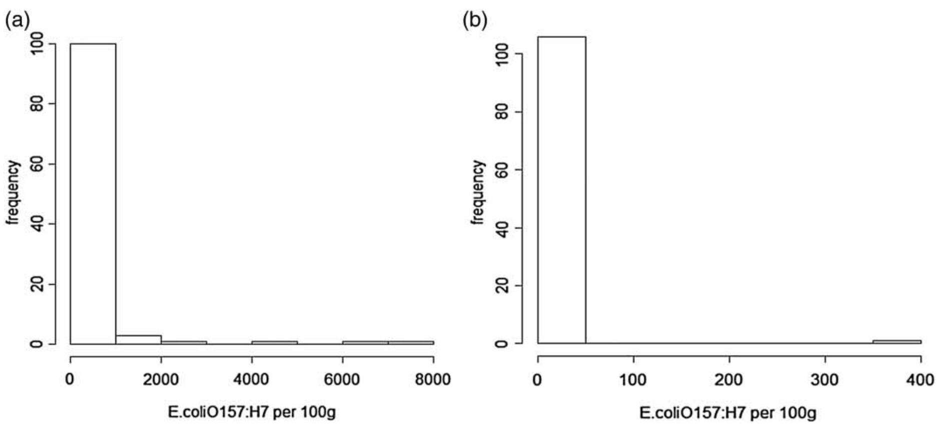

Figure 1 | Frequency distribution of *E. coli* O157:H7 in lettuce across the farm sites in the wet (a) and dry (b) seasons.

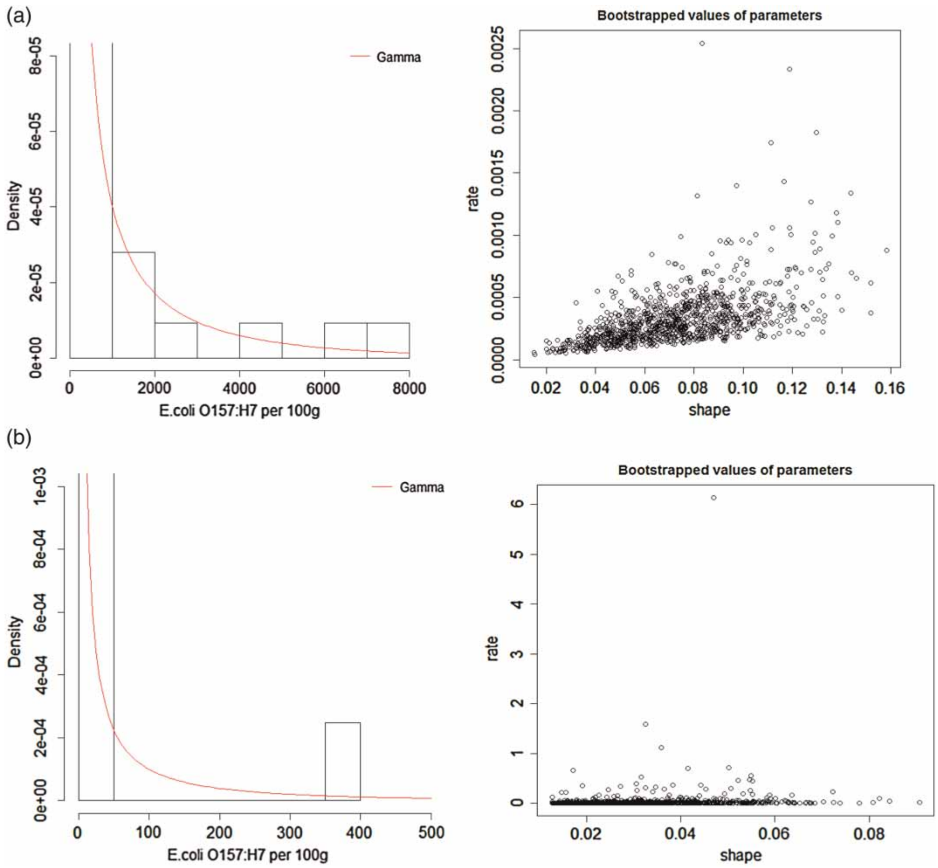

Figure 2 | Level of *E. coli* O157:H7 in wastewater-irrigated lettuce in the wet (a) and dry (b) seasons fitted with the gamma probability distribution function and the bootstrapped parameters of the distribution.

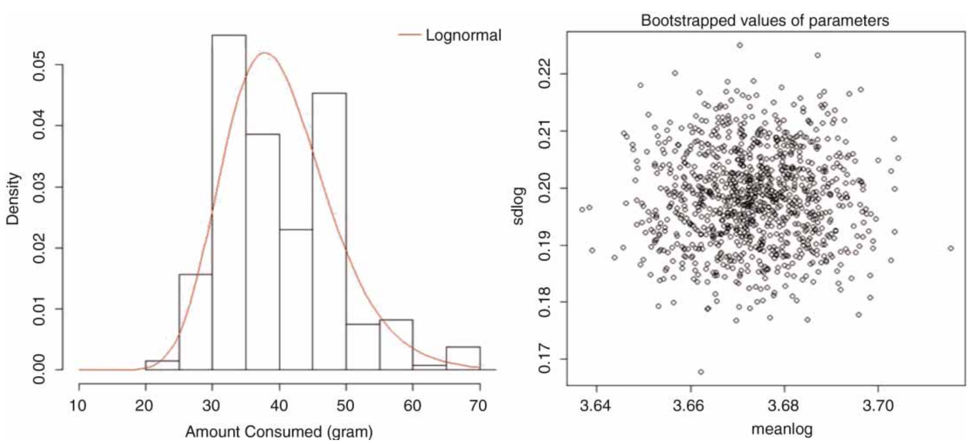

Figure 3 | Lognormal probability distribution fitted to the amount of lettuce salad consumed and the bootstrapped parameters of the distribution.

respectively 0.012 (95% CI: 0.000138–0.05589) and 0.0014 (95% CI: 5.26×10^{-6} –9.4×10^{-3}), respectively. In the same season, the corresponding diarrhoeal mortalities for the worst-case and best-case scenarios were 8.69×10^{-6} (95% CI: 9.6×10^{-8}–3.9×10^{-5}) and 9.9×10^{-7} (95% CI: 3.68×10^{-9}–6.6×10^{-6}), respectively.

Table 2 | Predicted infection risk, diarrhoeal disease cases and disability adjusted life years (DALYS) associated with a single consumption of *E. coli* O157:H7-contaminated lettuce salad in Kumasi, Ghana

	Wet season				**Dry season**			
Scenario	**Infection risk (P_{inf}) (mean (95% CI))**	**Diarrhoea morbidity (mean (95% CI))**	**Diarrhoea mortality (mean (95% CI))**	**DALYs (mean (95% CI))**	**Infection risk (P_{inf}) (mean (95% CI))**	**Diarrhoea mortality (mean (95% CI))**	**Diarrhoea mortality (mean (95% CI))**	**DALYs (mean (95% CI))**
Worst-case scenario	1.7×10^{-1} (2×10^{-2}–3.9×10^{-1})	1.2×10^{-1} (1.4×10^{-2}–2.7×10^{-1})	8.25×10^{-5} (9.6×10^{-6}–1.9×10^{-4})	2.8×10^{-3} (3×10^{-4}–6.5×10^{-3})	1.8×10^{-2} (2×10^{-4}–8.1×10^{-1})	1.2×10^{-2} (1.4×10^{-4}–5.6×10^{-2})	8.69×10^{-6} (9.6×10^{-8}–3.9×10^{-5})	3×10^{-4} (3×10^{-6}–1.3×10^{-3})
Best-case scenario	4.2×10^{-2} (1.3×10^{-3}–1.8×10^{-1})	2.8×10^{-2} (8.9×10^{-4}–1.2×10^{-1})	2×10^{-5} (6.28×10^{-7}–8.6×10^{-5})	7×10^{-4} (2.2×10^{-5}–2.96×10^{-3})	2.05×10^{-3} (7.63×10^{-6}–1.36×10^{-2})	1.4×10^{-3} (5.2×10^{-6}–9.38×10^{-3})	9.9×10^{-7} (3.68×10^{-9}–6.6×10^{-6})	3.4×10^{-5} (1.2×10^{-7}–2.3×10^{-4})

E. coli O157:H7 DALYs

The estimated disability adjusted life years (DALYs) for the wet season were 2.8×10^{-3} (95% CI: 3×10^{-4}–6.5×10^{-3}) and 7×10^{-4} (95% CI: 2.2×10^{-5}–2.96×10^{-3}) for the worst-case and best-case scenarios, respectively (Table 2). In the dry season the DALYs were 3×10^{-4} (3×10^{-6}–1.3×10^{-3}) and 3.4×10^{-5} (95% CI: 1.2×10^{-7}–2.3×10^{-4}) for the worst-case and best-case scenarios, respectively (Table 2).

DISCUSSION

The study shows that the occurrence of *E. coli* O157:H7 in wastewater-irrigated lettuce was seasonally dependent, with potential determinants including precipitation, temperature and humidity. On average, wastewater-fed lettuce collected from the farms was nearly six times (28% in wet season compared to 5% in the dry season) as likely to be contaminated with *E. coli* O157:H7 in the wet season as in the dry season. In the wet season, run-offs from animal farms and grazing sites adjacent to the farms are a potential source of *E. coli* O157:H7 contamination of the irrigation water. Also, almost all positive farms used chicken manure, a source of *E. coli* O157:H7, as a soil ameliorant (Griffin & Tauxe 1991; Griffin 1995). *E. coli* O157:H7 can survive longer in manure-amended soils (Kudva *et al.* 1998; Gagliardi & Karns 2000; Islam *et al.* 2004) and even longer in the wet season when the soil is either moist or wet (Solomon *et al.* 2002). Islam *et al.* (2004) demonstrated that *E. coli* O157:H7 in compost could survive for more than 5 months after application to the soil; and can contaminate the leaves of low-growing vegetables such as lettuce in the event of rainfall through soil splashes. The survival of *E. coli* O157:H7 on the leaves of lettuce is also likely to be longer in the wet season due to favourable environmental conditions (e.g., temperature and humidity) compared to the dry season. Temperature is an important determinant of the persistence of pathogens in the environment. The average temperature across the study sites was lower in the wet season (25.2–31.5 °C) than in the dry season (29.5–37.6 °C). The effect of temperature and humidity on the survival of *E. coli* O157:H7 as a function of season was recently

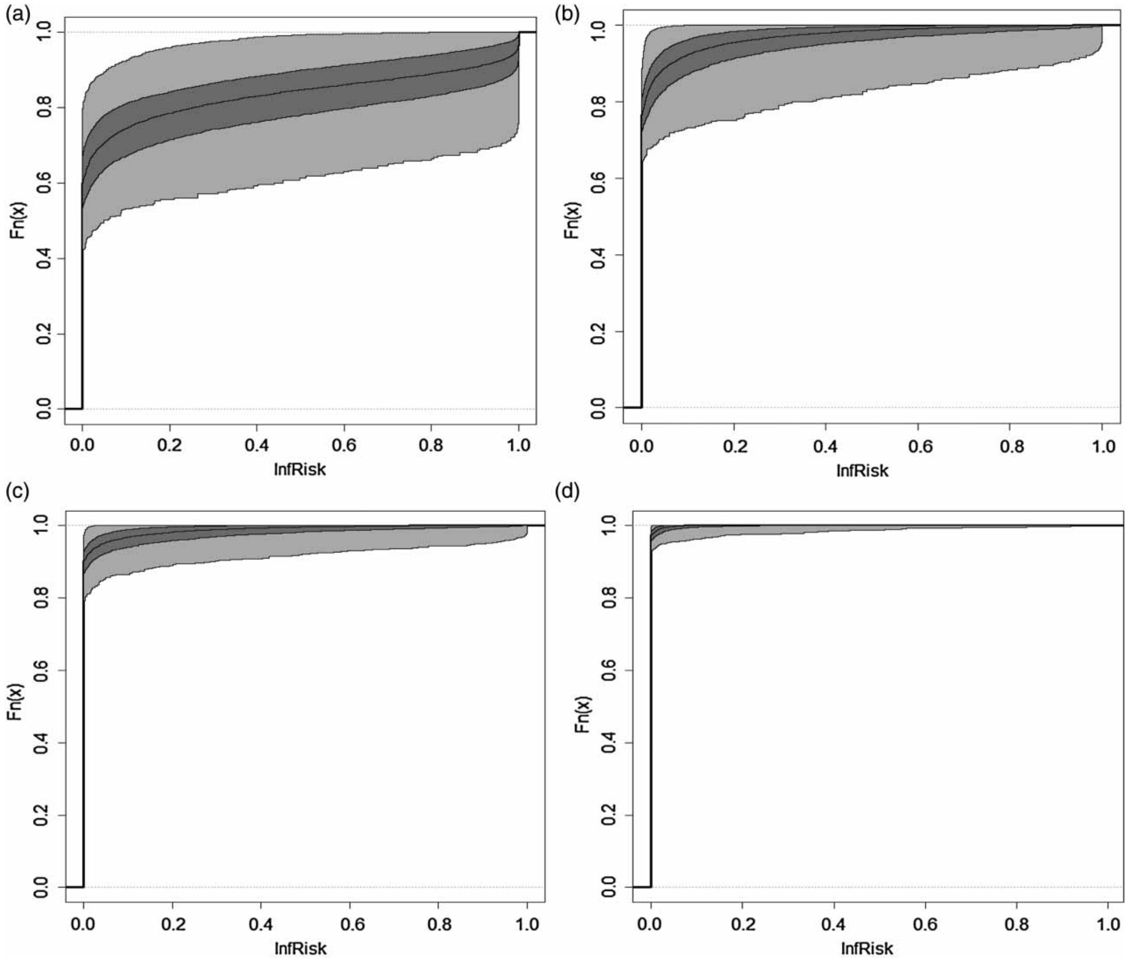

Figure 4 | *E. coli* O157:H7 infection risk associated with the consumption of wastewater-irrigated lettuce in the wet season, worst-case (a) and best-case (b), and dry season, worst-case (c) and best-case (d) scenarios. Grey bands correspond to the 95% uncertainty range on each quartile of variability and dark grey band corresponds to the 50% uncertainty range on each quartile of variability.

demonstrated (Oliveira *et al.* 2012). Oliveira *et al.* (2012) found that *E. coli* O157:H7 was more persistent on lettuce leaves in the fall when the humidity and temperature were 10 °C and 82%, respectively, and less persistent in the spring when humidity and temperature were 17 °C and 62%.

The study has shown that wastewater-irrigated farms are contributing to the transmission of *E. coli* O157:H7 with associated diarrhoeal disease and secondary complications (i.e., HUS and ESRD) in the city of Kumasi. Under the worst-case scenario in the wet season, the *E. coli* O157:H7 infection risk was 1.7×10^{-1} per single consumption of wastewater-irrigated lettuce. This is about 6 log greater than the World Health Organization (WHO) tolerable diarrhoeal disease infection risk of 2.7×10^{-7} per consumer per day (~10^{-4} per consumer per year) (WHO 2006). The wet season worst-case scenario *E. coli* O157:H7 infection risk resulted in 0.12 diarrhoeal disease cases per consumer per exposure, which is comparable with the diarrhoeal disease prevalence of 0.8–1.3 per person per year for developing countries with a potentially dire economic consequence (Mathers *et al.* 2002). The relatively high *E. coli* O157:H7 infection risk and accompanying DALYs has implications for the local economy and health systems of the city in particular. Currently, there are no epidemiological data on the incidence and cost of *E. coli* O157:H7-related diseases in Ghana in general, and Kumasi in particular. However, studies from the United States suggest that *E. coli* O157:H7-related diseases could have a devastating impact on the local economy. Mead *et al.* (1999) estimated that US$405 million was lost annually through *E. coli* O157 (STEC) diseases related to food or other sources in the United States. Another estimate from the United States revealed that the average cost of *E. coli* O157:H7-related disease can range from US$26 for individuals who did not obtain medical care to US$5.2 million for cases that were hospitalized after developing HUS and ESRD (Frenzen *et al.* 2005). Based on Frenzen *et al.*'s (2005) study, the social and economic costs will be too prohibitive in the event that consumers of wastewater-irrigated lettuce salad in Kumasi develop HUS and ESRD. For those consumers suffering from kidney failure, the treatment options are kidney transplant or dialysis. The costs of paying for thrice-weekly dialysis would be unaffordable for the vast majority of consumers in Kumasi if they needed to pay out of their own pocket (Callegari *et al.* 2013). This seriously questions the continuous use of wastewater for vegetable irrigation given that the existing irrigation and farm practices lead to the contamination of vegetables with *E. coli* O157:H7.

From the foregoing information, the most critical question that policymakers should address is how to ensure that wastewater use in urban vegetable production does not result in significantly high economic and public health burdens. Several on-farm and post-harvest risk interventions have been piloted in Kumasi and other cities in Ghana to reduce the health risk (including diarrhoeal disease cases) associated with wastewater irrigation. These include on-farm interventions such as drip irrigation (Keraita *et al.* 2008a), irrigation cessation (Keraita *et al.* 2007), use of simple filters (Keraita *et al.* 2008a), sedimentation ponds (Keraita *et al.* 2008b) and post-farm interventions such as vegetable disinfection (Amoah *et al.* 2007). On-farm interventions such as drip irrigation, irrigation cessation and simple filtration have been effective in the reduction of faecal coliforms on wastewater-irrigated lettuce, and yet have proven difficult to implement due to economic reasons and farmers' perceptions of health risk. In the short to medium term, these on-farm interventions are not likely to be implemented by farmers without a comprehensive enforcement backed by effective local guidelines and regulations. At the same time, additional efforts should be made towards improving post-harvest handling practices in the kitchens of street-food vendors and restaurants. For example, the study shows that a 0.5–2 log reduction in *E. coli* O157:H7 in the wastewater-irrigated lettuce would reduce the wet season worst-case diarrhoeal disease cases by a little over 90%.

In Ghana, surface disinfection of lettuce with salt solution is widely practised by street-food sellers and restaurants. Chlorine and vinegar are also used, but sparingly, because food sellers consider them to be expensive (Amoah *et al.* 2007). While the efficacy of salt solution and vinegar in the removal of *E. coli* O157:H7 on vegetables has not been documented in the literature, studies have reported a 1.5–2 log reduction of *E. coli* O157:H7 on vegetables (Beuchat 1999; Keskinen *et al.* 2009; Al-Nabulsi *et al.* 2014). Beuchat (1999) showed that the treatment of lettuce with 200 ppm chlorine solution or deionized water was effective in removing *E. coli* O157:H7 from lettuce. However, chlorine disinfection may prove ineffective if *E. coli* O157:H7 internalized in the vegetables is not significantly exposed to the active disinfectant agent, as has been demonstrated in several studies. *E. coli* O157:H7 can be internalized into lettuce through roots (Solomon *et al.* 2002; Erickson *et al.* 2010), attach inside stomatal pores (Seo & Frank 1999) and invade interior tissues via cut leaf edges (Kazue & Frank 2000; Erickson *et al.* 2010). Internalization of *E. coli* O157:H7 can render the surface treatment of lettuce with disinfectants such as chlorine ineffective (Kazue & Frank 2000). For example, a study showed that treatment of *E. coli* O157:H7 internalized in iceberg lettuce with 300 and 600 ppm chlorine solutions resulted in reductions of less than 1 log (Niemira 2008). Irradiation has been found to be more effective in the reduction of *E. coli* O157:H7 internalized in the tissues of lettuce. Niemira (2008) found a 5 log reduction of *E. coli* O157:H7 in iceberg lettuce treated with 1.5 kGy of irradiation. Nthenge *et al.* (2007) also showed that *E. coli* O157:H7 in the leaf tissue of lettuce was effectively removed by gamma irradiation while 200 ppm aqueous chlorine had no effect. Electrolyzed oxidizing water for the removal of *E. coli* O157:H7 on vegetables has also been demonstrated to be more effective than chlorine (Keskinen *et al.* 2009). These alternative disinfectants are relatively expensive and are not currently available to the vast majority of food-sellers and restaurants in Kumasi. It is therefore important that food sellers are introduced to less costly but effective disinfection measures. The introduction of any disinfection measure has to be backed by robust legislative instruments with objectively verifiable monitoring indicators to ensure its full implementation.

This study is not without limitations. The culture-based method used to enumerate *E. coli* O157:H7 could have underestimated the number of *E. coli* O157:H7 in the wastewater-irrigated lettuce from the study farms. The limitation of culture methods for *E. coli* O157:H7 enumeration was recently demonstrated by Moyne *et al.* (2013). The study by Moyne *et al.* (2013) showed that within 2 h of application, the culturable number of *E. coli* O157:H7 decreased by 1,000-fold, while molecular methods (polymerase chain reaction) showed the number of cells was equivalent to the inoculum levels. It was concluded that *E. coli* O157:H7 either died immediately after application or were no longer culturable. According to Moyne *et al.* (2013), sub-injured cells, which are rarely captured using culture-based methods, can be infectious, and have the ability to regrow under favourable temperature and moisture conditions. Without any further studies to assess the recovery rate of the method, the risk assessment models assumed full recovery (100%) for the method used in this study. This assumption can lead to the underestimation of the infection risk estimates arrived at in this study. It is recommended that molecular methods are used to enumerate *E. coli* O157:H7 in wastewater-irrigated vegetables.

CONCLUSION

This study has shown that wastewater-fed agricultural produce in Kumasi is contributing to the transmission of *E. coli* O157:H7 among consumers of wastewater-irrigated lettuce with potential dire economic and public health burdens. Irrespective of the season, both the infection risk and disability adjusted life years (DALYs) exceed WHO tolerable levels. A reduction of *E. coli* O157:H7 by 0.5–2 log to reflect the current disinfection used by street-food sellers and restaurants did not result in tolerable infection risk and disease burden. It is recommended that legislation that ensures that both farmers and food-sellers adopt effective risk mitigation measures is implemented. The legislation should have clear, verifiable monitoring indicators with a penalty for those who do not comply.

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