

Short Communication

Parasitic contamination on vegetables irrigated with Awash River in selected farms, Eastern Showa, Ethiopia

Girmaye Benti* and Fekadu Gemechu

Department of Biology, Dilla University College of Natural and Computational Science, P. O. Box-419 Dilla, Ethiopia.

Received 13 March, 2014; Accepted 24 June, 2104

This study was conducted from February, 2013 to April, 2013 to evaluate parasitic contamination of vegetable at Melka Hida and Wonji Gefersa wastewater irrigated vegetable farms that are found in Adama Woreda. A total of 72 vegetables samples were collected from both farms using a random sampling technique. Among all vegetables examined, *Ascaris lumbricoides* eggs were detected in 22.22% (16/72) of spinach (*Spinacea oleracea*), 16.67% (12/72) of lettuce (*Lactuca sativa*) and 23.61% (17/72) of cabbage (*Brassica oleracea* Linn). *Giardia intestinalis* cysts were detected in 18.06% (13/72) of spinach, 19.44% (14/72) of lettuce and 15.28% (11/72) cabbage samples. Of all parasitic contaminants *Entamoeba histolytica* cyst was the least detected parasite; 8.33% (6/72) of spinach, 12.5% (9/72) of lettuce and 8.33% (6/72) of cabbage samples from both farms. The high parasite contamination rates associated with these vegetable samples indicated poor farming practice employed in the overall production in the study area.

Key words: Awash River, contamination, parasite, vegetables.

INTRODUCTION

Vegetables are essential for good health, and they form a major component of human diet in every family. They are vital energy contributors that are depended upon by all levels of human as food supplement or nutrient (Duckworth et al., 1996). Food safety regardless of the specific food product should be a paramount concern to everyone, with parasites from contaminated vegetables being a potential health risk. According to Speer (1997), vegetables can become contaminated with enteric bacterial, viral and parasitic pathogens throughout the

process of planting to consumption. The extent of contamination depends on several factors that include, among others, use of untreated wastewater and water supplies contaminated with sewage for irrigation (Amoah et al., 2007; Beuchat, 2002; Simões et al., 2001).

Intestinal parasites are common in fresh vegetables. Vegetables are reported to harbor intestinal parasites such as *Ascaris lumbricoides*, *Taenia* species, *Fasciola hepatica*, *Hymenolepis nana*, *Echinococcus* species, *Trichuris* species, *Enterobius vermicularis*,

*Corresponding author. E-mail: gurmuukoo@gmail.com.

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Trichostrongylus species, *Toxocara* species, *Strongyloides stercoralis*, *Giardia intestinalis*, *Entamoeba* species, *Iodamoeba butschlii*, *Blastocystis hominis* and *Cryptosporidium parvum* (Gharavi et al., 2002; Gupta et al., 2009; Abougrain et al., 2009; Uga et al., 2009). Intestinal parasitic infections are the top global health problems, whereas amoebiasis and ascariasis, are among the ten most common infections (WHO, 1987). Hence, consumption of raw vegetables plays an important role in the transmission of human parasitic infection (Amoah et al., 2005; Choi et al., 1982; Coelho et al., 2001; Daryani et al., 2008; Erdogrul and Sener, 2005).

Contamination of vegetables may occur through contacting with the soil, raw manure and sewage used as fertilizer on vegetable farms. In most cases, contamination of vegetables is associated with the water used for irrigation (Simoes et al., 2001). Use of sewage contaminated water for irrigation of vegetables is a common practice in developing countries. The access to clean water for irrigating vegetables is a major challenge. As an alternative, urban and peri-urban vegetable farmers in search of water for their crops have no other choice than to use water from these highly polluted sources. This raises public health concerns due to possible crop contamination with pathogens where vegetables are eaten uncooked (Amoah et al., 2006).

Unhygienic sewage disposal and absence of its treatment facilities pose potential health hazards through contaminating irrigated food crops with parasites in urban and suburban areas of African countries including Ethiopia (Damen et al., 2007; Nyarango et al., 2008; Srikanth and Naik, 2004; Weldesilassie et al., 2009). Many farm households in Eastern Showa that are irrigating their farmlands with wastewater are not aware of the risks or the potential harmful environmental consequences. This may be attributed to illiteracy, lack of adequate information and exposure to poor sanitary conditions for most of their lives. Several studies documented prevalence of intestinal parasites in different parts of Ethiopia including Oromia region through microscopic examination of stool samples collected from suspected human population (Legesse and Erko, 2004; Tadesse, 2005; Dejenie and Petros, 2009). However, research endeavors to detect parasites from environmental sources such as vegetables are scarce in Ethiopia (ROSA, 2009). Therefore, the aim of this study was to assess the degree of parasitic contaminations on selected vegetables irrigated with Awash River at Melka Hida and Wonji Gefersa farms.

MATERIALS AND METHODS

Description of the study area

This study was conducted at Melka Hida and Wonji Gefersa wastewater irrigated vegetable farms that are found in Adama Woreda. Wonji Gefersa is located in East Showa zone, Oromia

region, 107 km away from Addis Ababa. It is located at a latitude of 8° 26' 59" North and longitude of 39° 16' 48" East. It has an elevation of 1588 masl and its temperature and rain fall is 23°C and 500 to 800 mm, respectively (Environmental Protection Authority, 2005). While Melka Hida is found in Adama town administrative zone, Oromia region, 99 km away from Addis Ababa. It is located at latitude of 8° 33' 0" North and longitude 39° 16' 12" East. It has an elevation of 1620 masl (Environmental Protection Authority, 2005) (Figure 1).

Study Design

Preliminary surveillance study was conducted before starting the test experiment at Melka Hida and Wonji Gefersa vegetable farms using Awash River for irrigation. Accordingly 160 sample respondents were purposively selected and provided with already prepared questionnaire. The questions were interpreted for few producer respondents who can't understand the matter. Out of the selected producer 135 were males and 25 were females, all of which are taken as literates. According to the answer obtained and analyzed from the respondents most of the problems that frequently faced them were recognized by the professional physicians and so related with the parasitic contaminations from the vegetables and fruits. Based on these the following three parasitic contaminants namely giant roundworm (*A. lumbricoides*), *Giardia* (*Giardia intestinalis*) and parasitic protozoan (*Entamoeba histolytica*) have seen to be the most dominant and leading parasite diagnosed. Then, a cross sectional survey test experiment was conducted to assess contamination of specific parasites on the main leafy vegetables [lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea* Linn), and spinach (*Spinacea oleracea*)] that were grown in Melka Hida and Wonji Gefersa vegetable farms. The samples were regularly collected three times at three week interval from February, 2013 to April, 2013.

Sample collection

A total of 72 samples comprising three types of fresh vegetables (cabbage, lettuce and spinach) were collected from Melka Hida and Wonji Gefersa vegetable farms using a random sampling technique. Recently, mature leaves of lettuce, cabbage and spinach were sampled at early maturity according to methods used by Itanna (1998). All samples were collected aseptically in a sterilized universal container and plastic bags and transported to Dilla University using a cooler box for laboratory processing. Analysis was conducted within 24 h of arrival at the Parasitology Laboratory of Dilla University.

Parasitological analyses of vegetables

In the laboratory, 100 g of each fresh vegetable sample was chopped into small pieces and put into a clean beaker containing enough physiological saline solution (0.85% NaCl), to wash the sample. After removing fragments of the vegetable sample from the washing saline using clean forceps, it was kept about 24 h for sedimentation to take place. After 24 h sedimentation, the top layer of the washing saline was carefully discarded leaving 5 ml of the sediment. This was finally centrifuged at 2000 rotations/min for 5 min by HERMLE Z200A centrifuge. After discarding the supernatant, the residue was mounted on slides, stained with Lugol's iodine solution and examined under the compound light microscope to examine the samples for intestinal parasites: *A. lumbricoides* eggs, *E. histolytica* cysts and *G. intestinalis* cysts (Abougrain et al., 2009; Uga et al., 2009). To increase the chance of parasite detection, three slides were prepared for each vegetable sample.

Data analysis

For data analyses, descriptive statistics in percentage was computed for the pre surveillance data and parasitic contamination of vegetable samples. Chi-square test was used for comparison between vegetable contamination rates using SPSS version 16.0. A P-value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The study results of the pre surveillance and test experiment data on parasitological analysis of leafy vegetables were explained as shown in Tables 1 and 2, consecutively.

The aforementioned results (Table 1) show that 67.5% (108) of the respondents said that their income depends on the vegetable production. Producers also asked for why and which types of vegetables they produce and so, 50% (80) and 64.4% (103) explained that they were produced for the purpose of income generation and vegetable types like cabbage, lettuce and spinach, respectively. Similarly, 87.5% (140) of the producer said that the disease agents are caused by different parasitic worms and other pathogenic microbes. Among the parasites *A. lumbricoides*, *E. histolytica* and *G. intestinalis* are the predominantly mentioned by respondent.

Based on the information obtained from the surveillance, regular experiment was conducted to test for the presence of the aforementioned parasite. The results (Table 2) showed that cabbage samples collected from both Melka Hida and Wonji Gefersa farms, 17 (23.61%) were found to be positive for *A. lumbricoides* eggs. In developing countries, intestinal parasites are very common. Fresh vegetables are an important route of their transmission. Daryani et al. (2008) reported the detection of intestinal parasites in 29% (13/45) of native garden vegetables. The distribution of parasites on vegetables between Melka Hida and Wonji Gefersa was significantly different ($\chi^2=3.772$, $P=0.042$). Variation may be due to different sections of the wastewater drainage canal in Wonji Gefersa farm which receive different load of faecal contamination.

A. lumbricoides eggs were the predominant intestinal parasite in this work. These include 22.22% (16/72) in spinach, 16.67% (12/72) in lettuce and 23.61% (17/72) in cabbage. The results showed that cabbage is relatively highly contaminated and lettuce is the least contaminated (Table 2). This might be due to cabbage nearby the soil as compared to the other vegetables that was directly contaminated with irrigation water or indirectly by contact with contaminated soil, may aggravate the rate of contamination. Other studies show that vegetables with dense foliage were most contaminated than those growing on surface (Idrissa et al., 2010). The dense foliage would protect the helminth eggs against unfavourable conditions to their survival and persistence,

such as sunlight, drying, and wind (Dssouli 2001; Dssouli et al., 2006).

In relation to this finding, Abougrain et al. (2009) reported on other vegetables that helminth eggs and *G. intestinalis* cysts were detected in 58% (73/126) of vegetables examined. These include 14% (5/36) of tomato, 42% (15/36) of cucumber, 96% (26/27) of lettuce and 100% (27/27) of cress samples. This shows that the use of sewage water plays important role in the transmission of parasitic disease to human through consumption of such vegetables (Gupta et al., 2009). *A. lumbricoides* eggs were detected from lettuce with a value of 25% (9/36) from Melka Hida and 8.33% (3/36) from Wonji Gefersa. There was significant variation between the sites ($\chi^2=3.600$, $P=0.050$), Melka Hida having the highest rate. This may be due to contamination during production and application of human excreta, animal manure as a fertilizer. The results of this study is comparable with the previous finding that showed Chinese cabbage (*Brassica pekinensis*) had the highest degree of contamination (91.1%) by *A. lumbricoides* eggs (Choi and Lee, 1992).

G. intestinalis cysts were detected in 18.06% (13/72) of spinach, 19.44% (14/72) of lettuce and 15.28% (11/72) of cabbage samples from both farms. There was also significant difference in the prevalence of parasites stages ($\chi^2 = 5.675$, $P = 0.017$). This indicates that the prevalence of *G. intestinalis* cyst was more prevalent on lettuce collected from Wonji Gefersa farm than the other vegetables. This may be due to the production related sources of parasitic contaminants associated with practices of using untreated wastewater for irrigation.

On the other hand, the prevalence of *G. intestinalis* cyst examined on cabbage and spinach from both farms were not significantly different ($\chi^2 = 2.683$, $P = 0.101$; $\chi^2 = 2.347$, $P = 0.126$), respectively (Table 2). The finding of this study is in line with Daryani et al. (2008) who reported detection of intestinal parasites in 29% (13/45) of native garden vegetables consumed in Ardabil city, Iran. Similarly, Abougrain et al. (2009) examined 126 samples of four different types of fresh salad vegetables from wholesale and retail markets in Tripoli, Libya of which 58% were positive for helminth eggs and *G. intestinalis* cysts. The variation in the prevalence might be due to sample size difference.

Moreover, *E. histolytica* cyst was detected in 8.33% (6/72) of spinach, 12.5% (9/72) of lettuce and 8.33% (6/72) of cabbage samples from both farms. The results of the study indicated that the prevalence of *E. histolytica* cyst was the least. However, there was significant difference ($\chi^2 = 3.175$, $P = 0.045$). The result of the study revealed that lettuce collected from Wonji Gefersa was more contaminated by *E. histolytica* cysts. As shown in Table 2, *E. histolytica* cysts was not significantly different ($\chi^2 = 2.683$, $P = 0.101$; $\chi^2 = 2.347$, $P = 0.126$) between cabbage and spinach, respectively.

In connection with this finding, Damen et al. (2007)

Table 1. The percentage and frequency of pre surveillance data collected.

Parameter	Frequency	%
Sex		
Male	135	84.4
Female	25	15.6
What is your source of income?		
Vegetables production	108	67.5
Other crops production	15	9.4
Both	37	23.1
Which type of vegetables do you cultivate?		
Cabbage	8	5.0
Lettuce	10	6.2
Spinach	12	7.5
Beetroot	4	2.5
Tomato	23	14.4
Cabbage, lettuce and spinach	103	64.4
Purpose of production		
Income generation	80	50
Home consumption	24	15.0
Both	56	35.0
Source of water for the production of vegetables		
Awash River	153	95.6
Under ground water	7	4.4
Would you think this water (Awash River) is contaminated?		
Yes	136	85
No	24	15
If you say yes for above question, what is the possible sources for contamination		
Wastewater from the municipality directly join Awash River	34	21.2
Community nearby Awash River defecated on open space	47	29.4
Both are the source of contamination of Awash River	79	49.4
Did you get sick in last one year?		
Yes	115	71.9
No	45	28.1
What is the possible source for disease occurrence?		
Consumption of improperly cooked vegetables	110	68.8
Personal hygiene	28	17.5
Utensil used	22	13.2
Which types of disease causing agents are identified in laboratory diagnosis?		
Parasite	140	87.5
Bacteria	20	12.5
If you say parasite for above question, which types of the parasites predominantly observed?		
<i>Ascaris lumbricoides</i>	40	25
<i>Entamoeba histolytica</i>	20	12.5

Table 1 cont'd

<i>Giardia intestinalis</i>	15	9.4
<i>Fasciola hepatica</i>	4	2.5
<i>Strongyloides stercoralis</i>	10	6.2
<i>Cryptosporidium parvum</i>	6	3.8
<i>Ascaris lumbricoides</i> , <i>Entamoeba histolytica</i> and <i>Giardia intestinalis</i>	65	40.6
What types of recommendation recommended by the physician about the quality of food consumptions?		
Not to eat improperly cooked vegetables	90	56.2
Wash the vegetables properly with clean water before consumption	50	31.2
Treat the water used for vegetables production	20	12.5

Table 2. Prevalence of three purposively selected parasites from cabbage, lettuce, and spinach samples of both farms (Melka Hida and Wonji Gefersa).

Vegetable type	No. of examined sample	Detected organisms	Site			χ^2 test	P-value		
			MH	%	WG			%	Total (%)
Cabbage	12	<i>A. lumbricoides</i> eggs	5	13.89	12	33.33	23.61	3.772	0.042
		<i>G. intestinalis</i> cyst	3	8.33	8	22.22	15.28	2.683	0.101
		<i>E. histolytica</i> cyst	1	2.78	5	13.89	8.33	2.909	0.088
Lettuce	12	<i>A. lumbricoides</i> eggs	9	25.0	3	19.44	16.67	3.600	0.050
		<i>G. intestinalis</i> cyst	3	8.33	11	30.56	19.44	5.675	0.017
		<i>E. histolytica</i> cyst	2	5.56	7	19.44	12.50	3.175	0.045
Spinach	12	<i>A. lumbricoides</i> eggs	6	16.67	10	27.78	22.22	1.286	0.257
		<i>G. intestinalis</i> cyst	4	11.1	9	25.00	18.06	2.347	0.126
		<i>E. histolytica</i> cyst	2	5.56	4	11.11	8.33	0.727	0.394

MH: Melka Hida, WG: Wonji Gefersa.

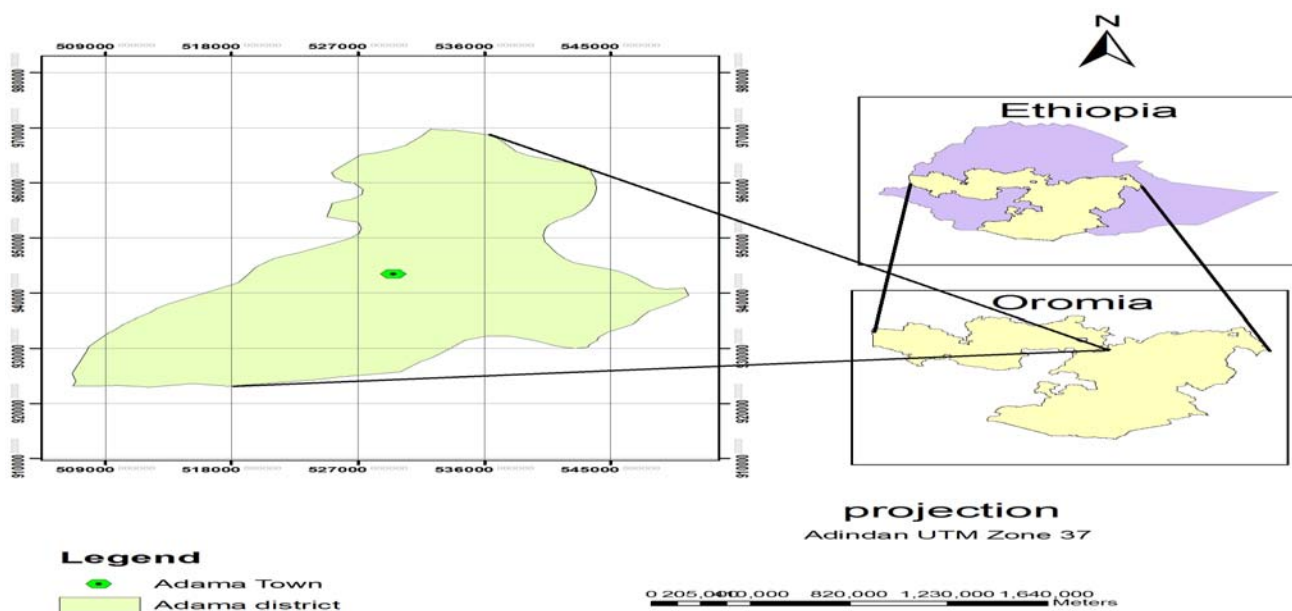


Figure 1. Location of the study area (Aynalem, 2010).

isolated ova of *A. lumbricoides* eggs (17.1%) and *E. histolytica* (14%) from different vegetables in Jos, Nigeria. In general, the study showed significant differences in the occurrence of pathogenic intestinal parasites detected from fresh vegetables. Several factors may include geographical location, type and number of samples examined, methods used for detection of the intestinal parasites, type of water used for irrigation, and per harvesting handling methods of such vegetables may contribute to such differences. According to Bethony et al. (2006) environmental factors play a great role in the incidence of intestinal parasitic infection as hot and humid tropical climate favour increased parasite prevalence. Based on variation in climatic and geographic zones in Ethiopia, it should be evident that there are macro and micro environmental factors contributing to the differences in prevalence of intestinal parasites (Jemaneh, 2000).

Conclusions

This study showed that *A. lumbricoides* eggs, *G. intestinalis* cysts and *E. histolytica* cyst were recorded on vegetable samples of both farms. Taking account of results obtained, it is clear that irrigation process of these vegetables fields has been performed using contaminated water sources (wastewater). Therefore, great attention should be given in using contaminated water for production of vegetables for the public health perspective. An adequate treatment of the sewage water and banning wastewater use for irrigation of plants intended for human consumption, among others, should be implemented. Health promotion and education on the mode of transmission of disease, environmental sanitation and eating habits will enhance the prospect for the control of parasitic infections.

ACKNOWLEDGEMENTS

The authors thank Dilla University College of Natural and Computational Science for the financial, material support and laboratory facilities provision. They also appreciate the efforts of Wonji Gefersa Administrative for helping in sample collection.

Conflict Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Abougrain AK, Nahaisi MH, Madi NS, Saied MM, Ghenghesh KS (2009). Parasitological contamination in salad vegetables in Tripoli-Libya. *Food Control* 21(5):760-762.

- Amoah P, Drechsel P, Abaidoo C (2005). Irrigated urban vegetables production in Ghana: Sources of pathogen contamination and health risk elimination. *Irrigation Drainage* 54(1):49-61.
- Amoah P, Drechsel P, Abaidoo RC (2006). Pesticide and microbiological contamination of vegetables in Ghana's urban markets. *Arch. Environ. Contam. Toxicol.* 50(1):1-6.
- Amoah P, Drechsel P, Abaidoo RC, Klutse A (2007). Effectiveness of common and improved sanitary washing methods in selected cities of West Africa for the reduction of coliform bacteria and helminth eggs on vegetables. *Trop. Med. Int. Health* 12(Suppl)2:40-50.
- Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A (2006). Soil transmitted helminth infections: Ascariasis, trichuriasis, and hookworm. *Lancet* 367(9521):1521-32.
- Beuchat LR (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microb. Infect.* 4:413-423.
- Choi DW, Lee S (1992). Incidence of parasites found on vegetables collected from markets and vegetables gardens in Taegu area. *Korean J. Parasitol.* 10:44-51.
- Choi DW, Ock MS, Suh JW (1982). Recent demonstration of helminth eggs and larvae from vegetables cultivating soil. *Korean J. Parasitol.* 20:83-92.
- Coelho LM, Oliveira SM, Milman MH, Karasawa KA, Santos RD (2001). Detection of transmissible forms of enteroparasites in water and vegetables consumed at school in Sorocaba, Sao Paulo state, Brazil. *Rev. Soc. Bras. Med. Trop.* 34(5):479-482.
- Damen JG, Banwat EB, Egah DZ, Allanana JA (2007). Parasitic contamination of vegetables in Jos, Nigeria. *Ann. Afr. Med.* 6:115-8.
- Daryani A, Ettehad GH, Sharif M, Ghorbani L, Ziaei H (2008). Prevalence of intestinal parasites in vegetables consumed in Ardabil, Iran. *Food Control* 19(8):790-794.
- Dejenie T, Petros B (2009). Irrigation Practices and Intestinal Helminth Infections in Southern and Central Zones of Tigray. *Ethiop J. Health Dev.* 23(1):48-56.
- Dssouli K (2001). Traitement et réutilisation des eaux usées en agriculture au Maroc oriental: Etude des helminthes parasites. Thèse de doctorat en sciences, Faculté des Sciences, Oujda. French.
- Dssouli K, El Halouani H, Berrichi A (2006). L'impact sanitaire de la réutilisation des eaux usées brutes de la ville d'Oujda en agriculture: Etude de la charge parasitaire en oeufs d'helminthes au niveau de quelques cultures maraichères. *Biol. Santé* 6(1):51-58.
- Duckworth RB (1996). Farming systems for the production of fruits and vegetables. *Fruits and vegetables oxford: Pergama press.* pp. 48-62.
- Erdogrul OR, Sener H (2005). The contamination of various fruit and vegetable with *Enterobius vermicularis*, *Ascaris* eggs, *Entamoeba histolytica* cysts and *Giardia lamblia* cysts. *Food Control* 16:557-560.
- Gharavi MJ, Jahani MR, Rokni MB (2002). Parasitic contamination of vegetables from farms and markets in Tehran. *Iranian J. Publ. Health* 31(3-4):83-86.
- Gupta N, Khan DK, Santra SC (2009). Prevalence of intestinal helminth eggs on vegetables grown in wastewater irrigated areas of Titagarh, West Bengal, India. *Food Control* 20(10):942-945.
- Idrissa S, Belghyti D, Kharrim KE, Yoro C (2010). Parasitic Contamination of the Mint and Turnip Irrigated by untreated wastewater in Sidi Yahia Gharb (Morocco). *BALWOIS 2010 Conference, Ohrid, Republic of Macedonia.*
- Itanna F (1998). Metal concentrations of some vegetables irrigated with industrial liquid waste at Akaki, Ethiopia. *Ethiop. J. Sci.* 21(1):133-144.
- Jemaneh L (2000). The Epidemiology of Schistosomiasis mansoni and soil transmitted helminths in elementary school children from the South Gonder zone of the Amhara National Regional State, Ethiopia. *Ethiop. Med. J.* 38:105-118.
- Legesse M, Erko B (2004). Prevalence of intestinal parasites among schoolchildren in a rural area close to the southeast of Lake Langano. *Ethiop. J. Health Dev.* 18(2):116-120.
- Nyarango RM, Aloo PA, Kabiru EW, Nyanchongi BO (2008). The risk of pathogenic intestinal parasite infections in Kisii Municipality, Kenya. *BMC Public Health* 8:237.
- ROSA (2009). A preliminary survey of the microbiological and parasitological quality of some locally produced and marketed vegetables done as part of the ROSA research activity in Arba Minch,

- Ethiopia. pp. 43-44.
- Simoës M, Pisani B, Margues EGL (2001). Hygienic sanitary conditions of vegetables and irrigation water from kitchen gardens in the municipality of Campinas, SP. *Braz. J. Microbiol.* 32:331-333.
- Speer CA (1997). Protozoan parasites acquired from food and water. *Food Microbiology: Fundamentals and Frontiers*, Washington DC, American Society for Microbiology. pp. 478-493.
- Srikanth R Naik D (2004). Prevalence of Giardiasis due to wastewater reuse for agriculture in the suburbs of Asmara City, Eritrea. *Int. Environ. Health Res.* 14:43-52.
- Tadesse G (2005). The prevalence of intestinal helminthic infections and associated risk factors among school children in Babile town, Eastern Ethiopia. *Ethiop. J. Health Dev.* 19:140-147.
- Uga S, Hoa NTV, Noda S, Moji K, Cong L, Yaoki SK, Rai Fujimaki Y (2009). Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam. *Nepal Med. Coll. J.* 11:75-78.
- Weldesilassie AB, Frör O, Boelee E, Dabbert S (2009). The economic value of improved wastewater irrigation: A contingent valuation study in Addis Ababa, Ethiopia. *J. Agric. Resour. Econ.* 34:428-44.
- World Health organization (WHO) (1987). Prevention and control of intestinal parasitic infections. Technical Report Series 749. WHO, Geneva.