RESEARCH ARTICLE

The Effect of Washing Procedures on Contamination of Raw Vegetables with Nematodes Larvae

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Abstract

Background and Objective: Vegetables can be contaminated with bacteria, viral, and parasitic pathogens during their cultivation, collection, and transportation processes, and consumption of unsafe vegetables is considered a risk factor for human parasitic infections. This study aimed to evaluate the contamination of raw vegetables with nematodes larvae, and the effect of washing procedures on their elimination in Shahrekord city, Iran. **Materials and Methods:** This study carried out on 160 samples of different vegetables including mint, basil, garden cress, parsley, satureja, radish, chives, and wild leek. Each sample was divided into four groups including unwashed and washed with pure water, vinegar, and germicide groups. Then, the samples were examined for nematodes larvae using Baermann funnel technique. Data were analyzed using Chi-square test. **Results:** Among 40 samples on each group, contamination was detected in 33 (82.5%) of unwashed group, 13 (32.5%) of washed with pure water group, five (12.5%) of washed with vinegar, and two (0.5%) of washed with germicide group. There was a significant difference in the rate of contamination in the washed groups compared to the control group ($P < 0.05$). Contamination rate in the group washed with water was significantly higher than the vinegar and germicide groups ($P < 0.05$) Moreover, our study showed that the highest rate of contamination was in chives and wild leek and the lowest one in radish. **Conclusion:** This study concluded that vegetables could be a potential source of nematodes larvae and proper washing and disinfecting procedures before consumption of raw vegetables should be performed to avoid transmission of nematodes larvae.

Key words: Nematode larvae, raw vegetables, washing method

INTRODUCTION

Fresh raw vegetables provide a significant part of human nutrition, as they are important sources of vitamins, minerals, and dietary fiber.[1,2] They are highly beneficial for the maintenance of health and prevention of diseases. Furthermore, many vegetables are good sources of Vitamin C, carotene and mineral elements such as iron, and vitamins including thiamine, niacin, and riboflavin.[3] There is a substantial amount of evidence that high consumption of vegetables is associated with a decreased risk of many chronic diseases such as obesity, heart diseases, cancer, and childhood malnutrition.[4-8] On the other hand, vegetables can be contaminated with bacteria, viral and parasitic pathogens during their cultivation, collection, and transportation processes, and consumption of unwashed, raw, and unsafe vegetables is considered a risk factor for human parasitic infections. Parasitic diseases can be considered among the most common diseases in the world, which are transmitted to humans through the soil, water, and foodstuffs such as vegetables.[9] Contamination of vegetables may occur through contacting with soil, raw manure, and sewage used as a fertilizer on farms.

In recent years, several studies in different parts of the world have shown that the vegetables can be the agent

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of transmission of eggs and larvae of nematode species (*Ascaris lumbricoides, Trichuris trichiura, Toxocara* spp., *Trichostrongylus, and* hookworm).[10] Nematodes incidentally infect humans who consume contaminated, uncooked, or improperly washed vegetables and fruits.[11] According to the WHO report in 1975, 700 million people (26% of the world population) have been infected with *A. lumbricoides*.[12] It is estimated that in 2010, 819 million people are infected with *A. lumbricoides*, 465 million with *T. trichiura*, and 439 million with hookworms globally.[13] Iran is a country with a high prevalence of parasitic infections; thus, identifying the source of infection, methods of transmission, spread of such infections, and prevention methods are health priorities. Several studies conducted in Iran have found that the level of parasitic contamination in vegetables is considerable in different cities in Iran such as Yazd, Tehran, Mazandaran, and Tabriz.[9,11,12] Identification of parasitic contamination transmitted through vegetables in each region may help public health authorities in the control and prevention of these infections in the region. However, little study has been done on comparison the effect of various washing procedures on the elimination of parasitic contamination. Therefore, the main aim of this study was to investigate the contamination with nematodes larvae in raw vegetables and influence of various washing procedures on their elimination in Shahrekord city, Iran.

MATERIALS AND METHODS

Sample collection

This experimental study was done on 160 fresh samples (100 g of each) from eight types of commonly consumed vegetables including mint (*Mentha piperita*), basil (*Ocimum basilicum*), garden cress (*Lepidium sativum*), parsley (*Petroselinum crispum*), satureja (*Satureja hortensis*), radish (*Raphanus sativus*), chives (*Allium schoenoprasum*), and wild leek (*Allium ampelorasum*) were collected randomly from the ten vegetable markets in Shahrekord, Iran. Each vegetable sample was placed in a separate sterile nylon bag, and then, immediately transferred to the laboratory of parasitology of the faculty of veterinary medicine of Shahrekord University, Shahrekord, Iran.

Sample preparation and examination

Vegetable samples were divided into four groups (with five replicate) as follows: The first group was unwashed as a control group, the second group was put in a dish filled with pure water, the third group was put in 1% vinegar/water solution, and the fourth group was dipped in a dish containing five litters water and five drops of germicide. After 30 min, vegetables were rinsed in tap water and examined for nematodes larvae using Baermann funnel technique.

Baermann funnels

Vegetable roots and leaves put on the filter, and the filter is placed on the funnel of water, which has an evacuating valve. After 12 h, the soil larvae and nematodes moved toward water and accumulated inside the funnel tube. At last opening, the funnel tap leads the nematodes and larvae in the glass bottle. This procedure used for all samples in our study.[14]

Data analysis

Statistical analyses were carried out using Chi-square test of the SPSS software version 22 for Windows (SPSS Inc., Chicago, IL, USA) to compare the rate of contamination of vegetables among different groups. A $P < 0.05$ was considered statistically significant.

RESULTS

The results of this study demonstrated that 53 cases (33%) showed contamination with nematodes larvae. Among 40 samples on each group, nematodes larvae contamination was detected in 33 (82.5%), 13 (32.5%), 5 (12.5%), and 2 (0.5%) in control washed with pure water, vinegar and germicide groups, respectively. Washing procedures significantly reduced the rate of contamination comparing to control groups ($P < 0.05$). Group washed with pure water showed a significant difference with other three groups ($P < 0.05$). Although contamination in germicide group (0.05%) was less than vinegar group (12.5%), it was not significant ($P > 0.05$). Differences between the nematodes larvae prevalence due to the washing and the control groups are presented in Table 1, and the mean number of the nematodes larvae contamination in each type of vegetable is represented in Table 2.

Furthermore, our study shows that the highest rate of contamination in control and washed with pure water groups were determined in chives and wild leek (100%). Furthermore, chives contamination in group which washed with vinegar

Table 1: The nematodes larvae prevalence based on the washing procedures

Methods	Nematodes larvae prevalence		Percentage
	Number of examined	Number of positive	
Unwashed	40	33[a]	82.5
Water	40	13[b]	32.5
Vinegar	40	5[c]	12.5
Germicide	40	2[c]	0.5
Total	160	53	33

Values in the same column with different letters are significantly different among each washing procedures ($P<0.005$)

was detected in 100% of samples, however, wild leek had no larvae in this group. In all types of vegetables, wild leek was only vegetable groups which had larvae in the germicide group. In comparison between all eight types of vegetables, significant differences between wild leek and chives with other six types of vegetables were observed ($P < 0.05$). The lowest level of contamination was seen in radish [Figure 1].

DISCUSSION

The results of the present study showed that contamination with nematodes in the washed groups was significantly different compared to the control group. Vegetables may pollute with pathogenic nematode such as *T. trichiura*, *Trichostrongylus*, *Ascaris* spp., and hook worms, that are primarily transmitted to vegetables through the soil.[10] Furthermore, the soil is a basic and important problem because nematode larvae are developed until they become infective.[12] Studies show that the incidence of intestinal parasites in developing countries probably due to higher consumption of raw vegetables.[15] In this study, unidentified nematode larvae contamination were detected in 33% of vegetable samples, in agreement with the present study, a high prevalence of parasitic contamination of vegetables has been reported in the Shahrekord, Iran.[11] The rates of vegetable contamination with parasites in different cities of Iran were 15.5% in Ahvaz,[12] 65% in Tehran,[12] 30.4% in Zabol,[16] 13.76% in Yazd,[17] and 2% in a study conducted in Isfahan.[18]

Table 2: The mean number of nematode larvae in each kind of vegetable

Vegetable	Unwashed	Pure water	Vinegar	Germicide
Wild leek	44[a]	9[b]	11[b]	2[b]
Mint	4[b]	-	-	-
Basil	5[b]	6[b]	-	-
Garden cress	6[b]	-	-	-
Parsley	4[b]	-	-	-
Satureja	9[b]	-	-	-
Radish	3[b]	-	-	-
Chives	41[a]	32[c]	-	-
Total	116	47	11	2

Values in the same column with different letters are significantly different among each kind of vegetable in the mean number of nematode larvae (P<0.005). Values in the same row with different letters are significantly different among the washing procedures (P<0.005)

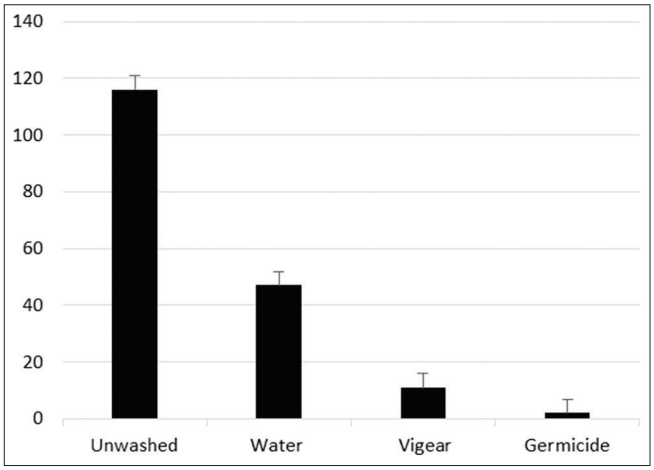

Figure 1: Values of the nematodes larvae contamination in different washing procedures. Different letters in the columns are significantly different in washing procedures ($P < 0.005$)

Siyadatpanah *et al.* reported that the rate of nematode larvae on raw vegetables was 25.8% in Amol, North of Iran.[19] In another study, the low frequency of free-living larvae contamination (7%) was reported in vegetables consumed in Malayer city, West of Iran.[20]

Parasite contamination in vegetables has also been reported in several countries such as Turkey (36%) and Nigeria (5.9%).[21,22] In Morocco, 50% of vegetables from farmland were contaminated by helminthic larvae.[23] Luz *et al*. reported that the percentage of parasite contamination in vegetables marketed in Brazil was 50.9%, with a predominance of nematode larvae (36.5%).[24] In Sudan, nematodes larvae and adult were detected among 34 samples of 260 vegetable samples.[25]

In a study conducted that the poorly washed and undisinfected vegetables are a main route for transmitting intestinal parasitic infections.[26] Our study indicated that washing by vinegar or germicide significantly reduced the rate of parasitic contamination comparing to poorly washed samples ($P < 0.05$). It has been determined that proper washing and disinfecting of vegetables before consumption can prevent transmission of the parasites to human.[2,17,26] In a study in Turkey, 0% of helminthic eggs were observed in washed vegetable samples.[17] Ashrafi *et al*. reported that there was no significant difference between washing vegetables with water and washing with dish soap for parasitic or bacterial contamination.[9]

In the present study, the highest rate of contamination was detected in chives and wild leek samples (100.0%) which it corresponds to the study conducted in Kerman.[27] Furthermore, the lowest rate of contamination in this study was in radish (39.1%). It could be concluded that the shape and surface of vegetables are associated with the rate of contamination. According to our results, like many other studies, the parasite prevalence in leafy vegetables was considerably higher, compared to root vegetables.[28-32]

CONCLUSION

The result of this study demonstrated that raw leafy vegetables consumed by people are often contaminated with nematodes

larvae. These types of vegetables should be considered as a potential source of nematodes larvae in Shahrekord, Iran. Furthermore, our study revealed that proper washing and disinfecting procedures (usage of vinegar or germicide) significantly reduce the nematodes larvae contaminations and people should be trained in how to properly disinfect vegetables before consumption.

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