Study on the parasitic and microbial contamination of vegetables, and the effect of washing procedures on their elimination in Ilam city

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ABSTRACT

The risk of disease spread through water, fruits and vegetables contaminated through unclean waters varies from region to another. Springs and underground water resources are essentially free of germs or they have tolerable levels of germs; unless they are infected by surface water or human excreta. Scientific reports have shown that outbreaks of food-borne illnesses especially foods such as fresh fruit and vegetables are rising. The aim of the study is to evaluate the effect of washing procedures in eliminating of microbial and parasitic contamination of vegetables consumed in Ilam city. Multiple samples of vegetables were collected from five regions of the city (North- South- West- East- Center) about 1 kg from each shop and transferred to the laboratory. Then we put apiece in a dish containing five liters water; vegetable was washed into it and a sample was collected. Then we added to dish, 20 drops of dish soap and mixed it for a few minutes so that all of vegetable dipped in the foam. After a few minutes, the vegetable were washed with pure water. Another sample was collected from sewage of this water. For detection of microbes, we used two methods: formalin eater and direct microscopic observation. 86 samples were collected from five regions of city. 40.6% of them had parasitic contamination in scantling water of washing with pure water, and 43% had parasitic contamination in scantling water of washing with dish soap. 70.9% of them had parasitic and bacterial contamination in both wastewaters. There was no significant difference between washing with water and washing with dish soap for parasitic or bacterial contamination. Methods of washing in some types of vegetable may reduce effectively parasitic infection; but we found that washing with dish soap didn't have any significant effect on reduction of microbial contamination.

Key words: Vegetable; Parasitic Contamination; Microbial Contamination; Washing Method; Ilam city.

INTRODUCTION

Fresh fruits and vegetables are essential nutrients in the human diet; and is now well established that they have a high nutritional value, as their consumption is effective in human health. People in many countries have recommended that have fresh fruits and vegetables at least five times a day in their routine diet [1, 2]. Statistics show that consumption of fresh vegetables (ready for use) in different countries are not the same; it's different from 1 to 30 Kg/year per person (Spain: 1-1.5 Kg, British: 2 Kg, Germany and Belgium 3 Kg, Italia 4 Kg, France 6 Kg, and in the U.S 30 Kg) [3]. The risk of disease spread through water, fruits and vegetables contaminated by infected water varies from a region to another, because, microbial and parasitic propagation has variable degrees in different regions [4]. Springs and underground water resources are essentially free of germs or they have tolerable levels of germs; unless they are infected by surface water or human waste. So, before watering fruits and vegetables, the water are needed to get healthy. Bacteria and protozoa, primarily survive very short outside the human body. But the worms and viruses have much greater survival there; so they

can persist for months or even years contaminants. Scientific reports have shown that the outbreaks of food-borne diseases are rising, especially with fresh fruits and vegetables [4]. There are several reports that raw vegetables are the pathogenic porter [5, 6]. Salmonella and E.coli have been separated from raw vegetables [6, 7]. These bacteria may contaminate the vegetables in the planting, harvesting, storing, and distributing stages. Some epidemics due to consumption fresh vegetables and fruits are reported every year [7, 8]. Pathogens that are often associated with these diseases include: bacteria (Salmonella, E.coli, and Listeria monocytogenes), viruses (norovirus, hepatitis A), parasites (cryptosporidium, cyclospora) [9, 10]. As a example, 200 patients with urinary tract infection due to consumption of raw vegetables (spinach) contaminated with E.coli 0157:H7 resulted in the deaths of three people [11]. Therefore, Fresh fruits and vegetables are a major risk to human health, because they are used as raw, and are exposed to contamination from the farm to the table food [12]. According to the same study in different countries and continents, we have to evaluate parasitic and microbial contamination of vegetables, and the effect of washing procedures in elimination of the contaminants, in Ilam city. It is a mountainous district located in Iran-West, and has specific requirements from view of point the development in the animal husbandry and particular epidemic & environmental conditions.

MATERIALS AND METHODS

We randomly selected 86 shop of greengrocery throughout five regions of city (North- South- West- East- Center) and collected about 1 kg vegetables (Including leek, radish, onion, basil, cress, dill, cilantro and parsley) from each store. The samples were separately got into nylon bags, and were transferred to the laboratory during two hours. For each sample, we did separately following procedures. We washed vegetable in a dish filled with water and collected a sample from muddy water. Then we put vegetable in a dish containing five liters water, and added 15-25 drops of dish soap into it, and then mixed it for a few minutes so that all of vegetable dipped in the foam. After a few minutes, the vegetable were washed with pure water. Other samples were collected from foams & sewage of this water. For detection of microbes, we used two methods: formalin eater and direct microscopic observation. To decontamination and elimination pathogenic germs, we dissolved 1 gr chlorine in 5 liters of water inside the container. At this stage, a sample was collected. After 5 minutes, we washed it and collected another sample. Direct microscopic examination was used for detection of parasites. At first, we centrifuged 25 ml of water (sewage of vegetables' washing) with 2000 rpm for 10 minutes. Then, we made a thin spread from sample, stained with logol. We observed protozoa cyst or parasitic seed directly with 40x or 100x magnification of microscope. We centrifuged 10 ml of sewage with 2000 rpm for 10 minutes, 8 ml of the supernatant discarded; the remainder (2 ml) mixed and transferred to different culture environments (includes: Blood agar , Nutrient agar, Eosin Methylene blue agar, MacConky agar). The number of colonies was recorded as the number of CFU that was conducted after 48 hours incubation at 37 ° C at aerobic conditions. For the detection of pathogenic bacteria, such as Staphylococcus aureus and E.coli, we used diagnostic methods such as colony morphology, hemolytic pattern on blood agar, gram stain characteristics, mannitol fermentation, and slide & tube coagulase test. For detection of some microbes, we used oxidase reactions, chemical activities on MacConky agar, and IMViC[1] biochemical tests.

RESULTS

Most parasites found on vegetables in the northern part of Ilam were Ascaris (18.8%). Washing with pure water or dish soap showed no significant difference in parasitic infection. But Giardia lamblia in wastewater of dish soap was more than pure water. Washing with pure water or dish soap had not any significant difference in microbial contamination. There was 82.4% bacterial infection in the consumption vegetable at this region; E.coli was the commonest (25%).Most parasites found on vegetables in the southern region of Ilam were Taenia (42.9%). Prevalence of parasitic contamination was high

(more than 76%), but for microbial contamination was 23%. Only 21.4% of the samples were positive for both bacterial and parasitic contamination.

In the center region of city, Giardia was the most common parasite. Total of parasitic contamination was 44.4%, and microbial contamination was 77.4%. E.coli was the most common microbe (34.8%). Washing with pure water or dish soap had not any significant difference in microbial infection. Although, parasitic contamination in wastewater of dish soap was more than pure water, but it wasn't significant. Parasitic contamination in the west region of Ilam was 58.8% and microbial contamination was 35.2%. Taenia, E.coli and Enterobacter aerogenes were the most common parasitic and microbial contaminations in this area.

Table 1. parasitic and microbial contamination of vegetables in different areas of Ilam city

Class	**Taenia**		**Tricocefalo**		**E.coli**		**Ascaris**		**Giardia**		Total sample	
Region	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
North	4	29	2	14	3	21	3	21	2	14	14	100
south	3	37.5	1	12.5	1	12.5	2	25	1	12.5	8	100
East	3	25	1	8	2	16	3	25	3	25	12	100
west	5	35	1	7	4	29	2	14.5	2	14.5	14	100
Center	2	25	0	0	2	25	1	12.5	3	37.5	8	100
Total	17	30	5	9	12	21	11	20	11	20	56	100

Table 2. Parasitic and microbial contamination in wastewater of dish soap and pure water

Region	**Total samples**		**Parasites**				**Microbes**			
	Water	*Dish Soap*	*Water*		*Dish Soap*		*Water*		*Dish Soap*	
			Number	*%*	*Number*	*%*	*Number*	*%*	*Number*	*%*
North	17	17	7	20	6	16	14	23	14	23
south	17	17	4	11	5	14	13	21	13	21
East	17	17	6	17	6	16	14	23	14	23
west	17	17	10	29	11	30	6	10	6	10
Center	18	18	8	23	9	24	14	23	14	23
Total	86	86	35	100	37	100	61	100	61	100

Table 3. Microbial contamination of vegetables in Ilam city

Region	Escherichia coli	Agglomerans	Enterobacter aerogenes	providencia	Acinetobacter freundii	Klebsiella pneumonia	Shigella Dysenteriae	Salmonella Arizona	Pseudomonas	Salmonella paratyph	Klebsiella oxytoca	Shigella boydii	Acinetobacter	Agglomerans	Total samples
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
North		2	1	2	3	2	1	1	0	1	0	0	1	0	24
south	2	0	2	1	5	6	0	1	1	1	0	0	0	0	22
East	3	0	0	0	2	1	0	1	2	0	0	0	0	0	14
west	2	0	2	0	1	0	0	1	1	1	1	0	0	0	10
Center	8	0	2	0	2	3	1	1	1	2	0	0	2	0	23
Total	21	2	7	3	13	12	2	5	5	5	1	0	2	0	93

Although, parasitic contamination in wastewater of dish soap was more than pure water, but it wasn't significant. In the east region of city, the prevalence of parasitic contamination was 35.3%. Tinea, Giardia and Ascaris were the most common. 82.4% of samples had microbial contamination; the most common contaminations were Escherichia coli and Enterobacter aerogenes. 29.4% of samples had both microbial and parasitic contamination; while, 11.8% hadn't any contamination. at whole samples, parasitic contamination in wastewater of dish soap was 43%, and in pure water was 40.6%. Contamination rate in both wastewater was 70.9%. The most parasitic contamination among five regions of Ilam city was Giardia (17.6%); and the most bacterial contamination among them was E.coli (22.5%).

DISSCUSSION

Information about that fresh vegetables and fruits may be as sources of bacteria, viruses, protozoa and worms causing diseases in different populations are rising [13]. One way of contamination, unsanitary irrigation waters those are pathogens on.Bacterial and parasitic contaminations have been reported in the consumption vegetables in the most countries.

Johnston and et al at 2006 in Mexico compared microbial contamination of Mexican products with imported products. They reported E.coli, Entrococcus faecium , and Entrococcus fecalis. But they did not report any cases of Salmonella or Shigella. The most common contamination was E. faecium [14].Johannessen in Norway at 2002 showed that there wasn't salmonella in the samples, because of microbial outbreaks in animals very low in the area are listed [15]. This finding is in contra with our study that we have 9% contamination of salmonella, because of high prevalence of infection in our animals.In a study by Kozan in 2004 at Turkey, the prevalence of worm eggs in raw vegetables used in salad was evaluated. Contamination in washed vegetables was 5.9%; while in the unwashed vegetables wasn't reported [16]. The most commonly reported infections were tinea infection which was highly consistent with our research. Ascaris and Toxocara were the other parasitic contaminations were reported. In our study, only Ascaris have been found. Contamination of vegetables by worm eggs due to irrigation and vegetable curry with unsanitary water and wastewater of human and animal contaminations has been rising, especially in developing countries where the contaminations isn't controlled [17].Proper washing the vegetables may reduce the microbial and parasitic contaminations. Although, in reducing the contamination load of Listeria, E.Coli, and Pseudomonas, the usage of chlorine with high concentrations and increasing the duration of decontamination have not been effective significantly as pure water [18]. Our study stated that detergents (dish soap) have little effect in reducing microbial contamination.World Health Organization recommends that vegetables should be washed with water that biologically affected by the disinfectants so that less than 100 coliformis in each 100 ml of water in excess of 80% of samples were found [19].Shojaei and et al showed that washing hands reduces 50% the microbial contamination of conventional food distributors [20].

CONCLUSION

Although, washing the vegetables with enough water at the proper time may have a significant role in reducing contaminations, but Proposed researches on water resources, irrigation of vegetables and a variety of microbial and parasitic contamination of vegetables must be designed and implemented.

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