Original Article

Detection of Parasitic Contamination in Ready-to-Eat Fresh Packaged Herbs Sold in Tehran, Iran

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Abstract

Introduction: Consumption of raw herbs plays a major epidemiological role in the transmission of some parasitic food-borne diseases. Some manufacturers are producing different kind of fresh ready-to-eat packaged herbs in Iran. Therefore, this study was carried out to evaluate the parasitic contamination in fresh ready-to-eat packaged herbs from one of major manufacturers of this kind of product in Tehran, Iran.

Materials & Methods: In this descriptive study, a total of 200 packages of fresh ready-to-eat herbs were purchased from a distributor in Tehran, Iran during July 2014 to April 2015. The total content of each package was used as the sample which, was placed into an Erlenmeyer flask. Then, the detergent solution was added and the flask content was shaken. The herbs were removed and the washing solution was filtered through 0.2 µm filter. The filtrate was discarded, the filter was eluted by detergent solution, and the content was shaken for one minute. The filter was discarded. The solution was centrifuged at 7000 g for 10 minutes and the sediment was examined via microscope.

Results: In the current study, 8.5% of samples were reported to have parasitic contamination. Cyst and oocyst of *Cryptosporidium* spp. (4%), *Giardia* spp. (3%) and *Entamoeba* spp. (1.5%) were identified in the present study.

Conclusion: The study findings highlighted the potentiality of fresh ready-to-eat packaged herbs to serve as transmission vehicle for parasites. In addition, this study demonstrated that the washing method used by the manufacturer of this product was not appropriate.

Keywords: Food-borne diseases, Vegetables, Iran, Cryptosporidium, Giardia, Entamoeba

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Introduction

The prevalence of some important parasitic diseases such as Cryptosporidiosis, Hydatidosis and Toxoplasmosis is reported high in Iran. Consumption of improperly washed vegetables can be mentioned as one of the reasons for this high prevalence. In Iran most families are not cognizant of hazards that can put their lives in danger via consuming of improperly washed raw vegetables. Each year, dozens of people in Iran undergo the surgery due to Hydatidosis. In addition, a high number of individuals, including immunocompromised ones may lose their lives due to Toxoplasmosis and Cryptosporidiosis. Treatment of the mentioned diseases is regarded hard and even impossible in some cases and has a high economic cost for the families and countries, whereas prevention of such diseases seems to be easy.

In recent years, production of packaged ready-to-eat vegetables has increased, specifically in large cities of Iran. Fresh herbs, as a highly consumed vegetable [1, 2], could be introduced as a mean for transmission of some parasites [3, 4]. As a matter of fact, contaminated fresh herbs can normally considered as a potential source of human infection, during production, collection, transport, preparation, processing or even at home [5].

In many developing countries as well as Iran, several factors are reported to be responsible for the high rates of contamination with pathogenic parasites, including use of insufficiently treated wastewater to irrigate herbs and increase in highly susceptible persons, which can be related to their, aging, malnutrition, HIV infection and other underlying medical conditions [6, 7].

Many studies have demonstrated parasitic contamination in fresh washed or unwashed herbs, whereas no study has been conducted on the fresh packaged herbs in Iran. The prevalence of specific parasites in herbs varies between various countries [8] so as the findings indicate different levels of parasitic contamination of raw vegetables in different regions [9]. Therefore, this study was carried out to evaluate the parasitic contamination of fresh ready-to-eat packaged herbs from one of the major manufacturer of this product in Tehran, Iran from July 2014 to April 2015.

Materials and Methods

In this descriptive study, a total of 200 packages of fresh ready-to-eat herbs were investigated from a distributor in Tehran, Iran within 10 months from July 2014 to April 2015. Every month, 20 samples were selected via the simple random sampling method using the random number table (http://www.randomizer.org/form.htm). Each package (approximately 200 grams) consisted of leek *(Allium porrum)*, cress *(Lepidium sativum)*, basil *(Ocimum basilicum)*, tarragon *(Artemisia*

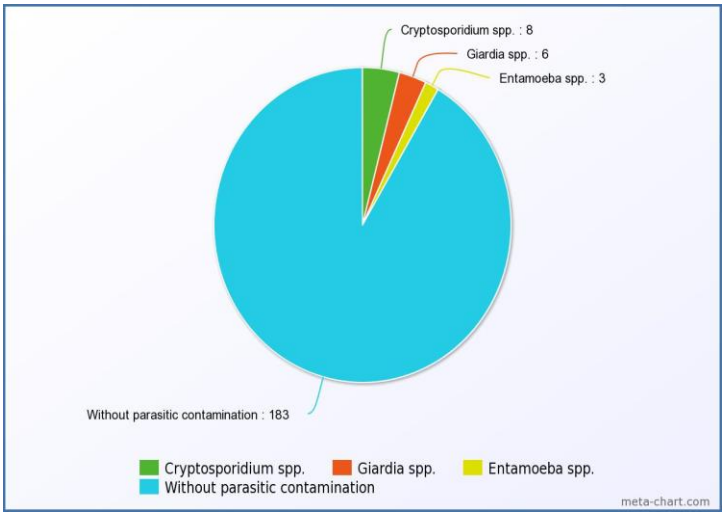
dracunculus), mint (*Mentha kopetdaghensis*), radish *(Raphanus sativus)* and scallion *(Allium fistulosum)*, which were used as the study sample.

The sample was placed into a 1000 ml sterile Erlenmeyer flask, and then the volume was increased to 1000 ml using a detergent solution (containing 10 mM of Phosphate-buffered saline (PBS), 0.1% of sodium dodecyl sulfate (L3771 Sigma-Aldrich, USA), and 0.1% of Tween-20 (P1379 Sigma-Aldrich, USA)) [10]. The content was shaken for one hour on an automatic shaker (Wise Cube WIS-20, Wisd Laboratory Equipment, Germany), the herbs were removed by a sieve [11, 12], and the washing solution was filtered through a 0.2 µ filter (Z222593 Aldrich, Zap Cap bottle-top filter, USA). Moreover, the filtrate was discarded, and the filter was eluted by a detergent solution in a 15 ml tube. The content was shaken for one minute, and the filter was discarded. Ultimately, the solution was centrifuged at 7000 g for 10 minutes, and its sediment was examined by a light microscope (BH2 Series**,** Olympus, Japan). Modified Ziehl-Neelsen acid-fast stain was applied for staining of coccidian protozoa oocysts. The prevalence of each detected parasite was calculated using descriptive statistics via SPSS (ver. 16) [5].

Results

In the current study, 17 samples (8.5%) were reported to have parasitic contamination, including cyst and oocyst of *Cryptosporidium* spp. (4%), *Giardia* spp. (3%) and *Entamoeba* spp. (1.5%), among which *Cryptosporidium* spp. was the most prevalent parasite (Figure 1). Moreover, the results of the present study revealed no helminth contamination. The total time needed to examine each sample lasted two hours approximately from sample preparation to microscopic examination.

Figure 1. Number of positive and negative samples according to each detected parasite



Discussion

In developing countries, intestinal parasites are very prevalent and consumption of raw vegetables can play an important role in the transmission of such parasitic contaminations [2]. Recovery of parasites from vegetables may be helpful in indicating the incidence of intestinal parasites among a community [4]. All studies on parasitic contamination of vegetables in Iran used traditional sedimentation and floatation method regarding the first stage isolation of parasites from vegetables which is a time consuming process. In this study, parasites' cysts or oocysts were isolated via applying the filters.

This study represented that, 8.5% of considered herbs underwent parasitic contamination which is lower than that of other studies conducted in other cities of Iran such as Ahwaz [13], Kerman [14], Shahrekord [1], Golestan [15], Semnan [16], Khoramabad [17] and Ardabil [4]. The results of the present study demonstrated that, oocyst of *Cryptosporidium* spp. was found in 4% of the total samples, which is lower than the results declared by other researchers such as Razavi *et al* in Shiraz [18] and Bahadori *et al* in Tehran [19]. Moreover, *Giardia* spp. was observed in 3% of the total samples, which was found to be lower than the rate reported by some scholars such as Daryani *et al* in Ardabil [4], Saki *et al* in Ahwaz [13] and Garedaghi *et al* in Tabriz [20]. In the current study, *Entamoeba* spp. was identified in 1.5% of the total samples. This result is lower than the results announced by Ezatpour *et al* in Khoramabad [17], Esboei *et al* in Golestasn [15], and Saki *et al* in Ahwaz [13]. Several factors such as geographical location, type of water used for irrigation, post-harvesting handling methods of vegetables and level of development of various countries may contribute to differences in reported prevalence rates [2, 21].

The findings of this study provide evidence for the risk of acquiring parasitic contamination in the fresh ready-to-eat herbs. Therefore, effective measures are necessary to be taken in order to reduce the parasitic contamination in this product [9]. As a matter of fact, findings of the current study could be used to ameliorate control of the food-borne parasitic diseases in Iran. Although the prevalence of parasites in the present study was reported lower than that of other studies, more control measures and standards necessitate to be applied with respect to quality control of fresh ready-to-eat packaged herbs. The local authorities are demanded to take more controls on washing method used by the manufacturers of this product.

Utilizing filtration method for isolation of parasites can be stated as the strength of the current study, whereas applying a light microscope as the only tool used for the detection of parasites can be mentioned as the study limitation, since microscopic methods

seem to have low sensitivity which could be solved by applying molecular methods.

Conclusions

The results of the current study showed that more hygienic control should be established for manufacturers of fresh ready-to-eat vegetables in Iran. In addition, quality of packaging was very bad for most of these products in Iran and it is possible that contamination takes place during transportation. The author suggested that producers of fresh ready-to-eat vegetables use modern automatic systems for washing and packaging process instead of obsolete and manual ones.

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