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Short communication

Contamination of commonly consumed raw vegetables with soil transmitted helminthes eggs in Mazandaran province, northern Iran.

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Abstract:

Soil-transmitted helminthes (STHs) infections are responsible for significant burden of morbidity and mortality worldwide. Consumption of raw vegetables without proper washing is one of the major routes of such infections. We evaluate the prevalence of STH contamination in commonly used vegetables in Mazandaran province, northern Iran. A total of 772 fresh raw vegetables were obtained from retail markets. Each sample was divided into two groups. One group was used as the unwashed sample and the second group was washed with standard washing procedures. Then, samples were examined for helminth eggs by using standard methods. Data analysis was performed using SPSS. The overall prevalence of STHs was 14.89% (115/772). The rate of STH contamination was significantly higher in warm seasons (20.5%, 79/386) than in cold seasons (9.32%, 36/386) among the unwashed vegetables (OR = 2.50; CI 95% = 1.64-3.8; P < 0.001). No parasites were observed in standard washed samples (OR= 271.40; CI 95% = 16.84-4373.64; P < 0.001). Prevalence of STH contamination was significantly higher in leafy vegetables than root vegetables (OR = 1.67; CI 95% = 1.09-2.55; P < 0.05). The prevalence of STHs species in all the vegetables were as follows: *Ascaris lumbricoides* (3.36%), *Trichuris trichiura* (2.2%), hookworms (2.9%), *Toxocara* spp. (1.68%), *Trichostrongylus* spp. (1.55), *Taenia* sp. (0.9%) and *Hymenolepis nana* (2.2%). The results of the present study emphasized that vegetables are potential risk factor for transmission of helminthes infection to human in Northern Iran. It is necessary that health authorities trained the consumers to proper and standard washing of vegetables before consumption.

**Keywords:** Vegetables, Contamination, Soil-transmitted helminthes, Northern Iran
1. Introduction

Soil-transmitted helminthes (STHs), food-borne parasitic infections, are responsible for significant burden of morbidity and mortality worldwide. It is estimated that 819 million people are infected with *Ascaris lumbricoides*, 465 million with *Trichuris trichiura*, and 439 million with hookworms globally (Pullan et al., 2014). An estimated 4.98 million or more disability-adjusted life years have been attributed to STH infections (Pullan et al., 2014). Moreover morbidity due to STH infections has primarily been associated to a range of complications, including gastroenteritis, malnutrition, anemia, intestinal obstruction, poor physical and cognitive development, nutrient absorption and iron loss (Hotez et al., 2008; Nokes and Bundy, 1994).

Vegetables and fruit are an important part of a healthy and balanced meal, providing vitamins, minerals, and phytonutrient (Losio et al., 2015). The increased global tendency for eating raw or slightly cooked vegetables and also rapid transport of foods especially soft fruit and vegetables may increase the risk of foodborne infections (Fallah et al., 2012; Kozan et al., 2005). Direct ingestion of human-infective eggs due to consumption of raw vegetables and fruits without proper washing is one of the major routes of infection (Adenusi et al., 2015; Kozan et al., 2005). In recent years, several studies have shown STH contamination of fresh vegetables and fruits, suggesting their important role in human infection (Adamu et al., 2012; Adanir and Tasci, 2013; Daryani et al., 2008; Ezatpour et al., 2013; Fallah et al., 2016; Fallah et al., 2012; Gupta et al., 2009; Kozan et al., 2005; Maikai et al., 2012).

Northern Iran is a highly endemic area for intestinal parasites (Daryani et al., 2012; Omrani et al., 2015). Up to our knowledge, there is no previously published study on the STH
contamination of vegetables in northeastern Iran. Therefore, the main aim of this study was to
determine the prevalence of STH contamination in commonly used vegetables in Mazandaran province, north of Iran and influence of season and standard washing procedure on burden of the
STH in the vegetables.

2. Materials and methods

2.1. Study area

This cross sectional study was carried out in four cities (Noor, Amol, Babol and Sari) in
Mazandaran Province, Northern Iran, from May 2014 to February 2015. This area (36.5° 25´N
53° 21´E) has a hot-summer Mediterranean climate, a mean annual temperature of 16°C and
about 900 mm of precipitation falls annually. According to Statistical Centre of Iran (SCI), the
number of total population in this area is about 3,100,000 (SCI, 2012).

2.2. Sample collection

A total of 772 samples of commonly consumed raw vegetables (193 samples in each city,
386 samples in warm seasons [193 spring and 193 summer] and 386 samples in cold seasons
[193 autumn and 193 winter]) were obtained (200-300 gr each) from retail markets (Table 1).
The included vegetables for this study were Radish (Raphanus sativus), Scallion (Allium
wakegi), Spinach (Spinacia oleracea), Parsley (Petroselinum crispum), lettuce (Lactuca sativa),
Green onion (Allium ascalonicum) and Mint (Mentha piperita). Vegetables were collected
separately in nylon sterile polythene and transported to the laboratory for parasitological
examination.
2.3. Sample preparation and examination

Each sample was divided into two groups. One group was used as the unwashed sample and the second group was washed with standard washing procedures according to Iranian Ministry of Health and Medical Education protocol. Briefly, in first washing stage, the leaves of leafy vegetables were separated and immersed in tap water inside a sink. Thus, mud and dust of these vegetables were removed. In the second stage, separation of helminth eggs was performed by 3 to 5 droplets of detergent per liter for 5 min. Then, disinfection of vegetables was conducted by calcium hypochlorite solution (with 200 mg/l free chlorine) for 5 min; and finally the disinfected vegetables were washed with tap water (Ministry of Health and Medical Education of Iran, 2010; Yarahmadi et al., 2012). The unwashed vegetable samples were tested for presence of STH eggs and larvae using the formalin ether concentration technique according to the methods described previously (Adenusi et al., 2015; Organization, 1991, 1994). Helminth eggs were identified by microscopic observation (Zeiss, Germany, 100× and 400× magnification).

2.4. Analysis of data

The data were analyzed using the SPSS software version 20 for windows (SPSS Inc., Chicago, IL, USA). The chi-square test was used to compare the differences in rate of contamination among warm and cold seasons and between unwashed and washed vegetables. At expected frequencies less than five, the statistical significance was calculated using simulation by the Monte Carlo method bases on 10,000 replicates. Associations were tested using odds ratios (OR) and 95% confidence intervals (CI) after adjustments. A $P$-value <0.05 was considered statistically significant. The mean number of STH eggs was calculated as described previously (Choi and Lee, 1972).
3. Results

Across the 772 vegetable samples, the overall prevalence of STH was 14.89% (115/772). The distributions of the vegetables sampled according to the each season and percentage of contaminated samples with STH eggs in different seasons are presented in Table 1. The rate of parasitic contamination in vegetable samples was the highest in summer (48.3%) and the lowest in winter (6.7%) \((P < 0.001)\). Table 2 and 3 summarizes the results of parasitic contamination of vegetables during warm and cold seasons. The rate of STH contamination was significantly higher in warm seasons (20.5%, 79/386) than in cold seasons (9.3%, 36/386) among the unwashed vegetables \((\text{OR}= 2.50; \text{CI}_{95\%}=1.64-3.8; \text{P} < 0.001)\). No parasites were observed in standard washed samples \((\text{OR}= 271.40; \text{CI}_{95\%}=16.84-4373.64; \text{P} < 0.001)\). The prevalences of STH species in all the vegetables were as follows: \textit{Ascaris lumbricoides} (3.36%), \textit{Trichuris trichiura} (2.2%), hookworms (2.9%), \textit{Toxocara} spp. (1.68%), \textit{Trichostrongylus} spp. (1.55), \textit{Taenia} spp. (0.9%) and \textit{Hymenolepis nana} (2.2%). Prevalence of STH contamination was significantly higher in leafy vegetables (spinach, parsley, lettuce and mint; 17.39% \([80/460])\) than root vegetables (radish, scallion and green onions; 11.2% \([35/312])\) \((\text{OR}= 1.67; \text{CI}_{95\%}=1.09-2.55; \text{P} < 0.05)\). There was no significant difference in the incidence of STH contamination among the four cities \((P = 0.72)\) (fig. 1). Table 4 shows a comparison of the mean number of STH contamination per 200 grams of vegetables. The mean number of STH contamination was 0.42 from radish, 0.34 from scallion, 0.46 from spinach, 0.43 from parsley, 0.46 from lettuce, 0.33 from green onion and 0.26 from mint.
4. Discussion

A downside to the potential health benefits is that raw, leafy, green vegetable products are responsible for the transmission of foodborne pathogens, including bacteria, protozoa and Soil-transmitted helminthes (STHs) (Losio et al., 2015). Recent outbreaks of foodborne diseases in both of Europe and the USA demonstrated that there are a significant relationship between pathogen contamination and vegetable consumption (Mercanoglu Taban and Halkman, 2011). Studies on helminthological contamination of vegetables in Iran are rare. This survey is first study investigating only helminthological contamination of vegetables in Iran. The results obtained in this study have shown moderate STH contamination (14.89%) of vegetables in Northern Iran that is lower than the prevalence of parasitic contamination reported in Shahrekord, southwest Iran (Fallah et al., 2016; Fallah et al., 2012), Khorramabad, western Iran (Ezatpour et al., 2013), and in Ardebil, northwest Iran (Daryani et al., 2008). The use of rivers and springs water to irrigate vegetables in this area could be a possible explanation to this lower prevalence of STH in this region, whereas due to water shortage in other areas of Iran, sewage effluent or water supplies contaminated with sewage are used to irrigate vegetables. In addition, previous studies have shown that uncontrolled use of water contaminated with sewage or human and animal faeces to irrigate of vegetables (at least 20 million hectares in 50 countries) is responsible for their high rates of contamination with STH eggs, especially in developing countries (Kozan et al., 2005; Hussain et al., 2001). It is important, because that a proportion of the vegetables cultivated in these developing countries are exported to the developed world (Kozan et al., 2005; Robertson & Gjerde, 2001). Also, the overall prevalence of STH in our study (14.89%) is higher than the 6.3% in Burdur, Turkey (Adanir and Tasci, 2013), 3.5% in
Maiduguri, northeast Nigeria (Adamu et al., 2012), and 8.44% reported in Ogun State, southwest Nigeria (Adenusi et al., 2015).

Our finding revealed that the majority of the contaminated vegetable samples were leafy vegetables (10.36%), especially in spinach, parsley and lettuce. A possible description could be that vegetables like lettuce have broad leaves and large surface areas, leading to more contact with the sewage contaminated soil surface (Adamu et al., 2012). The rate of STH contamination was smaller in root vegetables (4.53%), suggesting that broad leaves and more contact with soil are important predictors to STH contamination. Similar with our study, lettuce and spinach were the vegetables contaminated most commonly in Ankara, Turkey (Kozan et al., 2005), Benha, Egypt (Eraky et al., 2014), Maiduguri, northeast Nigeria (Adamu et al., 2012), Riyadh, Saudi Arabia (Al-Megrini, 2010), and in Kaduna State, Nigeria (Maikai et al., 2012).

Another important objective of this study was to ascertain whether standard washing procedures and climate changes have influence in rate of STH contamination. The standard washing procedures is differing substantially from what is called traditional washing. In traditional procedure, the vegetables are immersed in tap water inside a sink. After a short period to sedimentation of mud and dust, they are gently collected, put in a wood or plastic basket, and rinsed for 1.5-2 min with tap water (Fallah et al, 2012). In fact, unlike standard washing procedures, no detergent or disinfectant solution is used in traditional washing. No parasites were observed in standard washed samples. In agreement with our study, Kozan et al. in Ankara, Turkey, founded STH contamination in 5.9% of unwashed vegetable samples and none in washed samples (Kozan et al., 2005). Also, Fallah et al. have reported that rate of parasitic contamination is significantly lower in pre-washed and standard washed samples than unwashed samples. They founded intestinal parasites from 32.6% of unwashed, 1.3% of traditionally
washed and not in any standard washed samples (Fallah et al., 2012). Although it should be noted that the possible unsafety of the water used for the washing of vegetables may be responsible for some reported contaminations in our investigation and studies conducted by other researchers. In addition, results of the present study have showed that rate of contamination was significantly higher in warm seasons. This result is consistent with previous studies in Iran and the world (Al-Megrin et al., 2010; Eraky et al., 2014; Ezatpour et al., 2013; Fallah et al., 2012). A possible explanation could be higher shedding of helminthes eggs in warm seasons by humans and animals. In addition, the majority of days in cold seasons are rainy in northern Iran, resulted in probable wash of helminthes eggs from the surface of vegetables (Fallah et al., 2016). Climate change is an important determinant of transmission of STH and moisture and warm temperature are essential for larval development and eggs survival in the soil. Taking into account that many of developing countries and countries with not appropriate hygienic condition (such as India and African, Middle East and Southeast Asian countries) have warm weather in during the year, the more rate of STH contamination in warm season cannot be overlooked. As reported by Pullan et al. (2014) the vast majority of STH infections (>75%) and years lived with disability (>70%) occurred in Asian and African countries. Moreover, de Silva et al. have reported that highest rates of Ascaris infection occur in China and Southeast Asia, in the coastal regions of West Africa, and in Central Africa. Trichuris infections reach their highest prevalence in Central Africa, southern India and Southeast Asia. Hookworm infections, however, are common throughout much of sub-Saharan Africa, in addition to South China and Southeast Asia (Silva et al., 2003).

Despite sustained improvement in sanitary conditions over the past three decades, intestinal parasitic infections are still highly prevalent in Iran. It is estimated that the overall prevalence of
The prevalence rate for some of the more common helminthes parasites in Iran is as follow: *A. lumbricoides* 10.9%, and *H. nana* 2.3%, hookworms <1%, *T. trichiura* <1%, *Strongyloides stercoralis* (in endemic areas) 4.9-42%, *Taenia* spp. <1% (Omrani et al., 2015; Pullan et al., 2014; Rokni, 2008, 2009). In this study, the most detected STH eggs were *A. lumbricoides*, hookworm eggs, *T. trichiura* and *H. nana*, respectively. The high fecundity of *A. lumbricoides* and *T. trichiura*, high resistant these helminthes eggs that can persist in the environment for months to years and high rate of these helminthes infections in the humans could be significant reasons for high STH contamination of vegetables in this area and other areas in the world with hot and humid climates.

Northern Iran is highly endemic area for hookworm infections (Necatoriasis) and Taeniasis (Hydatidosis) (Rokni, 2008, 2009). High prevalence of these infections in human and animals and poor sanitation in area with agricultural and pastoral culture can be source of vegetables contamination. In addition, results of this study indicate that vegetables can be an important risk factor for such infections in human.

The vegetables surveyed in our study are untreated and unwashed and are usually sold unpacked in variable values as chosen by the customers. Moreover, vegetables from the northern Iran are exported to neighboring provinces in Iran and neighboring countries. Therefore, this study is also important with regard to this point of view.

**5. Conclusion:**

In conclusion, the results of the present study emphasized that if standard washing and disinfecting procedures of the raw eaten vegetables be neglected, they could be considered as a potential risk factor for transmission of helminths infection to human. It is necessary an
integrated approach to improve the sanitary conditions in the areas where the vegetables are cultivated. In addition, the health authorities should train the consumers to proper and standard washing of vegetables before consumption.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Health Organization.

Health Organization.

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Figure 1: Distribution of contaminated raw vegetables with soil transmitted helminthes (STHs) eggs according to different cities in Mazandaran province, northern Iran.
Table 1: Number of vegetables and percentage of contaminated samples with soil transmitted helminthes (STH) eggs in different seasons.

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>$P$ Value$^A$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>Infected (%)</td>
<td>number</td>
<td>Infected (%)</td>
<td>number</td>
</tr>
<tr>
<td>Radish</td>
<td>29</td>
<td>5 (17.2)</td>
<td>29</td>
<td>7 (24.1)</td>
<td>29</td>
</tr>
<tr>
<td>Scallion</td>
<td>24</td>
<td>2 (8.3)</td>
<td>25</td>
<td>3 (12)</td>
<td>24</td>
</tr>
<tr>
<td>Spinach</td>
<td>29</td>
<td>7 (24.1)</td>
<td>29</td>
<td>11 (37.9)</td>
<td>29</td>
</tr>
<tr>
<td>Parsley</td>
<td>29</td>
<td>8 (27.5)</td>
<td>29</td>
<td>8 (27.5)</td>
<td>29</td>
</tr>
<tr>
<td>Lettuce</td>
<td>28</td>
<td>6 (21.4)</td>
<td>28</td>
<td>9 (32.1)</td>
<td>28</td>
</tr>
<tr>
<td>Green onion</td>
<td>25</td>
<td>2 (8)</td>
<td>24</td>
<td>2 (8.3)</td>
<td>25</td>
</tr>
<tr>
<td>Mint</td>
<td>29</td>
<td>4 (13.7)</td>
<td>29</td>
<td>5 (17.2)</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>193</td>
<td>34 (36.5)</td>
<td>193</td>
<td>45 (48.3)</td>
<td>193</td>
</tr>
</tbody>
</table>

$^A$ = Chi-square Test or Chi-square Test with Monte Carlo simulation

* $P \leq 0.05.$
Table 2: Contamination of vegetables with soil transmitted helminthes (STH) eggs in spring and summer (warm seasons).

<table>
<thead>
<tr>
<th></th>
<th>Common STH</th>
<th>Uncommon STH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascaris</td>
<td>Toxocara</td>
</tr>
<tr>
<td></td>
<td>eggs no. (%)</td>
<td>ra eggs no. (%)</td>
</tr>
<tr>
<td>Radish (Raphanus sativus)</td>
<td>3 (5.17)</td>
<td>1 (1.72)</td>
</tr>
<tr>
<td>Scallion (Allium wakegi)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Spinach (Spinacia oleracea)</td>
<td>4 (6.89)</td>
<td>3 (5.17)</td>
</tr>
<tr>
<td>Parsley (Petroselinum crispum)</td>
<td>2 (3.44)</td>
<td>5 (8.62)</td>
</tr>
<tr>
<td>Lettuce (Lactuca sativa)</td>
<td>5 (8.92)</td>
<td>2 (3.57)</td>
</tr>
<tr>
<td>Green onion (Allium ascalonicum)</td>
<td>0 (0.00)</td>
<td>1 (2.04)</td>
</tr>
<tr>
<td>Mint (Mentha piperita)</td>
<td>1 (1.7)</td>
<td>2 (3.44)</td>
</tr>
<tr>
<td>Total</td>
<td>38 (3.88)</td>
<td>14 (3.62)</td>
</tr>
</tbody>
</table>
Table 3: Contamination of vegetables with soil transmitted helminthes (STH) eggs in autumn and winter (cold seasons).

<table>
<thead>
<tr>
<th>N o</th>
<th>Common STH</th>
<th>Uncommon STH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascaris eggs no. (%)</td>
<td>T. trichiura eggs no. (%)</td>
</tr>
<tr>
<td>Radish (Raphanus sativus)</td>
<td>2 (3.44)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Scallion (Allium wakegi)</td>
<td>1 (2.04)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Spinach (Spinacia oleracea)</td>
<td>1 (1.72)</td>
<td>1 (1.72)</td>
</tr>
<tr>
<td>Parsley (Petroselinum crispum)</td>
<td>1 (1.72)</td>
<td>1 (1.72)</td>
</tr>
<tr>
<td>Lettuce (Lactuca sativa)</td>
<td>2 (3.57)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Green onion (Allium ascalonicum)</td>
<td>3 (6.12)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Mint (Mentha piperita)</td>
<td>1 (1.72)</td>
<td>1 (1.72)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (2.84)</td>
<td>3 (0.77)</td>
</tr>
</tbody>
</table>
Table 4: Total and mean number of soil transmitted helminthes (STH) eggs found per 200 gram from each vegetable.

<table>
<thead>
<tr>
<th></th>
<th>Radish</th>
<th>Scallion</th>
<th>Spinach</th>
<th>Parsley</th>
<th>Lettuce</th>
<th>Green onion</th>
<th>Mint</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of vegetable examined</td>
<td>103</td>
<td>84</td>
<td>110</td>
<td>106</td>
<td>96</td>
<td>82</td>
<td>112</td>
</tr>
<tr>
<td>Total vegetable weight (gram)</td>
<td>27600</td>
<td>20800</td>
<td>29000</td>
<td>25200</td>
<td>29200</td>
<td>21400</td>
<td>30400</td>
</tr>
<tr>
<td>Total number (mean number per 200 gr) of <em>Ascaris</em> eggs from vegetable</td>
<td>22 (0.15)</td>
<td>8 (0.07)</td>
<td>23 (0.15)</td>
<td>11 (0.08)</td>
<td>21 (0.14)</td>
<td>8 (0.07)</td>
<td>5 (0.03)</td>
</tr>
<tr>
<td>Total number (mean number per 200 gr) of <em>T. Trichiura</em> eggs of vegetable</td>
<td>7 (0.05)</td>
<td>0 (0.0)</td>
<td>9 (0.06)</td>
<td>14 (0.11)</td>
<td>13 (0.08)</td>
<td>4 (0.03)</td>
<td>8 (0.05)</td>
</tr>
<tr>
<td>Total number (mean number per 200 gr) of Hookworm eggs of vegetable</td>
<td>11 (0.07)</td>
<td>7 (0.06)</td>
<td>14 (0.09)</td>
<td>15 (0.11)</td>
<td>9 (0.06)</td>
<td>9 (0.08)</td>
<td>13 (0.08)</td>
</tr>
<tr>
<td>Total number (mean number per 200 gr) of other helminthes eggs of vegetable</td>
<td>19 (0.13)</td>
<td>21 (0.2)</td>
<td>22 (0.15)</td>
<td>15 (0.11)</td>
<td>25 (0.17)</td>
<td>13 (0.12)</td>
<td>15 (0.09)</td>
</tr>
<tr>
<td>Total helminthes eggs</td>
<td>59 (0.42)</td>
<td>36 (0.34)</td>
<td>67 (0.46)</td>
<td>55 (0.43)</td>
<td>68 (0.46)</td>
<td>36 (0.33)</td>
<td>41 (0.26)</td>
</tr>
</tbody>
</table>
Highlights

- We evaluate the prevalence of Soil-transmitted helminthes in vegetables in northern Iran
- The overall prevalence of STHs was 14.89% (115/772)
- The rate of STHs contamination was associated with climate changes
- The rate of STHs contamination was associated with washing procedures
- No parasites were observed in standard washed samples