Intervention and Control Measures of Soil -Transmitted Helminths from Some Salad Vegetables by Washing

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Abstract

The study conducted from July to December 2018, to determine the effectiveness of thorough washing of raw vegetables to eliminate helminth contamination, included of three different kinds of salad vegetables (15 samples of each vegetable kind), namely *Coriandrum sativum, Mentha arvensis* and *Allium cepa* obtained from Meiktila Market, and these vegetables were washed in tap water, purified water and common salt solution for five times and examined the parasite each time. Although helminth eggs and larvae were still detected in the samples washed for the first, second and third times, eggs and larvae were not totally observed at fourth and fifth times. It is observed that interventions were essential to prevent contamination of raw vegetables prior to consumption. It is suggested that thorough washing at least (4 to 5 times) before consumption of raw vegetables need to be practiced as a measure to curb transmission of helminths.

Key words: Soil-transmitted helminths, salad vegetables, washing methods, Meiktila Market

Introduction

Vegetables in its broadest sense, refers to any kind of plant life or plant products. It is also commonly referred to as the fresh edible portion of herbaceous plants, roots, stems, leaves or fruits. These plants are either eaten fresh or prepared in a number of ways (Damen *et al*., 2007).

Fresh vegetables are regarded as important part of a healthy diet. Fruits and vegetables normally become potentially sources of human infection through their contamination, during their growth, collection, transport, processing, and preparation or during processing and the sources of helminth contamination are usefully contaminated soils or water (Wafa and Megrin, 2010).

The present cropping practices in many developing countries, where sanitation in general and wastewater treatment in particular remain challenges, cannot assure vegetables that are free from pathogens. Many West African studies have shown high levels of pathogen contamination in irrigation water; on both farm and market vegetables (Amoah *et al.,* 2007).

In recent years, there has been an increase in the number of reported cases of food-borne illness linked to consuming fresh vegetables. The consumption of raw vegetables plays a major epidemiological role in the transmission of parasitic food-borne diseases. Intestinal parasites are widely prevalent in developing countries, probably due to poor sanitation and inadequate personal hygiene. Several surveys in different parts of the world showed that the vegetables can be agent for transmission of helminth eggs and larvae (Altekruse *et al.*, 2007).

Vegetables are important sources of nourishment and a major part of human food for good health. In many parts of the world vegetables have been reported to be contaminated by parasites. These parasites infest vegetables while still on the field and are usually transmitted by contaminated water and spread by poor hygiene practices. The resistant cysts or eggs of these helminths enhanced their survival in the natural environment. In developing countries of the world, intestinal parasites have been regarded as a major source of public health and socio-economic problems. Different species of soil-transmitted helminths are widely distributed in tropical and subtropical parts of the developing world (Edosomwan *et al.,* 2011).

The salad vegetables sold at the Meiktila municipal market were found to be contaminated with parasites hence the inhabitants needed to be for educated on food safety, good distribution practices and improvement on sanitary conditions. Control of soil-transmitted helminth is a constant object of public health strategies. These information lead to conduct this research on the three salad vegetables commonly consumed uncooked by the people in Meiktila Township.

The objectives of this study are

- to determine the effectiveness of washing the raw vegetables contaminated with helminths
- to analyze and improve the effectiveness of various washing methods for the reduction of helminth parasites from the surface of vegetables.

Materials and methods

Study area and sample collection site

Meiktila was selected as a study area and collection site of vegetable samples. Meiktila is located between 20° 51' and 20°55' N and between 95° 49' and 95° 54' E. The vegetables are usually brought to the market by rural farmers from the environs (Plate 1).

Plate 1. Map of study area and location of Meiktila Market
Source from Google Earth, 2010

Study period

The duration of study period was from July to December 2018.

Collection of vegetable samples

Fresh salad vegetable samples of *Coriandrum sativum*, *Mentha arvensis* and *Allium cepa* were obtained from this market in early hours of morning between 6:00 and 7:30 hours directly from the farmers. The vegetable traders buy from farmers and subsequently wash off the dirt and sand from these vegetables before displaying the wares for sale (Plate 2).

Wastes damped behind the vegetable hawkers and congested nature of vegetables on sale

Plate 2. Condition of the market involved in the study

Sample preparation and detection of helminth eggs / larvae

One hundred gram weight of each vegetable sample was taken and the edible part of each vegetable sample was separated according to household practice. First, each three same kind of vegetable sample was immersed separately in three plastic bowls containing each bowl with 1 litre of running tap water, purified water and common salt (NaCl, half of one tea spoon) solution for 5 minutes. Then, these vegetable samples were taken out of the separate bowls and were washed with tap water, purified water and common salt solution for second, third, fourth and fifth times respectively in similar manner.

At each washing, the washing water was left for 15 minutes for sedimentation to take place. Afterward, the top water was discarded and the remaining washing water was centrifuged at 1000 rpm for 15 minutes (modified from method of Fallah *et al.,* 2012). The supernatant was removed and 3ml residue was left in a test tube. A drop of residue was placed on the center of a clean glass slide and mixed with a drop of Lugol's Iodine solution and then gently covered with a clean cover slip. The preparation was examined under a light microscope for detection of helminths using x100 and x400 magnification. The process was systematically repeated until the residue in each test tube was exhausted. Helminth eggs and larvae were examined under the light microscope as described by Downers and Ito (2001).

For statistical analysis, analysis of variance (ANOVA) was used test to evaluate the effectiveness of common salt solution samples on contaminated vegetables. The differences were considered at 0.05 level of significance.

Results

A total of three different kinds of salad vegetables (15 samples of each vegetable kind) were examined for the helminth contamination. The results of helminth contamination in vegetable samples washed with common salt solution, running tap water and purified water during studied period are summarized as shown in Tables 1, 2 and 3. The numbers of parasites gradually declined from first time to successive times and no parasites were observed at 4^{th} time in all samples of salad vegetables.

Comparison on the helminth eggs/larvae in *Coriandrum sativum* sample by various washing methods

The result of elimination of helminth eggs/larvae by common salt solution revealed that the contamination of *Ascaris lumbricoides* was found 40% after the first wash and declined to 16% in second and 2% in third wash. However, not a single egg was found in the vegetables washed into fourth and fifth times. The contamination of *Strongyloides starcoralis* was also found 22% in first wash, 9% in

second and 2% in third wash. In contrast, no helminth larvae were found in vegetables washed with fourth and fifth times. Eggs of *Taenia* sp., was found 5% in first time and no egg was observed in second time. Contamination of each of *Trichuris trichiura* and *Enterobius vermicularis* was observed 2% in first time. But, no helminth was found in second time (Table 1, Figure 1). A significant difference ($p<0.05$) was found between the number of helminth eggs/larvae of *A. lumbricoides*, *S. starcoralis* and *Taenia* sp., recovered from common salt solution washing methods. However, no significant difference was found in the eggs of *T. trichiura* and *E. vermicularis.*

The elimination of helminth eggs/larvae by running tap water showed that the contamination rate of *A. lumbricoides* was 36% after the first wash, declined to 16% in the second and 6% in third wash specimens. However, not a single egg was found in the vegetables washed with fourth and fifth times. The contamination rate of *S. starcoralis* was also found 26% in first wash and 8% in second. In contrast, no helminth larvae were found in vegetables washed with third time. Although the infection of *Taenia* sp., was found 8% in first time, no single egg in second time. But, no eggs of *T. trichiura* and *E. vermicularis* were found after first time washing (Table 1, Figure 1). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides*, *S. starcoralis* and *Taenia* sp., recovered from running tap water washing methods. No significant difference was found in the eggs of *T. trichiura* and *E. vermicularis* in the washing method of vegetable samples.

The result of elimination of helminth eggs/larvae by using purified water with washing procedure was observed that the contamination rate of *A. lumbricoides* was 33% after the first wash, declined to 13% in the second and 4% in third wash. However, not a single egg was found in the vegetables washed with fourth and fifth times. The contamination rate of *S. starcoralis* was also found 22% after first wash, declined to 13% in the second and 2% in the third times. In contrast, no helminth larvae were found in vegetables washed with fourth and fifth times. Although the infection of *Taenia* sp., was found 9% in first time, it was not completely found in second time. Although the infection of *T. trichiura* was detected 4% in only the first time, it was not observed in remaining washing times. But, *E. vermicularis* was not found even after first time (Table 1, Figure 1). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides*, *S. starcoralis* and *Taenia* sp., recovered from purified water washing method. However, differences were not significant in *T. trichiura* and *E. vermicularis*

Table 1. Comparison on helminth eggs / larvae in *Coriandrum sativum* sample by various washing methods

Washing Methods	Counts on helminth (%)																					
	Ascaris					*Strongyloides*					*Taenia*					*Trichuris*					*Enterobius*				
	Times					Times					Times					Times					Times				
	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth
A	40	16	2	0	0	22	9	2	0	0	5	0	0	0	0	2	0	0	0	0	2	0	0	0	0
B	36	16	6	0	0	26	8	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	33	13	4	0	0	22	13	2	0	0	9	0	0	0	0	4	0	0	0	0	0	0	0	0	0

A = Common salt solution,
B = Running tap water,
C = Purified water

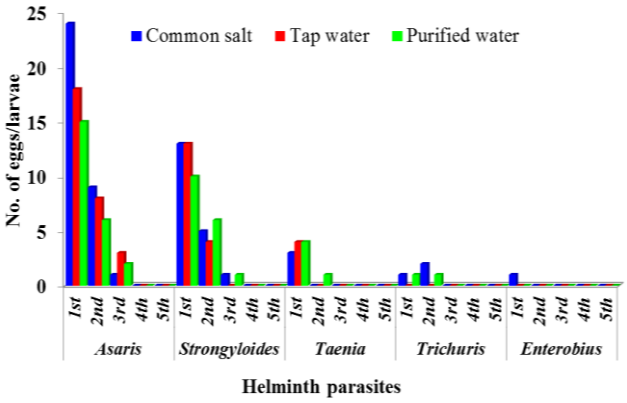

Figure 1. Comparison on the effectiveness of helminth eggs/larvae collected from *Coriandrum sativum* by various washing methods

Comparison on the effectiveness of helminth eggs/larvae in *Mentha arvensis* sample by various washing methods

For the *M. arvensis* sample, the elimination of helminth eggs/larvae by using common salt solution with washing procedure were observed that the contamination rate of *A. lumbricoides* was found 48% in first time and 7% in second time. However, helminth eggs were not found in the vegetable samples washed for third time. The contamination rate of *S. starcoralis* was also found 14% in first time and 10% in second time. In contrast, no helminth larvae were found in vegetables washed in remaining times. Although the percentage of *Taenia* sp., was found 9% in first time, it was not completely found in second time. The contamination of *T. trichiura* was 7% in first time. But, no helminth was found in wash with second time. Eggs of *E. vermicularis* were not found in wash with first time (Table 2, Figure 2). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides*, *S. starcoralis* and *Taenia* sp., recovered from washing methods. However, there was no significant in the number of eggs of *T. trichiura* and *E. vermicularis* observed.

In *M. arvensis* sample, the removal of helminth eggs/larvae by using running tap water with washing procedure was observed that the contamination rate of *A. lumbricoides* was 36% after the first wash, dropped to 14% in the second wash time. However, not a single egg was found in the vegetables washed with third time. Although the percentage of *S. starcoralis* larvae was found 29% and 7% in washed with first and second times respectively, it was not completely found in third time. The contamination rate of *Taenia* sp., and *T. trichiura* were observed 6% and 3% respectively washed with first time. However, these eggs were not detected in wash with remaining times. But, no *E. vermicularis* was found in wash with first time (Table 2, Figure 2). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides, S. starcoralis* and *Taenia* sp., recovered from running tap water washing method. No significant difference was found in the eggs of *T. trichiura* and *E. vermicularis* in the washing method of vegetable samples.

In *M. arvensis* sample, the removal of helminth eggs/larvae by using purified water with washing procedure were observed that the contamination rate of *A. lumbricoides* was 27% after first wash, dropped to 16% in the second and 3% in third wash times. However, not a single egg was found in the vegetables washed with fourth and fifth times. Although the incidence of *S. starcoralis* larvae was found 24%, 15% and 3% in wash with first, second and third times respectively, they were not completely found in fourth and fifth times. The contamination rate of *Taenia* sp., and *T. trichiura* were observed 9% and 3% respectively in wash with first time. However, these eggs were not detected in washed with remaining times. But, no *E. vermicularis* was found in wash with first time (Table 2, Figure 2). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides, S. starcoralis* and *Taenia* sp., recovered from purified water washing method. No significant difference was found in eggs of *T. trichiura* and *E. vermicularis* in washing of vegetable samples.

Table 2. Comparison on helminth eggs/ larvae in *Mentha arvensis* sample by various washing methods

Washing Methods	Counts on helminth (%)																							
	Ascaris					*Strongyloides*					*Taenia*					*Trichuris*					*Enterobius*				
	Times					Times					Times					Times					Times				
	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth
A	48	7	0	0	0	14	10	0	0	0	9	0	0	0	0	7	0	0	0	0	0	0	0	0	0
B	36	14	0	0	0	29	7	0	0	0	6	0	0	0	0	3	0	0	0	0	0	0	0	0	0
C	27	16	3	0	0	24	15	3	0	0	9	0	0	0	0	3	0	0	0	0	0	0	0	0	0

A = Common salt solution,
B = Running tap water
C = Purified water

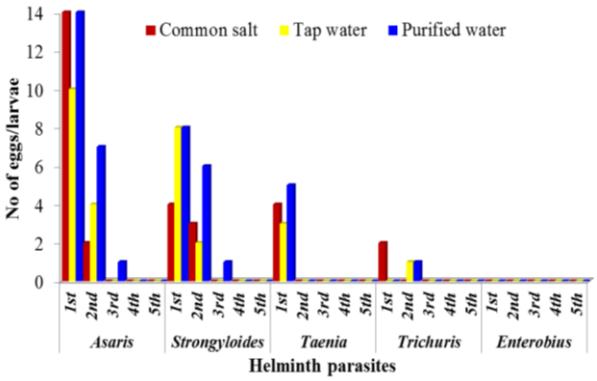

Figure 2. Comparison on the effectiveness of helminth eggs/larvae collected from *Mentha arvensis* by various washing methods

Comparison on the helminth eggs/larvae in *Allium cepa* sample by various washing methods

Among the *A. cepa* sample, the removal of helminth eggs/larvae by using common salt solution with washing procedure were observed that the highest contamination rate of *A. lumbricoides* eggs was found 32% in first time and 18% in second time, but not detected in third time. Although the larvae of *S. starcoralis* was found 26% and 9% in first and second times respectively, it was totally not found in third time. The contamination of *Taenia* sp., *T. trichiura* and *E. vermicularis* eggs was observed 6%, 6% and 3% respectively in first time wash. However these eggs were not detected in next wash (Table 3, Figure 3). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides*, and *S. starcoralis* recovered from common salt solution washing method. No significant difference was found in the eggs of *Taenia* sp., *T. trichiura* and *E. vermicularis* in the washing method of vegetable samples.

In *A. cepa* sample, the removal of helminth eggs/larvae by using running tap water with washing procedure were observed that the contamination rate of *A. lumbricoides* was 38% after the first wash, dropped to 12% in the second and 3% in third wash times. However, not a single egg was found in the vegetables washed with fourth and fifth times. Although the percentage of *S. starcoralis* larvae was found in 24% and 12% in wash with first and second times, it was not completely found in third time. The contamination rate of *Taenia* sp., and *T. trichiura* was observed 8% and 3% respectively in washed with first time. But, no of *E. vermicularis* was found in wash with first time and also in successive times (Table 3, Figure 3). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides, S. starcoralis* and *Taenia* sp., recovered from running tap water washing method. No significant difference was found in the eggs of *T. trichiura* and *E. vermicularis* in the washing method of vegetable samples.

Among the *A. cepa* sample, the removal of helminth eggs/larvae by using purified water with

washing procedure were observed that the highest contamination rate of *A. lumbricoides* eggs was found 29% in wash with first time and 21% in second time, but these were not detected in third time. Although the percentage of *S. starcoralis* larvae was found 21% and 15% in wash with first and second times, it was not completely found in third time. The contamination rate of *Taenia* sp., and *T. trichiura* eggs were observed 9% and 2% respectively in wash with first time. However, these eggs were not detected in washwith remaining times. But, no of *E. vermicularis* was found in wash with first time (Table 3, Figure 3). There was a significant difference ($p<0.05$) between the helminth eggs/larvae of *A. lumbricoides, S. starcoralis* and *Taenia* sp., recovered from purified water washing method. No significant difference was found in the eggs of *T. trichiura* and *E. vermicularis* in the washing method of vegetable samples during studied period.

Table 3. Comparison on helminth eggs / larvae in *Allium cepa* sample by various washing methods

Washing Methods	Counts on helminth (%)																					
	Ascaris					*Strongyloides*					*Taenia*					*Trichuris*					*Enterobius*				
	Times					Times					Times					Times					Times				
	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth
A	32	18	0	0	0	26	9	0	0	0	6	0	0	0	0	6	0	0	0	0	3	0	0	0	0
B	38	12	3	0	0	24	12	0	0	0	8	0	0	0	0	3	0	0	0	0	0	0	0	0	0
C	29	21	0	0	0	21	15	0	0	0	9	0	0	0	0	2	0	0	0	0	0	0	0	0	0

A = Common salt solution,
B = Running tap water,
C = Purified water

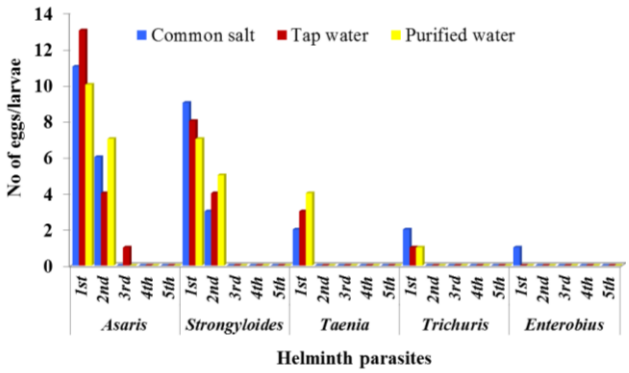

Figure 3. Comparison on the effectiveness of helminth eggs/larvae collected from *Allium cepa* by various washing methods

Discussion

This study was conducted with intention to control the helminth parasites contaminated in three kinds of fresh salad vegetables (*Coriandrum sativum, Mentha arvensis* and *Allium cepa*) by different washing methods (common salt solution, running tap water and purified water).

The results clearly indicated to be aware for the people regarding the eating of raw vergetables, because all fresh salad vegetatbles used in this experiment are contamined with helimnth parasites in raw condition. Therefore, the raw salad vegetables should be thoughly washed before eating.

Wafa and Megrin (2010) reported that the consumption of raw vegetables plays an important role in transmission of human parasitic infection. The consumption of vegetables is regarded as a major way for transmission of parasitic contamination.

The results showed that the helminth parasites from vegetables were eliminated by continuous washing with common salt solution, running tap water and purified water. Amoah *et al.* (2007) reported that salt solutions, water and vinegar are the dominant means used for washing vegetables in Ghana, whereas in the cities of all its francophone countries surveyed, chlorine bleach and potassium permanganate are well established disinfectants.

Beuchat (1998) reported that the reduction depending on the nature of surface of leaves can be achieved when washed vigorously in tap water. Moreover, washing sanitizer solution and rinsing with clean water reduce helminth parasites (WHO, 2006) (cited by Amoah, 2007).

Ogban *et al.* (2010) reported the contamination of helminth in some fruits and vegetables sold in markets, in Calabar, Nigeria. All the vegetables and fruits were found contaminated by helminth eggs. *A. lumbricoides* was the most frequently encountered (76.4%), followed by *T. trichiura* (15%), *Taenia* sp., (14.1%), *S. starcoralis* (7.3%) and *E. vermicularis* (1.3%). When compared to this study the infection is similar but the prevalence rates are lower in present study.

Edosomwan *et al.* (2011) stated the contamination of helminth in some vegetables sold in markets in Benin City, Edo State, Nigeria. All the vegetables were found contaminated by helminth eggs and *A. lumbricoides* was the most frequently encountered, (33.5%). In this study *A. lumbricoides* was also the most prevalent parasite (36.6% after washing first time).

Edosomwan *et al.* (2011) stated that the eggs of *A. lumbricoides* were difficult to wash off due to their adhesive nature. The presence of eggs in the vegetables is of public health significance considering the fact that communities are at risk of infection. Consumers get infected through ingestion of parasitic eggs adhering to these leafy vegetables that may be eaten raw such as Coriander, Onion tops and mint or made in preparation for rice noodles, rice noodles with gravy and varieties of fried or roasted food. It has been determined that proper washing and disinfecting of vegetables prior to consumption can prevent transmission of parasites to human.

This finding is supported by Uga *et al.* (2009) who stated that vegetables sold in markets were highly contaminated by helminth parasites. Therefore, there

is a need to educate farmers and marketers on hygienic handling of vegetables, the need for public enlightenment of consumers for the necessity to wash leafy vegetables and disinfect salad vegetables before use (cited by Endosomwan *et al.*, 2011).

Avcioglu *et al.* (2011) reported that six types of vegetable samples at a catering service in Bursa, Turkey were subjected to helminth eggs. Helminth eggs were detected in six (3%) unwashed sample and not in any washed samples. The eggs of *A. lumbricoides* were detected in four (2%) and *Toxocara* sp., two (1%) in unwashed vegetables, mostly among leafy vegetables such as lettuce and parsley. In the present study, helminth eggs and larvae were detected in various washing vegetable samples for first and second times, mostly among *C. sativum* sample, however *Toxocara* eggs were not detected. However, no significant effectiveness was observed among washing solutions.

Conclusion

The result of the present study emphasizes the importance of raw vegetables in the transmission of intestinal helminth parasites to human. It is necessary to improve the sanitary conditions in the area where the vegetables are cultivated and consumed. The washing vegetables for only one time are not sufficient for consumption. It is recommended for the safety of consumers, vegetables should be washed at least four or five times before consumption.

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