

Microbial Contamination of Raw Vegetables in Ahvaz, Iran during 2014-2015

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Background & Aims of the Study: Vegetables are useful for humans as they contain minerals, vitamins, fiber and other nutrients. Eating raw vegetables are a nutritional habit in Iranian families. Raw eating vegetables is the main source of parasitic infections. The aim of this study was to determine microbial contamination of raw vegetables in Ahvaz, Iran during 2014-2015.

Materials and Methods: In this study, 20 samples collected from markets of Ahvaz. Average weight of collecting raw vegetables was 1 to 2 kilograms. Then, raw vegetables were washed by 4 to 5 liter tap water. For parasitic ova washed water leaved for 24 hours for sedimentation and then the supernatant poured and about 50 to 100 milliliter of settled water transferred to 15 ml centrifugal tubes. After centrifugation, pellet floated and finally parasitic ova were observed microscopically (corrected Bailenger method). The multiple tube method used for Coliform bacteria (Total & Faecal) examination.

Results: Maximum Coliform bacteria was in Kootabdullah samples (total Coliform was 25893319.52 MPN/100ml and Fecal Coliform was 15054572.83 MPN/100ml). Maximum Ascaris ova in Hamidieh was 43.3 per liter and Sheiban 36.66 per litter.

Conclusion: Microbial contamination of raw vegetables, especially in Kootabdullah, possibly was due to Karoon river water pollution by sewage discharge of Ahvaz city, and also in Hamidieh possibly due to Karkkeh river water pollution by sewage discharge of Hamidieh city. Thus, sewage treatment of these cities before discharging in rivers is necessary.

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Background

Vegetables are useful plants which have a key role in providing food nutrients such as vitamins, minerals, proteins and fibers. According to vegetable daily frequently consumption may prevents different diseases and it has a special value in Iranian nutritional culture (1). Although these vegetables may

spread parasitic and bacterial infection (2,3). Vegetables, especially those which are consumed rawly, are the most important factors of parasite transmission to human body(4). The most frequent globally illnesses which can be through water and Soil transmitted to nutritional foods such as Vegetables are parasitic infections (5). Struggling with contagious diseases through water, foods requires understanding the microorganism bio-

ecology and improving people's knowledge about transmission routes of these illnesses to find a suitable solution beside considering economic aspects and parasites spoilage in foods (6). Eating raw vegetables with food is a common nutritional habit in some Iranian communities. In spite of providing a considerable quantity of essential vitamins and minerals for the body, it threatens people's health to parasitic infections in unsuitable washing. The number of parasitic infections cases in Iran are considerable. Therefore, identifying parasitic infections sources and its contagion and preventing their transmission are specific hygienic priorities (7). The most important of its transmission route is soil, Water, foods and vegetables. Different ways contaminated Vegetables with parasites and other microorganisms consist of:

1. Using human fertilizer in agriculture (which can lead to Giardia, Amoeba, Cryptosporidium, Isospora, Toxoplasma, Hymnolepis, Teania, Hydatid cyst, Terishoris, Terikostronjelios, Ascaris, hookworm and Strongyloides).
2. Using animal fertilizer, which is contaminated with common zoonosis parasites.
3. Using sewages for farm irrigation.
4. Vegetables pollutions at production, collection, transporting and preparing stages for sale.
5. Traffic of infected animals such as dogs, cats, and wild carnivorous in farm fields (Toxoplasma, Hydatid cyst) (8-10)

There are several researches on raw vegetables as a factor in transmission of contagious diseases. Salmonella & E. coli can be isolated from raw vegetables. These bacteria can contaminate vegetables in plantation, cultivation, irrigation, maintaining, washing and distribution stages. Eating raw infected vegetables are important for human health. Because, possibility of microbial contamination is high from farm fields to consumption (11). Previous studies shown that vegetable parasitic infections is about 20% in Kermanshah, 68.3% in Arak, 21% in Jiroft, 55.9% in Tehran's

countryside, 41.3% in Tehran, 29.6% in Kerman, 53.62% in Sabzevar, %8 in Shahrood and 13.76% in Esfahan (12,13) Parasitic diseases as the result of raw vegetable consumption have been studied in developing and development countries. In a study conducted in northern Nigeria, parasitic infection from vegetables was varied in different regions from 3.5% to 68.8% and *Ascaris ova* was reported 0.5% (14). In another study in Saudi Arabia, *Ascaris* infection was reported 16% (15) that arised from unsuitable washed vegetable (16). Parasitic diseases cause digestive disorders such as diarrhea, stomachache, losing weight, appetite, nausea and vomiting, anemia, and brain and mental problems (17). Human beings are usually infected through eating fetus ova which are reached in infection stage.

Many researchers have investigated vegetables parasitic and microbial contamination in different regions in Iran (2,18-26). Ahvaz County with approximately more than 1 million populations is located in western south of Iran. Its temperature is usually high and about 50 degrees salacious in warm seasons (19,20). The temperature rarely reaches to 10 degrees salacious in winter (19,20). The annual rain precipitation rate is about 25 milliliter. The highest rain rate is about 5 millimeters Azar (November) and Dey (December) months. Humidity is relatively low, about 20% -30%. Since pollution control and infection prevention programs are very important for executing such plans, identification of transmission routes can be effective and helpful.

Aims of the study:

The purpose of this study was to assess to determine whether vegetables are one of transmission routes or not in Ahvaz, Iran during 2014-2015. According to high consumption of vegetables and its sale in distribution centers, we attempted to investigate microbial and parasitic contamination.

Materials & Methods

3.1. Methods

This study was a cross sectional study. Samples collected based on cultivation farmlands areas. 20 vegetable samples collected from all farmland areas as resources in Khuzestan province (2014-2015), including ShahrakeBargh, Pol 5(Bridge No. 5) and Tasfieh in Ahvaz City and Hamidieh, KootAbdollah, Ramhormoz and Shoushtar cities. Vegetables were spearmint, basil, pansy, leek, Chicory, two types of radish, and two types of fumitory. One Kilogram of vegetables was collected for each sample (totally 20 kg).Vegetable samples were collected from grosser market which provided from all farmlands around Ahvaz (Khuzestan province).Vegetables weight was 1 kg and those were washed by 3 litter tap water. After being transferred to the lab, the collected vegetables were washed with a little detergent (2 drops per liter) to prevent acceleration transmission of parasite ova. We used jam dishwashing fluid (ingredient for used: Alkylbenzenesulfonic acids, Diethanolamide, Cocamidediethanolamine, Isothiazolinone, distilled water).Then the liquid for their washing was maintained in special containers in the Microbial lab of the health college for 24 hours for the reason of parasite ova sedimentation. For testing Coliform bacteria and Fecal Coliforms were separated from the water and 0.01 and 0.001 substances were made with the vegetables and the experiment was done on the Lactose broth culture setting. After 24 hours the surface liquid was poured in the pails and the sediment was divided in centrifugal tubes and was centrifuged (2000 rpm) for 15 minutes. The flouting, sedimentation and direct methods were used for investigating parasitic pollution. We calculated the Coliform in washed water. A microscope (Luminous) was used for identifying and counting the parasite ova in the samples. For investigating the total Coliforms and the fecal Coliforms 9-tube method was used based on the WHO method for parasite examination and

standard method for Coliform examination (27, 28). Results of Coliforms are reported as most probable number (MPN/100ml). Bailenger method (28) was used For studying the parasite ova which is conducted through Ether Buffer stock, zinc sulfate and centrifuge system and its calculation is based on $N = AX/PV$ formula.. The microbial tests were done in slinked water of vegetables washing process.

Results

According to the Figure 1, the largest amount of Total Coliform and Fecal Coliform in washed water of the vegetables were observed in Kott Abdullah. In water of washed samples with water and detergent it has the highest pollution,which the number of total Coliforms was 25893319.52 MPN/100ml and number of Fecal Coliforms was 15054572.83 MPN/100 ml. The second most polluted area with Fecal Coliforms was the Pol 5. In terms of Ascaris ova parasite, Hamidieh and Shaiban had more Ascarisova Parasite ova than other regions.

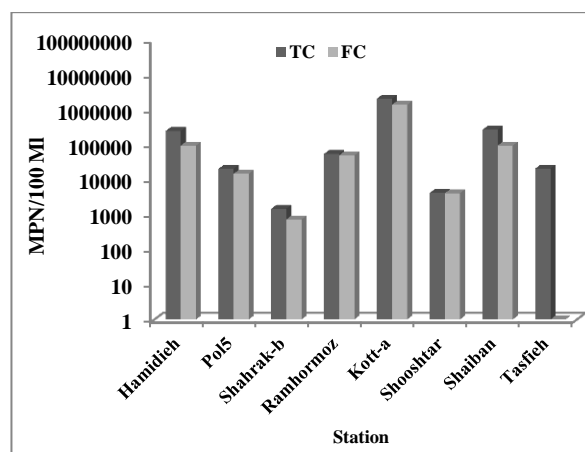


Figure 1) Fecal Coliform and Total Coliform in the washed samples with water only, MPN/100ml

As indicated in figure 2 maximum total and fecal Coliform was in kottabdollah cultivation area and then Sheiban area. Minimum total and fecal Coliforms was in Shushtar cultivation area.

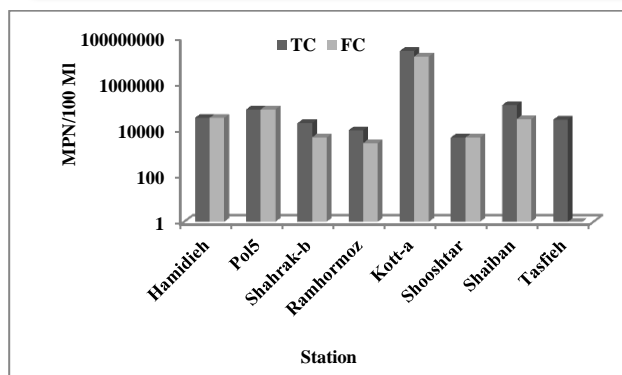


Figure 2) Fecal Coliform and Total Coliform in the washed samples with water and detergent, MPN/100ml.

In table number 1 various parasite ova observed in washed water with water only and washed water with water and detergent.

Table 1) Number of observed parasite ova per liter

Site	Ascaris	Diphyllobothriumlatum	Ova mite	Animal.nematode	Hymenolepis Nana	Hookworm	Not seen	Giardia	Sanitation
Hamidieh	43.3	0	0	173.3	0	0	0	0	Water only
Pol-5	0	0	133.33	26.6	0	0	13.3	0	Water only
Shahrak.Bargh	0	26.66	280	13.33	40	0	0	0	Water only
Ramhormoz	0	0	266.66	306.66	0	0	0	0	Water only
Kottabdollah	0	0	0	13.3	0	0	0	0	Water only
Shooshtar	0	0	0	0	0	0	0	0	Water only
Shaiban	36.66	24.44	134.44	0	12.22	0	0	12.22	Water only
Tasfieh	0	26.66	0	160	0	0	0	0	Water only
Hamidieh	14.66	0	0	69.66	3.66	0	0	0	Water&detergent
Pol-5	0	0	92	24	0	0	0	0	Water&detergent
Shahrak.Bargh	0	24	328	32	0	0	0	0	Water&detergent
Ramhormoz	0	0	144	168	0	0	0	0	Water&detergent
Kottabdollah	0	0	0	16	0	0	0	0	Water&detergent
Shooshtar	0	0	6.66	0	0	0	0	0	Water&detergent
Shaiban	0	0	0	0	6.66	0	0	0	Water&detergent
Tasfieh	0	32	0	0	0	16	0	0	Water&detergent

Table 2) Fecal Coliform observed in washed water with water only and washed water with water and detergent.

Site	FC	Sanitation
Hamidieh	95	Water only
Pol-5	14.8221	Water only
Shahrak.bargh	0.73394	Water only
Ramhormoz	50	Water only
Kott.a	1400	Water only
Shooshtar	4.1	Water only
Shaiban	94.9367	Water only
Tasfieh	0	Water only
Hamidieh	32.7332	Water&detergent
Pol-5	75.9878	Water&detergent
Shahrak.bargh	4.5714	Water &detergent
Ramhormoz	2.5893	Water&detergent
Kott.a	15000	Water&detergent
Shooshtar	4.5714	Water&detergent
Shaiban	28.6259	Water&detergent
Tasfieh	0	Water&detergent

Discussion

Maximum of Total Coliform and Fecal Coliforms were found in Kouttabdollah vegetables. This indicates that discharging Ahvaz sewage to Karoonriver cause water pollution; this region is located in downstream which irrigating vegetables with the polluted water of Karoonriver with sewage was main reason of vegetables microbial contamination. A leak of partially treated sewage water into a creek used for irrigation of commercial produce resulted in contamination of a crop of cabbages with at least six different serotypes of *Escherichia coli*(29). The second place with the highest pollution based on Coliform is the Pol 5 which due to pouring urban sewage and watering vegetables with Karoonriver. Irrigation and fertilization of vegetable crops with raw human and raw animal wastes or contaminated surface water can contribute the etiological agents of infectious hepatitis,

typhoid fever, shigellosis, salmonellosis, listeriosis, viral gastroenteritis, cholera, amoebiasis, giardiasis, and other enteric as well as parasitic diseases (29) Hamidieh and Sheiban had more *Ascaris* parasite ova than other areas which were due to watering with polluted water or using animal fertilizer. Vegetables are important sources of pollution in transmission of disease agents such as virus, bacteria and parasite. Consumption of the raw vegetables has a key role in their contagion. Detergents can separate contamination and fat from vegetables leaves (30). Raw vegetables, especially lettuce, have been identified as a common cause of travelers' diarrhea (31) contamination of vegetables with wide leaves such as lettuce is more than others (32). There are many studies conducted in Iran in relation to the transmission significance of parasite illnesses. In these researches the reported amount of contamination is high to compare others. Most separated varieties related to animal parasites such as Nematode, Mite, Hymenolep is Nana and *Ascaris*. According the literature 5.5% of the polluted vegetables in Turkey (32-35) and 2% in Norway (29) were polluted with *Giardia*. The obtained results of the previous studies are different from that of the present study. The difference may be due to geographical, Factors, climate, the number of collecting samples, the employed method for identifying parasite, the kind of irrigating region, using human and animal fertilizer, the amount of parasite pollution in human, and transportation of vegetables. In 2011 the amount of pollution with Larvae and nematode was reported 40% and 60%, respectively but *Ascaris*, *Trichoris*, *Strongyloides* and hookworms, nematodes were not observed (18). Other studies conducted in Iran investigating Nematode pollution revealed that there is some pollution with the human nematodes, e.g. in Esfahan the amount of pollution with parasite ova reported 24.2% (36), in Ramhormoz it was 4.8% (19), and in Zabol it was reported 10.9% (18).

Comparison with standards, According to New Zealand fecal Coliform bacteria standard recorded the acceptable amount of 100 (MPN/g) and the maximum acceptable amount of 1000 (MPN/g (37). Standard of Canada reported the maximum acceptable amount of 1000 MPN/g. (38)

Conclusion

According to the results of the study, the highest microbial pollution of Ahvaz vegetable consumption is found in Kouttabdollah in Ahvaz downtown. In the second place, the Pol 5, Hamidieh and Sheiban had the highest microbial pollution. The pollution reason is discharging urban wastewater without treatment to Karoon and Karkheh rivers. Therefore, complete wastewater treatment plant in Ahvaz, Hamidieh, and Sheiban should be taken into account seriously.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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