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Helminth Contamination of Lettuce and Associated Risk Factors at Production Sites, Markets and Street Food Vendor Points in Urban and Peri-Urban Kumasi, Ghana

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Abstract: The study assessed contamination levels of lettuce with helminth parasites and associated practices that may influence contamination levels at farm, market and street food vendor points in urban and peri-urban Kumasi. Three farms, three market sites that sold lettuce purchased from the selected farms and 20 street food vendors, who purchase their lettuce from these markets, were studied. Samples of lettuce, irrigation water and refreshing water (water used for keeping lettuce fresh throughout the day) were collected from these sites and analyzed for helminths eggs/larvae using standard methodology. Helminths on the lettuce leaves, irrigation water and refreshing water in the farms and markets were mostly *Ascaris lumbricoides*, with some *Shistosoma*, *Hookworm*, *Trichuris trichura*, *Taenia*, *Clonorchis* and *Strongyloides* larvae. Helminths eggs on lettuce leaves ranged between 4 and 14 100^{-1} g wet weight and 3 and 25 eggs L^{-1} in irrigation water on the farms and between 2 and 7 100^{-1} g wet weight and 4 and 15 eggs L^{-1} in refreshing water in the markets. Helminths egg counts on lettuce leaves on two farms were 40-52.9% more when compared with the farms' irrigation water but one farm had 40.5% more in irrigation water when compared with the lettuce leaves and these differences were significant. Helminths eggs on lettuce from the two farms were 50 and 60% higher when compared with its corresponding market samples and 23.5% higher in one market when compared with its farm source. Helminths eggs in street food lettuce samples analysed from the selected areas were only *Ascaris* and *Shistosoma* eggs ranging between 0 to 2 eggs 100^{-1} g wet weight. Helminths eggs for both farm and market samples exceeded the recommended level of <1egg L^{-1}. Education on farm practices, post harvest handling and washing methods at both market and street food vendor sites and improved hygienic practices at consumer level may help reduce their numbers and minimize the risk.

Key words: Helminths, wastewater, health risk

INTRODUCTION

The persistence in the use of wastewater resource has been attributed to several factors, including the decreasing availability of water resources for irrigation as a result of the increasing demand for potable water in urban and peri-urban communities, the high cost of artificial fertilizers and the realization that nutrients in wastewater can increase crop production and the social acceptance of the practice and dependence by large populations for their livelihoods (Mara and Cairncross, 1991).

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In Ghana, wastewater irrigated agriculture in peri-urban communities contributes to about 90% of the vegetables consumed in the cities and also provides a major source of income for the households (Drechsel *et al*., 2001). The vegetables commonly grown include cabbage (*Brassica oleracea capitata*), spring onion (*Allium fistulosum*), carrot (*Daucus carota* sp. *sativus*), tomato (*Lycopersicon esculentum*), onion (*Allium cepa*), Shallots (*Allium escalonicum*), egg plant (*Solanum melongena*) local spinach (*Amaranthus* sp.), cucumber (*Cucumis sativa*) and lettuce (*Lactuca sativa*) which are eaten raw (Sanduri, 2004) and which may be contaminated with microorganisms at both the production and distribution points due to wastewater irrigation (Drechsel *et al*., 2000).

The sources of contamination extend beyond the use of wastewater on farms. Post harvest treatment of vegetables includes handling, washing, storage, transportation, sorting, packing, cutting and further processing equipment, both poor hygienic and poor personal hygiene practices during food preparation, and/or contact with contaminated soil or fecal matter. Wild animals too may contribute to the contamination. The common micro-organisms isolated from vegetable samples include *E. coli*, *Pseudomonas*, *Enterobacter cloacae*, *Salmonella arizonae* (Sonou, 2001; Jones and Heaton, 2006). Other organisms (helminthes and protozoan) have also been detected in vegetables collected from the production-consumption chain (Amoah *et al*., 2005).

World Health Organization (1989) documented the health hazards associated with the direct and indirect use of wastewater on rural health and safety for those working on the land or living on or near the land where the water is used and the risk that contaminated products from the wastewater use area may subsequently infect humans or animals through consumption or handling of the foodstuff or through secondary human contamination by consuming foodstuffs from animals that used the area.

World Health Organization (1989) also recognizes that the highest health risk associated with the use of waste water is theoretically for helminths infections, since helminths persist for long periods in the environment (from a few months up to 30 years) and host immunity ranges from low to non-existent and the infective dose is small.

The objectives of the study were to assess contamination levels of helminths parasites in lettuce at selected farm sites, markets and street vendors in urban and peri-urban Kumasi and to identify or assess risk factors that may have an influence on contamination levels of lettuce at farm sites, markets and street food vendors.

MATERIALS AND METHODS

Sampling Sites

The study was conducted on three selected vegetable production (farms) sites, three vegetable selling markets and 20 street food vendor points in and around Kumasi, the second largest city in Ghana. The farms were Karikari, Badu and Deduako. The Karikari and Badu farms are located close to Gyenyase, a suburb of Kumasi. They obtain their irrigation water from shallow hand dug wells and irrigation is done by means of watering cans. Deduako is located 10 km from the centre of Kumasi and obtains its irrigation water from an impounded stream running close to the farm and irrigation is done with overhead motorized sprinklers.

The three market sites from where the selected study farm produce is sold were identified as from Karikari farm to the Railway market, Badu farm to the European and French line markets and from Deduako to the Asafo market.

Twenty street food vendor points that purchase lettuce from the selected study market sites were chosen from the four sub-metropolitan (Subin, Asokwa, Manhyia and Tafo) areas in the Kumasi Metropolis. Based on the four sub-metros, one suburb from each of the four sub-metros where the sale of chopped lettuce leaves with rice is popular was selected (Ayigya in Asokwa; Adum in Subin; Tarkwa Maakro in Tafo; Krofrom in Manhyia) for sampling.

Sampling

Using a sketch map, on each sampling date, three lettuce heads per bed were randomly harvested from six beds per farm site and placed separately in labeled sterile plastic bags. In all, 162 lettuce samples were collected from the three production (farm) sites (54 lettuce heads per farm site).

Three sellers were selected from each market site and three lettuce heads were collected from each seller around mid-day to ensure that the lettuce had undergone some splashing/refreshing with water. Each lettuce was placed separately in separate labeled plastic bags. In all, 81 lettuce heads were collected from the markets (27 from railways, 27 from Asafo and 27 from European and French line markets).

From each street food vendor point, about 120 g of already sliced and prepared lettuce leaves were collected and placed in labeled food bags. A total of 40 lettuce samples were collected from the street food vendors.

From each of the three farms, weekly irrigation water samples (from either the hand dug well or stream) and water samples from the markets refreshing/splashing (sellers at Asafo market displayed lettuce without refreshing water) were collected in one liter Duran Schott bottles in triplicate for nine weeks. All samples (water and lettuce leaves) were transported to the laboratory in an ice chest and analysed for helminths eggs/larvae within 24 h.

Sampling was done between June 2005 and 2006 at all the selected farms, markets and street food vendor points.

Preparation of Samples and Quantification of Helminths Eggs

One hundred grams of lettuce was washed under running water into a bowl and then transferred into a sterile container. The water was allowed to settle in a two litre container overnight. Two litres of irrigation, splashing/refreshing water samples were also made to settle in a container overnight. All samples were quantified for helminths eggs/larvae using the modified USEPA methodology (Schwartzbrod, 1998).

Identification of Helminths Eggs

The helminths eggs were identified on the basis of their shape and size and compared with standard eggs on charts (Guerrant, 1995). The counting was done under a light microscope in both chambers of a haemocytometer at X40 magnification. Presumptive identification of the helminths eggs was done based on morphological characteristics (Guerrant, 1995).

Health Risk Assessment and Perception

Perception of risk and assessment of health risk practices were evaluated using structured questionnaires, focus group discussions and observation check lists at all stages of sampling (farm sites, markets and street vendor points) to document prevailing hygienic practices. The specific health risks examined included irrigation practices, use of personal protective equipment and lettuce processing (handling, washing, serving and preservation of leftovers).

Statistical Analysis

The Statistical Package for Social Scientists (SPSS) version 11.0 was used for testing the various statistical relationships between and within variables.

RESULTS

Helminths Eggs Numbers and Types on Lettuce Leaves and in Irrigation Water at the Production (Farm) Sites

Mean total helminths egg/larvae counts were 3, 25 and 3 eggs L^{-1} of irrigation water from Karikari, Badu and Deduako farms, respectively (Table 1). There were no statistically ($p \leq 0.215$)

Table 1: Types and population densities (per litre) of helminths eggs/larvae in irrigation water and on lettuce leaves (per 100 g) in three production sites in peri-urban/urban Kumasi

Farm sampling site	*Strongyloides*[1]	*Ascaris*	*Shistosoma*	*Hookworm*	*Trichuris*	*Taenia*	*Clonorchis*	Mean total egg count
Karikari farm	1 (1)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (5)
Badu's farm	4 (1)	17 (12)	1 (0)	1 (0)	1 (0)	1 (1)	0 (0)	25 (14)
Deduako farm	0 (1)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (4)

Values in parenthesis represent mean numbers of eggs/larvae on lettuce leaves; [1]These were larvae and not eggs; N for Lettuce = 54 for all farm sites; n for Irrigation Water samples = 9 for Karikari and Badu farms and 3 for Deduako farm

Table 2: Types and population densities (per litre) of helminths eggs/larvae in refreshing/splashing water and on lettuce leaves (per 100 g) in three market sites in peri-urban/urban Kumasi

Market sampling site	*Strongyloides*[1]	*Ascaris*	*Shistosoma*	*Hookworm*	*Trichuris*	*Taenia*	*Clonorchis*	Mean total egg count
Railways	1 (0)	5 (4)	0	0	0	0	0	6 (4)
European/french line	1 (4)	6 (10)	0	0	0 (1)	0	0	7 (15)
Asafo2	1	1	0	0	0	0	0	2

Values in parenthesis represent mean numbers of eggs/larvae in refreshing water, [1]These are larvae and not eggs, N for lettuce = 27 for railways, asafo market and European/French line market. N for refreshing water samples = 9 for railways and european/french line, [2]Asafo market displays lettuce leaves without refreshing water

significant differences between counts on Karikari and Deduako farms but there were significant differences between Karikari and Badu ($p \leq 0.001$) and between Badu and Deduako ($p \leq 0.001$).

Ascaris lumbricoides eggs were the dominant helminths in irrigation water from all the farms with their mean counts ranging between 2-17 eggs L^{-1} compared to 0-4 larvae L^{-1} for *Strongyloides*, 0-1 eggs L^{-1} for *Shistosoma* and 0-1 eggs L^{-1} for *Trichuris trichura* (Table 1). However, Taenia, Hookworm and *Clonorchis* ranged between 0-1 eggs L^{-1} (Table 1).

Mean counts of helminths eggs on lettuce leaves were also high, ranging from 4 to 14 eggs 100 g^{-1} wet weight with highest (14) counts recorded at Badu's farm and lowest (4) at Karikari farm (Table 1). Mean counts on Badu's farm were significantly ($p \leq 0.001$) higher compared to Karikari and Deduako farms.

Similarly, *Ascaris lumbricoides* eggs were the dominant helminths on lettuce leaves in all three farm sites, ranging between 3-12 eggs 100 g^{-1}. However, *Strongyloides* varied from 0-1 larvae 100 g^{-1} and *Taenia* between 0-1 eggs 100 g^{-1} wet weight (Table 1).

On all the farms, *Ascaris lumbricoides* eggs were dominant with population densities of 75% for Karikari, 69% for Badu and 89% for Deduako. *Strongyloides* larvae numbers represented 25% of the helminths eggs at Karikari, 19% at Badu and 11% at Deduako. However, there were other helminths types on Badu's farm; *Shistosoma* (3.2%), Hookworm (2.9%), *Trichuris trichura* (2.7), *Taenia* sp. (2.7%) and *Clonorchis* (0.5%).

Helminths Eggs/Larvae on Lettuce Leaves and Refreshing/Splashing Water from Three Market Sites

Mean helminths eggs/larvae numbers (100 g^{-1}) on lettuce leaves were 6, 7 and 2 for Railway, European and Asafo markets, respectively (Table 2). There were no statistically significant differences in counts between the Railway and European ($p \leq 0.652$) markets but there were significant ($p = 0.001$) differences between the Railway and Asafo and between Asafo and European/French line markets.

Similarly, mean helminths eggs counts (per litre) in the refreshing/splashing water were 4 for Railway and 15 for European/French line markets (Table 2). Differences in counts between these two markets were statistically ($p \leq 0.001$) significant. Sellers in the Asafo market do not use refreshing/splashing water.

Ascaris lumbricoides were again the most dominant helminths eggs on market lettuce leaves and refreshing water with mean counts (100 g^{-1} wet weight) ranging between 1 and 6 for lettuce leaves and 4 and 10 eggs L^{-1} for refreshing water (Table 2).

Ascaris lumbricoides represented 70% of all helminths eggs counted on lettuce leaves from Railway (2-10 eggs 100 g^{-1} wet weight), 78% from European (2-11 eggs 100 g^{-1} wet weight) and 60% from the Asafo markets (1-4 eggs 100 g^{-1} wet weight) (Table 2).

Helminths egg numbers in refreshing water were also predominantly *Ascaris lumbricoides*; 76% in European/French Line samples ranging from 4 to 25 eggs L^{-1} . They however represented only 24% in the Railway samples ranging from 1 to 9 eggs L^{-1}.

There were no statistically significant differences in helminths egg numbers on lettuce leaves between the Railway and European markets ($p \leq 0.652$) but counts in refreshing water at the European market were significantly ($p \leq 0.001$) higher than at the Railway market site.

Relationship Between Helminths Eggs/Larvae Counts in Irrigation Water, Farm Lettuce, Refreshing Water and Market Lettuce

Helminth egg counts on lettuce leaves from the Karikari and Deduako farms were 40 to 52.9% more than counts in the irrigation water used. However, in Badu farm, helminths egg numbers were 40.5% more in irrigation water when compared to lettuce leaves (Fig. 1). Numbers of helminths eggs on lettuce leaves and in irrigation water in the different farms revealed a weak positive correlation (r^2), 0.437 ($p \leq 0.001$) for Karikari, 0.268 ($p \leq 0.050$) for Badu and 0.048 ($p \leq 0.729$) for Deduako.

In the markets, the population density of helminths eggs were 20% more on lettuce leaves compared to the refreshing water at the Railway market but the counts in refreshing water from the European market were 26% higher than counts in the lettuce leaves (Fig. 2). A weak positive correlation also existed between the lettuce leaves and refreshing water samples ($r = 0.285$) ($p \leq 0.149$) for Railways and ($r = 0.211$) ($p \leq 0.218$) for the European/French Line markets.

Along the production-market chain, helminths egg numbers either reduced or increased. From the Karikari farm to the Railway market, egg counts increased by 23.5% but from the Badu's farm to the European/French line markets they decreased by 50%. Egg numbers also decreased by 60% for produce from Deduako farm to the Asafo market (Fig. 3).

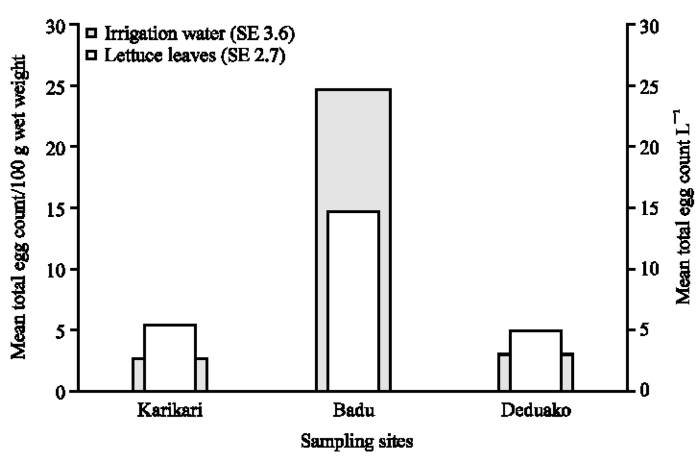

Fig. 1: Mean total count of helminths eggs on lettuce leaves and in irrigation water

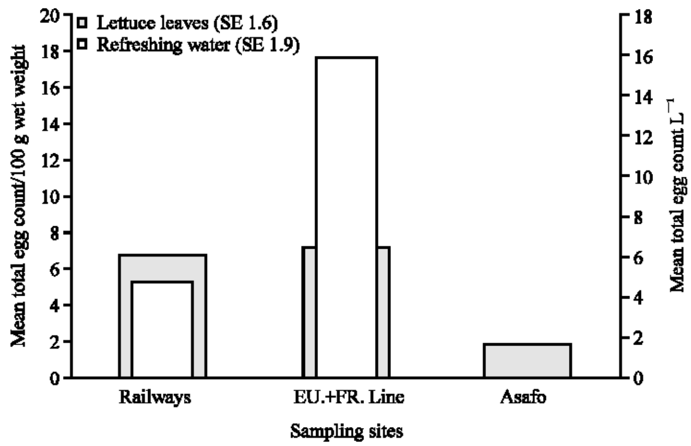

Fig. 2: Mean total count of helminth eggs on lettuce leaves and in refreshing water

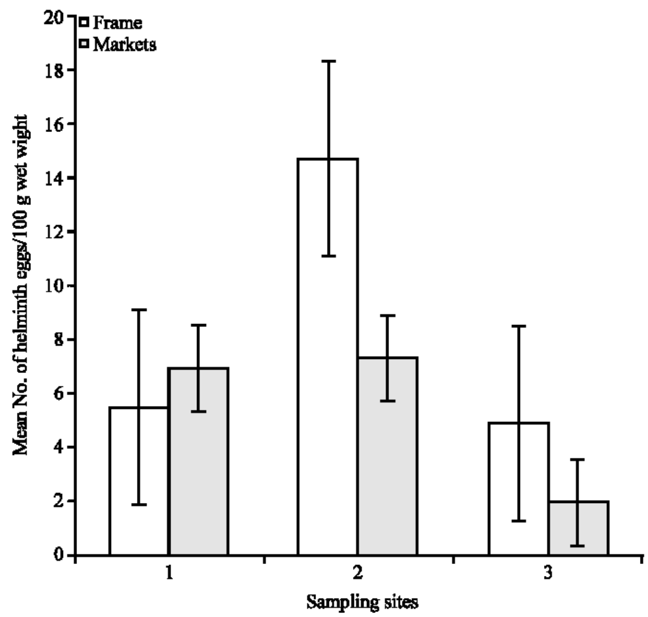

Fig. 3: Total helminth egg count on lettuce leaves on farm and their corresponding markets. (1) Karikari farm-railway market, (2): Badu farm-european/french line market (3): Deduako farm-asafo market

Helminth Numbers at Street Food Vendor Sites

Chopped lettuce leaves sold as part of the most ready to eat street foods had helminths eggs on them. Mean counts (100 g^{-1} wet weight) varied from 1 to 3 with no significant ($p \leq 0.47$) differences between the four sub metropolitan areas (Table 3). *Ascaris lumbricoides* and *Shistosoma haematobium* were microbial contaminants in all the samples analysed (Table 3).

Table 3: Mean number and types of helminths eggs on lettuce leaves (per 100 g) from selected street food vendor sites

Street food vendor site	*Ascaris lumbricoides* (range)	*Shistosoma haematobium* (range)	Mean total egg count
Adum	0-1	0-3	2
Krofrom	1-4	1-4	3
Ayigya	0-1	1-2	1
Tarkwa maakro	1-2	0-1	2

No. of Vendors sampled = 5 for Adum, 5 for Krofrom, 5 for Ayigya and 5 for Tarkwa Maakro

Health Risk Assessment

Production (farm) Site

Farmers from all three sites were males (90%) with most of them living close to their farms. Each of the farms had an average of 15 farmers who were all producing mainly for sale. During informal Focus Group Discussions, farmers claimed to have experienced some health problems in the past year, some of which were attributed to their exposure to wastewater. Although most of the farmers acknowledged the risk posed by wastewater use in irrigation, 80% also indicated that their health problems could be due to pesticides and poultry manure use. Farmers mostly reported of malaria (95%), skin and foot rot diseases (50%) and diarrhoea (10%), with other complaints being back ache. Although some farmers had family members with similar health problems, they did not attribute this to the consumption of their produce or exposure to wastewater.

During sampling, observed unhygienic practices on the farms included the disposal of waste in irrigation water channels, farmers walking indiscriminately through the irrigation water channels without use of Personal Protective Equipment (PPE) and washing of their body parts in the dug-wells after the day's work.

Market Sites

Of the 15 lettuce and other exotic vegetables sellers interviewed, all of them were female. Nine of the 15 (60%) of them displayed their produce in receptacles with water for refreshing the produce. Although most of the sellers did not associate any health risk with their handling of the produce, they admitted the source of irrigation water and the pesticides used could lead to cholera and other diarrhoeal diseases among the costumers. Some of the possible causes of contamination in the markets could be the practice of washing several types of vegetables in the same bowl of water and the use of refreshing water for other activities such as washing of hands before and after eating. The environment surrounding the vegetable market was unclean.

Street Food Vendor Sites

Out of the 20 street food vendors, sixty-three percent of the street food vendors were between the ages of 21-30 years, 79% females and 21% males. Seventy-nine percent had either primary or secondary education and twenty-one percent had no formal education. In getting rid of soil particles on their produce, 96% of the sellers washed/cleaned their lettuce with pipe borne water and 4% with well water. Nearly 50% of the sellers added salt and 13% added vinegar to the washing water. Chemical treatment is perceived by sellers to kill pathogens that may be present in the produce. Other vendors simply wash with these chemicals to impact flavour of the lettuce. Most of vendors prefer salt because it is cheaper and easy to get but knowledge on water to salt ratio was unknown.

DISCUSSION

This study has shown that lettuce leaves produced, sold and consumed (from farm to fork) using irrigation water, which is mainly wastewater from domestic sources that are channelled into water holes; shallow hand dug wells and urban rivers and streams, used in vegetable farming in urban/peri-urban Kumasi are contaminated with helminths eggs and the numbers are far above the WHO recommended level. Helminths numbers (100 g^{-1} wet weight) on farm lettuce ranged between 4 and 14 eggs and between 2 and 15 eggs in the market. However, there were significant decreases between farm/market and that on the chopped lettuce leaves sold by street food vendors which ranged between 1 and 3 eggs (WHO acceptable limit is less than one egg). Helminths numbers in this study were higher compared to reported numbers on farms in Turkey (7 eggs) (Özlem and Şener, 2005) and Iran (1-8 eggs) (Amahmid *et al*., 1999). The high helminths numbers in this study could be attributed to the quality of irrigation water on the three farms. Badu and Karikari farms, which had counts ranging between 3 to 25 eggs L^{-1}, exceeding the recommended level of <1 egg L^{-1} for unrestricted irrigation, use unprotected shallow hand dug wells for irrigation (World Health Organization, 1989). However, the Deduako farm which uses stream water for irrigation had average counts of between 0 and 3 eggs L^{-1} in their irrigation water. Amoah *et al*. (2005) also recorded lower numbers (2 to 4 eggs L^{-1}) in stream irrigation water sources in Kumasi and Accra.

Secondly, the farms were all close to built up residential areas and domestic livestock was often found feeding on these farms. Sanitary facilities were also absent, thus encouraging farmers to defecate in nearby bushes. The location of poultry manure heaps also allows for possible runoff into the irrigation water sources and onto vegetable beds (Drechsel *et al*., 2000; Amoah *et al*., 2005). Seventy five percent of the poultry manure used by the farmers in the management of soil fertility has shown to contain high numbers of helminths eggs/larvae (Drechsel and Kunze, 2002). Fresh uncomposted poultry manure is broadcasted on vegetable beds one to two weeks after transplanting lettuce or whenever it is available. However, in Iran, vegetable farms were sited far from residential quarters and farmers who use chemical fertilizers and irrigation water from deep wells had lower contamination levels (Gharavi *et al*., 2002).

Ascaris lumbricoides were the dominant of the varied helminths eggs detected in the production-consumer chain, accounting for 74% of the helminths counted (farms 76%, market 71% and food vendors -56%). Similarly, *Ascaris* has been shown to be the dominant helminths on farms and on fruits and vegetables in Marrakech, Morocco (Bouhoum *et al*., 1997; Amahmid *et al*., 1999), in Iran (Gharavi *et al*., 2002) and in Turkey (Özlem and Şener, 2005).

In this study, the species variation of helminths eggs in the irrigation water and on lettuce leaves from Badu's farm was wider (*Ascaris lumbricoides*, *Strongyloides*, *Shistosoma*, *Hookworm*, *Trichuris*, *Taenia* and *Clonorchis*) compared to those at Karikari and Deduako which were predominantly *Ascaris lumbricoides* and *Strongyloides*.

Lettuce leaves sold at the vegetable markets in Kumasi were also contaminated with helminths eggs/larvae (2-7 eggs 100 g^{-1} wet weight) but the numbers were 21.6% lower when compared to the numbers on farms (Table 2). It is believed that the splashing of water on the vegetables to keep them fresh from the scorching sun in the open receptacles they are displayed in contributes to the contamination. This study shows that the refreshing water contains helminths eggs (4-10 eggs L^{-1}). Sellers use one bucket of water to wash a sellers' consignment of vegetables all day, thus contaminating the vegetables over time. There was a positive correlation between helminths egg numbers on the lettuce leaves and the refreshing water. Cross contamination can also be considered since several kinds of vegetables are washed and displayed in the same receptacle. Poor handling and cleaning practices by the market women also contribute to the contamination. Sellers in Kumasi wash their vegetables with the irrigation water at the farm gate before sending them to the markets (Drechsel *et al*., 2000).

Helminths eggs in market lettuce leaves were also mainly *Ascaris lumbricoides* (1- 6 eggs 100 g^{-1} wet weight) (Table 2). Similar findings have been reported in vegetable markets in Turkey (Ulukanligil *et al*., 2001) and in Penang, Malaysia (Anuar, 1977). *Ascaris* survival is attributed to the production of a cyst or ova stage (Feachem *et al*., 1983).

Variation in helminths egg/larvae types in market lettuce leaves and in refreshing water samples were the same as for the farms.

Helminths eggs were predictably low (1 to 3 eggs 100 g^{-1} wet weight) but not absent in the chopped lettuce with rice sold by the street food vendors in the Kumasi metropolis (Table 3). This reduction is 67% compared to farm samples. Solar radiation, temperature, humidity and rainfall and desiccation directly affect the persistence of microorganisms (World Health Organization, 2002).

Ascaris lumbricoides and *Shistosoma haematobium* were the only types of helminths eggs identified (Table 3). Although the street food vendors wash their vegetables with salt to taste water or diluted vinegar solution, ready to eat lettuce is still contaminated with helminths. The difficulty may be in determining the correct concentration of the salt-water or vinegar-water ratio and the quantity of lettuce leaves to be washed in them. Often the same volume of salt-water or vinegar-water is used several times probably due to the ignorance of the vendors.

Most of the farmers had a good knowledge of the health hazards associated with wastewater use in vegetable farming, but could not afford the cost of potable water for irrigation. Besides, wastewater was readily available and some farmers were of the opinion that it contains nutrients (Khouri *et al*., 1994). The World Health Organization (1989) and Cifuentes *et al*. (1993) have shown that there is a risk of infection of people exposed to wastewater and it is highest for round worms such as *Ascaris lumbricoides*, *Trichuris trichura* and Hookworms. The major concern is the evidence that all excreted pathogens can survive in the soil long enough to pose a potential risk to farm workers. The incidences of diarrhoea, stomach ache and body itch suffered by the farmers could be associated to their direct contact with the wastewater. The farmers did not wear any Personal Protective Equipment as some of them felt uncomfortable in ‘Wellington’ boots and hand gloves and claimed that wearing these slowed down their work. Personal hygiene practices like washing of hands with clean water and detergent following farm work were not observed.

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