



Contamination of food-borne parasites from green-leafy vegetables sold in public markets of San Jose City, Nueva Ecija, Philippines

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Abstract Vegetables contribute a significant portion of the typical Filipino diet. Hence, it is a concern that based on previous studies, vegetables could potentially act as vectors for parasitic transmission. This study aimed to further investigate these occurrences by assessing the food-borne parasitic contamination of vegetables sold in the public markets of San Jose City, Nueva Ecija. A total of 288 vegetable samples (cabbage, lettuce and Chinese cabbage) were collected from March to August 2018 in the wet market and supermarkets of San Jose City, Nueva Ecija. Results showed that 116 out of 288 (40.28%) vegetable samples were positive of food-borne parasites such as *Ascaris* sp., *Hymenolepis diminuta*, *Isospora* sp., Hookworm/*Strongylid* sp./free-living nematode larvae, *Strongylid* sp. egg, *Taenia* sp., and *Trichuris* sp. *Isospora* sp. has the highest contamination rate (16.67%) and mean density (13 eggs/g) from the sampled vegetables. Possible sources of these identified parasites are contaminated soil and water harbored by the sampled vegetables during cultivation, transport and handling/preparation by vendors. Further studies are needed to establish a more reliable information that could lead to public health improvement and food safety awareness for the community.

Keywords Parasitic contamination · Vegetables · Unwashed versus treated · Food-borne parasites

Introduction

Vegetables contribute a significant portion of the Filipino diet. They come in various forms such as leafy green, cruciferous, marrow, root, edible plant stem and allium. Green-leafy vegetables, in particular, contain nutrients, vitamins, minerals, protein and fiber that are vital to human body to support good health and to protect against diseases. They usually have crisp green leaves and stalks which are usually eaten raw or lightly cooked when served in salad to retain most of their nutrients. These, however, could cause serious health concerns as these fresh vegetables could potentially act as vectors for parasitic transmission which may lead to food-borne illnesses (Su et al. 2012).

In recent years, there have been increasing number of cases of food-borne illnesses linked to consumption of fresh vegetables. These were also observed to be a major medium of transmission of intestinal parasites (Ogbolu et al. 2009). These could be brought by their contamination in the production, collection, transportation, preparation or processing in farms or markets (Ebrahimzadeh et al. 2013). As reported by Su et al. (2012), contamination of green-leafy vegetables was due to poor sanitation processes in farming and improper handling that leads to transmission by humans through oral route.

Philippines, as one of the agricultural countries in Asia, could grow and cultivate various kinds of vegetables. However, as a developing country associated with poor and low awareness on food-borne diseases, studies like this may sometimes be neglected. Hence, it is necessary to conduct such research on parasitic contamination of vegetables, especially in provincial and municipal areas. Nueva Ecija, considered as the “Rice Granary of the Philippines,” is a province in Region III. Since the main concern of this study is public health, this study aimed to

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assess the food-borne parasites from green-leafy vegetables sold in the markets of San Jose City, Nueva Ecija, Philippines. Information obtained may help in the development of prevention strategies and could raise food safety awareness in the community, especially the local government units of the city as well for the general public's health and welfare.

Materials and methods

Ethical consideration

No animal was used or harmed during the entire conduct of this study. The survey was conducted with informed consent from the selected vendors as respondents, as well as proper consent from the Local Municipal Office. All information obtained during the survey were provided by the respondents voluntarily. Only the information relevant to the study were presented. All personal and business information pertaining to the respondents shall remain anonymous in compliance to research ethics and confidentiality.

Study area

The specified green-leafy vegetables were collected at San Jose City Public Market and supermarkets within the city situated at 15°48'13.326"N and 120°59'56.772"E. The samples were processed and analyzed at the Interactive Laboratory of the Department of Biological Sciences in Central Luzon State University, Science City of Muñoz, Nueva Ecija between the months of March and August 2018.

Collection of vegetable samples

A total of 288 vegetable samples were collected including 96 samples with three replicates for each, namely; cabbage (*Brassica oleracea* var. *capitata*), lettuce (*Lactuca sativa* L.), and Chinese cabbage (*Brassica rapa pekinensis*). These were bought from three selected wet market vendors, and three different supermarkets in San Jose City, Nueva Ecija. The collection was carried out twice a month with one (1) week interval between the months of March and August 2018. Three replicates of each vegetables were collected from top-shelf (displayed), middle shelf, and vegetables kept at storage.

Recovery and identification of food-borne parasites in vegetables

This study used modified sedimentation technique adapted from Ordoñez et al. (2018) to harvest and identify the food-

borne parasites. Vegetable samples were cut into smaller pieces and stirred in a beaker containing one (1) l of 0.95% saline solution for 10 min. The solution was then strained to remove large debris of vegetable samples then left to stand for 12 h. After this time, the supernatant was decanted and the sediment was transferred into micro-centrifuge tubes and centrifuged for 5 min at 13,000 rpm. After centrifugation, the supernatant was decanted again, and the remaining sediment was added with few drops of 10% formalin to preserve the food-borne parasites collected.

For the identification, wet-mount of the sediment were prepared on glass slides. It was then examined for presence of parasites under light microscope (10x – 40x).

Survey on knowledge, attitude and practices (KAP) of vendors on vegetable handling

An initial surveillance of the local wet market and supermarkets was performed prior the conduct of the study; only three vendors from the wet market and three supermarkets were selected as sources of vegetables since they offered all the three leafy vegetables (cabbage, lettuce and Chinese cabbage) on a regular basis. Other vendors were not surveyed due to irregularity in the availability of vegetables. Collection of vegetables was performed from March to August 2018. A total of nine (9) respondents from the three selected wet market vendors were interviewed and answered the structured questionnaire; these included the owner of the store and the hired personnel that assisted in handling/selling vegetables. However, supermarkets were not surveyed/interviewed to maintain confidentiality of information.

The structured questionnaire includes questions about the respondent's age, sex, and educational attainment, as well as practices in handling, preparation, storage and displaying of vegetables, personal hygiene and awareness on diseases related to food safety and sanitation. These questions may provide insight as to whether the respondent's awareness and practices in vegetable handling influence the distribution/contamination of parasites.

Data analysis

The contamination rate and mean density of the food-borne parasites collected were analyzed using Quantitative Parasitology version 3.0 (QP3). Comparison among the three vegetables, between the supermarkets and wet market, and among the different washing agents and control were tested using Chi square test for homogeneity at 95% level of significance.

Results

A total of 288 vegetable samples were obtained from wet market (n = 72) and supermarkets (n = 216) in San Jose City, Nueva Ecija. 116 out of the total 288 samples (40.28%) were found contaminated with food-borne parasites namely *Isospora* sp., *Ascaris* sp., *Hymenolepis diminuta*, *Trichuris* sp., *Strongylid* sp., Hookworm/*Strongylid* sp./free-living nematode larvae and *Taenia* sp.

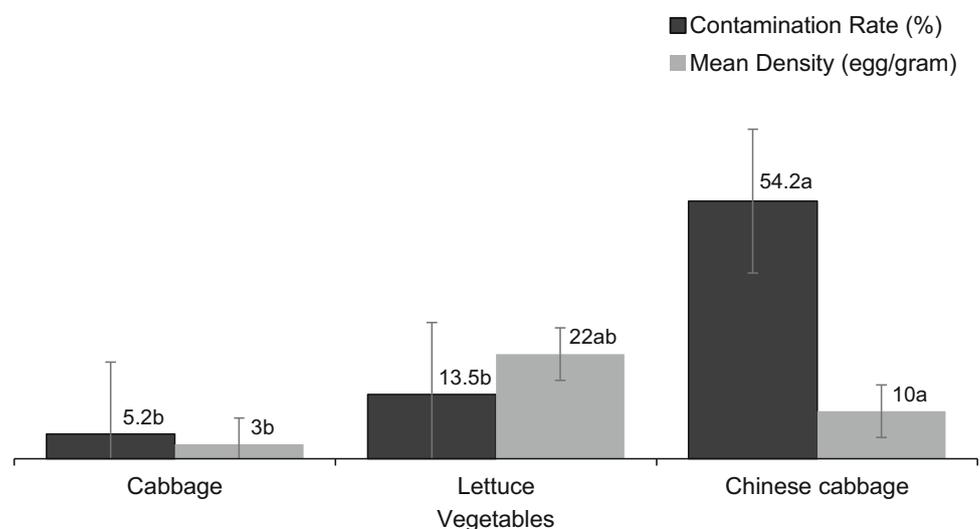
Contamination rate and mean density of parasites collected in green-leafy vegetables

Among the leafy vegetables, Chinese cabbage (*B. rapa pekinensis*) had the highest contamination rate with 54.2% and found to be significantly different ($p > 0.05$), followed by lettuce (*L. sativa*) with 13.5%, and the least with 5.2% was cabbage (*B. oleracea capitata*) with no significant difference ($p > 0.05$). However in terms of mean density, lettuce had the highest with 22 eggs/g followed by Chinese cabbage with 10 and cabbage with mean density of 3 eggs/g (Fig. 1).

Contamination rate and mean density between markets

Wet market samples reported an average contamination rate of 31.9% with mean density of 13 (eggs/g), while supermarket samples reported an average contamination rate of 21.75% and 11 mean density (eggs/g) with no significant difference ($p > 0.05$) (Fig. 2).

Fig. 1 Contamination rate (%) and mean density (egg/g) of parasites from three different green-leafy vegetables collected from the markets of San Jose City



Distribution and mean density of collected parasites from Green-leafy vegetables

Food-borne parasites collected were determined as helminthic and protozoan. Five (5) helminths identified were *Ascaris* sp., *Hymenolepis diminuta*, *Trichuris* sp., *Strongylid* sp. egg, Hookworm/*Strongylid* sp./free-living nematode larvae, *Taenia* sp. and one (1) protozoan, *Isospora* sp. Among these parasites, *Isospora* sp. had the highest contamination rate with 16.67% and mean density of 13 (eggs/g veg) followed by *Ascaris* sp. with 12.8% and 5 (eggs/g veg), *Strongylid* sp. egg (5.90% and 1 egg/g veg), *Trichuris* sp. (2.43% and 2 egg/g veg), *Hymenolepis diminuta* (1.04% and 1 egg/g veg), Hookworm/*Strongylid* sp./free-living nematode larvae (0.69% and 1 egg/g), and *Taenia* sp. (0.35% and 1 egg/g veg). All food-borne parasites except *Taenia* sp. were found in Chinese cabbage. *Isospora* sp. and *Ascaris* sp. were present in the three vegetables sampled; while *Trichuris* sp. was only present in Chinese cabbage and *Taenia* sp. in lettuce (Table 1) (Fig. 3).

Knowledge, attitude and practices (KAP) of vendors to food safety

A total of nine (9) respondents from the wet market vegetable stores were surveyed. Three (3) of them were the owners of each store, whereas the six (6) were hired personnel who assist in handling/selling vegetables. Based on the obtained information regarding the preparation and handling practices, it showed that all the surveyed vendors use table/tables for display of vegetables for sale. Green-leafy vegetables such as cabbage and Chinese cabbage were washed with tap water or ground water prior to display. According to the vendors the water that they usually

Fig. 2 Comparison between supermarkets and wet market of San Jose City in terms of contamination rate (%) and mean density (egg/g) of food-borne parasites. Note: Values are not significantly different from each other

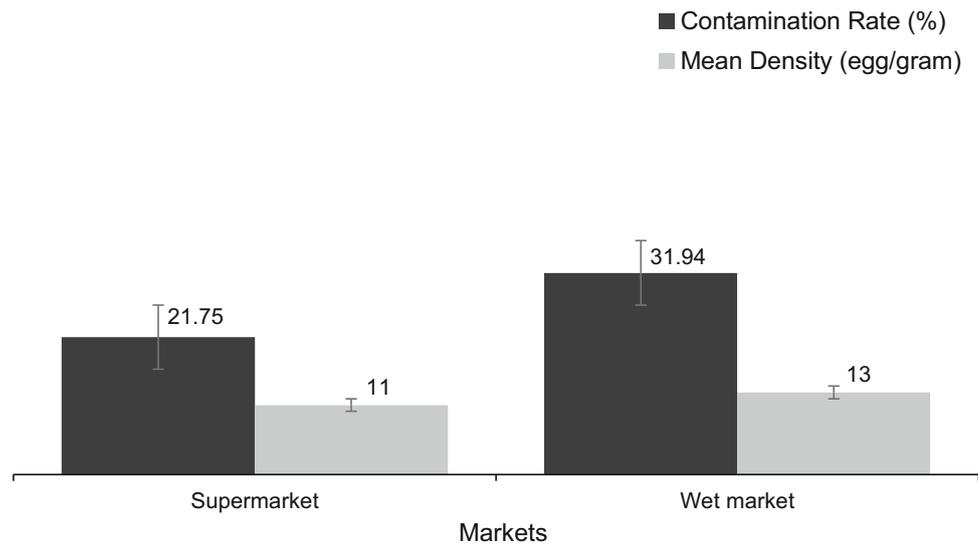


Table 1 Distribution of different species of parasites and its mean density on all vegetables sampled collected from wet market and supermarkets in San Jose City

Parasites	Contaminated vegetable			Total no. of parasites (egg/larvae)	Contamination rate (%)	Mean density (egg/g)
	Cabbage	Chinese cabbage	Lettuce			
Protozoa						
<i>Isospora</i> sp.	4	33	11	601	16.67	13
Helminths						
<i>Ascaris</i> sp.	1	29	7	178	12.85	5
<i>Hymenolepis diminuta</i>	0	1	2	4	1.04	1
<i>Trichuris</i> sp.	0	7	0	12	2.43	2
<i>Strongylid</i> sp. egg	0	10	7	23	5.90	1
Hookworm/ <i>Strongylid</i> /free-living larvae	0	3	0	3	0.69	1
<i>Taenia</i> sp.	0	0	1	1	0.35	1
Total	5	83	28	822	40.28	3

use in washing the vegetables comes from the public water supply of the wet market. However, green-leafy vegetables such as lettuce are not washed to minimize deterioration of outer leaves. Beforehand, tables were also cleaned by brushing off dirt and removing rotten parts of the previous sold produce. Left over produce are left on display tables overnight under cloth cover, disposed or brought home for consumption. The respondents were also asked on their practices when leafy vegetables fell off the ground. Two (2) respondents answered that they just pick it up immediately and return to the shelves; four (4) respondents answered that they just removed the dirt by hands; and three (3) respondents washed again the vegetables except the lettuce before putting the vegetables in the shelves again.

In terms of personal hygiene, six (6) vendors wash their hands when selling vegetables using tap water or ground water. Other than this, only one vendor reported of

refraining from selling when suffering from sickness while others still continue selling if they only have mild cough, cold or fever. Respondents' answers suggest that most of them had little to no knowledge regarding illnesses caused by consuming unhygienic vegetables.

Discussions

Vegetables are part of the Filipino diet. It is commonly known to be good for the health. However, it can act as a vector for transmission of parasites when contaminated vegetables were consumed. Mostly, young and immune-compromised members of the community are being affected. This study may help the public by raising awareness on food safety and for the improvement of the community's public health.

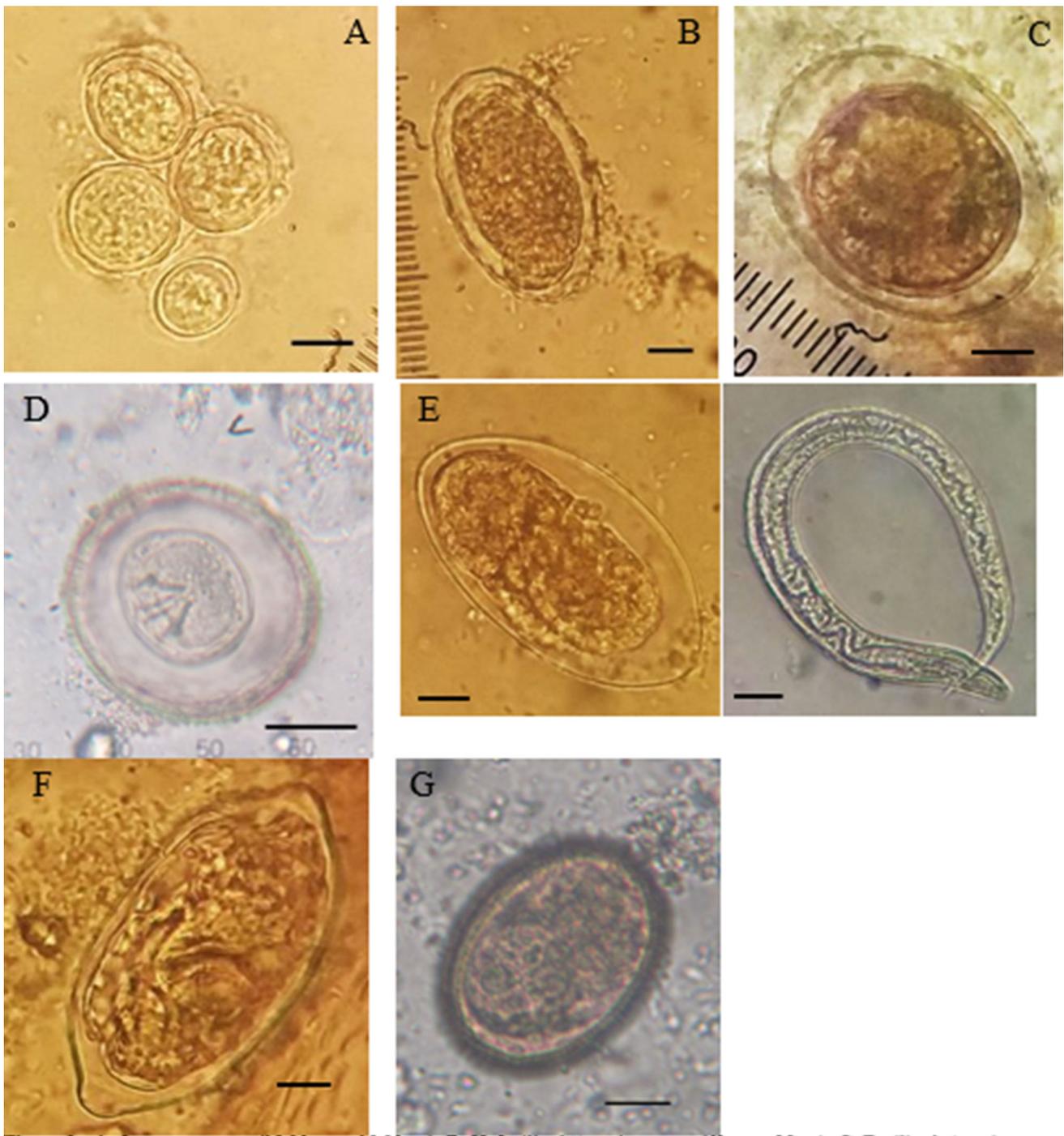


Fig. 3 **a** *Isospora* sp. egg (15–20 μm × 15–20 μm); **b** Unfertilized *Ascaris* sp. egg (60 μm × 35 μm); **c** Fertilized *Ascaris* sp. egg (52.5 μm × 40 μm); **d** *Hymenolepis diminuta* egg (35 μm × 30 μm); **e** *Trichuris* sp. egg (67.5 μm × 35 μm); **f** *Strongylid* sp. egg

(75 μm × 12.5 μm) and Hookworm/*Strongylid* sp./free-living nematode larvae (300 μm × 12.5 μm); **g** *Taenia* sp. egg (47 μm diameter). Note: Scale bar is equal to 10 μm

Findings in this study shows 40.28% contamination rate of food-borne parasites from green-leafy vegetables which are comparable to previous studies. The highest contamination rate was found in Chinese cabbage (*B. rapa pekinensis*) having 54.2% and mean density of 10 (eggs/g)

followed by lettuce (*L. sativa*) with 13.5% and 22 (eggs/g), and cabbage (*B. oleracea capitata*) as the least contaminated with 5.2% and 3 (eggs/sample). High contamination rate in Chinese cabbage is associated to high occurrence of parasite eggs/larvae in sampled vegetables during sampling

periods. Meanwhile, density of food-borne parasites was higher in lettuce.

Based on observation during sampling, low contamination rate in cabbage and lettuce may be associated to the removal of large amount of its outer layers, particularly the unwanted and rotten parts, before being displayed. For Chinese cabbage, on the other hand, it was observed to take longer time to rot compared to the other two vegetables. This could give sufficient time for parasites to reproduce on the leaves during display.

Chinese cabbage also has the highest number of different species of parasites present. All the parasites specified above were present in the sampled Chinese cabbage except for *Taenia* sp., which is only present in lettuce. Most surveys had studied lettuce and cabbage, particularly in Nigeria (Abe et al. 2016), Poland (Kłapeć and Borecka 2012), Philippines (Ordoñez et al. 2018), and Morocco (Hajjami et al. 2013). Meanwhile, Chinese cabbage was studied by only few in Laos (Maipanich et al. 2011). Another study by Choi and Lee (1972) conducted in Korea, wherein they found out that Chinese cabbage also showed highest contamination of *Ascarid* egg with (91.9%) from the vegetable gardens. In the case of filariform larva, Chinese cabbage was found to be twice as high as that of lettuce and young radish that were sampled from the study.

Further, contamination rate of food-borne parasites vegetables from wet market and supermarkets were compared. Based from the observation in the wet market, vendors were not using gloves or face mask when selling which could lead to cross contamination in handling vegetables. Meanwhile in the supermarkets, vegetables sold were packaged individually with cling wrap but the preparation before being displayed was not observed. The exposure of vegetables to open-air may also contributed to increase in contamination. According to previous study, vegetables from open-aired markets have higher contamination than in supermarkets but it can also be at risk of contamination during post-harvest activities including packaging and display of fresh produce, transport of produce from farm to supermarkets (Duedu et al. 2014). However, despite these observations, results showed that there was no significant difference in contamination rate was between the two stores ($p > 0.05$). Hence, further research on this needs to be done.

Among the food-borne parasites recovered in this study, *Isospora* sp. has the highest contamination rate and mean density. This type of protozoan parasite is very resistant to environmental conditions and disinfectants (Hall 2013). Its primary sources are contaminated food and water (Suh et al. 2015). Meanwhile, *Ascaris* sp., along with *Trichuris* sp. and Hookworm/*Strongylid* sp. belong to Soil-Transmitted Helminths (STH). These are intestinal worms infecting humans that are transmitted through

contaminated soil (CDC 2018). *Ascaris* sp. are also considered most resistant enteric pathogens (Fallah et al. 2015) due to its thick shell that allows it to withstand environmental conditions (Ordoñez et al. 2018). Hookworms/*Strongylid* sp. commonly infects animals like livestock, birds, reptiles, amphibians (CDC 2018). These livestock are found in farms, thus, there is possibility of these animals to defecate near crops that leads to soil contamination that results to contamination of produce during cultivation. Further, possible source of *H. diminuta* can be the transmission of fecally contaminated food, water and soil. Same features goes with *Taenia* sp. which usually infects cattle and pigs leading to soil contamination as well.

Soil and water were the possible sources of green-leafy vegetables sampled in this study. The use of wastewater for cultivating irrigating vegetables is one possible cause of contamination (Kozan et al. 2007). The use of sewage water plays an important role in the epidemiology of transmission of parasitic diseases to human through consuming such vegetables (Gupta et al. 2009). In addition to this, post-harvest fecal contamination of vegetable may also occur during handling and transport of vegetables. This occurs through splashing the vegetables with contaminated water to keep the vegetable fresh. Through this, some of the viable parasitic cysts or oocysts will be easily transmitted to humans (Al-Megrin 2010). Also, avoiding contact with fecal matter or sewage can help prevent parasitic contamination. Proper sewage disposal and fecal management are keys to prevention (CDC 2018).

Sources of contamination in vegetables can also be attributed to the texture and distance of the vegetable in soil. Vegetables with rough, highly textured surfaces with deep crevices would be more likely to harbor soil, hence leading to increase contamination. Moreover, vegetables are susceptible to contamination the more it is near to the ground, especially during heavy rains, floods and even during watering the crops. To minimize the causes of contamination of green-leafy vegetables and reduce the risk of infection to humans, certain remedies should be established.

Summary and conclusion

This study showed that the three different green-leafy vegetables sampled were positive of food-borne parasites. Chinese cabbage had the highest contamination rate while lettuce had the highest mean density. Parasites identified were *Isospora* sp., *Ascaris* sp., *Hymenolepis diminuta*, Hookworm/*Strongylid* sp., *Trichuris* sp., and *Taenia* sp. with *Isospora* sp. and *Ascaris* sp. being the predominant species. Possible factors that lead to contamination were the handling and practices of vegetables, exposure of

produce prior or during purchase, water used in irrigation and soil harbored before being harvested. Limited knowledge and awareness regarding diseases caused by contaminated vegetables were also observed from the surveyed vendors. Hence, increase in public information and monitoring on food safety by local government units is suggested for the benefit of the community to improve health and safety and to minimize the risk of infection. This will also serve as a baseline for future researchers particularly in areas of food safety and public health.

Recommendation

The following recommendations are given based from the findings: 1. Direct sampling of vegetables from farms to monitor the contamination rate of parasites prior to transportation and distribution to vendors and markets, 2. Before consumption, remove damaged or rotten parts of green-leafy vegetables then wash using running tap water or using the different treatments used in this study, 3. Conduct a proper coordination and monitoring with the local government units to increase public awareness and best practices on food safety.

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Authors contribution Conceived and designed the experiments: KCCV, ZGB, DSCC; Performed the experiment, Data analysis: KCCV; All authors participated in writing the final paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Abe E, Ajah L, Ayuba S, Mogaji H, Ekpo U (2016) Geohelminths contamination fruits and vegetables sold in Lafia Markets. *Annu Res Rev Biol* 11(2):1–8
- Al-Megrin WAI (2010) Prevalence of intestinal parasites in leafy vegetables in Riyadh, Saudi Arabia. *Int J Trop Med* 5(2):20–23
- Choi DW, Lee S (1972) Incidence of parasites found on vegetables collected from markets and vegetable gardens in Taegu Area. *Korean J Parasitol* 10(1):44–51
- Duedu KO, Yarnie EA, Tetteh-Quarco PB, Attah SK, Donkor ES, Ayeh-Kumi PF (2014) A comparative survey of the prevalence of human parasites found in fresh vegetables sold in supermarkets and open-air markets in Accra, Ghana. *BMC Res Notes* 7(1):1–6
- Ebrahimzadeh A, Jamshidi A, Mohammadi S (2013) The parasitic contamination of raw vegetables consumed in Zahedan. *Iran. Health Scope* 1(4):202–206
- Fallah AA, Makhtumi Y, Pirali-Kheirabadi K (2015) Seasonal study of parasitic contamination in fresh salad vegetables marketed in Shahrekord, Iran. *Food Control* 1:20. <https://doi.org/10.1016/j.foodcont.2015.08.042>
- Gupta N, Khan DK, Santra SC (2009) Prevalence of intestinal helminth eggs on vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. *Food Control* 20:942–945
- Hajjami K, Ennaji MM, Amdioui H, Fouad S, Cohen N (2013) Parasitic contamination fresh vegetable consumed in Casablanca City (Morocco) and risk from consumer. *Int J Sci Technol* 2(7):543–549
- Hall EJ (2013) Chapter 57: small intestine. In: Washabau RJ, Day MJ (eds) *Canine and feline gastroenterology*. Elsevier-Saunders, North York, pp 651–728
- Kłapeć T, Borecka A (2012) Contamination of vegetables, fruits and soil with geohelminths eggs on organic farms in Poland. *Ann Agric Environ Med* 19(3):421–425
- Kozan E, Sevimi FK, Kose M, Eserm M, Cicek H (2007) Examination of helminth contaminated wastewaters used for agricultural purposes in Afyonkarahisar. *Turkiye Parazitoloj Derg* 31:197–200
- Maipanich W, Chaisiri K, Yoonuan T, Sato M, Sato MO, Pongvongsa T et al (2011) Zoonotic helminth contamination of the environment in rural villages of Southern Lao PDR. *J Trop Med Parasitol* 34(2):54–61
- Ogbolu D, Alli O, Ogunleye V, Olusoga-Ogbolu F, Olaosun I (2009) The presence of intestinal parasites in selected vegetables from open markets in South Western Nigeria. *Afr J Med Med Sci* 38(4):319–324
- Ordoñez K, Paller VG, Goh XT, Lim Y (2018) Parasite contamination of vegetables from selected organic and conventional farms in Laguna and Benguet Province, Philippines. *Trop Agric Sci* 41(4):1741–1746
- Parasites (2018) <https://www.cdc.gov/parasites/az/index.html>. Accessed 1 May 2019
- Su GLS, Mariano CMR, Matti NSA, Ramos GB (2012) Assessing parasitic infestation of vegetables in selected markets in Metro Manila, Philippines. *Asian Pac J Trop Dis* 2(1):51–54. [https://doi.org/10.1016/S2222-1808\(12\)60012-7](https://doi.org/10.1016/S2222-1808(12)60012-7)
- Suh KN, Kozarsky P, Keystone JS (2015) *Cyclospora cayentanensis*, *Cytoisospora (Isospora) belli*, *Sarcocystis* species, *Balantidium coli*, and *Blastocystis* species. In: Bennett JE, Dolin R, Blaser MJ (eds) *Mandell Douglas, and Bennett's principles and practice of infectious diseases*, 8th edn. Elsevier-Saunders, North York, pp 3184–3191

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