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Intestinal Parasitic and Bacteriological Contamination of Raw Vegetables From Selected Farms and Markets in Nekemte, Ethiopia

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Abstract

This study was conducted to assess the extent of intestinal parasitic and bacterial contamination of raw vegetables sold at Nekemte town and collected from selected farms near to Nekemte town. Samples of three different vegetables; cabbages, lettuce and carrots were collected from Nekemte town and selected farms and all samples were examined for intestinal parasites and bacterial contamination, to determine bacterial load and prevalence intestinal parasites. The results show that cabbages were found to be the most heavily contaminated vegetable in both farms and in markets by aerobic bacteria. The highest total coliform count was also recorded from lettuce in all vegetable farms. The highest faecal coliform count was recorded in cabbage sampled from selected farms. The high microbial contamination rates associated with these vegetable samples indicated poor water quality for irrigation employed in the overall production of vegetables in the study area.

Keywords: Heavy metals, Indicator bacteria, Parasites, Vegetables

1. Introduction

Parasites and bacteria are among the main public health problems around the world especially in tropical and sub-tropical countries. The prevalence is highest among the inhabitants of towns in developing countries, where there is improper disposal of garbage and untreated sewage into streams and rivers, poor health systems and overcrowding. Globally, it is estimated that some 3.5 billion people are affected, and that 450 million are sick from intestinal parasite infections, with an estimated 200,000 deaths annually (Wakid, 2009). Parasite infections such as *Ascaris lumbricoides* (1.2 billion), *Trichuristrichiura*(795 million), hookworms (*Ancylostomaduodenale*and *Necatoramericanus*) (740 million) and so many others affect people all over the world (De Silva *et al.,* 2003; Bethony*et al.,* 2006).

Bacterial contamination of vegetables caused by Salmonella in human are divided in to typhoid fever, caused by salmonella typhi and salmonella parathyhi and range of clinical syndromes, including diarrheal diseases caused by large number of salmonella serovars (Gordon, 2008). Shigellosis is an acute invasive enteric infection accused by Shigella; it is clinically manifested by diarrheal that frequently bloody. Major obstacles of controlling shigellosis includes the eases with Shigella spread from person to person rapidly with which it develops antimicrobial resistance(WHO,2005). It is well established that the use of excreta-polluted irrigation water to grow vegetables is a health risk to the farmer and consumers (Zavadil, 2009). In spite of this, the use of wastewater has been used for farming purposes for many types of vegetables (Agamid *et al.*, 1999). Wastewater frequently contains high numbers of protozoan and helminthes parasites, which are of primary public health concern for wastewater reuse. An important characteristic of these organisms is the production of highly resistant cyst and ova that can survive for very long in the wastewater (Erdogrul and Sener, 2005).

Over 60% of the communicable diseases in Ethiopia are due to poor environmental health conditions arising from unsafe and inadequate water supply and poor hygienic and sanitation practices (Abebe, 1986). However, there is little information available on the risks of parasite infections associated with the consumption of contaminated vegetables in Ethiopia (Erko*et al*., 1995) have shown vegetable contamination with amoeba cyst and *Ascaris* eggs on vegetables grown in faecally contaminated gardens. As these parasites are highly resistant and able to withstand harsh conditions, it is important to assess the risk of human infections associated with the consumption of raw vegetables that might be contaminated with these parasites during cultivation, transport, washing or storage.Although a number of studies have been conducted on the distribution and prevalence of parasites and bacterial contamination in raw vegetables, there is a limited study addressing the importance of vegetables as contributing source for the high prevalence especially in Nekemte town. Therefore, this study, attempts to determine the extent of raw vegetable contamination with parasites that could be transmitted to humans and to determine the bacterial loads in raw vegetables.

2. Materials and Methods

2.1. Study Area

The study was carried out in Nekemte, the capital city of East Wollega Zone, which has a total population of 2,979,086 according to the 2009 population census (http:// www. csa. gov.et/doc/ cen, 2010). There are agricultural farms in and around the city, which are irrigated with river water. These rivers receive numerous discharges of raw sewage, community refuse and urban wastewater. The main vegetables grown include cabbage potato, carrot, lettuce, cauliflower, red beet and tomato which are sold in the nearby markets in the city vegetables sold in the major markets in Nekemte town at different places.

2.2. Sampling locations

Raw salad vegetable, lettuce, which is cultivated on the sire along the major rivers within Nekemte was picked from different farms which were selected after a preliminary survey of finding farms in and around Nekemte out of these, three irrigated farms were selected based on their accessibility. These farms use water from rivers which receive contaminated raw sewage, waste refuse and polluted water from the community (Itanna, 2002). Raw vegetables, lettuce, cabbage, carrot and tomato were also picked from three major markets, Darge, Bordi and M/Sefar, to determine possible parasite contamination of market vegetables as well as bacterial loads.

2.3. Collection of samples for the study

In estimating the sample size (n), 50% prevalence, 95% confidence interval for Z^2, conventionally 1.96 , *e* is the desired level of precision (taken to be 5%, e=0.05) and P is the estimated proportion of an attribute that is present in the population 0.5. A representative sample for proportions will be thus calculated using the following statistical formula (Cochran 1963 cited in Kasiulevičius*et al*., 2006). Vegetables was picked six times during the study period, 96 samples of each vegetable type will be taken. One sample will be taken as a portion of vegetable weighing 250 grams. All samples were collected on clean polyethylene bags while water samples will be collected on clean 30 liters plastic containers. Samples were then brought to Biology Laboratory, Wollega University for analysis of vegetables quality (Parasitic and bacteria loads).

2.4. Vegetable analysis

2.4.1. Parasitological Samples Analysis

Parasites from vegetable was analyzed by taking a portion of vegetables weighing (250 g) and washed with 1000ml physiological saline solution (0.85% NaCl) wearing gloves in a bucket. The washing was left for 24 hours for sedimentation to take place. The top physiological saline solution was then discarded carefully without shaking and the remaining 5 ml washing physiological saline solution will be centrifuged (Gallenkamp Angle head centrifuge Cat.No CFB 700 0100 HZ50) at 2000 g for 5 min. The supernatant discarded and the residue will be agitated gently by hand in physiological saline solution for further distribution of the cysts and eggs in the residue, then examined in Lugol's iodine (10.5% in distilled water) by using light microscope (Bailenger,1962 cited in Daryani*et al.*,2008). The remaining will be preserved in sodium acetateacetic acid formalin (SAF) (15g sodium acetate, 20ml glacial acetic acid, 40ml formalin in 925ml distilled water).

2.4.2. Bacteriological samples analysis

All vegetables was collected from different sources and cultured on selenite F broth and incubated for 24hrs at 37^0c followed by subculture on XLD agar for isolation for 24hrs at 37^0c for isolation of Shigella and Salmonella species. The bacteria were identified by their characteristics appearance on their respective media and confirmed by the turn biochemical reaction used for identification of enterobacteriaceae.

2.5. Modified Ziehl – Neelsen staining method

Smears were prepared from the SAF preserved fractions and the samples for detection of *Cryptosporidium* cysts. After the smears have air-dried they were fixed with 70% methanol for 3 minutes. This was then stained with Carbol-fuchsin (0.34% fuchsine and 4% w/v phenol) for 30 minutes which was then washed off with tap water. The smears were then decolorized using acid alcohol (1% hydrochloric acid–ethanol 95%) for 1minute and were counter-stained with 1% methylene blue for another 1minute. The stain was again washed off with tap water and the smears were microscopically examined by using 1000x magnification (Hendrickson and Pholenz, 1981).

2.6. Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 17. P-values were calculated using Chi-square test appropriate. A P-value <0.05 was considered statistically significant.

3. Results and Discussion

2.7. Prevalence of intestinal parasites on vegetables in Nekemte town and Selected farms

In the present study different vegetables samples were collected from markets in Nekemte town and farms near to Nekemte town (Arjo, Bako and sire) analyzed to determine bacteria load and parasitic contamination of the raw vegetables and percentages of parasites. These percentages suggest a high risk of human infection, since parasites which exist in association with these vegetables are capable of infecting human; especially *Cryptosporidium and Giardia* cyst are highly prevalent from both farms and markets. Data showing the presence of *Giardia* cyst and *Cryptosporidium* in this study are similar to those identified by other investigators (Robertson and Gjerde, 2000, Amoah *et al*., 2007). Choi and Lee (1992) in Korea reported that ascaris eggs were found to be the highest (49.0%) among lettuce (*Lactuca sativa*), young radish(*Raphanussapivus*) and Chinese cabbage (*Brassica pekinensis*) where Chinese cabbage showed the highest degree of contamination (91.1%) and lettuce being next(49.0%) in positivity of ascarid eggs. A study from Saudi Arabia also reported the detection of *Ascaris lumbricoides* in 16% of leafy vegetables examined which was lower than the present study (Al-Binali*et al.*, 2006).

Recently, Daryani*et al.* (2008) reported the detection of intestinal parasites in 29% (13/45) of native garden vegetables consumed in Ardabil city, Iran. Similarly some previous studies have reported vegetable contamination with intestinal parasites ranged from 1.94% to 68.3% in different parts of Iran (Hamzavi, 1997; Sahebani*et al*., 2001; Seyed and Sajjadi, 1997). In line with this finding, Oliveira and Germano (1992) reported that from the parasites studied, *Ascaris* eggs were the highest in number which is in close agreement with this result. Ulukanligil*et al.* (2001) in Turkey detected soil-transmitted helminths (mainly *A. lumbricoides*) in 14% (14/100) of fresh vegetables, in 84% of soil samples where vegetables are cultivated and in 61% of irrigation water. Another study from Iran, reported prevalence of 25% and 29% for intestinal parasites in vegetables of markets and gardens, respectively with *A. lumbricoides* eggs being detected in 2% of samples examined which is very low compared to the present result (Daryani*et al*., 2008). Previous studies from different countries where STH infection is endemic have shown that vegetables were highly contaminated with *Ascaris lumbricoides* eggs and had similar findings with the present study, e.g. Malaysia (Sinniah, 1983), Thailand (Yodmani*et al*., 1983), and Philippines (de Leon *et al*., 1992).

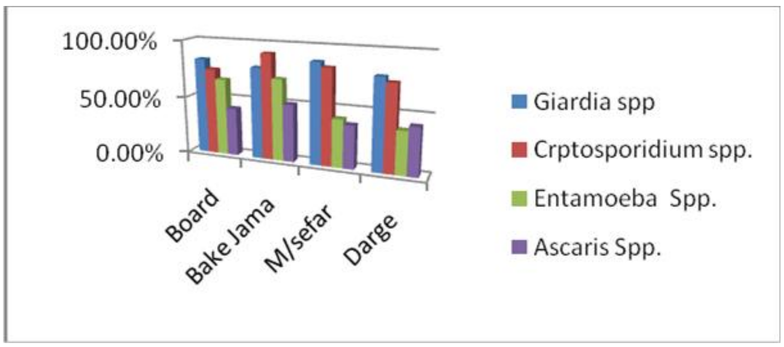
In previous study Abougrain*et al.* (2009) reported that helminth eggs and *Giardia* cysts were detected in 100% (27/27) of fresh lettuce samples which was higher than the present study. As shown from the results the degree of contamination for vegetables was different from each other depending on the kind of vegetable. This could be due to the difference in distance between the soil and the plant leaves. Damen *et al.* (2007) examined different types of vegetables for parasitic contamination and reported that cabbage had the highest contamination rate of (64%), followed by lettuce (40%) which were higher than the present study.

Contrary to these findings, in the present investigation *Giardia* cysts were detected in 25% of the total vegetable samples and the study revealed that *Giardia* cysts were found in the different vegetables examined with cabbage samples being most contaminated followed by lettuce Samples. The observed differences in prevalence rates of the different intestinal parasites from fresh vegetables reported in the present work and those reported by others is expected. Several factors may contribute to such differences. These may include, among other factors, geographical location, type and number of samples examined, methods used for detection of the intestinal parasites, type of water used for irrigation, and pre-harvest handling methods of such vegetables.

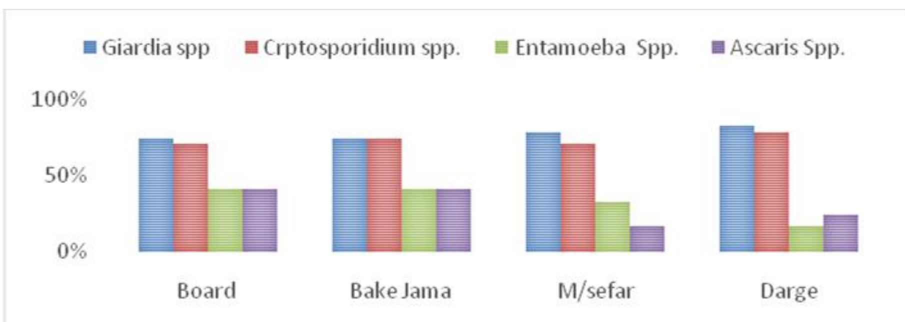
Table 1. Prevalence of intestinal parasites in raw vegetables in Nekemte town and selected farms, 2017

Nekemte town

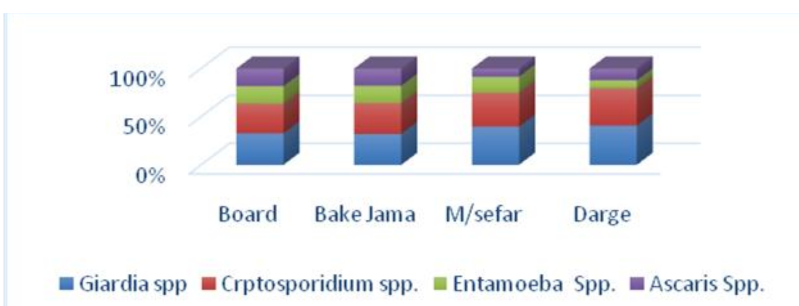
Cabbages

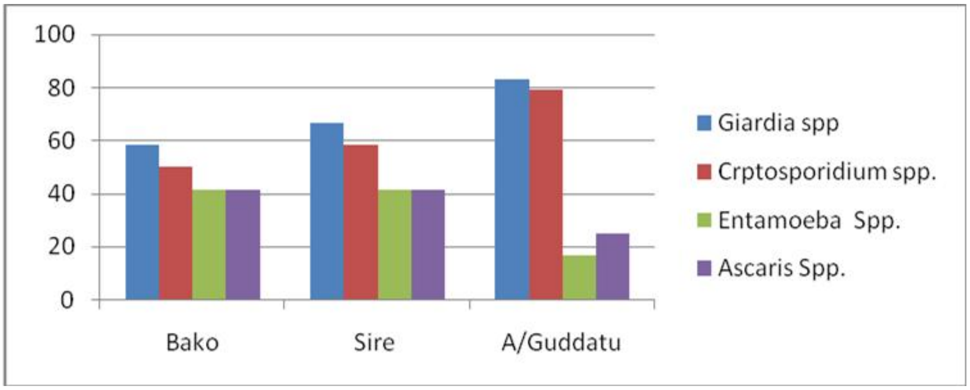


Lettuce

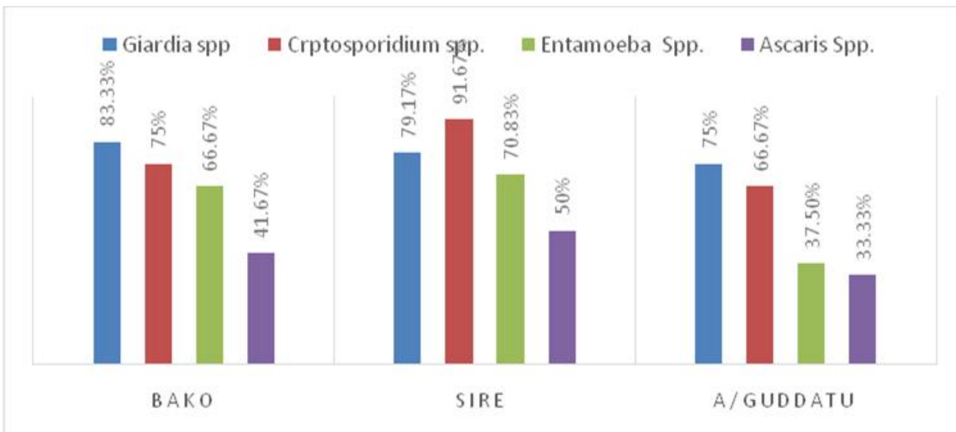


Carrot

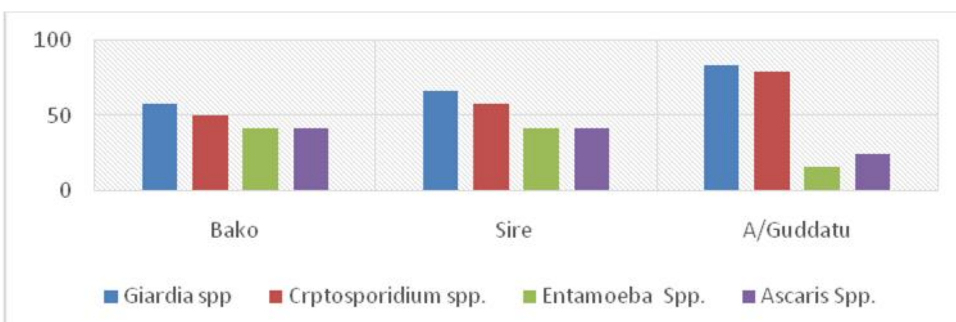




Farms
Lettuce



Cabbages



Carrots

Bacteriological analysis of raw vegetables in Nekemte town and selected farms, 2017

In this study the bacterial indicators in were randomly collected from raw vegetables (carrots lettuce and cabbage) were enumerated mainly to asses level of bacterial contamination. In the present study high percentages of total bacterial counts were observed in cabbages collected from both farms markets and farms. Pre-harvest conditions can come from irrigation water, improperly composted manure used as fertilizer, fecal contamination from human and domestic animals (Cornish *et al.*, 1999).

The mean values showed the following order for total aerobic count for the three vegetables. Total aerobic count was highest in cabbages followed by lettuce and carrots. The cabbages vegetable collected from farm was found to be the most contaminated based on the total aerobic count in terms of CFU/g. In the present study, fecal coliform counts were lower in all samples. This agreed with reports of earlier workers of Cornish *et al.* (1999), Keraita *etal.* (2003), and Amoah*et al.* (2005). All reported high bacterial numbers on lettuce produced in farms within Kumasi. This result was closer with the findings of Thunberg *et al.* (2004). They have reported total viable count as 5.0×108, 4.0×108, 3.1×107, 2.5×107, and 2.0×106 CFU g-1 for spinach samples collected from various farm sites. Bacterial numbers recorded in this study in all of the three crops exceed the International Commission on Microbiological Specifications for Food limits of 103 to 105 coliforms 100 g-1 wet weight of vegetables usually eaten raw (ICMSF, 1998)

Aerobic organisms reflect the exposure of the sample to contamination and the existence of favorable conditions for multiplication of microorganisms (Tortora, 1995). For export purposes, it is important that fresh vegetables should not have a total aerobic count exceeding4.9×106 CFU g-1 which is the acceptable limit by some countries (Nguyen-The and Carlin,1994). Therefore, reducing the total count on the products is a priority to ease the economic impact of such contamination. However, some studies showed low levels of contamination inEgypt, Turkey and Taiwan food Products reported by Lund (1993). In contrast, Albrecht *etal*. (1995) and Fang *et al*. (2003) reported high aerobic plate count on vegetable samples inTaiwan ranging from 2.0×103to 4.4×108 CFU g-1. Furthermore, Vural and Erkan (2008) inTurkey had a range of aerobic count of microorganisms from 2.7×106 to 4.3×107CFU g-1.Similarly for salad vegetables collected from Johannesburg in South Africa, Christison*et al*.(2008) reported an average aerobic plate count of 1.0×107CFU g-1. In other studies a rangefrom 1.0×102 to 1.0×106CFU g-1 was obtained by different scholars (Angelidis*et al*., 2006;Aycicek*et al*., 2006; Kubheka*et al*., 2001; Mosupye and von-Holy, 2000). In close agreement with the present result, a study in india (Viswanatha and Kaur, 2001) showed that the total aerobic plate count for cabbage and lettuce was found to be 2.8 ×106 and 1.2 ×108 CFU g-1 respectively. Whereas in the same study the total coliform count for these two vegetables was from 2.0×102-2.5×103 for cabbage and 1.2×105-7.0×106 for lettuce.

Table 2. Mean bacteriological counts (log_{10} CFU/g) of raw vegetables in Nekemte town, 2017

Sites	Vegetables	Total Aerobic Mesophilic count	Total coliform counts	Fecal colifrom counts	Total yeast and molds counts
Bordi	Cabbages	4.17	4.04	3.98	4.03
	Lettuce	4.40	4.04	3.98	3.44
	Carrots	4.26	4.35	3.59	3.83
Darge	Cabbages	4.43	4.23	4.12	4.04
	Lettuce	4.52	4.36	4.33	3.33
	Carrots	4.40	4.50	4.41	4.30
M/sefar	Cabbages	4.12	4.23	4.40	4.26
	Lettuce	4.05	3.66	3.65	3.88
	Carrots	3.76	4.54	4.10	4.28
B/jama	Cabbages	4.05	3.32	4.24	4.35
	Lettuce	4.05	3.05	4.13	4.35
	Carrots	4.10	4.31	4.12	4.31

Table 3. Mean bacteriological counts (log_{10} CFU/g) of raw vegetables in farms, 2017

Sites	Vegetables	Total Aerobic Mesophilic count	Total coliform counts	Fecal colifrom counts	Total yeast and molds counts
Bako	Cabbages	4.4	4.04	3.99	3.44
	Lettuce	4.18	4.26	3.73	3.82
	Carrots	4.32	4.04	4.06	3.36
Sibu sire	Cabbages	4.24	4.25	3.69	3.95
	Lettuce	4.24	4.04	4.02	3.68
	Carrots	4.35	4.15	3.84	3.44
ArjoGudatu	Cabbages	4.1	4.13	3.84	4.18
	Lettuce	4.4	4.04	3.99	3.44
	Carrots	4.16	4.31	3.82	4.04

4. Conclusion and Recommendations

The study revealed that there were bacterial, parasitic and heavy metals contamination of fresh leafy vegetables (lettuce, cabbage and carrots) grown Nekemte town vegetable farms. In production and processing of fresh produce, quality and hygiene are the most important criteria for reducing consumer risk. Leafy vegetables are often eaten raw or with minimal processing and, if contaminated with pathogenic bacteria, may lead to health hazard. In recent years several surveys of food-borne pathogens and indicator bacteria in these products have revealed their variable occurrence. Some reports are also available on the survival and transfer of the emerging food borne pathogens.

The result from this study also shows contamination of pathogenic intestinal parasites from both vegetable farms *Ascaris* eggs being the most prevalent in cabbage (41.6%). Therefore great attention should be given in using contaminated water for production of vegetables around Nekemte town for the public health perspective.

The following recommendations can be drawn from the results found in this study:

1. Due to the potential microbiological risks of vegetables, it should be treated directly with certain disinfectant before consumption and to develop highly effective treatments for removing pathogens from a wide range of raw produce.

2. As a consequence of this study, the government should impose strict measures to control or at least minimize the risk of microbial contamination by implementing the Hazard Analysis and Critical Control Point (HACCP).

3. Proper domestic and industrial waste disposal and sludge treatment plant is needed in the area; in addition further work has to be done about the current microbiological quality of the irrigation water in the area.

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