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Microbiological Quality of Leafy Vegetables Irrigated with Wastewater in Harar Town Vegetable Farm, Eastern Ethiopia

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Abstract
Food safety issues are of growing concern to consumers globally because of the risks associated with consumption of foods contaminated with pathogenic microbes. Harar town vegetable farm is known to produce vegetables irrigated with wastewater. To what extent these vegetables are contaminated with pathogenic microbes was not known. The study was aimed to examine the extent of microbial contamination of vegetables. Thus, a laboratory based cross sectional study was conducted from October 2016 to January 2017. Accordingly, a total of 72 samples from four leafy vegetables namely lettuce (Lactuca sativa), spinach (Spinacea oleracea), kale (Brassica carinata) and cabbage (Brassica oleracea) were examined. The results revealed that the mean values in all vegetables were $9.5x10^7$ CFU/g for total aerobic mesophilic bacterial count, $4.3x10^6$ CFU/g for total coliform and $4.6x10^5$ CFU/g for fecal coliform count. All the mean values exceeded the International Commission on Microbiological Specifications for Foods recommended levels for leafy vegetables. These leafy vegetables were also examined for some pathogenic bacteria (Salmonella, Shigella and Campylobacter species) and infective parasitic stages (Ascaris lumbricoides eggs, Entamoeba histolytica and Giardia lamblia cysts). Salmonella, Shigella and Campylobacter species were isolated in 12.5%, 9.7% and 2.8%, respectively, of all vegetables. Ascaris lumbricoides eggs was the predominant (43.5%) intestinal parasitic stage detected in the present study, followed by Entamoeba histolytica (25%) and Giardia lamblia cysts (15.3%). The findings of this study have important information on the implications of public health by transmission of pathogenic bacteria and infective parasitic stages among vegetable consumers of Harar town and the surroundings. Thus, it is recommended that the concerned public health authorities need to create awareness in the community and discouraging the use of untreated wastewater for cultivating vegetables.
Keywords: Parasites, Pathogenic bacteria, Vegetables, Wastewater

1. INTRODUCTION

Currently, it has been reported that there is an increasing number of cases of foodborne illnesses mainly linked to eating fresh vegetables (Alhabbal, 2015). Wastewater reuse in irrigation is largely considered an inevitable option to compensate water shortages in developing countries as a result crop irrigation with wastewater is a widespread practice in these countries (Sou *et al*., 2011). Municipal wastewater for the irrigation of vegetables by marginal farmers is a common practice in urban and peri urban ecosystems of many countries (Chang *et al*., 2013). Since the mid-1990s, there have been an increasing number of outbreaks of fresh produce associated food-borne illness identified internationally and efforts are being made to resolve these food safety problems (Berger *et al*., 2010).

Human health is affected by pathogens (bacteria, protozoan cysts and helminthes eggs) which are likely to exceed health protection standards (WHO, 2006). Among the main food borne illnesses are diarrheal conditions such as gastroenteritis, typhoid fever and shigellosis (Callejas *et al*., 2011). The study conducted by Sou *et al*. (2011) has demonstrated a very close relation between the consumption of vegetables irrigated with wastewater and many food borne diseases like gastroenteritis, cholera, chemical toxicity etc. Preference for eating raw vegetables to protect heat labile nutrients may increase the risk of food borne infections (Fallah *et al*., 2012). Population growth, inadequate sanitation and infrastructure are causing serious environmental pollution in Harar town. Wastewater is being generated in the city from domestic, commercial, health centers and industry. Many farm households that are irrigating their farmlands with wastewater in this area are not aware of the risks or the potential harmful environmental consequences. Altogether, the situation will put the consumers at high risk of contracting diseases.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

Harar town is the capital city of Harari People National Regional State and is located in eastern part of Ethiopia. Geographically, it is located at 9^0 18' 43''N latitude and 42^0 07' 23''E' longitude, at a road distance of 526 km east of Addis Ababa, the capital city of Ethiopia. The elevation of the town is approximately between 1800 and 2200 meters above sea level (masl). Harar is the administrative and commercial capital of the region and covers about 1,950 hectares of land which is using water sources for irrigation from different sources and its

geographical position is suitable for getting the entire water source that drains from the surrounding villages. Major vegetables grown in this area include cabbage (*Brassica oleracea),* lettuce (*Lactuca sativa*), Spinach (*Spinacea oleracea*), and kale (*Brassica carinata*).

2.2. Study Design and Sample Collection

A laboratory based cross sectional survey was conducted from October 2016 – January 2017 to assess the microbial contaminants on the main leafy vegetables [Lettuce (*Lactuca sativa*), Cabbage (*Brassica oleracea*), Spinach (*Spinacea oleracea*), and kale (*Brassica carinata*)] that are grown in Harar Town vegetable farm. The samples were regularly collected within three week intervals from October 2016 – November 2016 and analysed for indicator bacteria (total aerobic mesophilic bacteria, total coliform bacteria and faecal coliform bacteria), for detection of some pathogenic bacteria (*Salmonella*, *Shigella* and *Campylobacter* species) and for detection of infective parasitic stages (*Ascaris lumbricoides* eggs*, Giardia lamblia,* and *Entamoeba histolytica* cysts*)* following standard procedures. A random sampling procedure was adopted to collect the vegetable samples. A total of 72 samples comprising four vegetable types were collected from Harar Town vegetable farm. All samples were collected aseptically in a disinfected universal ice box.

2.3. Microbiological Analysis

The edible portions (leaves) of these vegetables were washed with running tap water. After draining for 1 min, the vegetables were aseptically transferred to a sterile stomacher bag for stomaching. For bacteriological analysis, 25 grams of leafy vegetable samples were aseptically removed from each vegetable sample using a sterile scalpel. Each 25 g of vegetable sample was weighed and homogenized in 225 ml of sterile 0.1% (w/v) bacteriological peptone water blended in a sterile blender for 2-3 minutes under sterile conditions (Biniam and Mogessie, 2010). The homogenate was used as a source of microbial inoculum for determining the indicator bacteria and also for detection of some selected pathogenic bacteria (*Salmonella*, *Shigella* and *Campylobacter* species). In addition to this, parasitological analysis was also undertaken for the vegetable samples using standard procedures.

2.3.1. Enumeration of indicator bacteria

A total of ten sterile test tubes were dispensed with 9 ml of bacteriological peptone water as a diluent (Girmaye *et al*., 2014). Ten fold serial dilutions of 10^{-1} to 10^{-10} were prepared from 1ml of the stomacher bag suspension with 0.1% buffered bacteriological peptone water using disposable pipettes. Then sterile molten agars were dispensed into petridish and allowed to solidify by leaving the petridish stand on the horizontal surface of the biological safety cabinet. After complete solidification, 0.1ml from each dilution (10^{-1} to 10^{-10}) was spread plated on to standard plate count agar (for total aerobic mesophilic bacteria), and Eosin methylene blue agar (for total coliform and fecal coliform) in duplicates. Then the medium were carefully mixed by rotating the petridish and inoculated by spread plate method. All the petridish were inverted and incubated in an incubator at required temperature (37 ℃ for total aerobic mesophilic and total coliform bacteria and 44.5 ℃ for fecal coliform bacteria for 24-48 hrs). After the incubation period, duplicate plates were selected from the appropriate dilution that contained 30-300 colonies per plate and were expressed as colony forming units per gram (CFU/g) (Hannan *et al*., 2014).

2.3.2. Detection of Salmonella and Shigella species

Twenty five grams of each washed vegetable sample was thoroughly mixed in 225 ml buffered peptone water and the suspension was incubated at 37 °C for 24 hour for the metabolic recovery and proliferation of cells (pre-enrichment). After incubation, 1ml of culture were transferred into two separate tubes containing 9 ml Selenite Cystein Broth (selective enrichment broth) and incubated at 37 ºC for 24 h (Biniam and Mogessie, 2010). After the selective enrichment broth a loop full of the samples were streaked onto *Salmonella-Shigella* (SSA) agar (selective agar) and incubated at 37 ºC for 24 h. Typical *Salmonella* colonies which appeared red/pink/colourless colonies with black centers were suspected as *salmonella* species (Abakpa *et al*., 2015) and colourless colonies were suspected as *Shigella* species. Suspected *Salmonella* and *Shigella* colonies on SSA plates were selected; subcultured on to nutrient agar and further confirmed with biochemical tests (Swagato *et al*., 2015).

2.3.3. Detection of Campylobacter species

Twenty five grams of each vegetable samples was thoroughly mixed in 225 ml of buffered peptone water and incubated at 42 ºC for 20 h (pre-enrichment). One ml of the suspension was pipetted to 9 ml of Bolton broth base (selective enrichment broth) and was incubated at 42 ºC for 24 h. A loop full of the sample suspension was streaked directly onto *Campylobacter* blood free charcoal-based selective medium, specifically mCCDA (modified cefperazone charcoal deoxycholate agar) which is selective for the isolation of *Campylobacter* species. After inoculation, the medium was kept in gas jar containing *Campylobacter* gas pack systems to maintain microaerophilic condition. The gas pack system containing the inoculated Petri-plates was incubated at a temperature of 42 ºC for 48 ± 2 h under microaerobic conditions (Cinzia *et al*., 2015). After 48 hours, growth of *Campylobacter* was identified by colonial morphology. Typical mucoid, spreading and convex colonies were

taken as suspect colonies and sub-cultured on to nutrient agar plates. Biochemical tests were also used for identification of *Campylobacter* species from sub-cultured colonies.

2.4. Biochemical Tests for the Identification of Pathogenic Bacteria

Standard biochemical tests were performed to identify pathogenic bacteria isolated from vegetable samples. The tests include Kligller iron agar test, Motility test, Indole test, Citrate utilization test, Urease test, and Catalase test (Swagato *et al*., 2015).

2.5. Parasitological Analysis of Vegetable Samples

In the laboratory, 100g of each vegetable sample was chopped into small pieces and put into a clean beaker containing 250 ml physiological saline solution (0.85% NaCl) enough to wash the samples. After removing fragments of the vegetable sample from the washing saline using clean forceps, it was kept for 24 hours to allow sedimentation to take place. Following sedimentation, the top layer of the washing solvent was carefully discarded leaving 5 ml of the suspension that contained the sediment. This was finally centrifuged at 2000 rotations for 5 minutes using a centrifuge (Dada and Olusola-Makinde, 2015). After centrifugation, the supernatant was carefully siphoned off without shaking, and discarded; and the remaining residue was agitated gently by hand in a drop of physiological saline solution for further distribution of the cysts and eggs in the residue. Then the residue was mounted on a slides, stained with Lugol's iodine solution and examined under the compound light microscope using 10X and 40X objectives (Daryani *et al.,* 2008) for the presence of *A. lumbricoides eggs* and cysts of *E. histolytica* and *G. lamblia* (Girmaye and Fikadu, 2014). Three slides were prepared for each sample to increase the chance of parasite detection.

2.6. Data Analysis

In this study, all statistical analyses were computed using SPSS software version 20. Descriptive statistics such as mean, percentage and frequency were used to describe the extent of pathogenic bacteria, infective parasitic stages and the loads of indicator bacteria. As the level of microbial contamination might vary with vegetable types, ANOVA was used to test the existence of significant difference between means. In statistical analyses, confidence level was held at 95% and $P<0.05$ (at 5% level of significance) were considered as significant.

3. RESULTS AND DISSCUSSION

3.1. Indicator Bacterial Contaminants in Leafy Vegetable Samples

Table 1 shows the percentage of vegetable samples contaminated with indicator bacteria from Harar town vegetable farm. The highest percentage was obtained for total aerobic mesophilic bacteria (100%) as demonstrated by its occurrence in all vegetable samples analyzed. Obviously such a result is expected as our oxygenated environment is largely occupied by aerobic microflora. Similar to this result, Girmaye *et al*. (2014) reported that total aerobic mesophilic bacteria (100%) occurrence in all vegetable samples collected from Melka Hida and Wonji Gefersa farms around Adama town (Ethiopia) and Getachew and Desalegn (2015) reported that all vegetable samples (100%) were contaminated with total aerobic mesophilic bacteria in Nekemte town, Ethiopia.

Table 1: Percentage of positive vegetable samples for indicator bacteria

	Leafy Vegetables									
Indicator organisms	Lettuce (N=18)		Spinach (N=18)		Kale (N=18)		Cabbage (N=18)		**Total (N=72)**	
	F	%	F	%	F	%	F	%	F	%
TAMB	18	100	18	100	18	100	18	100	72	100
TCB	18	100	17	94.4	16	88.9	18	100	69	95.8
FCB	17	94.4	17	94.4	15	83.3	16	88.9	65	90.3

TAMB=Total aerobic mesophilic bacteria, N=Number of examined samples, TCB=Total coliform bacteria, FCB=Fecal coliform bacteria, F=Frequency of positive samples, %=Percentage of positive samples, Kale=*Yegurage gomen* (Ethiopian local leafy vegetable) (*Brassica carinata*)

The mean values for TAMBC, TCBC, and FCBC were $9.5x10^7$, $4.3 x10^6$, and $4.6 x10^5$ CFU/g, respectively, for all positive samples (Table 2). Indicator bacterial count showed that the total aerobic mesophilic count, total coliform count and fecal coliform counts ranged from 1.1×10^6 to 9.2×10^8, 6.2×10^4 to 9.9×10^6 and 1.0×10^4 to 4.2×10^6 CFU/g, respectively.

Table 2 : Mean, maximum and minimum values (CFU/g) of indicator bacteria among the tested vegetable samples

Indicator organisms		Vegetable types				
		Lettuce	Spinach	Kale	Cabbage	For all positive samples
TAMBC	Mean	$5.1x10^{7\,b}$	$2.9x10^{8\,a}$	$7.0x10^{6\,b}$	$3.4x10^{7\,b}$	$9.5x10^{7}$
	Minimum	$5.4\ x10^{6}$	$3.7\ x10^{7}$	$1.1\ x10^{6}$	$1.0\ x10^{7}$	$1.1\ x10^{6}$
	Maximum	$8.8\ x10^{7}$	$9.2\ x10^{8}$	$7.7\ x10^{7}$	$9.7\ x10^{7}$	$9.2\ x10^{8}$
TCBC	Mean	$5.1\ x10^{6\,a}$	$4.9\ x10^{6\,a}$	$5.1\ x10^{5\,b}$	$6.2\ x10^{6\,a}$	$4.3\ x10^{6}$
	Minimum	$2.2\ x10^{5}$	$7.4\ x10^{5}$	$1.0\ x10^{5}$	$6.2\ x10^{4}$	$6.2\ x10^{4}$
	Maximum	$9.7\ x10^{6}$	$9.1\ x10^{6}$	$2.5\ x10^{6}$	$9.9\ x10^{6}$	$9.9\ x10^{6}$
FCBC	Mean	$3.4\ x10^{5\,bc}$	$9.2\ x10^{5\,a}$	$5.4\ x10^{4\,b}$	$4.8\ x10^{5\,c}$	$4.6\ x10^{5}$
	Minimum	$5.5\ x10^{4}$	$2.0\ x10^{5}$	$1.0\ x10^{4}$	$1.0\ x10^{5}$	$1.0\ x10^{4}$
	Maximum	$9.0\ x10^{5}$	$4.2\ x10^{6}$	$1.3\ x10^{5}$	$9.9\ x10^{5}$	$4.2\ x10^{6}$

[a-b-c] Means with different superscript letters across the row for the same parameter do significantly differ ($P<0.05$), TAMBC = Total aerobic mesophilic bacterial count, TCBC = Total coliform bacterial Count, FCBC = Faecal coliform bacterial count, Kale= *Yegurage gomen* (*Brassica carinata*)

The mean total aerobic mesophilic bacterial counts for the vegetables were $5.1x10^{7}$ (lettuce), $2.9x10^{8}$ (spinach), $3.4x10^{7}$ (cabbage) and $7.0x10^{6}$ CFU/g (kale). This result was in connection with Razzaq *et al*. (2014) who reported that the average total aerobic mesophilic count of cabbage was $3.0x10^{7}$ CFU/g.

In this study, total aerobic mesophilic bacterial counts ranged from $1.0x10^{7}$ to $9.7x10^{7}$ CFU/g for cabbage, $5.4x10^{6}$ to $8.8\ x\ 10^{7}$ CFU/g for lettuce, $3.7\ x\ 10^{7}$ to $9.2\ x\ 10^{8}$ for spinach and $1.1\ x\ 10^{6}$ to $7.7\ x\ 10^{7}$ for kale *(yegurage gomen)*. In connection with this result, Viswanatha and Kaur (2001) from India indicated that total aerobic mesophilic bacterial count for cabbage and lettuce were found to be $2.8x10^{6}$ to $1.2x10^{8}$ and $1.3x10^{7}$ to 2.3×10^{7}CFU/g, respectively. But it is lower than that of the study conducted by Girmaye *et al*. (2014) who reported that the TAMBC for spinach, lettuce and cabbage were in the range of 2.0×10^{8} to 2.2×10^{8}, 1.6×10^{8} to $1.7\times10^{8}$and 8×10^{7} to 9.3×10^{7}CFU/g, respectively from vegetable samples collected from Melka Hida and Wonji Gefersa farms irrigated with wastewater around Adama Town (Ethiopia).

The data showed that there was a highly significant difference ($p<0.0001$) in the average counts of TAMBC between spinach and the other vegetable types (Table 2). This investigation additionally showed that the vegetable samples collected from this farm were heavily contaminated by total aerobic mesophilic bacteria. Moreover, all the bacterial counts recorded in this study exceeded the recommended levels by the International Commission on microbiological Specifications for Food (ICMSF, 1998) standards (10 to 100 coliforms CFU/g, 10 faecal coliform CFU/g and 4.9×10^{6} aerobic counts CFU/g) wet weight vegetables.

Total coliform levels ranged from $2.2\ x\ 10^{5}$ to $9.7\ x\ 10^{6}$ CFU/g for lettuce, $7.4\ x\ 10^{5}$ to $9.1\ x\ 10^{6}$ CFU/g for spinach, $1.0\ x\ 10^{5}$ to $2.5\ x\ 10^{6}$ CFU/g for kale and $6.2\ x\ 10^{4}$ to $9.9\ x\ 10^{6}$ CFU/g for cabbage. In contrast to this study, Nma and Oruese (2013) revealed that vegetables in Port Harcourt, metropolis (Nigeria), total coliform counts ranged from $3.4\ x10^{5}$ to $5.6\ x\ 10^{5}$ CFU/g for cabbage and from $3.4\ x\ 10^{5}$ to $4.0\ x\ 10^{5}$ CFU/g for lettuce Furthermore, the mean value for cabbage in the present study ($6.2x10^{6}$ CFU/g) was higher than that of the data reported by Razzaq *et al*. (2014) who demonstrated that the average total coliform count in cabbage was $9.0\ x\ 10^{2}$ CFU/g.

According to Viswanathan and kaur (2001) who also reported that the total coliform counts of vegetables ranged from $1.0x10^{6}$ to$1.0\ x\ 10^{9}$ CFU/g, value which was higher than the present study ($6.2x10^{4}$ to $9.9\ x\ 10^{6}$ CFU/g). There was highly significance difference ($P<0.0001$) between kale (*yegurage gomen)* to the other vegetable types (cabbage, lettuce and spinach) (Table 2). In this study, the fecal coliform counts for lettuce, spinach, kale, and cabbage samples collected from the study site ranged from $5.5\ x10^{4}$ to $9.0\ x10^{5}$, $2.0\ x10^{5}$ to $4.2\ x10^{6}$, $1.0\ x10^{4}$ to $1.3\ x10^{5}$, and $1.0\ x10^{5}$ to $9.9\ x10^{5}$ CFU/g, respectively.

In line with this Girmaye *et al*. (2014) reported that the fecal coliform counts of cabbage, lettuce and spinach samples grown in wastewater ranged from 5.2×10^{5} to 5.7×10^{5}, 2.3×10^{5} to 3.1×10^{5} and 2.2×10^{5} to 3.7×10^{5} CFU/g from Melka Hida and Wonji Gefersa farms, respectively around Adama Town (Ethiopia). The mean fecal coliform values of all the four vegetable samples exceed the ICMSF recommended level of 10 fecal coliform g-1 fresh weight. The results of the analysis of variance for fecal coliform count showed that there was a significant difference amongst vegetable types (Table 2).

3.2. Detected Pathogenic Bacteria in Leafy Vegetable Samples

A total of seventy two (72) samples of leafy vegetables from wastewater irrigated farm were examined for *Salmonella*, *Shigella* and *Campylobacter species* from Harar town vegetable farm. These selected pathogenic bacteria were detected from four different leafy vegetable types (Lettuce, Spinach, Kale and Cabbage) with the

overall prevalence of *Salmonella* species (12.5%), *Shigella* species (9.7%) and *Campylobacter* species (2.8%) (Table 3). The presence of *Salmonella* species in each vegetable type was 22.2% (4/18), 16.7% (3/18), 11.1% (2/18) and, 0% in lettuce, spinach, cabbage and kale, respectively.

Table 3: Prevalence of selected pathogenic bacteria in some leafy vegetables irrigated with wastewater in Harar town vegetable farm

Leafy Vegetables	**Number of examined samples (N)**	**Detected Pathogenic bacteria**					
		Salmonella species		*Shigella* Species		*Campylobacter* specie	
		F	%	F	%	F	%
Lettuce	18	4	22.2	3	16.7	1	5.6
Spinach	18	3	16.7	1	5.6	0	0
Kale	18	0	0	2	11.1	1	5.6
Cabbage	18	2	11.1	1	5.6	0	0
Total	72	9	12.5	7	9.7	2	2.8

F=Frequency of positive samples, %=Percentage of positive samples, Kale=*Yegurage gomen* (*Brassica carinata*) (Ethiopian leafy vegetable)

Of the 72 leafy vegetable samples 9 (12.5%) had *Salmonella* species which was in connection with the results of Nma and Oruese (2013) who demonstrated that the frequency of occurrence of *Salmonella* species associated with vegetables in Port Harcourt metropolis (Nigeria) was (13.6%); but higher than the study conducted by Tambekar and Mundhada (2006), Abadias *et al*. (2008) and Oliveira *et al*. (2011) who reported that 5.8%, 0.7% and 1.2%, respectively, of the total vegetable samples contaminated by *Salmonella* species. In contrast to this result, Adjrah *et al*. (2013) and Moayed *et al*. (2013) did not detect *Salmonella* species in any of the vegetable samples examined. The higher detection of *Salmonella* from these produce might be related to the large surface area of these vegetables exposed to contact with the effluent from irrigation water and the type of irrigation system used. *Shigella* species was isolated in 9.7% (7/72) of the 72 leafy vegetable samples and its presence in each vegetable type was 16.7% (3/18), 5.6% (1/18), 11.1% (2/18), and 5.6% (1/18) in lettuce, spinach, kale and cabbage, respectively. This result (9.7%) was higher than the findings of Nma and Oruese (2013) who reported that the frequency of *Shigella* species was 2.5%, from Port Harcourt, metropolis (Nigeria); Tambekar and Mundhada, (2006) from India indicated that *Shigella species* was isolated in 3.4% of vegetable samples; Wahla and Devi (2015) also revealed that the percentage of occurrence of *Shigella* species was 5% from salad vegetables in India and Cinzia *et al*. (2015) revealed that from 125 vegetable samples collected from Italy, *Shigella* species were not detected at all.

Among all leafy vegetables analyzed for pathogenic bacteria 2.8% were contaminated by *Campylobacter* species. This result was in connection with Kumar *et al*. (2001) who reported low isolation rates of *Campylobacter* in 2 (3.6%) of 56 fresh vegetables. In the present study, low detection rate of *Campylobacter* species compared to other pathogenic bacteria (*Salmonella* and *Shigella* species) from vegetables may be because of the fact that these bacteria are microaerophilic food borne pathogens; survive poorly on plants, perhaps because of lack of microsites with sufficiently low oxygen concentrations in that habitat. According to PHLS (2000) guide line in 25 grams of raw vegetables, pathogenic bacteria should not be detected. However, pathogenic bacteria were detected in leafy vegetable samples. Generally, the results of this study also confirmed that the level of contamination was high in vegetables that were irrigated with wastewater in Harar town vegetable farm. It was also shown that thorough washing was not sufficient to reduce pathogen levels to safe limits in leafy vegetable types studied.

3.3. Parasitological Analysis of Leafy Vegetables

Infective parasitic stages in this study (*A. lumbricoides* eggs, *E. histolytica* and *G. lamblia* cysts) were detected. As can be seen from Table 4, the percentage of detection of *Ascaris lumbricoides* eggs, *Entamoeba histolytica* and *Giardia lamblia* cysts were 43.1%, 25% and 15.3%, respectively in all vegetable samples.

Table 4: Percentage of positive samples for parasites detected in spinach, lettuce, kale, and cabbage

Detected parasitic stages	Leafy vegetables									
	Lettuce (N=18)		Spinach (N=18)		Kale (N=18)		Cabbage (N=18)		Total (N=72)	
	F	%	F	%	F	%	F	%	F	%
***A. lumbricoides* eggs**	8	44.4	9	50	4	22.2	10	55.6	31	43.1
***E. histolytica* cyst**	6	33.3	1	5.6	5	27.8	6	33.3	18	25
***G. lamblia* cyst**	3	16.7	4	22.2	1	5.6	3	16.7	11	15.3

F=Frequency of positive samples, N=Number of samples examined, %=Percentage of positive samples, kale=*Yegurage gomen* (*Brassica carinata*)

Ascaris lumbricoides eggs was the most prevalent parasitic stage, followed by *Entamoeba histolytica*, and *Giardia lamblia* cysts. In contrast, Abougrain *et al*. (2010) reported that eggs of *Ascaris lumbricoides* was detected in 68% (85/126) of vegetables examined a proportion that was greater than the present finding. In addition, Al-Binali *et al*. (2006) reported the detection of *A.lumbricoides* in 16% of leafy vegetables; Daryani *et al*. (2008), reported the prevalence of 25% and 29% for pathogenic parasites in vegetables of markets and gardens, respectively with *A. lumbricoides* eggs being detected in 2% of samples examined in Iran. In line with this, study done in Shahrekord (Iran) had shown that *Ascaris lumbricoides* eggs were the most predominant parasitic stages in vegetables (Fallah *et al*., 2012).

In the present study, among protozoan parasites detected from vegetables was a cyst of *Entamoeba histolytica* (Table 4). It was detected in 25% (18/72) of fresh leafy vegetables examined including 33.3% (6/18) of lettuce, 5.6% (1/18) of spinach, 27.8% (5/18) of kale and 33.3% (6/18) of cabbage samples from this farm. In contrast to this study, Damen *et al*. (2007) isolated *E. histolytica* (14%) from different vegetables in Jos (Nigeria), which showed lower value than the current finding. The presence of the *Entamoeba* species in the vegetable samples could be the result of inappropriate agricultural practices; during cultivation, when cultivated vegetables come in a direct contact with soil and water that is contaminated with human and animal feces (Silva *et al*., 2014).

Giardia cyst was also detected in 15.3% (11/72) of all leafy vegetables examined in the present study (Table 4). These included 16.7% (3/18) of lettuce, 22.2% (4/18) of spinach, 5.6% (1/18) of kale and 16.7% (3/18) of cabbage. As can be seen from Table 4 *Giardia lamblia cyst* was the least prevalence among the other parasites detected. However, it was higher than the study reported by Erdogrul and Sener (2005), Abougrain *et al*. (2010) and Ali and Ameen (2013) who revealed that the prevalence of *Giardia lamblia* cysts attached to vegetables in some developing countries ranging from 3% to 10%.

Occurrence of more than one parasite per sample in this study reflects the possibility of multi faecal contamination of vegetables which could lead to multiple parasitic infections in human. It might also indicate the persistence of intestinal parasitic infection in the area (Tamirat *et al*., 2014). The presence of infective parasitic stages poses a greater health risk from handling and consuming the contaminated vegetables. The differences were seen in the relative abundance of the parasites found in the vegetables.

4. CONCLUSION

As most farmers irrigate different leafy vegetables before harvesting them with the same water again and again, it is likely that there is pre-harvest contamination of vegetables. This water can easily spread pathogenic bacteria and parasites from the contaminated source to leafy vegetables. Data obtained from the microbial analysis suggested that, there were more risks from consumption of vegetables. These were illustrated by high contamination of vegetables by indicator bacteria, pathogenic bacteria, and parasites from this farm. The high contaminations of vegetables with total and fecal coliforms suggest high risk of acquiring infectious diseases through the consumption of vegetables. The occurrence of such indicator microorganisms is an indication of the contamination of the vegetables with faecal matter derived from humans and other animals. The higher number of coliforms also indicates that pathogenic bacteria may exist. High total coliform bacteria levels are known to be related to lack of proper hygiene practice in and around the farms and the quality of water used for irrigation.

The results of this study also showed that vegetables cultivated in Harar town vegetable farm may posed a great public health problems, due to the presence of pathogenic bacteria like *Salmonella* species, *Shigella* species, *and Campylobacter* species, despite the vegetables did not show any visible signs of contamination. This study is a preliminary, on-going research; it showed the presence of organisms that have serious public health significance in this site. High numbers of pathogenic bacteria in raw consumed vegetables would lead to the consumer's illness with symptoms of the particular or combined microbial presence. Washing of vegetables with just water is inadequate to remove all contaminating pathogens. The contaminating pathogens are responsible for various types of enteric diseases as well as serious intoxications in human. This finding also raised the concern of public health being at high risk of infection with amoebiasis, giardiasis and ascariasis. Biologically, the highest health risk is for helminth infections compared with other pathogens because helminthes persist for

longer periods in the environment, host immunity is usually low to non-existent, and the infective dose is small.

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