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DETECTION OF *E. COLI* AND ENTERIC PARASITES IN WASTEWATER IRRIGATED VEGETABLES

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Abstract

In developing countries, including Pakistan, irrigation system of crop is achieved using untreated wastewater and domestic animal raw compost as fertilizer. Hence, vegetables are believed to be the most important cause of human infection with protozoan, bacterial and helminthes parasites. Therefore the aim of the present study was to investigate the prevalence of pathogenic bacteria (*E. coli*) and enteric parasites in wastewater irrigated vegetables of suburb of Quetta city irrigated by wastewater. In this study 20 wastewater and 150 different leafy vegetables including spinach, tartezak, maithili, salad, coriander, jamboo, mustard, mint and green onion were examined for the presence of bacteria and parasites. Out of 150 leafy vegetables, n=45 (30%) samples were contaminated with *Giardia lamblia* (n=16; 10.6%), tape worms (n=3; 2.0%), hook worms (n=10; 6.6%), round worms (n=15; 10%,) *Coccidia lamae* (n=3; 2.0%), pin worms (n=9; (6%), adult lung worms (n=3; 2%), whip worms (n=1; 0.6%), *E. histolytica* (n=1; 0.6%), *Camillid cappilaria* (*n*=3; 2.0%), *Entomoeba coli* (n=7; 4.6%), *i*ntestinal fluke (n=1; 0.6%), stomach worms n=1; 0.6%). Jambo (50%) was the most frequently contaminated vegetable followed by mint (45%), spinach (44%), salad (37%), tartezak (33%), coriander (25%), green onion (22%) while maithili and mustard (Sarsun) significantly showed less contamination. Total 20 wastewater samples were analyzed out of which, n=12 (60%) were contaminated with coliform bacteria and n=8 (30%) were contaminated with *E. coli*. Wastewater coliform composition ranged from 4.5 to 1.2 log with an average of 2.7 log CFU/100 ml, while *E. coli* ranged from 3.0 to 1.5 log CFU/100 ml with a mean of 1.9 log in wastewater.

Keywords: *Contamination, parasite, Giardia lambia, wastewater, vegetable*

INTRODUCTION

For human health, green leafy vegetables are an essential component having a great dietary importance. Leafy plants are softly cooked to maintain the savor of flavor. Vegetables are the primary source of human food, but they become the source of bacterial and parasitic infections when these vegetables are irrigated by untreated wastewater (1). These parasites enter the human body easily and transfer by oral-fecal route from one person to other and cause various diseases in human beings. In dry climate regions, due to shortage of water, citizens mostly use polluted water for irrigation (2). In developing countries, usage of unprocessed wastewater, raw composts as fertilizer for irrigation and the routine consumption of raw or undercooked vegetables increase the peril of microbial infection (3). In developed as well as under developing states, due to deprived sanitation and inadequate personal hygiene, significant outbreaks of intestinal parasitic infections allied with lightly cooked vegetables have been documented (4). Globally, a number of studies have been performed including Ethiopia, Iran, Syria, India, Vietnam, Pakistan, Ghana, Nigeria and Egypt; showing that the vegetables can be a foremost source of protozoan oocysts transmission like *Cryptosporidium* species, cyst *Giardia lamblia, E. histolytica* and larvae or helminthes' eggs e.g. *Taenia species , T. trichiura, Fasciola hepatica, A. lumbricoides, Toxocara species* and *Strongyloides stercoralis* (5, 6). Furthermore, in water, a wide variety of microbial pathogens has been found and can be transferred during irrigation to crops. Okafo (2003) detected *Salmonella* species, *E. coli* and *Vibrio* species from irrigation stream water in Nigeria and found that these microbes were also present on the irrigated plants (7). On daily basis consumption of contaminated green vegetables, human are at high risk of getting infections such as amoebiasis, giardisis, strongyloidiasis, ascariasis and other bacterial infections. Hence, the purpose of this study was to verify the prevalence of pathogenic microorganisms and parasitic contagions of vegetables cultivated with the municipal wastewater and raw manure (dung & droppings) as fertilizer on pre-harvest vegetables in Quetta city and Suburbs.

METHODOLOGY

STUDY AREA

This study was conducted in Quetta city and suburbs during November 2016 to March 2017. Randomly fresh vegetables were collected from field and also some samples were bought by shops of Sariab area.

COLLECTION OF VEGETABLE SAMPLES

Wastewater samples were obtained from four sampling points in each plot in a sterile 100-ml screw-capped bottle from the feeder furrow. Furthermore, total 150 different leafy vegetables including spinach, tarteeza , manthali, salad , coriander, janboo, mustard, mint, green onion were collected in sterile labeled bags and transported immediately to the Microbiology laboratory of University of Balochistan for parasitic and microbial analysis.

PARASITIC IDENTIFICATION

In the laboratory, a sample of 100g of each fresh vegetable was chopped into diminutive pieces and put into a sterile beaker contained normal saline solution (10% NaCl), sufficient to wash the sample vegetable. For detection of parasitic stage, (cysts, ova, oocytes and larvae) of protozoan and helminthes, it was kept all night for sedimentation. Later, the upper layer of the washing saline was carefully discarded after overnight sedimentation and 12 ml of the sediment was then centrifuged for 30 minutes at 3000 rpm. The supernatant was then gently discarded without trembling after centrifugation. The residue was then placed on slides after discarding the supernatant, stained in the iodine solution of Lugol, and then examined using light microscopy to examine the samples for infectious stages of intestinal parasites such as larvae, protozoan cysts and helminth eggs (8). Two slides for each vegetable sample were prepared to increase the probability of parasite detection.

TOTAL *COLIFORM* BACTERIAL COUNT

Standard total *coliform* was enumerated by the membrane filter method (Millipore Corp). The sterile membrane filters were used to filter approximately 50 ml of untreated wastewater (diameter, 47 mm, pore size, 0.45 rimn addition, M-Endo agar LES (difcoLaboratories) was used as the TC count culture medium (9).

IDENTIFICATION OF *COLIFORM* LIKE BACTERIA

Membranes with 20 to 50 well-spaced coliform-positive colonies were selected. All coliform-positive colonies were picked from these membranes and cultured. In addition to IMVic (indole, methyl red, Voges-Proskauer, citrate), cytochrome oxidase tests were performed on all these isolates.

STATISTICAL ANALYSIS

Statistical analysis of data was performed using SPSS 20.0. In order to establish the relation between the acquisition of parasitic infection in fresh vegetables and the type of parasite, the Chi square test and the study of variance ANOVA were used to ensure the result between the type of infected parasite and the type of vegetable and water samples used for sprinkling vegetables. The p value <0.005 was calculated as significant.

RESULTS

In this study 20 wastewater and 150 different leafy vegetables including spinach, tartezak , maithili, salad , coriander, jambo, mustard, mint, green onion were examined for bacterial and parasitic examination.

Briefly, these vegetable samples were collected from shrubs of Quetta city which were irrigated with wastewater. Furthermore, different parasites and *coliform* bacteria were detected in leafy vegetables and wastewater vegetables.

Table I. Frequency distribution of parasitic contamination among vegetables

Positive parasites	Total Positive	Percentage %	Total Samples
Giardia lamblia	45	30 %	150
Round Worms	10	6.6 %	150
Tape Worms	16	10.6 %	150
Hook Worms	3	2.0 %	150
CoccidiaLamae	15	10.0%	150
Pin Worm	3	2.0 %	150
Aadult Lung Worms	9	6.0 %	150
Whip Worms	3	2.0 %	150
E. Histolytica	1	0.6 %	150
Camillid Cappilaria	1	0.6 %	150
Entomoeba Coli	3	2.0 %	150
Intestinal Fluke	7	4.6 %	150
stomach Worms	1	0.6 %	150
Lubricoide Surface Focus	1	0.6 %	150

Out of 150 leafy vegetables n=45 (30%) samples were contaminated with *Giardia lamblia*, n=16 (10.6%) *Tape worms*, n=3 (2.0 %) *Hook worms*, n=10 (6.6 %) *Round worms*, n=15 (10%) *Coccidia lamae*, n=3 (2.0%) Pin worms, n=9 (6%) *Adult lung worms*, n=3 (2%) *Whip worms*, n=1 (0.6%), *E. histolytica*, n=1 (0.6%), *Camillid cappilaria*, n=3 (2.0%) *Entomoeba coli*, n=7 (4.6%) *Intestinal fluke*, n=1(0.6%) *Stomach worms*, n=1(0.6%) *Lubricoide surface focus* shown in Table I.

VEGETABLES ASSOCIATED WITH PARASITIC CONTAMINATION

As analyzed using the chi-square test, kind of vegetables were significantly associated with parasitic contamination (P value <0.005). Jambo (50%) was the most frequently contaminated vegetable followed by mint (45%), spinach (44%), salad (37%), tartezak (33%), coriander (25%), green onion (22%) while maithili and mustard (Sarsun) significantly showed less contamination.

VEGETABLES ENUMERATION OF TOTAL *COLIFORM* BACTERIA AND *E. COLI* IN WASTEWATER SAMPLES

Total 20 wastewater samples were analyzed out of which n=12 (60%) were contaminated with *coliform* bacteria and n=8 (30%) were contaminated with *E. coli* . In the present study, wastewater *coliform* composition ranged from 4.5 to 1.2 log with an average of 2.7 log CFU/100 ml, while *E. coli* ranged from 3.0 to 1.5 log CFU/100 ml with a mean of 1.9 log, in wastewater.

DISCUSSION

Incidence of high rate of intestinal parasites have been found that consumed raw or semi cooked vegetables, particularly irrigated with polluted water (10). In addition, preference of consumption of raw or undercooked green vegetables to protect heat labile nutrients and possibly will enhance the threat of food borne contagions. To evaluate the prevalence of parasitic contamination in vegetables a inadequate number of research studies have been done in the study area.

The present study highlighted the contamination of enteric parasites in vegetables and salad of Quetta city which irrigated by wastewater aimed to control or minimize the risk of enteric parasites infections and coliform bacteria from wastewater irrigated vegetables.

This study showed a significantly high level of contamination of green vegetables involving intestinal parasites. Out of 150 leafy vegetables, n=45 (30%) samples were contaminated with *Giardia lambia* , n=16 (10.6%) with tape worm, n=3 (2.0%) with hook worms, n=10 (6.6 %) with round worms, n=15 (10%) with *Coccidia lamae*, n= 3(2.0%) with pin worms, n=9 (6 %) with adult lung worms, n=3 (2%) with whip worms, n=1(0.6%) with *E. histolytica*, n=1 (0.6%) with *Camillid cappilaria*, n=3 (2.0%) with *Entomoeba coli*, n=7 (4.6%) with intestinal fluke, n=1(0.6%) with *stomach worms*, n=1(0.6%) with *lubricoide surface focus*.

Table II. Prevalence of parasites among vegetables

Samples	**Spinach**		**Tartezak**		**Salad**		**Coriander**		**Maithili**		**Jambo**		**Mustard**		**Mint**	
150	18	%	12	%	16	%	20	%	14	%	16	%	12	%	20	%
Giardia	8	44%	4	33%	6	37%	5	25%	0	0%	8	50%	0	0%	9	45%
Round Worms	6	33%	0	0%	0	0%	2	10%	0	0%	1	6%	1	8%	0	0%
Tape worms	0	0%	2`	16%	2	12%	5	25%	0	0%	0	0%	0	0%	5	25%
Hook Worms	1	5%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	2	10%
Coccidia	2	11%	2	16%	2	12%	0	0%	2	14%	3	18%	0	0%	2	10%
Pin Worm	0	0%	1	8%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
L. Worms	0	0%	1	8%	3	18%	0	0%	2	14%	1	6%	0	0%	0	0%
Whip Worms	0	0%	2	16%	0	0%	0	0%	0	0%	1	6%	0	0%	0	0%
E.Histolytica	0	0%	0	0%	1	6%	0	0%	0	0%	0	0%	0	0%	0	0%
C. Cappilaria	0	0%	0	0%	1	6%	0	0%	0	0%	0	0%	0	0%	0	0%
E.Coli	0	0%	0	0%	0	0%	0	0%	1	7%	2	12%	0	0%	0	0%
I. Fluke	0	0%	0	0%	0	0%	1	5%	0	0%	3	18%	1	08%	1	5%
S. Worm	0	0%	0	0%	0	0%	0	0%	0	0%	1	6%	0	0%	0	0%
Lubricoide	0	0%	0	0%	0	0%	0	0%	0	0%	1	6%	0	0%	0	0%

Table III. Bacteriological analysis of untreated wastewater

Parameters	**Max**	**Min**	**Mean**
Total coliform (log CFU 100 ml)	4.5	1.2	2.7
E.coli (log CFU 100 ml)	3.0	1.5	1.9
Helminthes egg	4	≤ 1	1

Giardia lamblia cysts were the most prevailing parasite contaminating the green vegetables with (30%). The least number of parasites were found on *E. histolytica*, n=1(0.6%). In Kenya a study performed by Nyarango in 2008, revealed higher rate (75.9%) of incidence of parasite in green vegetables (11). A study conducted in Asmara and its suburbs, Eritrea, documented high incidence of protozoan parasite, Giardia spp. (45%) in vegetables which were irrigated with untreated water (12). Both studies showed high prevalence rate of contamination of parasite in vegetable in contrast to the present study. Another study conducted in Iran and Nigeria illustrated less parasitic infestation rate with 25.1% than the present study (13). In 2016, astudy conducted in Sudan by Mona Ali, reported the presence of 22.9% *G. lamblia* (14) which shows less prevalence as compare to present study. These differences between the present study and previous results may be due to the variations in climatic, geographical locations, and environmental status, the type of fruit and vegetable samples examined and number of fruits/vegetables observed, laboratory methods used, and hygienic condition of the society (15).

In the present study, jambo (50%) was the most frequently contaminated with *Giardia* followed by mint (45%), spinach (44%), salad (37%), tartezak or peppergrass (33%), corrode (25%), green onion (22%) while maithili (fenugreek) and mustard (sarsun) significantly showed less contamination . In this study, spinach (44%) showed high contamination with parasite as compared to other vegetables. A research documented by Amoros *et al,*. (2010) reported 52.6% *Giardia* in spinach which showed high incidence than present study.

In contrast to our study, a study conducted in Arba Minch town in 2017, showed high prevalence of *Giardia* (54.4%) in Green leafy vegetables (16). In addition, some studies showed less contamination of *Girdia spp.* in different cities. It was 9% in Ardabil, 8.2% in Shahrekord, 6.5% in Tehran, 4% in Qazvin and 14% in Jiruft (17-19). In Saudi Arabia, the research study was carried out by Al-Megrin 2010, reported 31.6% of green vegetables were contaminated with *Giardia* parasite (20). Furthermore, in Norway Robertson and Gjerde observed 2.1% contamination of *Giardia* parasite in vegetables (21). Contrary to these findings, another study in Libya detected *Giardia* cysts in 10% of the total vegetable samples examined (22). Another study documented by Getache *et al*.,2018 showed 15.3% *Giardia* cysts in leafy vegetables (23). In Ghana a research study documented 36% parasitic contamination in all vegetables studied. The variance in the frequency of *Giardia lamblia* can be due to the long survival times of the cysts in cool and humid environments, and to geographical distribution variations (24).

Ankylostoma deudenale, Necatrus americanis performed a study and revealed that hookworm was detected as the 2nd most commonly existed parasite in the vegetables (25), as helminthes contamination was found in 42% of the mint samples and 28% of coriander samples (26). In addition, the overlie leaves of the plant protects the eggs of helminthes from wind, sunlight and drought (26). *Hook worm* eggs were observed in 21% and *entamoeba coli* cysts were observed in 61% vegetables (27). Soil-transmitted helminthes (hookworms) are among the least reported parasites, our results were in contrast with studies documented in Ethiopia, Egypt, Nigeria, and Eretria, where the prevalence of hookworms was 12.5%, 20.83%, 16.7%, 56.31%, and 20.3% respectively (2, 4, 28), similarly, in Oyo State of Nigeria, the hookworms (32.3%) were predominant (29).

This is suggested that human population is at high risk of receiving infection by consuming unsanitary fruits and vegetable in their daily routine. These results elevate consternation of human health at high threat of infections with gardiasis, amoebiasis, ascariasis, strongyloidiasis and many others. Therefore, approved organize procedures should be applied that cover guiding principle of irrigation water quality, apply different procedure to decrease the vulnerability of transmitted disease mainly caused as a result of parasites, thwart entering of wild and domestic animals into the plant farms and evade usage of unprocessed soil as fertilizer.

Total 20 wastewater samples were analyzed for the presence of pathogens out of which n=12 (60%) were found positive with coliform bacteria and n=8 (30%) with *E. coli*. In our study, wastewater coliform composition ranged from 4.5 to 1.2 log CFU/100 ml with an average of 2.7 log CFU/100 ml, while *E.coli* in wastewater with a mean of 1.9 log CFU/100 ml, ranged from 3.0 to 1.5 log CFU/100 ml.

Another study, conducted in Mexico by Rosas (1984), revealed that the total coliform values found in irrigation water ranged from 4×10^4 to 29×10^4, and for *E. coli* the values ranged from 5×10^2 to 30×10^2 (30). Studies in Ghana, Israel and Mexico found faecal coliform concentrations at least 100 times higher than those in Faisalabad (31-33). The average composition of coliform ranged from 3.36 to 4.33 with a mean of 4.0

log CFU/100 ml and the mean of fecal coliform bacteria was greater than that WHO suggested average of 3 log CFU/100 ml (34).

Total coliform composition of wastewater ranged from 3.19 to 4.82 log CFU/100 ml with a mean of 4.4 log CFU/100 ml. With a mean of 4.0 log, faecal coliform bacteria ranged from 3.36 to 4.33 log CFU/100 ml (35). A study conducted in 2007 by Jeroenand Ensin in London Uk (36) , showed low concentrations of *E. coli* (1.8 · 107 CFU⁄100 ml) than our study. The bacteria may be stay alive and multiply more effectively under warm environment (36, 37), owing to gratis water residue interactions as well as less availability of dilution in summer (38). Fecal bacteria indicators are well-known to have ability to stay alive for long time when associated with soil sediment (39).

The hazard associated with the usage of wastewater for irrigating the vegetables was reviewed by Bain 2012 (40) and revealed that their concentration levels range from 3 to 4 log units of fecal coliforms in 100 ml and from 6 to 15 eggs of helminth per litre (41). In order to prevent a possible out-break, the local people and farmers too should be educate on food safety measurement. Although utilization of unhygienic vegetables and chicken might be responsible for causing different enteric infection comprise dysentery, typhoid and cholera (42, 43). Deprived quality of wastewater is the result of manmade activities for example the drainage of domestic waste into drains, runoff poultry muck, open defecation just about farms possibly be altered the soils condition in vegetable farms (44).

CONCLUSION

The purpose of the present research was to study the usage of untreated drained water for irrigation of green vegetables and its potential public health hazards. The microbial pathogens detected in the study may possibly cause contagious human diseases including diarrhea. Such results raised questions about the increased risk of infections with strongyloidiasis, amoebiasis, ascariasis, giardiasis, and others in public health. Therefore, in future, this work recommended the implementation of advanced treatment technologies to restrain the microbial contagion in the wastewater. Furthermore, farmers as well as local people are not aware, and should be educate about the health peril allied with consuming unhygienic green vegetables.

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