



Fresh Vegetables and Ready-to-eat Salads: Sources of Parasitic Zoonoses in Mampong-Ashanti, Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Author PKAR conceived and designed the study and drafted the manuscript. Author IG analyzed the data and supervised the laboratory analysis and also reviewed the manuscript. Author SS assisted in analysing and interpreting the data. Author DDY critically reviewed the manuscript. Author OYA reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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ABSTRACT

This study assessed parasitic contamination of fresh vegetables and ready-to-eat salads from Mampong Municipality in the Ashanti Region of Ghana. Water and soil samples from various farms were also assessed for possible sources of contamination. Fresh vegetables and ready-to-eat salads were examined for parasites using saline as floatation medium, stained with Lugol's iodine and Ziehl Neelsen and observed under X40 objective lens. Data gathered were analyzed using Microsoft Excel. Of the 271 fresh vegetables examined, *Ascaris lumbricoides* recorded the highest prevalence (26.94%), followed by *Giardia lamblia* (19.93%). However, of the 120 salad samples examined, *Giardia lamblia* was most prevalent (24.17%), followed by *Ascaris lumbricoides* (19.17%). *Fasciola* spp., *Moniezia*, *Toxocara* spp., *Trichuris trichiura* and *Entamoeba histolytica*, were other parasites recovered from both fresh vegetables and salads and also from soil and water on the farms. Fresh vegetables and ready-to-eat salads were contaminated with parasites of both human and animal origin, similar to those recovered from soil and water on the farms. Farm soils and water are potential sources of parasitic infestations on vegetables. Consumers of fresh vegetables and ready-to-eat salads are at risk of diarrheal diseases and parasitic zoonoses.

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1. INTRODUCTION

Globally, fresh vegetable production contributes significantly to the food market [1]. Farmers are motivated to produce them throughout the year due to the high demand and health benefits derived from the consumption of fresh vegetables [2]. Vegetables are mostly eaten raw or partially cooked in a bid to preserve heat-labile nutrients. Consumption of raw vegetables are increasingly recognized as important vehicles for the transmission of pathogens hitherto associated with foods of animal origin.

Sources of contamination can be traced to pre-harvest and post-harvest activities [3]. Farm soils [4], water used for irrigation and/or application of insecticides/fungicides, faeces, dust, improperly composted manure and some human activities at various points of production [5] have all been implicated in contamination of vegetables. Notably, the use of wastewater for irrigation and poor food hygiene practices are often associated with a higher risk of food-related diarrheal cases [6].

Contamination on the farm is most likely to be transferred to the consumers, particularly in areas where farmers rarely wash the vegetables before transporting to the markets. Parasitic infections from consumption of such foods may contribute significantly to morbidity in developing countries [7]. Reports from Ghana Health Service suggests a steady increase in intestinal worm infestations, from 2.1% in 2011 to 3.6% in 2016, seventh of the top twenty causes of morbidity at the out-patients department[8]. Meanwhile, the Asante-Mampong Municipal alone contributes 0.5% to the national annual prevalence [9].

The Government of Ghana has recently initiated the Planting for Food and Jobs Programme to create food security and jobs [10]. This initiative includes the production of fresh vegetables and such mass production should, however, be accompanied by strict supervision both on the farms and vending sites to avoid compromising public health. While there have been several reports on health risks associated with consumption of vegetables in Ghana, only few have reported on parasitic contamination [11-13]. Duedu and co recovered human parasites from vegetables and further asserted a higher risk associated with patronage of vegetables from open-aired markets [11]. The data from this

previous study may however, not be transferrable to our study location considering the differences in the epidemiological settings.

So far, there are no previous reports on the parasitological quality of vegetables in the Asante-Mampong Municipality. Given the numerous vegetable farms and the proliferation of street-food vendors in this area, it is imperative to assess the quality of vegetable products. Here, the open-aired markets remain the main sources of vegetables to consumers, suggesting a greater threat to public health. Moreover, there is paucity of up-to-date data on the epidemiology of parasitic contamination, route of contamination, and sources of contamination of fresh vegetables in this area. In this write-up, we discuss the farm-related risks associated with vegetable production and further report on the parasitological quality of both fresh vegetables and street-vended, ready-to-eat salad samples.

2. METHODS

2.1 Study Settings and Design

A cross-sectional study was conducted to assess the parasitological quality of fresh vegetables and ready-to-eat vegetable salads in the Asante-Mampong Municipal of Ashanti Region, Ghana.

Asante-Mampong is one of the largest cities in the Ashanti Region of Ghana with an estimated population of about 88,051 [14]. The Municipality has 69 settlements of which 58% are rural, covering a total land size of about 782 km². Its geographical coordinates are 7° 4'0" North, 1° 24' 0" West. It is located at the north—eastern part of Kumasi, the Ashanti regional capital. The inhabitants of the Municipality are predominantly farmers who cultivate vegetables, including carrots, lettuce, cabbage and green bell pepper.

2.2 Sample Size and Collection

For the purpose of this study, the following vegetables were sampled: cabbage (*Brassica oleraceae*), carrot (*Daucus carota*), lettuce (*Lettuce sativa*) and green pepper (*Capsicum annum*). For qualitative determination of parasites that contaminate vegetables, 70 Cabbages, 67 Carrots, 69 Lettuce and 65 Green peppers making a total of 271 samples were collected for this study while a sample size of 120 of ready-to-eat vegetable salads was

estimated based on prevalence of 3.6%, with 5% margin of error at 95% confidence level.

2.3 Sampling Procedures

Soil and water samples from four major and six small vegetables farms were collected. About 100 to 200 grams of soil dug at 3 to 5 cm from the surface of the ground using a small hand shovel [15] and collected with a clean spoon into small plastic bags labeled with the date, the farm name, and sample sites. Water samples from farms used to irrigate these vegetables were also collected after stirring into clean 500 ml plastic clean bottles. These samples were kept in bags and bottles and transported to the laboratory for parasitological analysis.

Vegetable samples were randomly collected from retailers at the Mampong Municipal market by purchasing the various types of vegetables and purposively from the different farms. The market was divided into three (zones) clusters, out of each, a third of each vegetable types were collected. The vegetable salads were bought from randomly selected street food vendors from three clusters between 6.00 and 11.00 GMT. The salads comprised a combination of two or more of the following vegetables: Cucumber (*Cucumis sativus*), Carrot (*Daucus carota*), Lettuce (*Lactuca sativa*), Onions (*Allium cepa*), Tomatoes (*Lycopersicon esculentum*) and Cabbage (*Brassica oleracea*). Each ready-to-eat vegetable salads sampled was put into a separate labeled sterile plastic bags to prevent cross contamination [14] and stored under ice until examined. These vegetable products were all sampled from the period of February to April 2019.

2.4 Sample Preparation and Detection of Parasites

2.4.1 Vegetables samples

Each vegetable sample was put into a 100 ml round bottom clean plastic container and washed thoroughly with hand after vigorous agitation with formal-saline. For a tight head vegetable like cabbage, it was separated into units and for leafy vegetables like lettuce they were separated into individual leaves, while the rest were washed whole. Each part of the samples was removed, rinsed and drained with formal-saline into the 100 ml round bottom clean plastic containers respectively and allowed to stand on the bench for one hour to allow proper sedimentation. The

supernatant was carefully discarded using a Pasteur pipette leaving about 20 ml at the bottom. The 20 ml deposits were transferred into a 50 ml centrifuge tubes and centrifuged at 300 rpm for about 10 minutes. The supernatant was decanted while the deposit re-suspended with 10 ml formal-saline and centrifuged again. The supernatant was then discarded and the remaining pellet with 5ml of the saline examined [15]. Three smears of the deposits were prepared from each sample and stained with Lugol's iodine and examine for protozoan cyst and helminth eggs using light microscope as previously described [16].

2.4.2 Soil samples

Soil samples were treated with sucrose floatation technique as previously described by Uga and co [16]. Each soil sample was sieved with a 150 µm sieve to remove large particles. A weight of 2 grams of powder soil was mixed with 8 ml of distilled water in a test tube and centrifuged at 1000 rpm for 5 min. The supernatant was discarded and the pellet re-suspended with 8 ml of sucrose solution as previously described [4]. The mixture was vigorously vortexed and centrifuged at 2000 rpm for 10 min. The rest of the procedures were as previously described [4, 16].

2.4.3 Water for irrigation of vegetables

Each of the water samples from the farms was well shaken and poured into 50 ml centrifuge tube and centrifuged at 3000 xg for 15 minutes. The supernatant was then discarded and the remaining pellet was collected and examined [15]. Three smears were then prepared from each sample and stained with Lugol's iodine to examine for protozoan cyst and helminth eggs using light microscope. Smears were made and examined using X 40 objective lens as previously described [4].

2.5 Ready-to-eat Vegetable Salads

Each sample weighing about 100 g was thoroughly washed in 250 ml of sterile normal saline solution (0.85% NaCl) for 5 minutes in 500 ml conical flask. The samples were agitated vigorously (manually) in the normal saline for about three minutes. The vegetables were removed and drained into the normal saline solution. The normal saline solution was then transferred into a conical flask and then left to sediment. The supernatant was discarded and

50 ml of the remaining solution transferred into sterile conical tubes and centrifuged at 3000 xg for 15 minutes. The supernatant was then discarded and the remaining pellet with 5 ml of the saline was collected and examined [17]. Three smears were then prepared from each sample and stained with Lugol's iodine to examine for protozoan cysts and helminth eggs using light microscope [18].

2.6 Microscopy and identification of Parasites

Each stained smear slide was examined repeatedly (at least 200 fields) first with X10 objective lens and for identification of parasites with X40 using microscope objective lens. Identification of parasites were further supported using bench aids [19]. Quality in microscopic examination was achieved through the use of three independent microscopists who examined smears each from the same samples.

3. RESULTS

Table 1 shows the mean parasite counts of the different vegetables from the Mampong Municipal Market. The mean parasite counts of 9.88, 18.18, 2.65, and 1.35 were computed for cabbage, lettuce, carrot and green pepper respectively. There was significant difference in

the mean parasite count between lettuce and carrot and green pepper ($p < 0.05$, $F = 3.390$).

Of the 271 vegetable samples, 132 (48.7%) tested positive for intestinal parasites of which the most contaminated was lettuce, 54 (78.3%), followed by cabbage 48 (68.6%), carrot 19 (28.4%) and green pepper 11 (16.9%). *Ascaris lumbricoides* had the highest prevalence of 26.94%, followed by *Giardia lamblia* (19.93%), while *Toxocara* spp. recorded the least prevalence of 1.48% (Table 2). There was a significant difference between the prevalence of parasites contamination detected in each group of vegetables examined in the Mampong Municipal Market ($p < 0.05$).

Out of 120 vegetable salads examined, 80 (66.7%) of the samples were contaminated with parasites. *Giardia lamblia* recorded the highest prevalence (24.2%), followed by *Ascaris lumbricoides* (19.2%) while *Toxoplasma gondii* recorded the least prevalence of 0.8% (Table 3).

Of the soil and water samples from the farms examined, a total of two protozoans and thirteen helminths species were recovered. Some of the parasites recovered include, *Entamoeba histolytica*, *Giardia lamblia*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, hookworm and

Table 1. Mean parasite count of the different vegetables from the Mampong municipal market

Parasite species	Cabbage	Lettuce	Carrot	Green pepper	Total count
<i>Toxocara</i> spp.	3	1	1	0	5
<i>Ascaris lumbricoides</i>	71	129	26	17	243
<i>Fasciolopsis</i> spp.	7	29	0	1	37
<i>Hymenolepis nana</i>	5	13	2	0	20
<i>Strongyloides stercoralis</i>	8	22	2	2	34
<i>Schistosoma haematobium</i>	4	1	0	0	5
<i>Taenia</i> spp.	14	12	0	0	26
Unidentified Egg	3	5	0	0	8
<i>E. histolytica</i>	14	27	1	1	43
<i>Diphyllobothrium latum</i>	4	16	2	0	22
<i>E. vermicularis</i>	3	11	0	0	14
<i>G. lamblia</i>	53	65	14	9	141
<i>Moniezia</i> spp.	7	10	3	0	20
<i>Fasciola</i> spp.	2	4	1	0	7
<i>Trichuris trichiura</i>	8	3	0	1	12
Hookworm Ova/filariform	12	22	6	1	41
Mean Parasite Count	9.88^a	18.18^{ab}	2.65^a	1.35^a	
95% CL	1.58 – 18.18	2.85 – 33.50	-0.51 – 5.80	-0.74 – 3.45	

There was significant difference in the mean parasite count between "lettuce and carrot and green pepper" [$p < 0.05$; $F = 3.390$] as indicated by the different symbols

Table 2. The prevalence of different parasite species recovered from the vegetables from the Mampong municipal market

Parasites	Cabbage	Lettuce	Carrot	Green pepper	Vegetables contaminated (n)	% prevalence (n/271) *100
<i>Toxocara</i> spp.	2	1	1	0	4	1.48
<i>A. lumbricoides</i>	17	33	15	8	73	26.94
<i>Fasciolopsis</i> spp.	3	5	0	1	9	3.32
<i>H. nana</i>	3	8	1	0	12	4.43
<i>S. stercoralis</i> filariform	3	18	1	2	24	8.86
<i>S. haematobium</i>	4	1	0	0	5	1.85
<i>Taenia</i> spp.	11	7	0	0	18	6.64
Unidentified Egg	1	3	0	0	4	1.48
<i>E. histolytica</i>	12	25	1	1	39	14.39
<i>D. latum</i>	3	9	2	0	14	5.17
<i>E. vermicularis</i>	3	11	0	0	14	5.17
<i>G. lamblia</i>	13	28	9	4	54	19.93
<i>Moniezia</i> spp.	4	7	3	0	14	5.17
<i>Fasciola</i> spp.	1	4	1	0	6	2.21
<i>T. trichiura</i>	6	3	0	1	10	3.69
Hookworm spp.	5	9	3	1	18	6.27
# of vegetables examined	70	69	67	65	271	100
# of vegetables contaminated	48	54	19	11	132	48.71
% Prevalence	68.6	78.3	28.4	16.9	48.71	

There was a significant difference between proportions of the different vegetables contaminated and vegetables not contaminated [pv< 0.005]

Table 3. The prevalence of different parasite species recovered from Salad from the Mampong municipal market

Parasites	Contaminated Salad sample (n)	% Prevalence (n/120) *100
<i>Giardia lamblia</i>	29	24.2
<i>Ascaris lumbricoides</i>	23	19.2
<i>Moniezia</i> spp.	14	11.7
<i>Entamoeba coli</i>	7	5.8
<i>Toxocara</i> spp.	7	5.8
<i>Taenia</i> spp.	5	4.2
<i>Fasciola hepatica</i>	7	5.8
<i>Enterobius vermicularis</i>	5	4.2
<i>Trichuris trichiura</i>	4	3.3
<i>Toxoplasma gondii</i>	1	0.8
# of Salad samples examined	120	100
# of Salad samples contaminated	80	66.67
% Prevalence	66.67	

Enterobius vermicularis, etc. as shown in Table 4.

4. DISCUSSION

Given that our study location is predominantly a farming community, where vegetables are commonly cultivated, we first examined soil and

water samples from selected farms for the presence of parasites. We recovered fifteen genera of parasites, including ten nematode species of both human and animal origins from the farm soil and water samples. Geo-helminth eggs, including *Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Strongyloides* and hookworms were recovered from the soil

samples. This suggests contamination from human faeces from practices including open-defecation among farmers, the use of improperly composted manure, and the use of dirty water or waste water for irrigation. In a related study in West Cameroon, Nkouayep and her colleagues recovered five genera of nematodes (*Ascaris*, *Trichuris*, *Capillaria*, *Cooperia*, and hookworms) from soil samples collected from around latrines, at playgrounds, and behind classrooms in schools[4] fairly similar to ours.

This study reports for the first time on the parasitological quality of fresh vegetables and ready-to-eat salads in the Mampong Municipality.

The presence of *Strongyloides* and hookworms in farm soils suggests a risk of infection for farmers who may walk bare-footed on their farms. The use of animal droppings as sources of manure is also evident for the recovery of *Toxocara* spp. and *Moniezia* spp.; the former a common parasite of dogs [20] and the latter, commonly found in ruminants. The recovery of these animal parasites points to a potential source of parasitic zoonosis. Parasitic infestation in both farm soils and water samples suggests contamination occurs at the production sites [3]. Since the farmers often do not engage in washing or any form of processing before transporting the vegetables to the market, the quality of the vegetables, thus, reflects the prevailing farm conditions. However, instances when farmers wash fresh produce, water from ponds and rivers around the farms are usually used further increasing the risk of contamination

since these waters are mostly likely to contain some pathogenic microbes [21].

In countries like Ghana, the consumption of vegetables including cabbage, lettuce, onions, tomatoes and others has recently increased largely due to their readily availability and affordability in the market as compared to that of fruits like apples, grapes and others [22]. This high demand for these fresh vegetables has placed pressure on the chain of production right from farmers to the sellers and finally, the consumers [23]. In the process, care for safety reduces and many vegetables produced become contaminated with pathogens.

Post-harvest sources of contamination include harvesting equipment, unhygienic handling by farmers/vendors, insects, droppings of wild and domestic animals, methods of transportation, processing equipment dust and rinse water [3]. Parasites recovered from fresh vegetables in this study were similar to those found in farm soils and waters. In our study, we recorded an overall prevalence of 48.7%(132/271) vegetables testing positive for intestinal parasites, with varied parasite species recovered ranging between 1.5% and 27.0% of which *Ascaris lumbricoides*, *Giardia lamblia* and *Entamoeba histolytica* predominated. Infestation of vegetables with intestinal parasites, particularly *G. lamblia*, suggests that consumption of these vegetables could result in diarrheal cases. Water for irrigation of vegetables as well as unhygienic handling practices by vendors (including poor hand hygiene and/or poor wash methods) could

Table 4. Parasites recovered from soil and water from the farms in Mampong Municipality

Parasites	Soil		Water	
	Yes	No	Yes	No
<i>Toxocara</i> spp.	√			√
<i>Ascaris lumbricoides</i>	√		√	
<i>Fasciolopsis</i> spp.		√	√	
<i>Hymenolepis nana</i>	√		√	
<i>Strongyloides stercoralis</i>	√		√	
<i>Schistosoma haematobium</i>	√		√	
<i>Taenia</i> spp.	√			√
Unidentified Egg	√		√	
<i>E. histolytica</i>		√		√
<i>Diphyllobothrium latum</i>		√	√	
<i>E. vermicularis</i>	√			√
<i>G. lamblia</i>		√	√	
<i>Moniezia</i> spp.	√		√	
<i>Fasciola</i> spp.	√		√	
<i>Trichuris trichiura</i>	√		√	
Hookworm Ova/filariform	√			√

have favoured the transmission of *Giardia lamblia* in the present location. Other parasites recovered included *Toxocara canis* and *Diphyllobothrium latum*, both of which have been previously reported in dogs in the area [20], highlighting the risk of zoonotic infection. A study conducted in Egypt by Eraky, et al. [24] reported 29.6% positive for intestinal parasites in fresh vegetables with *Giardia lamblia* cysts, *Entamoeba* spp. cysts and *Enterobius vermicularis* eggs been the three most prevalent parasites recovered respectively while *Ascaris lumbricoides* eggs [24], the least reported contrary to our findings.

A significantly lower prevalence of infestations was seen in green bell pepper and carrot compared to the leafy vegetables. Green bell pepper and carrots have relatively smoother surfaces, and the fact that the former is not in direct contact with the soil further limits parasite infestations. Conversely, the higher prevalence seen in the leafy vegetables (lettuce and cabbage) could be attributed to their broader surfaces and rough uneven edges which provide attachment sites for parasites and their direct contact with the soil. This assertion supports previous study by Eraky and co in which the highest contaminated vegetable was lettuce, followed by other leafy vegetables watercress and leek with the least contamination [24]. Also, lettuce was reported with the highest multiple parasitic contamination while carrot and garden egg with smooth edges had the least [25] in a similar study conducted in Jos, Plateau State in Nigeria.

Although carrot is a root crop in direct contact with the soil, its smooth surface coupled with the rigorous washing to remove soil particles, tends to reduce parasite infestations on them. The dirty leafy outermost coverings of cabbages that come into direct contact with parasite-infested soil are often peeled off after harvest, a practice that reduces parasite infestations [24,25]. This could have accounted for the marginally lower prevalence in cabbage, as compared to lettuce, a vegetable which unlike carrot, is often subjected to tender washing to protect the soft and fragile leaves from damage [11].

We further assessed the safety of street-vended salads sold as accompaniments to mainly rice dishes. These salads were prepared from a combination of vegetables, including lettuce, cabbage, carrots and onions. Given, the high prevalence seen in fresh vegetables, we

anticipated high contamination in the salads, considering washing remains the only, notable processing method the vegetables are subjected to prior to sale. In areas with scarcity of water, vendors tend to wash vegetables in a bowl, rather than under running water, a process which may allow for cross-contamination. The results showed a high prevalence (66.0%) in salads, albeit with a lower parasite diversity (10 species) compared to the fresh vegetables (16 species). Compared to a similar study in Accra [26], we recorded a higher prevalence of infestation in salads in the present location (66.0% vrs 32.0%), with *Giardia lamblia* being the most predominant parasite in both studies. Differences in handling practices by the vendors and the parasite loads on the fresh vegetables may have accounted for the varying prevalence between the two locations. These results suggest patronage of street-vended salads may contribute to diarrheal cases and the spread of parasitic zoonoses.

5. LIMITATIONS

In this study, there were inherent limitations. Although this study reflects some crucial public health concerns with regards to food safety and hygiene standard practices among street food vendors, our sampling was limited only to the study area and may not reflect the entire region. The processes of recovering parasites from water, soil, vegetables, and salads samples could destroy some cysts, larvae or ova. Meanwhile we relied solely on the experience of the microscopists examination of stained smeared. There is need for further studies using a larger sample size from more diverse sampling sites and also sampling vegetables sources.

6. CONCLUSION

Contamination of vegetables occurs at the farms, through direct contact of vegetables with parasite-infested soils and water. Parasitic contamination in both fresh vegetables and street-vended salads were high, suggesting a public health threat. *Giardia lamblia* and *Ascaris lumbricoides* were the two most predominant parasites recovered from vegetable samples. Consumption of vegetables could be a source of diarrhea and parasitic zoonosis.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study will be made available upon request.

ETHICAL APPROVAL

This study was done in accordance with ethical guidelines of University of Education, Winneba.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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