Parasitic Contamination of Common Vegetables Sold in Lusaka, Zambia

S.S. Nyirenda [1]*✉ ⓘD, K.M. Bukowa [1], W.R. Banda [2], J. Mbewe [1], F. Hamankolo [1], F. Banda [1], G. Kwenda [3], M. Mumba [1], E. Zulu [3]

1. Central Veterinary Research Institute, Ministry of Fisheries and Livestock, Lusaka, Zambia
2. Occupational Health and Safety Institute, Kitwe, Zambia
3. Department of Biomedical Sciences, School of Health Sciences, The University of Zambia, Lusaka, Zambia

HIGHLIGHTS

- The overall parasitic contamination of fresh vegetables was 35.8% (34 out of 95).
- *Giardia lamblia* was the most prevalent parasite found in the vegetable samples.
- Some vegetables sold at the Lusaka City market (Zambia) were a potential source of parasitic infections for consumers.

ABSTRACT

Background: Consumption of raw or unhygienically prepared vegetables is a potential source of parasitic infection. This study aimed to establish the prevalence and types of intestinal parasites on the freshly sold vegetables at the market.

Methods: Totally, 95 vegetable samples were randomly procured from vendors at Lusaka City market (Zambia). The samples were examined for parasitic contamination using floatation and sedimentation methods. Data were analyzed using Epi Info version 7.2.4.0.

Results: The overall parasitic contamination of fresh vegetables was 35.8% (34 out of 95). The highest parasitic contamination rate was found in Chinese cabbage with 7.4% (7 out of 95), followed by rape with 6.3% (6 out of 95), chilli with 5.3% (5 out of 95), pigweed with 5.3% (5 out of 95), pumpkin leaves with 5.3% (5 out of 95), tomatoes with 4.2% (4 out of 95), and cabbage with 2.1% (2 out of 95). There was a significant (p=0.001) statistically difference between the type of vegetables and the presence of the parasites. The identified helminths had different prevalence rates, including *Taenia* spp. with 9.5%, *Trichuris trichuria* with 5.3%, *Ascaris lumbricoides* with 3.2%, *Clonorchis sinensis* with 2.1%, *Diphyllobothrium latum* with 2.1%, *Paragonimus westermani* with 2.1%, and *Strongyloides stercoralis* with 2.1%. Also, *Giardia lamblia* with 24.2% and *Balantidium coli* with 4.2% were the protozoan parasites identified.

Conclusion: This study showed that some vegetables sold at the Lusaka City market (Zambia) were a potential source of parasitic infections for local consumers. *G. lamblia* was the most prevalent parasite found in the vegetable samples.

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Introduction

Contaminated food harboring viable microorganisms is the most important source of infectious diseases. A variety of pathogenic microorganisms such as bacteria, parasites, and viruses can contaminate foods (Ahmadi

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* Corresponding author (S.S. Nyirenda)
✉ E-mail: stanleynyirenda@yahoo.co.uk
ORCID ID: https://orcid.org/0000-0002-0374-457X

et al., 2015; Bintsis, 2017; Chau et al., 2014; Hajimohammadi et al., 2014; Heredia and García, 2018). Despite vegetables and fruits being essential for good health in the daily diet (Abougrain et al., 2010), they are potential sources of several intestinal parasitic infections in humans and intensively managed small ruminants such as goats and sheep. Contamination of vegetables with intestinal parasites can arise during the processes of cultivation in gardens due to the utilization of organic fertilizer and contaminated irrigation water. Others may include during the handling, storage, transportation, selling, and buying (Al-Megrin, 2010; Faour-Klingbeil et al., 2016). The ingestion of raw and inadequately pre-pared vegetables plays an indispensible epidemiological role in the maintenance and transmission of intestinal parasites (Al-Megrin, 2010). Some common parasites identified as biohazards on fresh vegetables include *Ascaris lumbricoides*, *Giardia intestinalis*, *Enterobius vermicularis, Trichuris trichuria*, *Cryptosporidium* spp., *Toxocara* spp., *Fasciola* spp., and hookworms (Kozan et al., 2007). Studies conducted in different parts of the world indicated that more than 1.5 billion people of the world's population, infected with intestinal parasites, posed a significant health challenge (Duedu et al., 2014; Fallah et al., 2012).

Intestinal helminth contamination has been reported in sub-Saharan Africa (Abougrain et al., 2010; Adamu et al., 2012; Tefera et al., 2014), where the prevalence rates vary in regions with different types of parasites as a result of unhygienic practices. The study conducted by Adamu et al. (2012) in Nigeria suggested that proper washing of vegetables before consumption could reduce the infections due to intestinal helminth contamination, particular those consumed raw such as cabbage and lettuce. On the other hand, a related study by Tefera et al. (2014) conducted in Ethiopia reported that a method of display for selling vegetables was a major factor assessed for association with parasitic contamination such as helminths and protozoa. Similar findings were also reported in Libya by Abougrain et al. (2010), where the highest intestinal helminth contamination was as a result of *Ascaris* spp. in cress (96%), lettuce (96%), cucumber (75%), and tomatoes (19%). Despite reported cases of food-borne infectivity linked to fresh vegetables, there is an increasing demand for the commodity globally, particularly in tropical and subtropical countries (Duedu et al., 2014).

In Zambia, however, based on our knowledge, there is no data available on the parasitic intestinal contamination of fresh vegetables sold on local markets. The absence of data may be due to inadequate or unavailable conventional diagnostic, surveillance, and reporting systems for many food-borne pathogens, where outbreaks caused through contaminated vegetables go undetected and this lead to a low incidence of the disease in the country.

The Lusaka City market, popularly known as Soweto, is the largest in Lusaka (Zambia) and source of different types of vegetables from different farmers, sold to consumers in the city. These vegetables are not only consumed by humans but also by small ruminants and other intensively managed animals. On the other hand, the contamination rates and types of parasites, which may contaminate the vegetables at the market, are unknown. Hence, this study aimed to establish the prevalence and types of intestinal parasites on the freshly sold vegetables at the market.

Materials and methods

Study site

The study was conducted at the Lusaka City market (Zambia) located in the Lusaka Central Business District (CBD) positioned at the junction of Los Angeles and Lumumba Roads. It is a trading centre, where farmers take their farm produce for sale from Lusaka and adjacent towns. The samples were processed at the Central Veterinary Research Institute (CVRI) Parasitology laboratory located off the Kafue Road on Chanyanya Road, about 25 km Southwest of Lusaka.

Samples collection

The study frame was all the vegetables taken to Lusaka City market (Zambia) for sale. Marketers with freshly intact vegetables, which came from different farmers, were randomly selected and included in the study from market stands or displayed on the floor after verbal consent. The vegetables that were not fresh (yellow instead of green) or/and not cleaned or/and broken, were excluded from the study.

From March to September 2017, a total of 95 bunches of raw vegetable samples were randomly collected from the vegetable stand and those displayed on the floor. However, no single farm was sampled more than twice of the same vegetable. Among 95 vegetable samples, 46 and 49 vegetable types were displayed on the floor and the tables, respectively. The vegetables included tomatoes (*Solanum lycopersicum*; n=10), ladies finger or Okra (*Abelmoschus esculentus*; n=10), cucumber (*Cucumis sativus;* n=10), bondwe or pigweed (*Amaranthus hybridus*; n=10), Impwa or African eggplant (*Solanum macrocarpon*; n=9), rape (*Brassica napus*; n=10), cabbage (*Brassica capitate*; n=6), Impili-pili or chilli (*Capsicum annum*; n=10), pumpkin leaves (n=11), and Chinese cabbage (*Brassica rapa*; n=9).

After collection, samples were kept separately in sterile polythene bags and labeled with an identification number, type of vegetable, and collection date. The polythene bags containing the vegetable samples were sealed, put in the cool box and transported to CVRI Parasitology Laboratory for analysis. The collected samples were immediately worked on or stored at 4 °C overnight to detect ova, cysts, eggs, larvae, or trophozoites.

Preparation of samples

Sample preparation was done according to the method described by Eraky et al. (2014), with modifications. About 200 g of each vegetable was sliced and soaked in 200 ml of lukewarm physiological saline in a clean and sterile beaker for 15 min, followed by vigorous shaking with a mechanical shaker while covered for 15 min. Vegetables were removed, discarded, and the remaining wash solution was left to stand for 1 h, which was analyzed by both floatation and sedimentation techniques.

Floatation method

The resultant wash solution was mixed thoroughly, filtered through a sterile sieve (425 μm pore size) to remove large debris. Fifty ml was transferred into a clean beaker containing an equal volume of concentrated sodium chloride. After mixing the solution thoroughly, two test tubes were filled to the brim with each sample, where a 22×22 mm coverslip was applied. The test tubes were left to stand for 20 min to allow the eggs, oocysts, and cyst to float. The coverslip was transferred onto the microscopic glass slide. The wet smear was examined under the microscope using a 10× objective to determine the presence of various eggs, cysts, or oocysts and a 40× objective to scrutinize their morphological structure (Abougrain et al., 2010).

Formal-ether sedimentation technique

The remaining resultant wash solution was suspended in an equal amount of formalin and ether solution and then centrifuged at 2000 rpm (447×g) for 15 min. The supernatant was cautiously decanted and a few drops of the sediment were placed on microscopic glass slides with a dropper to which a drop of Lugol's iodine solution was added. A coverslip was applied gently to avoid air bubbles and flooding for examination under a light microscope. The preparation was examined under a light microscope as described for floatation method (Al-Megrin, 2010).

Statistical analysis

Data were entered in Microsoft Excel datasheets and analyzed using Epi Info version 7.2.4.0. The chi-square test (x^2) and Risk Ratio (RR) was used to investigate the association between types of vegetable, mode of display, and the infectivity on the vegetables. The *p*-value of <0.05 was considered as statistically significant at 95% Confidence Interval (CI).

Results

The overall parasitic contamination of fresh vegetables was 35.8% (34 out of 95) which is shown in Table 1. Parasitic contamination was found in most vegetable species sampled except in African eggplants, okra, and cucumber. The highest parasitic contamination rate was found in Chinese cabbage with 7.4% (7 out of 95), followed by rape with 6.3% (6 out of 95), chilli with 5.3% (5 out of 95), pigweed with 5.3% (5 out of 95), pumpkin leaves with 5.3% (5 out of 95), tomatoes with 4.2% (4 out of 95), and cabbage with 2.1% (2 out of 95). There was a significant (*p*=0.001) statistically difference between the type of vegetables and the presence of the parasites.

The identified helminths had different prevalence rates that were included *Taenia* spp. with 9.5% (9 out of 95; *p*=0.006), *T. trichuria* with 5.3% (5 out of 95; *p*=0.002), *A. lumbricoides* with 3.2% (3 out of 95; *p*=0.024), *Clonorchis sinensis* with 2.1% (2 out of 95; *p*=0.067), *Diphyllobothrium latum* with 2.1% (2 out of 95; *p*=0.067), *Paragonimus westermani* with 2.1% (2 out of 95; *p*=0.067), and *Strongyloides stercoralis* with 2.1% (2 out of 95; *p*=0.067). Also, *Giardia lamblia* with 24.2% (23 out of 95; *p*=0.00001) and *Balantidium coli* with 4.2% (4 out of 95; *p*=0.009) were the protozoan parasites identified. The prevalence rate of *G. lamblia* was significantly (*p*<0.05) higher than other parasites.

Among vegetable samples, 19.0% (18 out of 95) of floor vegetable and 16.8% (16 out of 95) of table vegetable were contaminated with parasites. This showed a non-significant (*p*=0.51) association between the mode of display (floor or table) and the presence of parasites.

Discussion

Intestinal parasites are common in the low-income countries and are usually acquired through the ingestion of raw or improperly cooked foodstuffs and salads. These intestinal parasites include protozoa, cestodes, trematodes, and nematodes, which contaminate different species of vegetables (Abougrain et al., 2010; Kozan et al., 2005; Srikanth and Naik, 2004). Our findings showed that about one-third of the examined vegetables from Lusaka, Zambia were contaminated with different types of intestinal parasites. Similar results were reported in vegetables sampled from Libya (Abougrain et al., 2010),

Table 1: Parasites detected in vegetables sold in Lusaka, Zambia

Types of vegetables	Sample size	No. (%) of contaminated samples	Detected parasites	*p*-value
African eggplant	9	0 (0.0)	Not detected	
Cabbage	6	2 (2.11)	*Giardia lamblia* (n=1), *Balantidium coli* (n=1) *Clonorchis sinensis* (n=1)	
Chilli	10	5 (5.26)	*Trichuris trichuria* (n=5)	
Chinese cabbage	9	7 (7.37)	*Ascaris lumbricoides* (n=1), *Girdia lamblia* (n=6) *Taenia* spp. (n=2), *Strongyloides stercoralis* (n=2) *Clonorchis sinensis* (n=1)	
Cucumber	10	0 (0.0)	Not detected	X^2=27.91
Okra	10	0 (0.0)	Not detected	
Pigweed	10	5 (5.26)	*Giardia lamblia* (n=4) *Taenia* spp. (n=4)	df=9
Pumpkin leaves	11	6 (5.26)	*Ascaris lumbricoides* (n=2) *Giardia lamblia* (n=5) *Taenia* spp. (n=3)	*p*=0.001
Rape	10	6 (6.32)	*Girdia lamblia* (n=6) *Balantidium coli* (n=3)	
Tomatoes	10	4 (4.21)	*Giardia lamblia* (n=1) *Paragonimus westermani* (n=2) *Diphyllobothrium latum* (n=2)	
Total	95	34 (35.8)		

Ethiopia (Tefera et al., 2014), and Nigeria (Kozan et al., 2007). Vegetables are an essential source of vitamins and minerals served with the main dish made from maize meal (Nshima and Ugali) in most communities in Zambia and Central and East African countries (De Groote and Kimenju, 2008; Muzhingi et al., 2008). In middle income and high-income families, vegetables serve as a meal supplement to beef, chicken, pork, and mutton. In addition, vegetables are used as salads at most events such as weddings and fed to intensive managed small ruminants (goats and sheep), guinea pigs, rabbits, and backyard chickens (Kozan et al., 2005). Therefore, parasitic contamination of vegetables may affect the health of both human beings and animals. The parasitic infection, prevalent in tropics and subtropics, is mainly associated with poor sanitation described elsewhere. Poor sanitation and hygienic status of the consumers and producers, inadequate transportation and packaging of vegetables, besides the application of untreated human and animal manure, and contaminated water for irrigation of vegetables are sources of contaminants (Haq et al., 2014; Srikanth and Naik, 2004).

The detection of parasites in the vegetable samples observed in this study was lower than those reported in Ethiopia (Tefera et al., 2014) and Iran (Fallah et al., 2012), where the parasitic vegetable contamination was found to be 58.0 and 57.8%, respectively. In contrast, other studies on parasitic contaminations of vegetables conducted in Libya (Abougrain et al., 2010), Iran (Daryani et al., 2008), Sudan (Mohamed et al., 2016), and Turkey (Adanir and Tasci, 2013) showed lower contamination rates of 32.6, 29.0, 13.5, as well as 6.3%,

respectively. Such observed differences in prevalence rates of different pathogenic intestinal parasites reported in our present work and those by others from previous works on fresh vegetables could have been attributed to several factors. Geographic location, kind and the number of samples analyzed, procedures used to identify intestinal parasites, type of water used for irrigation, and post-harvest handling practices of such vegetables are likely to be among these factors (Abougrain et al., 2010; Ishaku et al., 2013).

In this study, *G. lamblia* was the most common detected parasite, followed by *Taenia* spp., *T. trichuria*, *B. coli*, *A. lumbricoides*, *C. sinensis*, *D. latum*, *P. westermani*, and *S. stercoralis*. Several other studies have been conducted around the world which are consistent with the findings of the current survey (Abougrain et al., 2010; Adamu et al., 2012; Avcioglu et al., 2011; Dorny et al., 2009). We found that *G. lamblia* were associated with Chinese cabbage, pumpkin leaves, rape, pigweed, and cabbage. These findings were consistent with those from Sudan (Mohamed et al., 2016), Iran (Ezatpour et al., 2013), and Egypt (Eraky et al., 2014); where prevalence rates of 22.9, 6.9, and 8.8% were reported, respectively. Our findings suggest that most vegetable gardeners used contaminated water either from sewer or flooded pit latrine. *Giardia* cysts may be found on surface water such as lakes and rivers, and their concentration is positively associated with water pollution employing residential or agricultural fecal contamination.

We showed that *Taenia* spp. were the second common parasitic contamination detected and were associated with Chinese cabbage, pumpkin leaves, and pigweed.

These findings were similar to those reported in studies from Saudi Arabia (Al-Megrin, 2010), Tripoli in Libya (Abougrain et al., 2010), and Iran (Fallah et al., 2012); where more than one type of vegetable was contaminated with *Taenia* spp. eggs. It is a known fact that parasitic infections are common vegetable contaminants, where animal manure is used as a fertilizer (Abougrain et al., 2010; Fallah et al., 2012). In Zambia, the most common animal manure used is cattle, goat, and sheep dung due to its abundance. In a previous study in Zambia, a prevalence of *Taenia* infection in cattle has been reported (Dorny et al., 2002). In low rainfall areas of the country, untreated animal manure is used as fertilizer for both vegetables and maize and becomes a source of zoonotic infections if not sanitized before application (Haq et al., 2014; Srikanth and Naik, 2004).

Similar to the present study, *T. trichuria* was detected in vegetable samples in some previous studies (Adamu et al., 2012; Chau et al., 2014; Mohamed et al., 2016; Punsawad et al., 2019; Rostami et al., 2016; Shahnazi and Jafari-Sabet, 2010). The prevalence rate of *T. trichuria* (5.3%) in our study is consistent with that found in Pakistan (Haq et al., 2014) and Nigeria (Adamu et al., 2012), which were 6.4 and 5.1%, respectively. In contrast, our result is higher than those found in Thailand (Punsawad et al., 2019), Nigeria (Adamu et al., 2012), Sudan (Mohamed et al., 2016), Ghana (Duedu et al., 2014), Mazandaran Province of Iran (Rostami et al., 2016), and Qazvin Province of Iran (Shahnazi and Jafari-Sabet, 2010), where the contamination rates were 2.6, 0.5, 2.9, 2.0, 2.2, and 0.9% respectively.

In the current research, 4.3% *B. coli* cysts were detected in vegetable samples. Our findings are not in agreement with the result from Bangladesh (Paul et al., 2019), where *B .coli* was the most abundant protozoan detected (51.5%). The presence of *B. coli* cysts suggests contamination of the environment by fecal matter of the porcine since the protozoan is commonly present in swine faeces. *B. coli,* a causative agent of zoonotic balantidiasis, transmitted via the fecal-oral contamination route (Paul et al., 2019).

A 3.2% rate contamination of *A. lumbricoides* eggs detected in this study was associated with Chinese cabbage and pumpkin leaves. However, in the previous studies conducted in Libya (Abougrain et al., 2010) and Ghana (Amoah et al., 2006), higher contamination rates of 68% and 85% were reported, respectively. Additionally, findings from Sudan (Mohamed et al., 2016) indicated 2.9% contamination of *A. lumbricoides*, which are consistent with our results. Other studies indicated that *A. lumbricoides* was the most common isolated contaminant in vegetables and fruits (Al-Shawa and Mwafy, 2007; Mohamed et al., 2016). Detection of *A. lumbricoides* in fresh vegetables suggests that contaminated water and untreated animal manure are used in vegetable gardens. *A. lumbricoides* eggs are highly resistant, and so they are frequently found in raw sewage (Dorny et al., 2009). Furthermore, unhygienic practices, from harvesting to the marketing of vegetables, may contribute to contamination of the products (Mohamed et al., 2016). The presence of *A. lumbricoides* eggs is used as a parasitological indicator for evaluation of hygienic quality of vegetables and fruits, and uncontrolled use of natural manure (Blaszkowska et al., 2011; Gupta et al., 2009).

In the present research, *C. sinensis*, *D. latum*, and *P. westermani* eggs were detected in cabbage and Chinese cabbage with a 2.1% contamination rate for each. Our finding is consistent with those reported in Korea, where clonorchiasis was prominent in those who consumed vegetables (Choi et al., 2006; Kim et al., 2010). *P. westermani* eggs were associated with Chinese cabbage and this finding is in agreement with the result from Seoul in Korea, where the parasite contamination rate was 11.8% (Chai and Jung, 2018). Eggs of *D. latum* were only detected in our tomato samples, which was lower than the result from Iraq (Al-Mozan et al., 2015) with a contamination rate of 5%. *D. latum* is often acquired through the consumption of raw or undercooked fish. The current work suggests that it may also be transmitted through consumption of infested and improperly cooked vegetables handled by infected individuals in Zambia. The findings on *C. sinensis*, *D. latum*, and *P. westermani* are contrary to those reported from Libya (Abougrain et al., 2010), Ghana (Duedu et al., 2014), Ethiopia (Tefera et al., 2014), Egypt (Eraky et al., 2014), and Sudan (Mohamed et al., 2016), where these parasites were not detected.

Rhabdatiform larvae of *S. stercoralis* were detected in the present survey with a prevalence rate of 2.1%. *S. stercoralis* larvae were detected only in Chinese cabbage. Our results is contrary to the findings by some other studies, where larvae of *S. stercoralis* parasite were predominantly detected in Ethiopia (Tomass and Kidane, 2012), Nigeria (Adamu et al., 2012), Thailand (Punsawad et al., 2019), and Sudan (Mohamed et al., 2016) with contamination rates of 63.6, 41.7, 10.6, and 8.6%, respectively. Our result of *S. stercoralis* larvae is consistent with those reported in Malaysia (Zeehaida et al., 2011) and Brazil (Vidigal and Landivar, 2018). *S. stercoralis* rhabdatiform larvae were found in water samples used to wash Pegaga, Kesum, and water spinach in Malaysia (Zeehaida et al., 2011). The presence of *Strongyloides* spp. in fecal from goats brought for slaughter at the abattoir in Lusaka suggests that their manure might be the source of the infection when used as fertilizer in vegetable gardens (Bukowa et al., 2020). *S. stercoralis* is a free-living or heterogenic soil-transmitted helminth infecting approximately 1.5 billion people worldwide in tropical,

subtropical, and temperate regions (Akyala et al., 2013; Keiser and Nutman, 2004). The infective stage, filariform, can penetrate the susceptible individuals while handling the vegetables as it can excess the body through the skin puncture causing the cutaneous phase characterized by slight haemorrhage, swelling, and ground itch. The filariform larvae may migrate to the lungs, where they cause pulmonary infection before settling in the intestinal (Bogitsh et al., 2005).

It should be noted that this study did not compare the contamination rates of vegetables during different seasons of the year. Also, a sensitive identification method such as molecular assay was not applied. These are two main limitations of our study that should be considered in the researches in future.

Conclusion

This study showed that some vegetables sold at the Lusaka City market (Zambia) were a potential source of parasitic infections for local consumers. *G. lamblia* was the most prevalent parasite found in the vegetable samples. It is, therefore, important that the general public is made aware of the importance of washing the vegetables thoroughly before they are consumed. Also, necessary hygienic measures need to be applied to reduce the chances of infection of individuals in the community.

Author contributions

K.M.B. and S.S.N. designed the research, analyzed the data, and also prepared the manuscript; W.R.B., K.M.B., J.M., and F.H. involved in sample collection and data analysis; F.B., G.K., M.M., and E.Z. edited the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interests.

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