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Full Length Research Paper

Hygienic Assessment of Pathogenic Contamination in Raw Vegetables in Local Markets: an Implication for Public Health

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Abstract

This cross-sectional study aimed to assess the hygienic status of commonly used raw vegetables through the identification of their possible contamination with parasites as well as to the hygienic capability of using tap water in washing raw vegetables. Overall, 240 vegetable samples were randomly collected from local market. Four common types of raw vegetable (60 for each type), 120 of them were washed with tap water while the other half were washed with normal saline, with the aim of comparing their efficiency for parasite extraction from raw vegetables. Among samples washed with tap water, 28 (23.3%) were contaminated, while different type of parasites were detected in 30 (25%) of the samples washed with normal saline solution. With the exception of radish, all other three types of vegetables were contaminated with ova of *Ascaris lumbricoides* and *Hymenolepis nana,* while *Trichuris trichiura* was isolated only from green onions. Cysts of *Entameba histolytica* and *Giardia intestinalis* were extracted from radish while *Giardia intestinalis* was detected only in leeks. Tap water showed similar efficiency as normal saline in extraction of pathogenic parasites. These findings have important implications for food safety. Raw vegetables may threaten public health by transmitting intestinal parasites to humans. Further studies are recommended for a better understanding of the occurrence of possible infectious pathogens in raw food, and the status of hygienic knowledge, attitudes and practices among vegetables producers.

Keywords: Public Health, Food Protection, Food Policy, Food Hygiene, Food Safety, Vegetables.

INTRODUCTION

Vegetables are essential part of a human diet. They are sometimes eaten raw, without cooking to destroy pathogens. Thus they are potential sources of food-borne illnesses, which are one of important public health problem. Parasitic diseases are example of these diseases, which can be transmitted through raw vegetables that have been contaminated by human or animal feces (CDC, 2010). Foodborne diseases are an important public health threaten and challenge. Evidence from United States indicated that foodborne diseases affect tens of millions of people and kill thousands, in U.S. each year, which cause billions of dollars in healthcare-related and industry costs annually (CDC, 2011), and it is assumed for the situation to be worse in many poor and developing country. Infection with pathogenic parasites has been associated with morbidity and mortality, especially in young malnourished children and immune-suppressed people (Sullivian *et al*., 1990; Guerrant and Bobak, 1991).

Indeed, severity and symptoms of foodborne parasitic infections vary greatly depending on the type of parasite. Protozoa such as *Cryptosporidium* spp., *Giardia intestinalis*, and *Cyclospora cayetanensis* most commonly cause diarrhea and other gastrointestinal symptoms. Whereas, helminthic infections can cause abdominal pain, diarrhea, malnutrition, weight loss, muscle pain, neurological and many other symptoms and that depending on the particular organism and burden of infection (CDC, 2010). Moreover, report from Centers for Disease Control and Prevention (CDC) summarizes 2012 preliminary surveillance data and describes trends since 1996. A total of 19,531 infections, 4,563 hospitalizations, and 68 deaths associated with foodborne diseases were reported in 2012. For most infections, incidence was highest among children aged <5 years (CDC-MMWR, 2013).The prevalence of parasitic infections varies largely among different population groups and the highest rate of infection is in tropical and sub-tropical countries, especially in those with low socio-economic level (WHO, 1987; Black, 1993; Shubair *et al*., 2000).

The distribution of these infections depends on conditions such as suitable climate and human behavior and activities including population movements as well as poor sanitation. Transmission of some helminthic parasites takes place throughout the year in regions with a temperate climate. The soil transmitted parasites, mainly, *A. lumbrcoides* and *T. trichura* are usually more prevalent where there is favorable soil, warmth and moisture together with poor sanitation.

Yemen as one of the poor developing countries and due several reasons suffer from a wide range of parasitic infections in humans, among them soil transmitted parasites have a significant impact on public health all over the country. However, studies have been conducted in Yemen on different parasites with direct life cycles, showing prevalence values of 24%-68% for ascariasis (Farag, 1985; Azazy & Al-Taiar, 1999; Raja'a *et al*., 2000), 1-21% for trichiuriais (Raja'a *et al*., 2001; Azazy *et al*., 2002), 4-36% for *E. histolytica* (Farag, 1985; Raja'a *et al*., 2000; Raja'a *et al*., 2001), 9-20% for *G. intestinalis*, 2-9% for *H. nana* and 2-4%, for *Enterobious vemicularis* (Azazy & Al-Taiar, 1999; Raja'a *et al*., 2000; Raja'a *et al*., 2001). In the United States, the most common foodborne parasites are protozoa such as *Cryptosporidium spp., Giardia intestinalis, Cyclospora cayetanensis, Toxoplasma gondii,* and *Entamoeba histolytica;* roundworms such as *Trichinella spiralis* and *Anisakis spp.;* and tapeworms such as *Diphyllobothrium spp.* and *Taenia spp* (CDC, 2010). Very low rate of infection has been reported (3-2%) for *Fasciola hepatica* and (2%) for *Taenai* species (Farag, 1985; Raja'a *et al*., 2000; Raja'a *et al*., 2001). Said in her study which was conducted in Egypt reported that intestinal parasites were detected in 31.7% of the examined vegetables samples. The parasites she detected were including *Ascaris lumbricoides eggs*, *Toxocara spp. eggs*, *Hymenolepis nana eggs, Giardia spp. cysts, Cryptosporidium spp. oocysts, Cyclospora spp. oocysts* and *Microsporidium spp. Spores* (Said, 2012).

It should be appreciated that individuals in poor and developing communities might be practicing a social habit of poor hygienic behaviors such as not washing vegetables properly and poor personal hygiene. Correspondingly, it is better to be stressed on that people might neither aware of the potential risks of the occurrence of pathogens in their food, the severity and cost of the illnesses they might acquire. Moreover, in developing countries communities might lack of efficient public health policies regarding food production and food marketing. Therefore, the outcomes of this study seek to support and highlight the need for health policy tackling food protection issues and related regulation. Therefore, the aim of this study is to assess the hygienic and pathogenic contamination in common vegetables used for raw consumption in Sanna market, Yemen. Special concern was given to discover the existence of pathogenic parasites in raw vegetables and the level of efficiency of using tap water for washing vegetables with compare to normal saline.

MATERIALS AND METHODS

This study was conducted on raw vegetables from local markets in Sana'a city, Yemen. Four different types of raw vegetables (N=240) namely, Lettuce, Green onions, Leeks and Radish. Sixty from each type of vegetables were purchased from a central local vegetable's market randomly. All samples were processed immediately on arrival to extract parasites elements using two different washing processes; namely normal saline solution and tap water in order to assess possible presence of parasites and the efficiency of different washing. Washing process included the use of tap water and normal saline solution (0.95%).

Before processing the vegetable samples were divided into halves in order to compare the extraction efficiency of tap water with that of saline solution. For this purpose, half of vegetable samples (30 for each vegetable type) were washed with tap water and the rest with normal saline solution. Each sample was soaked in 300 ml of tap water and another with the same volume of normal saline solution for 10 minutes. Next, each preparation was sieved through sterile gauze and left to sediment for one hour at room temperature. Subsequently, the supernatant was decanted while the sediment was collected in a 15 ml centrifuge tube.

The tube was then filled with each corresponding washing solution and centrifuged at 1500 rpm for 3minutes. The supernatant was decanted and the sediment was examined microscopically screening for the possible contamination for either ova or cysts of *helmenthic*

and *protozoal* parasites, respectively.

Data were compiled, analyzed specially to assess the pathogenic parasites in raw vegetables and the efficiency of using tap water with compare to normal saline. The results were presented in frequency table with numbers and percentage. Moreover, Z-value used to show the level of difference between the two methods used for washing vegetables. The differences were considered significant at $p< 0.05$.

RESULTS

A total of two hundred and forty samples of four different kinds of raw vegetables were collected from a local market and examined for possible occurrence of different parasites whose transmission is via Fecal-oral route. Table 1, demonstrates the rates of parasites detected in commonly used vegetables. Out of 120 samples of the four types of vegetables washed with normal saline solution, 30 (25%) were found to be contaminated with protozoal and helminthic parasites. Similarly, among 120 samples of raw vegetables washed with tap water has extracted 28 (23.3%) of the parasites which indicated the contamination of the raw vegetables.

Table 2, shows the distribution pattern of parasites extracted from the examined vegetables. *T. trichiura* was not detected in Lettuce that washed with tap water while it was detected in the samples washed with the normal saline solution. *H.s nana* was not detected when it washed using tap water but it has been found in the samples washed by normal saline in Green onion. Also in the same vegetable *A. lumricoides* was found when washed by tap water but not when washed using normal saline. In Leek, *A. lumbricoides* were extracted from the samples that have been washed by tap water but *G. intestinalis* was not, vice versa among the samples washed with normal saline. As in Leek, *E. histolytica* were not found in the samples that washed by normal saline but it was found in the samples which washed by tap water. In contrast, *G. intestinalis* has been detected in the Radish samples that washed by normal saline but were not found in the samples washed with tap water. With exception of Radish, all other three types of vegetables were contaminated with ova of *A. lumbricoides* and *H. nana*, while *T. trichiura* was isolated from Green onions and Lettuce. As shown in table 2, cysts of both pathogenic protozoal parasites namely*: E. histolytica* and *G. intestinalis* were extracted from radish and only *G. intestinalis* was detected in Leek. The non-pathogenic protozoan parasite; *E. coli* was present in all tested vegetables.

DISCUSSION

In this study, the pattern of parasites contamination of raw vegetables commonly used and sold in local markets was detected, identified and reported. As stated in (Table 1) raw vegetables were found to be contaminated with protozoal and helminthic parasites. Previously, several studies were conducted on identification of intestinal parasites from vegetables. Data from nearby developing country detected intestinal parasites in 31.7% of the examined vegetables samples. The parasites were detected including *Ascaris lumbricoides eggs*, *Toxocara spp. eggs*, *Hymenolepis nana eggs, Giardia spp. cysts, Crytosporidium spp. oocysts, Cyclospora spp. oocysts* and Microsporidium *spp. Spores* (Said, 2012). Moreover, an attempt to detect intestinal parasites from vegetables was carried out in Taiz, Yemen (Adam & Mohammed, 2004), but conducting only normal saline washed process. The study showed that the overall rate of contamination with parasites in fresh vegetables was 68.33%. The present study also focused on comparing the efficacy of tap water and normal saline for the extraction of parasites in daily used raw vegetables. The frequencies of parasite contaminations in the freshly used vegetables reported in the literature might be varied and perhaps relate to the level of environmental contamination as well as to the diagnostic methods used. It has been stated that, contamination of vegetables may occur in a variety of ways such as contact with soil as well as pre- and post-harvest contact, in most cases; however, it is associated with water used for irrigation (Vazquez *et al*., 1997; Robertson & Gjerde, 2001; Simoes *et al*., 2001).

Interestingly, data presented in this study revealed that tap water is an efficient in parasites extraction form vegetables, and supposed to be as good as normal saline solution. In line with that, previous experimental trail concluded that using tap water for hygienic purposes is efficient as normal saline in hygienic uses. Valente e*t al*. (2003) in their study of wound irrigation concluded that; "there were no clinically important differences in infection rates between wound irrigated with tap water or normal saline solution. Tap water might be an effective alternative to normal saline solution for wound irrigation in children" (Valente e*t al*., 2003). Similarly, in another trail study testing the hygienic efficiency of using tap water concluded that tap water appears to provide a safe alternative to normal saline for wound cleansing (Griffiths *et al.,* 2001). However, this finding in our study is in contrast to previous study which has been conducted on commonly used vegetables revealed that tap water showed very low efficiency in removal of parasites from vegetables as compared with saline (AL-Binali *et al.,* 2006). This could be due to a variety of factors associated with the quality of tap water used in both studies, the status of the selected tested vegetables. Indeed, tap water is more convenient to be used by individuals for vegetables wash. Therefore, this study stressed that individuals should not consume their vegetables or fruits without proper washing.

Interestingly, the data obtained in the present study

Table 1. Parasites detected in selected commonly used raw vegetables

Tested vegetables	Normal saline		Tap water	
	Positive (n)	Percentage (%)	Positive (n)	Percentage (%)
Lettuce	10/30	33.3	11/30	36.6
Green onion	9/30	30	7/30	23.3
Leek	7/30	23.3	4/30	13.3
Radish	4/30	13.3	6/30	20
Total	30/120	25	28/120	23.3

Test of significance between parasites detected by normal saline and tap water; *Z-value= 0.3016, P-value= 0.7630*

**For each type of vegetable, 60 samples were examined: 30 were washed with normal saline and 30 with tap water. The rate of contamination was calculated on 30 samples.

Table 2. Type of parasites extracted from selected commonly used vegetables

Tested Samples	Tap water washed	Normal saline washed
Lettuce	*- A. lumbricoides* *- H. nana* *- E. coli*	*- A. lumbricoides* *- H. nana* *- E. coli* *- T. trichiura*
Green onion	*- A. lumricoides* *- T. trichiura* *- E. coli.*	*- T. trichiura* *- E. coli* *- H.s nana*
Leek	*- A. lumbricoides* *- E. coli* *- H. nana*	*- E. coli* *- G. intestinalis* *- H. nana*
Radish	*- E. coli* *- E. histolytica*	*- E. coli* *- G. intestinalis*

revealed that *A. lumbricoides* was the most pathogenic parasite isolated in all examined samples. This finding is in agreement with other reports that showed *A. lumbricoides* as the most common parasites (De Oleivera & Germano, 1992; Mesquita *et al.,* 1999). A non-pathogenic parasite; *E. coli* was detected in all tested raw vegetables when both water and normal saline solution used for extraction which provide an evidence for fecal contamination. Although the both solutions were found to have similar efficiency in extraction of parasites from the raw vegetables as presented (Table 1), differences were exist in the type of the parasites in tested vegetables as Table 2 showed, this finding might be difficult to interpret. However, since the samples were randomly selected and distributed between both methods, the variation might be related to the sensitivity of the solutions or/and characteristics of the vegetables or the parasites themselves. This finding begs more questions regarding the interpretations for the differences in the extraction of specific parasite from the same type of vegetable using tap water compared to normal saline.

Surprisingly, in contrast to previous reports, ova of *Taenia species* or *F. hepatica* were absent in the tested vegetables of the present study. It was expected that *Taenia species* were to be detected from the tested raw vegetables. One possible source of soil contamination of *Taenia* species is the ova of *Echinococcus granulosus* from dogs. Supporting this statement, dogs which are the definitive host for *E. granulosus* are used as guards in the farms where cultivation is practiced. Previously, it has been stated that *hydatid* disease due to *E. granulosus* was found to be more prevalent in rural areas among people are in contact with dogs (Azazy & Abdelhamid, 2000).

Unfortunately, parasites are not often looked for routinely, beside most people in the communities use raw vegetables in their diet and are neither aware of the potential risks to their health posed by contaminated food nor they know the source of infection they acquire. Although the study highlights important information, the findings from this study are subject to at least three limitations. First, producers and vendors hygienic and food safety's knowledge and practices were not investigated. Second, many infections transmitted through food were not identified in this study. Third, the proportion of real impacts and illnesses transmitted by pathogen not determined for consumers, therefore, the data provided in this study do not exclusively relate to infections from foodborne sources.

CONCLUSION

In conclusion, the current study shows that raw vegetables were contaminated with various parasites. Thus, the study stressed that individuals should not consume their vegetables or fruits without proper washing using tap water as it is more convenient for all individuals in different socio-economic status.

Information cited from this presented study should increase awareness of public policy and health decision makers as well as should alert people knowledge and behaviors. As it has been recommended in previous study, in neighbor developing country, regarding food hygiene that policy makers should address and implement more safety and hygiene measurement and policy to promote healthy and safer food (Omer *et al.,* 2013). Whereas, a food manufacturer would need a fully documented Hazard Analysis and Critical Control Points (HACCP), local market vendors would need to learn and follow good hygiene routine and practice.

Media programs can be used to inform the community about good sanitation hygiene and the potential health risk of raw vegetables to prevent transmission of the foodborne diseases. In addition, health education program regarding food safety and food protection should be directed towards producers and vendors of food local markets, beside the importance of the quality of water which are used for irrigations should be monitored regularly. Since, the source of food contamination can be transmitted by workers who practice poor hygiene or work in unsanitary facilities more quality assurance procedures regarding food production are needed. Farmers' and local food markets should be subjected to routine checks for example by food safety officers from local environmental health and trading standards department. Farmers, food safety officers, as well as buyers and consumers, should be taught and know how to identify possible hazards to food safety, know which of hazards is actually important for the type of food that are prepared or sold. For example, food transported to a market must be wrapped, covered or placed in suitable containers to prevent any possible contamination. Vehicles and containers must be kept clean and in good repair. Eatable raw foods better to be washed after harvest and protected from the public touching, coughing or sneezing in the display area in the markets since there is tendency of trying, testing and eating products by customers. However, there are a lot of works to be done by policy makers. As Omer *et al.* recommended in their study for the need of proof of training in order to obtain license for slaughters and meet butchers (Omer *et al.,* 2013), the same policy can be adapted by the local authority responsible for food safety for vegetable's markets and businesses to apply upon greengrocer.

The findings highlight the need for effective health education, enforced health policy, clear vegetable production regulation and supervision, empowerment, capacity building and creating partnership for better healthy and safer food production and marketing. Further investigations on food safety, sanitation and hygienic behaviours are needed for better understanding.

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