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## **Parasitic Contamination in Ready-to-Eat Salads in the Accra Metropolis, 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 VA collected the data and supervised the laboratory analysis and reviewed the manuscript. Author IM assisted in analysing and interpreting the data.*

*Author DDY critically reviewed the manuscript. Authors FN and EAN reviewed and edited the manuscript. All authors have read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Globally, vegetables have become an essential part of the human diet due to their high nutritional benefits. They are usually eaten raw or partially cooked and therefore could become vehicles for transmission of food-borne illness. This study therefore assessed parasitic contamination of ready-to-eat vegetable salads from street vended foods in the Accra Metropolis of Ghana.

**Methods:** A cross-sectional study was conducted to determine the level of parasitic contamination of ready-to-eat vegetable salads in the Accra Metropolis, Ghana. A total of 313 ready-to-eat vegetable salads were randomly sampled, washed with physiological saline solution using concentration method and microscopically examined.

**Results:** Of the 313 samples examined, about a third (32%) was contaminated with at least one parasite. Overall, twelve genera of parasites were recovered with *Giardia lamblia* (6.7%),

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*Entamoeba histolytica* (6.4%) and *Moniezia* spp. (4.2%) predominating. Other parasites detected included *Trichuris trichiura* (3.8%) and *Entamoeba coli* (3.5%), with the rest recording low prevalence (<2%).

**Conclusion:** The presence of intestinal parasites point to a risk of food-borne illnesses from consumption of street-vended vegetable salads. Vegetable salads were found to be a potential source of parasitic infections.

**Keywords:** Vegetable salads; parasites; contamination; Accra metropolis.

## 1. BACKGROUND

Vegetables are considered essential for balanced diets as they serve as a source of vitamins, minerals, dietary fiber, and phytochemicals [1]. They confer a lot of health benefits through prevention of chronic diseases, reduction and maintenance of weight, among others [2]. As such, many people consume raw vegetables as salad to retain the natural taste and heat labile nutrients [3]. Notwithstanding these health benefits, consumption of parasite infested vegetables favors the transmission of food-borne illnesses [4].

Food borne-illnesses remain a serious threat to public health with an estimated 9.4 million cases reported each year in the United States alone [5]. This phenomenon is a common feature in developing countries where hygienic and post-harvest practices are poor. The use of untreated animal excreta and waste water for irrigation of vegetables contribute to the transmission of food-borne illnesses [6]. Multiple studies have assessed the prevalence of parasitic contamination of vegetables worldwide and have recovered several parasites, including *Ascaris lumbricoides*, *Entamoeba histolytica* and *Giardia intestinalis* [7-9].

While there have been studies on safety of vegetables in Ghana, these were largely limited to bacterial and chemical poisoning [9-11]. The only available report however, highlighted parasitic contamination of fresh vegetables and further assessed the efficacy of three washing methods, excluding ready-to-eat salads [12]. Antwi Agyei and co [11] also reported 99% faecal contamination in street vended foods.

A recent report by the Ghana Health Service points to 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 [13]. Based on these reports, we suspected that consumption of street-vended salad may be contributing to the

increasing prevalence of helminthiasis in Ghana. Therefore, we qualitatively assessed the level of parasitic contamination in ready-to-eat vegetable salads sold at different retail outlets in the Accra Metropolis, an indication of the hygienic practices employed by food vendors. Accra is the most populous city in Ghana, with a high number of street food vendors. Street foods are highly patronized in these densely populated areas, though some vendors tend to operate under unhygienic conditions, thus putting consumers at risk of worm infestation and diarrhea.

## 2. METHODS

### 2.1 Study Settings and Design

A cross-sectional study was conducted to determine the level of parasitic contamination in ready-to-eat vegetable salads in five different locations within the Accra metropolis. Accra Metropolis is part of the national capital, covering an area of 3,245 km<sup>2</sup> with an estimated population of about 2.4 million as at 2019. The population density of the Metro is approximately 1,300 people per square kilometer. The Greater Accra Metropolitan Area (GAMA) has about 4 million inhabitants, which makes it the 11<sup>th</sup> largest metro Area in Africa. It has a latitude Position of 9389 km (5834 mi) north pole and 618 km (384 mi) on the equator with a longitude position of 22 km (14 mi). The latitude longitude coordinates are 5°33'21.67"N, 0°11'48.84"E.

### 2.2 Sampling Procedures

The vegetable salads were bought from randomly selected street food vendors from five selected localities in some sub-Metros between 6.00 and 11.00 GMT, February to April 2018. The salads comprised a combination of two or more of the following vegetables: Cucumber (*Cucumis sativus*), Carrot (*Daucus carota*), Lettuce (*Lactuca sativa*), Onions (*Alium cepa*), Tomatoes (*Lycopersicum esculentum*) and Cabbage (*Brassica oleracea*). We estimated a sample size of 310 based on a prevalence of

3.6%, with 5% margin of error at 95% confidence level. Overall, 313 ready-to-eat vegetable salad samples were collected and examined for parasites. Each sample was put into a labeled sterile plastic bag to prevent cross contamination [14] and stored at 4°C until examined. The samples were then transported to the College of Agriculture Education Microbiology laboratory for examinations.

### 2.3 Sample Processing

Each sample weighing about 100 g was thoroughly washed in 250 ml of sterile normal saline solution (0.85% NaCl) for 5min in 500ml conical flask. The samples were vigorously agitated (manually) in the normal saline for about three minutes. The vegetables were removed and drained from 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 50ml of the remaining solution transferred into sterile conical tubes and centrifuged at 3000xg for 15 min. The supernatant was then discarded and the remaining pellet re-suspended with 8 ml of the saline and re-centrifuged [15]. Three smears were then prepared from each sample, stained with Lugol solution and examined for protozoan cyst and helminth eggs using light microscope [16].

### 2.4 Microscopy and Identification of Parasites

Each stained smear slide was examined repeatedly (at least 200 fields) first with 10X objective lens and for identification of parasites with 40X using microscope objective lens. Identification of parasites was further supported using bench aids [17]. Quality in microscopic examination was achieved through the use of three independent microscopists who examined smears each from the same samples.

## 3. RESULTS

Out of a total of 313 ready-to-eat vegetable salad samples examined, 99 (32%) were infested with at least one parasite. We identified twelve [12] parasite species, including protozoa (*Giardia lamblia*, *Entamoeba coli*, *Entamoeba histolytica*, *Cystoisospora belli* and *Toxoplasma gondii*), nematodes (*Trichuris trichiura*, *Ascaris lumbricoides*, *Enterobius vermicularis*, *Ancylostoma duodenale*) and cestodes (*Taenia* spp., *Moniezia* spp. and *Fasciola* spp.). Fig. 1

shows images of some of the recovered parasites.

Results from Table 1 show the distribution of parasites in the vegetable salads collected by study locations. The data revealed that vegetable salads collected in Nima(A) had the highest load of parasites; 24 out of 60 (40%) followed by Accra central (B), 48 out of 147 (32.7%), Kaneshie(C), 10 out of 36(27.8%), Mallam (D), 10 out of 38 (26.3%), while Lapaz(E) had the least contamination, 7 out 32(21.9%). The spatial distribution of the parasites is also presented in Fig. 2.

*Giardia lamblia* was the most prevalent parasite species with a prevalence of 21(6.7%), followed by *Entamoeba histolytica* 20 (6.4%), *Moniezia* spp 13 (4.2%), *Trichuris trichiura* 12 (3.8%) and *Entamoeba coli* 11 (3.5%) (Fig. 3). The percentage occurrence of *Ascaris lumbricoides*, *Enterobius vermicularis*, *Fasciola* spp., *Cystoisospora belli*, *Taenia* spp., *Ancylostoma duodenale* and *Toxoplasma gondii*, were generally low (<2%).

## 4. DISCUSSION

In this study, we reported 32% of at least one intestinal parasite contaminating the ready-to-eat salads sampled in the Accra Metropolis of Ghana. This finding compares similarly with previous studies that assessed the prevalence of parasites in vegetable salads in developing countries ranging between 16% and 58% [7,12, 18-20]. A survey conducted by World Health Organization on vended food along streets has established that fresh fruits and vegetables form about 86% of the total food market [21]. This is as a consequence of the numerous nutrients provided by fresh fruits and vegetables in our dishes or daily diets. However, these must absolutely be free from contamination [22] and safe for consumption.

Among the parasites identified in the present study, *Giardia lamblia* was the most predominant which is an indication of faecal contamination [23,24]. Antwi-Agyei et al., [11] reported 99% faecal contamination of street foods and Nkrumah et al., [25] reported *Giardia lamblia* prevalence of 89.0% among school children. *Giardia lamblia* is associated with diarrhea, typical of communities without proper sanitation and potable water [24,26,27]. Some suburbs in Accra Metropolis have very poor sanitary conditions and transmission occurs from person

to person and animal to person via the faecal-oral route from faecal contaminated surfaces [28,29].

This study also found *Entamoeba histolytica* as the second most prevalent parasite, a well-recognized pathogen associated with intestinal and extra-intestinal infections that may arise from vegetables processing into salads [30,31]. *Moniezia* spp. recovered in this study is a common parasite found in ruminants, therefore suggestive of the use of animal droppings as a source of manure for vegetables cultivation. Other parasites which have been found in ruminants include *Fasciola hepatica* [32-34] which was found in the present study. These findings raise concern of public health at high risk of zoonotic transmission.

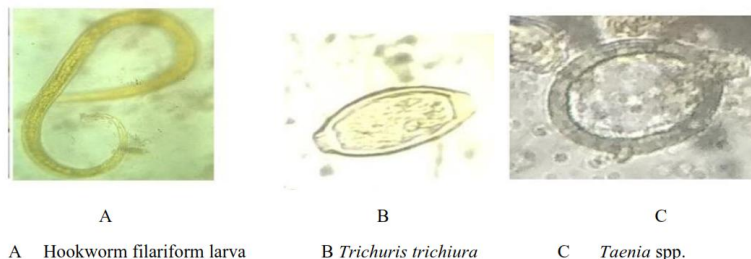
Globally, fresh fruits and vegetables importantly contribute to the food market and hence their safety is also a global issue [21]. The salads are sold as accompaniments to a variety of dishes at different retail points in the Accra Metropolis. Fresh vegetables are produced in urban and rural communities within and outside the Accra Metropolis. Access to clean water for irrigating vegetables is a major challenge in developing countries like Ghana. As an alternative, urban and peri-urban vegetable farmers without clean water for their crops, resort to the use of water from highly polluted sources [35,36]. Farmers also use wastewater and untreated animal and/or human excreta for these vegetables [6,37]. Moreover, unhygienic sewage disposal and the absence of its treatment facilities pose potential health hazards through contaminating irrigated fruit and vegetable crops with parasites in urban and sub urban of African countries [27,38].

Poor hygienic practices during production, transport, processing and preparation by handlers, including consumers, also contribute to vegetable contamination [39,40]. This raises public health concerns due to possible

contamination with pathogens as vegetables are often eaten uncooked or partially cooked [41]. Farm Contamination is most likely to be transferred to the consumer during processing and preparation of salads by food handlers. In Ghana and other developing countries, uneducated street food vendors use less or no disinfection of fresh vegetables before making them into salads [42].

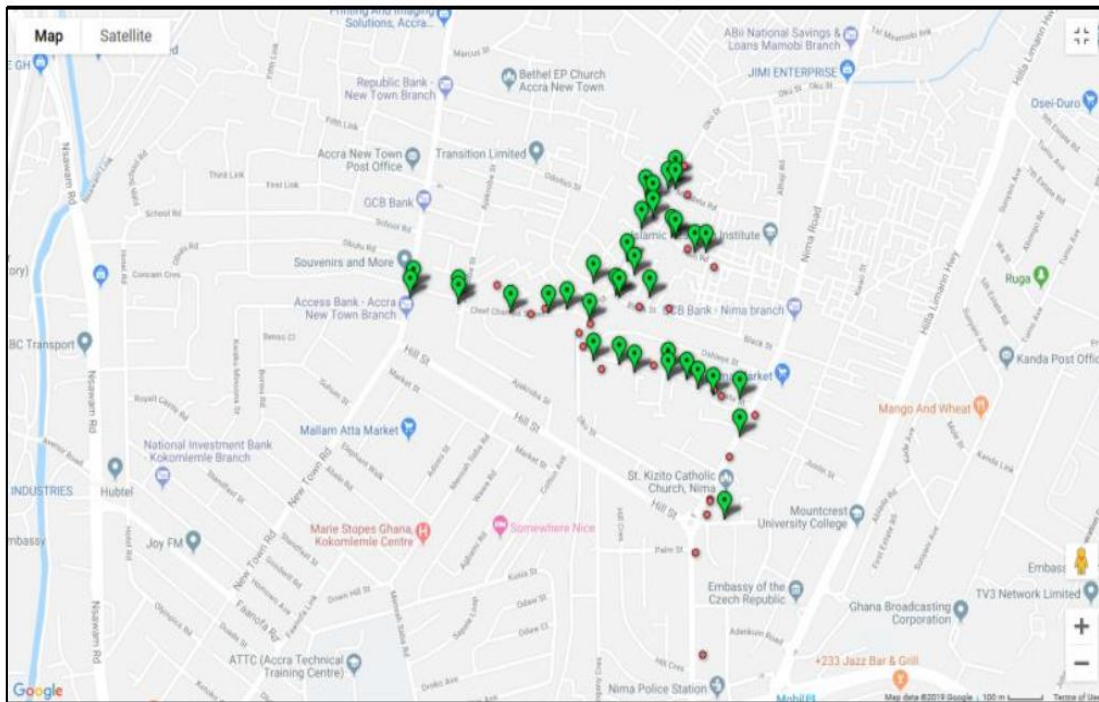
In the study conducted by Duedu et al. [12], the prevalence of protozoan and helminthes parasites in fresh vegetable chain in Accra was higher in open-aired markets than supermarkets. Fruits and vegetables for salad preparation are often prone to parasite contamination due to exposure to dusts and flies [43]. Most street food vendors purchase vegetables for salads from the open-aired markets and subject them to poor handling due preferential differences of buyers with the consequential cross contamination and improper washing methods adopted leaving some parasites for consumer [44].

The highest prevalence of parasites was recorded in Nima, an urban slum in Accra Metropolis. This location is characterized by inadequate access to safe water, poor sanitation and overcrowding [45,46]. The central business district (B), the area with the highest number of street vendors, recorded the next highest prevalence, with a third of the samples contaminated with at least one parasite. Generally, the washing process of vegetables was inadequate. Nearly all the vendors were faced with inadequate water supply. They mostly use one washing container with water to wash several vegetables, leading to gross and cross contamination, instead of disinfection [47]. The higher the number of cycles of washing, the more vulnerable the latter cycles are prone to contamination. Furthermore, improper storage of leftover salad may have contributed to the high prevalence recorded in the present study.

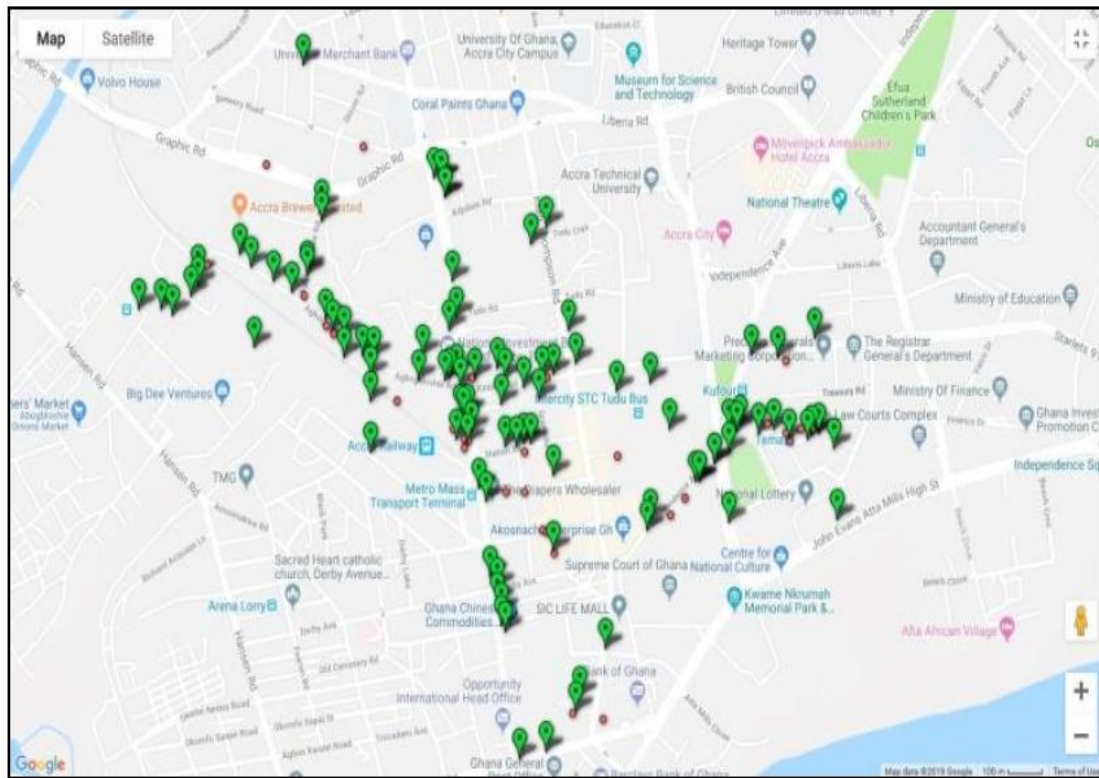


**Fig. 1. Images of some recovered parasites**  
[Source: Field data, 2018]

### LOCATION A

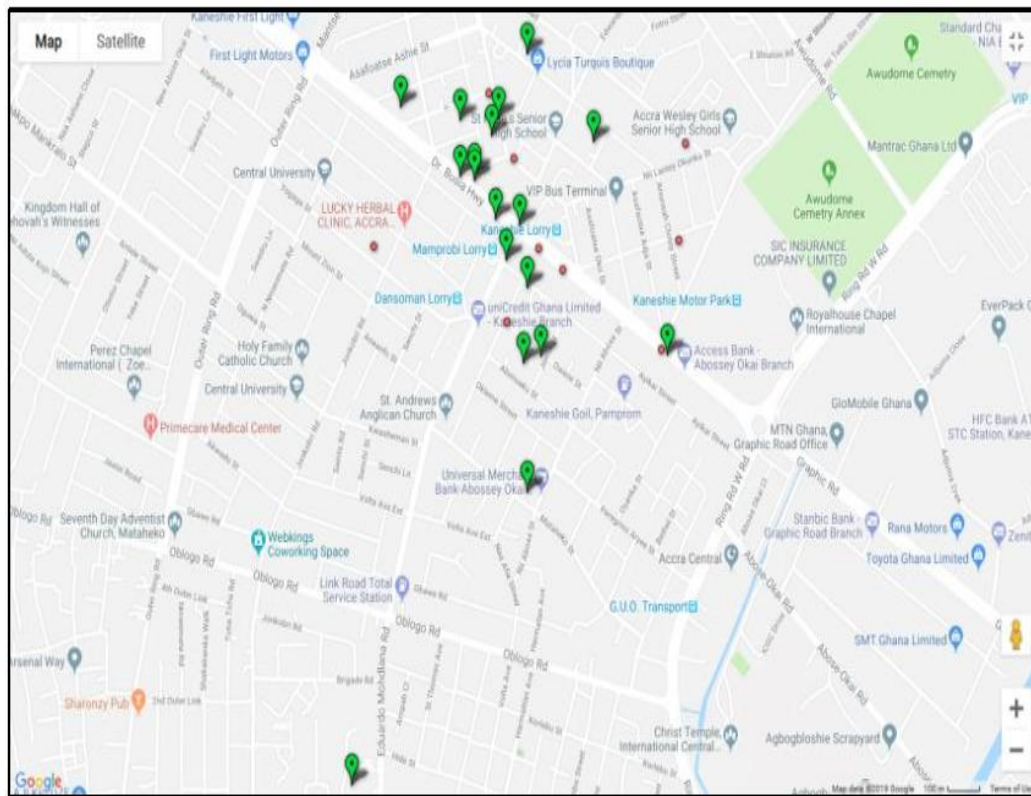


### Location B

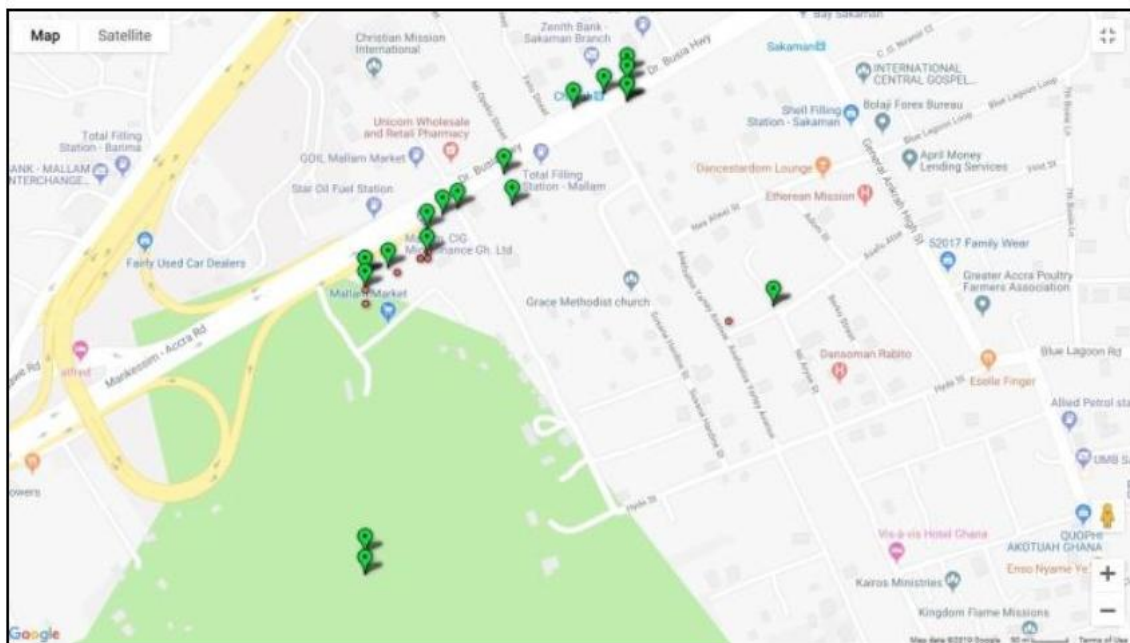




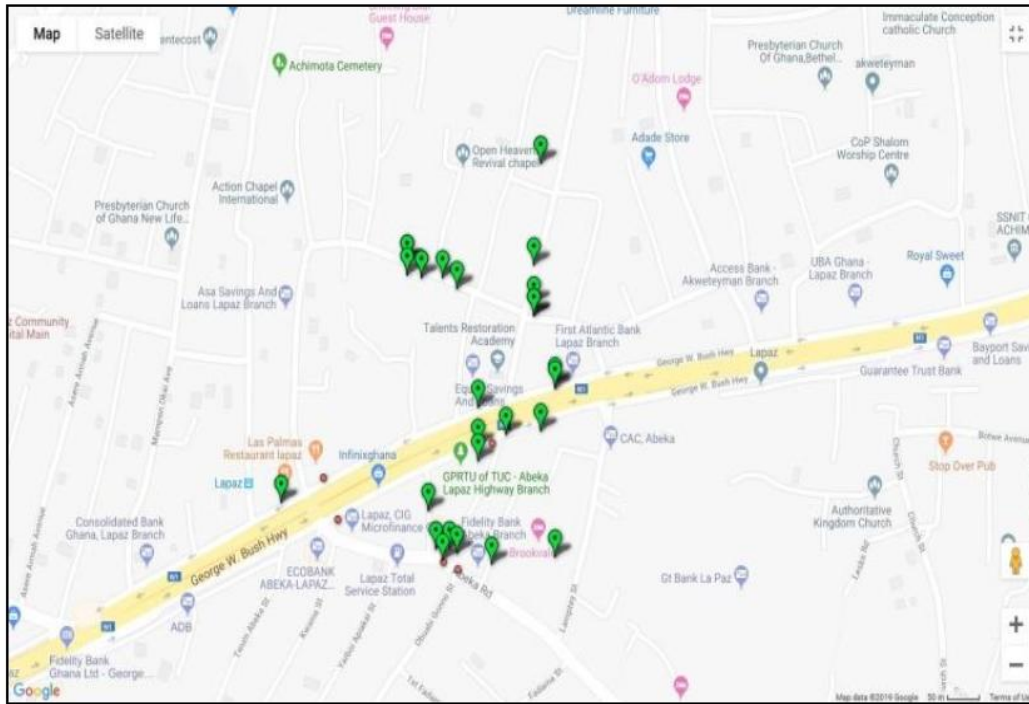
### Location C



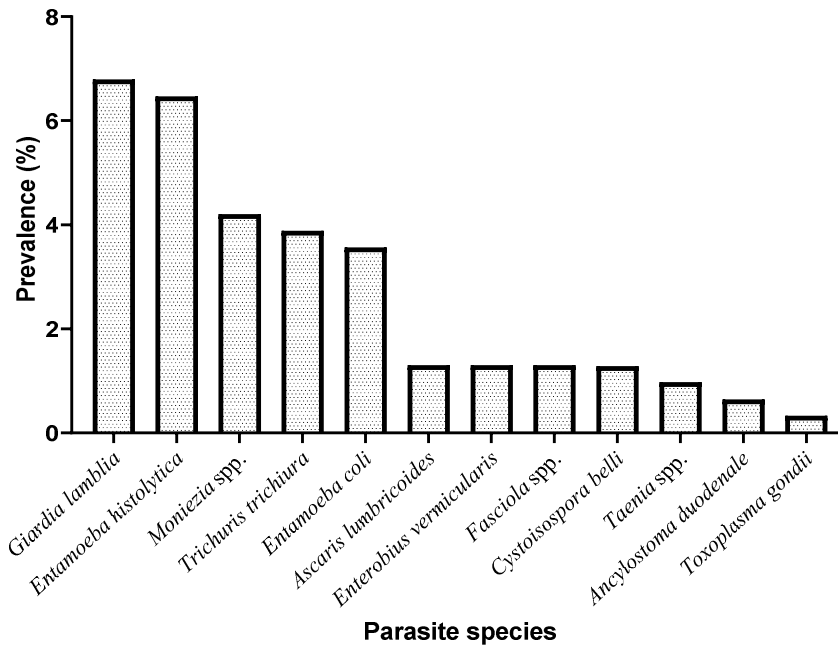
### Location D



Location E



**Fig. 2. Spatial distribution of sample locations and their contamination status (Green-no parasite detected Red – Parasites detected)**  
 [Source: Field data, 2018] – Location A (Nima), Location B (Central Business District), Location C (Kaneshie), Location D (Mallam), Location E (Lapaz)



**Fig. 3. Prevalence of parasite species from ready-to-eat vegetable salads**  
 [Source: Field data, 2018]



**Table 1. Parasitic prevalence by study location**

Sample location	Number of positive samples	Number of samples examined	Prevalence (%)
A	24	60	40 <sup>a</sup>
B	48	147	32.7 <sup>b</sup>
C	10	36	27.8
D	10	38	26.3
E	7	32	21.9
Total	99	313	32

Prevalence without symbols indicates no difference; alpha ( $\alpha$ ) and Beta ( $\beta$ ) indicate significant difference [P <0.05; Pearson Chi square 4.166] [Source: Field data, 2018]

Overall, sanitary conditions in and around the retail outlets were poor and the fact that the open-aired markets served as the sole source of vegetables for street-vended salads further heightened the risk of parasitic contamination especially in the absence of proper wash methods. These results suggest a serious threat to public health given the high patronage of these retail outlets and the seemingly lack of supervision of these street vendors.

## 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 sample size was relatively small. The processes of recovering parasites from salads samples could destroy some cysts, larvae or ova. Meanwhile we relied solely on the experience of the microscopists examination smeared staining. There is need for further studies using a larger sample size from more diverse sampling sites and also sampling vegetables sources.

## 6. CONCLUSION

The recovery of intestinal parasite species points to a risk of food-borne illnesses from consumption of street-vended vegetable salads which is an indication of failure of street food vendors to adhere to safe and hygienic food standard practices in their trade. *Giardia lamblia* was the most prevalent parasite and the recovery of *Moniezia* spp. is suggestive of the use of animal droppings as sources of manure for vegetable cultivation. Vegetable salads were found to be a salient source of contamination and therefore requiring urgent attention by policy makers.

### What is known about this topic?

- Safety of vegetables in Ghana in relation to bacterial and chemical poisoning.
- Human parasites largely recovered from fresh vegetables in Accra, Ghana.
- Microbial contamination of street vended foods in Accra.

### What is new about this topic?

- Large range of different species of intestinal parasites recovered from the samples.
- The recovery of ruminant parasites, including *Moniezia* spp. and *Fasciola* spp. indicates possible zoonotic transmission to humans from the consumption of ready-to-eat vegetable salads.
- The marked disparity of the prevalence of intestinal parasites at different locations in the Accra metropolis.

## ETHICAL APPROVAL

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

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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