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PARASITIC CONTAMINATION OF FRESHLY CONSUMED VEGETABLES SOLD IN THE MARKETS AND FARM FIELDS WITHIN AND AROUND ASMARA

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Abstract

Intestinal parasitic infection is one the factor responsible for food borne illness, it normally happens due to consumption of unclean, raw, or undercooked vegetables and it is a major public health problem in developing country like Eritrea. The main purpose of this study is to assess the presence of parasitic contamination in vegetables which are consumed raw in Asmara, Eritrea.

A cross-sectional study was conducted to evaluate the level of parasitic contamination of raw vegetables sold in two local markets and produce in seven farm fields of Asmara. 200 gm of four selected raw vegetables viz; lettuce, cabbage, carrot and girgir (local Name) were analysed by using sedimentation method, and the stages of parasites were observed under a light microscope. Statistical data was generated using Pearson chi-square test. A value of P <0.05 was considered as statistically significant. Of, 205 vegetable samples, 117 (57.07%) were contaminated with one or more parasites, the most common parasite was *Ascaris lumbricoides* 61(29.7%), followed by *Giardia lamblia* 55(26.8%). Of, four vegetables lettuce was the most contaminated 42 (60.8%), followed by carrot 30 (60%). From 88 samples collected from two Markets, *Giardia lamblia* 32(36.4%) followed by *Ascaris lumbricoides* 11(12.5%) was dominant. 117 samples from seven farms showed higher presence of *Ascaris lumbricoides* 50(42.7%) followed by *Giardia lamblia* 23(19.7%).This demonstration of parasites in raw vegetables which are frequently consumed by people of Asmara as part of their diet is alarming. So, health authorities must educate the farmers and retailers about implication of using faecal contaminated water for cleaning and for fertilization their farm land.

Keywords: *Contamination, Asmara, Vegetables, Parasites*

Introduction

Vegetables form a major component of healthy diet and they are very essential for human health and well-being. They are greatly beneficial to maintenance of good health and play a pivotal role in prevention of diseases. (1) Vegetables contain valuable nutritional factors which are very essential to build up and repair the body. Vegetables have presence of carbohydrates, vitamins and minerals and fibre contents. There is a recommendation of World Health Organization and joint Food and Agriculture Organization that the intake of vegetables and fruits must be minimum 400gms per day for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity as well as for the prevention and less severe deficiency of micronutrients which is especially common in less developed countries. (2) Consumption of raw vegetables and salads is a very common practice as they continue to have natural flavour and preserve heat labile nutrients.

On the other side consumption of raw vegetables can be a potential source of the spread of various infections and parasitic diseases. (3) Parasitic diseases are considered among the most common diseases on earth, these diseases are usually transmitted to human through water, soil, and food stuff such as vegetables. (4) Vegetables especially which are consumed raw are an important route of transmission of intestinal parasites and shown to be an important source of food borne outbreaks in developing countries. (5) There has been an increase in the number of reported cases of food borne illness linked to fresh vegetables (6) and the consumption of unwashed, raw, and un-hygienically prepared vegetables as a risk factor for parasitic infection. Vegetables during cultivation, harvest, transportation and further processing can also be contaminated with pathogens from human or animal sources. In addition, vegetables are washed after harvesting by producers and at markets and water used for this purpose if not clean can also lead to contamination. Furthermore, secondary contamination of vegetables may occur during transportation, ware housing and contamination by market vendors. In conditions where irrigation water is contaminated with human and animal wastes and where vegetables are washed with wastewater can also lead to secondary contamination, and the risk of getting parasitic diseases by workers that come in contact with or consume these products is high.

Like any other country consumption of selected raw vegetables is a very common practice in Asmara, Eritrea. Here because of inadequate or even non-existing systems for routine diagnosis and monitoring or reporting for many of the foodborne pathogens, the outbreaks caused by contaminated vegetables go undetected and their occurrence is underestimated.

The present study is designed to estimate the parasitic contamination in raw vegetables consumed in Asmara Eritrea. To our knowledge the findings of this study will help us to know the load of parasites in the selected vegetables and will also help to educate people about the role of raw vegetables in transmission of parasitic infection if they are grown, sold and consumed unhygienically.

Materials and Methods

A cross-sectional study was conducted to evaluate the level of parasitic contamination of some selected vegetables sold in the local markets of Asmara and the farm fields that produce and sell vegetables in the markets. In the present study, raw vegetables; lettuce, cabbage, carrot and girgir which are cultivated in and around Asmara were picked from seven major farm fields (Embaderho, Adi abeito, Paradiso, Sembel, Expo, Enda Quteba, Kehawta) and two major markets (Main market (Shuq) and Godaif Market), to determine parasitic contamination. Samples were collected according to their accessibility for the researchers from farm fields and markets.

A total of 205 samples were collected from farm fields within and around Asmara and two major markets in Asmara. Sample size was determined according to convenience of the researchers and availability of the sample. Different sample sizes from different farm fields and markets were collected as follows. Farm fields: Adi abeyto (9 samples), Emba derho (14samples), Enda Quteba (25 samples), Expo (18 samples), Kehawta (15 samples), Paradizo (13 samples) and Sembel (23 samples) and from two major markets Markato (42 samples) and Godaif (46 samples). The total number of each vegetable were also determined according to the availability of the

type of the vegetables and was collected as follows, Carrot (50 samples), Lettuce (69 samples), Cabbage (63samples) and girgir (23samples).

Fresh samples were collected in a clean disposable plastic bags which were properly labelled, and brought to the Clinical Parasitology Laboratory of Asmara College of health sciences for parasitological analysis. A portion (200 gm.) of each vegetable was washed separately in 500 mL of normal saline for detaching the parasitic stages (ova, larvae, cysts, and oocysts) of helminths and protozoan parasites commonly assumed to be associated with vegetable contamination. After overnight sedimentation of the washing solution, the supernatant was decanted leaving 15 mL of the sediment. Then the remaining sediment was transferred to a centrifuge tube using a sieve which allows the parasites to pass and to remove the undesirable matters. For concentrating the parasitic stages, the tube was centrifuged at 3500 rpm for five minutes (7). After centrifugation, the supernatant was decanted leaving 3ml carefully without shaking. Then the sediment was agitated gently by hand for re-suspending the parasitic stages. Lugol's iodine (1% for wet mount) was prepared in the lab using potassium iodide and iodine. Then it was added to the sediment samples on the slides to observe cysts of parasites.

Finally, the sediment was examined under a light microscope using ×10 and ×40 objectives. To increase the chance of parasite detection two slides were prepared for each sample.

Data was entered into cleaned and analyzed using statistical package for the social sciences (SPSS). The difference between frequencies of intestinal parasites among different categories was compared using Pearson chi-square test. A value of $P < 0.05$ was considered as a statistically significant difference. Also variation was expressed in terms of percentages.

Results

Prevalence of intestinal parasites on four vegetables

Out of 205 vegetable samples from seven farms and two markets, 117 (57.07%) vegetable samples were contaminated with one or more parasites. Of the 4 vegetable types examined, lettuce was the most contaminated 42 (60.8%), followed by carrot 30 (60%), girgir 13 (56.5%) and cabbage 32 (50.7%). Fig 1

Two species of parasitic protozoa, *Entamoeba histolytica, Giardia lamblia* and five species of helminthes, H*ookworm*, *Strongyloide stercoralis, Ascaris lumbricoides*, *Fasciola* spp and *Taenia* spp were detected. Table 1 & Fig 2

Out of these parasites the most frequently detected was *Ascaris lumbricoides* 61(29.7%), followed by *Giardia lamblia* 55(26.8%), *Strongyloide stercoralis* 22(10.7%), *Entamoeba histolytica* 9(4%), *Taenia* spp and *Fasciola* spp 4(1.9%).

On parasitic contamination frequency of lettuce samples, *A. lumbricoides* was the most common 27 (39.1%) followed by *Giardia lamblia* 25(36.2%), *Strongloide stercoralis* 4(5.8%), *Taenia spp* 2(2.9%), Hookworm 2(2.9%), *Entameba histolytica* 1(1.4%).

On parasitic contamination frequency of carrot samples, *Strongloide stercoralis* and *Giardia lamblia* were the most common 12(24%) each, followed by *A. lumbricoides* 10(20%), *Entameba histolytica* 3(6%).

The frequency of contamination of cabbage sample showed *A. lumbricoides* 18(26.6%), followed by *Giardia lamblia* 12(19%) was the dominant while *Entameba histolytica* and *Fasciola spp* each were 4 (6.3%). The presence of *Strongloide stercoralis* was 3(4.8%), and *Taenia spp* 2(3.2%). In girgir the most commonly detected parasites was *Giardia lamblia* and *A. lumbricoides* 6 (26.1%) each, followed by *Strongloide stercoralis* 3(13%), *Hookworm* and *Entameba histolytica* 1(4.3%) each. Fig 3

Single and multiple parasite contamination of vegetables

Out of 205 samples 78(38.04%) were contaminated by single parasites, 38(18.5%) were found to be contaminated with two parasites and 1(0.48%) was contaminated with four parasites. *A.lumbricoides/G.lambliae* was the most prevalent in double contamination 15 (39.5%),followed by *G.lamblia/E.histolytica* 6(15.7%). Lettuce was the most double contaminated vegetable 19(9.2%) followed by cabbage 8(3.9%). Table 2 & Fig 4

Relative prevalence of parasites between farm fields and market samples

Out of 88 samples collected from two Market places and 117 from seven farms, 44 (50%) and 73 (62.3%) were tested positive for parasites respectively.

Out of 44(50%) parasite positive from market samples *Giardia lamblia* was the most frequent contaminant 32(36.4%), followed by *Ascaris lumbricoides* 11(12.5%), and *E.histolytica* 5(5.7%). Similarly of 73(62.3%) parasite positive from farm field samples, *Ascaris lumbricoides* was the most dominant contaminant parasite occurring in 50 (42.73%) samples, followed by *Giardia lamblia* 23 (19.7%), and *S. stercoralis* 20(17.1%). Table 3 & Fig 5

Considering the overall contamination of parasites, the frequency of *A.lumbricoide* was 61 (29.7%) in the overall samples examined. 50(82%) of them were detected in the farm field vegetables and the remaining 11(18%) were detected in the market vegetables. This difference was significant with *P-value* <0.001. Similarly in the detection frequency of *S.stercoralis* there was a significant difference with *p-value 0.001*. Out of total 22 (10.7%) *S.stercoralis* detected in the total sample size, the 20(90%) were detected in the farm field samples and the remaining 2(9.1%) in the market samples. The frequency of *G.lamblia* also found significant with a *P-value* 0.008, out of 55 (26.8%) positive samples 23(41.8%) were detected in the farm field and the remaining 32(58.2%) were found in the market samples.

In our findings we did not found any significant difference with the *E.histolytica* contamination between the farm field and market samples. Table 4

Discussion

Vegetables may act as passive vehicles for transmission of pathogenic parasites and protozoa that are primarily transmitted through the fecal-oral route. (8)

Worldwide, parasites infect millions of people. In some regions, they are a major cause of childhood diarrhea and stunting of growth. They can also cause significant economic losses related to human, animal health and to agriculture (9).

The current study has shown that vegetables can get contaminated with eggs of *Ascaris lumbricoides, hookworms, Taenia* spp, *fasciola spp, larvae of Strongyloides stercoralis* and cysts of *Giardia* and *E. histolytica* that cause intestinal infections in humans. The presence of parasites on the vegetables is an indication that contamination may occur in a variety of ways such as contact with the soil, the water used for irrigation, non-hygienic conditions performed in markets to freshen up vegetables, and from contaminated hands of retailers; each of which were reported from cities in west Africa as a potential source of contamination with pathogenic organisms (10).

Although the scope of this study was to assess the level of parasitic contamination of the selected vegetables, the researchers have observed all the above mentioned activities are done routinely, a fact that may support the need of this study to be done.

The present study has attempted to assess the source and prevalence of different intestinal parasites from different vegetables sold and grown in different markets and farm fields of Asmara respectively. Out of total 205 sample size the overall parasitic contamination rate was (57.07%). The finding of our study is in agreement with the studies reported from Ethiopia, Nigeria and Libya which showed almost equal prevalence of (57.8%), (56.25%), and (58%) respectively.(11, 12) However, the prevalence of parasitic contamination is low in similar studies reported from, Egypt (31.7%) (13), Iran (29%) (14), Ghana (36%) (15) and Nigeria (36%) (16).

On the other hand, the rate of contamination in the current study is lower when compared with the findings of some studies conducted in Kenya (75.9%), (17) and Iran (79%) (18).

This discrepancy of prevalence between the present and previous studies might be as a result of the variation in geographical locations, climatic and environmental conditions, the kind of sample and sample size examined, the sampling techniques, methods used for detection of the intestinal parasites, and socioeconomic status. So as long as these factors differ, consequently the discrepancy between the results would be expected.

In the current study, *Ascaris lumbricoides* was the most frequently detected parasite with a prevalence of 61(29.7%). This result is in agreement with similar studies conducted in Addis Ababa, Ethiopia in June 2011 (20.3%), Akure Metropolis Ondo State, Nigeria (25.7%), while the study conducted in Hue City,

Vietnam the presence of *Ascaris lumbricoides* was higher (85.19%) (19, 20, 21). In some other studies conducted in Nigeria and Jimma Town, Southwest Ethiopia, *S. stercoralis* was the most frequent parasite detected (10). *Cryptosporidium spp*. was the most contaminating parasite in a study conducted in Alexandria, Egypt in 2012 (6).

According to Watson, *Ascaris lumbricoides* is known to have the most environmentally resistant eggs among the enteric pathogens in which around 200,000 eggs are produced per day for a year. The eggs can survive in the absence of oxygen, live for 2 years at 5-10° C and be unaffected by desiccation for 2 to 3 weeks. In favorable conditions of moist and sandy soil, they can survive for up to 6 years, even in freezing winter conditions. (22)

Out of four vegetables studied lettuce was the most contaminated (88.4%). The finding of our study is in accordance with the studies undertaken in Jimma Town, Southwest Ethiopia, Kaduna Metropolis, Nigeria and Benha, Egypt (10, 23-24). The high presence of parasite on lettuce may be due to its large surface area and uneven surface because of which the parasitic stages attach more easily to the surface of lettuce either on the farm or when washed with contaminated water.

Multiple species contamination was observed in all kinds of vegetables examined in this study. This indicates the possibility of high level contamination of vegetables, which perhaps results in multiple parasitic infections in human.

In comparing contamination of samples of markets and farm fields, contamination was higher in farm fields (62.3%). This could be due to the reason that samples of farm fields were not initially washed before being brought to the lab.

A.lumbricoides and *S.stercoralis* was mostly detected in the farm fields than in market samples with 50 (82%) out of 61 and 20(90.9%) out of 22 respectively. This could be due to the life cycle of these two parasites involving the soil.

G.lamblia was mostly detected parasite in the market samples than in the farm field with 32 (58.2%) out of 55. This result could be due to contamination after harvesting (i.e in transportation, contamination from hands of retailers, improper handling in the market or contamination from water used to freshen up vegetables).

However, this study did not address the effect of seasonal variation on the contamination of the vegetables. The findings of this study could not underscore the infectivity of the parasitic stages detected as viability study was not conducted except for *Strongloide stercoralis* in which a live infective larva was detected.

Conclusion

There is a potential source of infection to humans from contaminated vegetables in Asmara markets and farms that produce them. The most prevalent parasites detected in the present study in decreasing order are, *Ascaris lumbricoides, Giardia lamblia, Strongyloides stercoralis*, *Entamoeba histolytica*, *Hookworms*, *Taenia spp* and *fasciola spp*. *Ascaris lumbricoides* was the most frequent contaminant in the four vegetables considered in this study. The higher risk is on lettuce because of its larger surface and dense foliage which can bear more parasites.

Parasite contamination was higher in farm samples when compared to market samples. Therefore findings of the present study are of public health importance because most of the vegetables are eaten raw. In addition, people often pick up fallen fruits or vegetables like carrot and eat after merely dusting off the visible dirt with their hands or clothing. This might not have been well researched, but the results of the current study are alarming indicators of the fact. Therefore it requires an appropriate intervention to prevent transmission of parasitic infections that can be acquired through consumption of contaminated fruits and vegetables.

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Table 1: Frequency of parasites among four vegetables (Carrot, Lettuce, Cabbage, and Girgir) under study

Parasite detected	**Vegetable (N=205)**				Total
	Carrot	Lettuce	Cabbage	Girgir	
E.histolytica	3(6%)	1(1.4%)	4(6.3%)	1(4.3%)	9(4%)
G. lambliae	12(24%)	25(36.2%)	12(19%)	6(26.1%)	55(26.8%)
A.lumbricoides	10(20%)	27(39.1%)	18(26.6%)	6(26.1%)	61(29.7%)
S.stercolaris	12(24%)	4(5.8%)	3(4.8%)	3(13%)	22(10.7%)
Hook worm	0(0%)	2(2.9%)	0(0%)	1(4.3%)	3(1.4%)
Taenia spp	0(0%)	2(2.9%)	2(3.2%)	0(0%)	4(1.9%)
Fasciola spp	0(0%)	0(0%)	4(6.3%)	0(0%)	4(1.9%)
Parasite contamination	30(60%)	42(60.8%)	32(50.7%)	13(56.5%)	117(57.07%)
Frequency of parasites detection	37(74%)	61(88.4%)	43(68.2%)	17(73.9%)	

Table 2: Frequency of single and multiple parasite contamination of the four vegetables-carrot, lettuce, girgir and cabbage in farm fields and market of Asmara

Parasite detected	**Vegetable**				**Total**
	Carrot	**Lettuce**	**cabbage**	**Girgir**	
Single parasite contamination	23(11.2%)	23(11.2%)	23(11.2%)	9(4.3%)	78(38.04%)
Double parasite contamination	7(3.4%)	19(9.2%)	8(3.9%)	4(1.9%)	38(18.5%)
A.lumbricoides/G.lambliae	1	11	4	0	16(42%)
A.lumbricoides/E.histolytica	0	1	0	0	1(2.6%)
A.lumbricoides/Taeniaspp	0	2	1	0	3(7.8%)
A.lumbricoides/hook worm	0	1	0	0	1(2.6%)
A.lumbricoides/S.stercoralis	1	1	0	3	5(13.2%)
G.lamblia/E.histolytica	3	0	2	1	6(15.7%)
G.lamblia/S.stercoralis	2	3	0	0	5(13.2%)
S.stercolaris/E.histolytica	0	0	1	0	1(2.6%)
Quadruple parasite contamination	0	0	1(0.48%)	0	1(0.48%)
A.lumbricoides/G.lamblia/S.stercoralis/fasciola spp	0	0	1	0	1

Table 3: Comparison of parasites frequencies detected in farm fields and market places

Parasite detected	**Source of vegetable**	
	Farm fields	**Market**
E.histolytica	4(3.4%)	5(5.7%)
G.lamblia	23(19.7%)	32(36.4%)
A.lumbricoides	50(42.7%)	11(12.5%)
S.stercoralis	20(17.1%)	2(2.3%)
Hook worm	0(0%)	3(3.4%)
Taenia spp	4(3.4%)	0(0%)
Fasciola spp	3(2.6%)	1(1.1%)
Contamination	73(62.3%)	44(50%)

Table 4: Significance test of parasite contamination in farm field and market

Parasite detected	Market	Farm field	*P*-value
A.lumbricoides (N=61)	11(18%)	50(82%)	<0.001
G.lamblia (N=55)	32(58.2%)	23(41.8%)	0.008
S.stercoralis (N=22)	2(9.1%)	20(90.9%)	0.001
E.histolytica (N=9)	5(55.6%)	4(44.4%)	0.434

Figure 1: Frequency of vegetable contamination

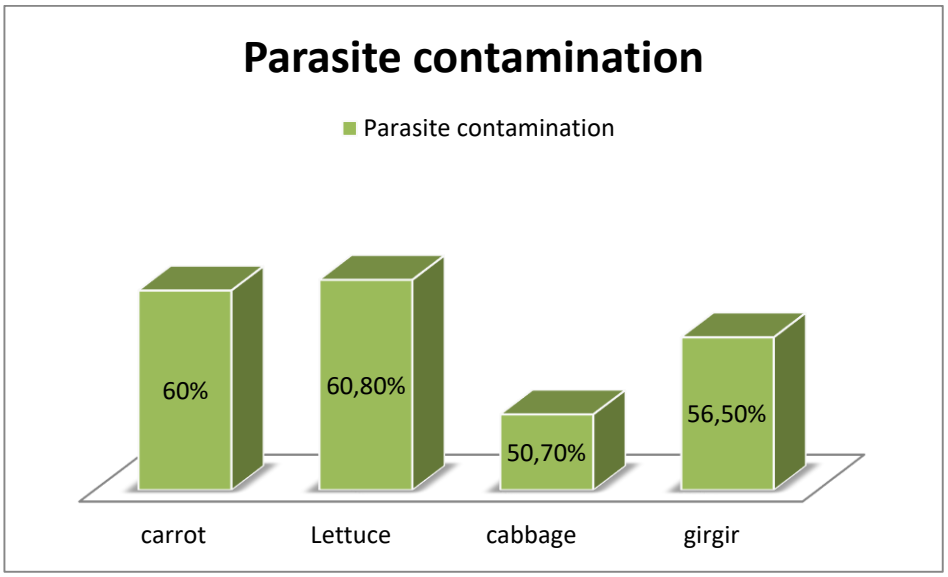

Figure 2: Frequencies of Parasites Detected

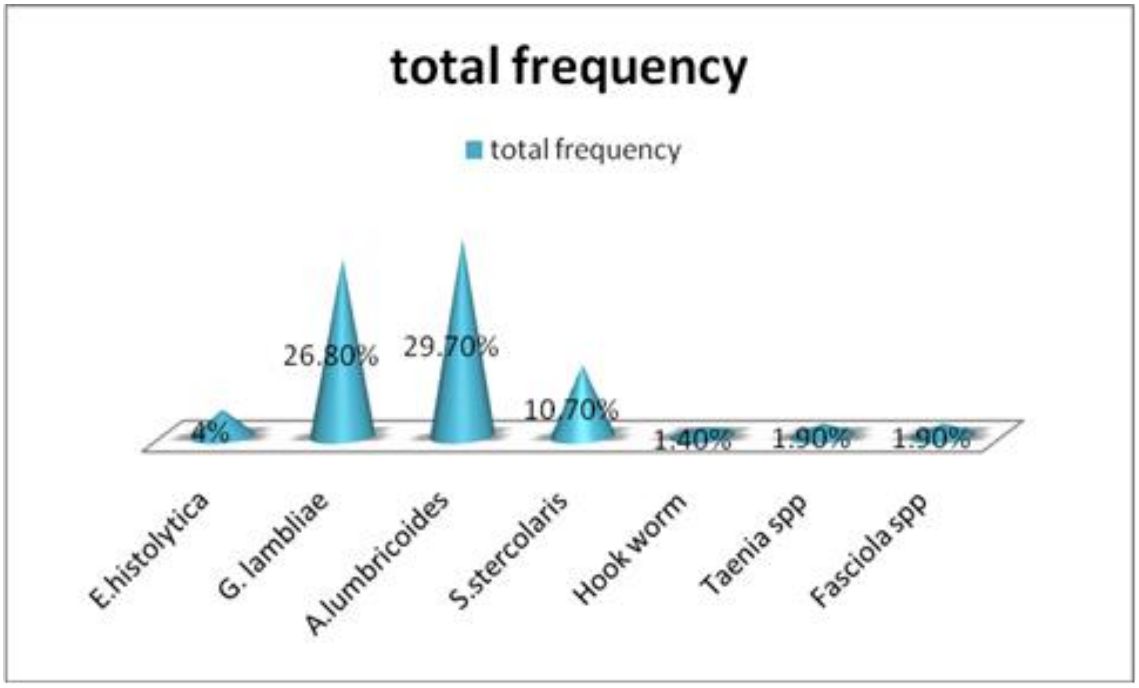

Figure 3: Frequency of parasites among the four vegetables in terms of percentage

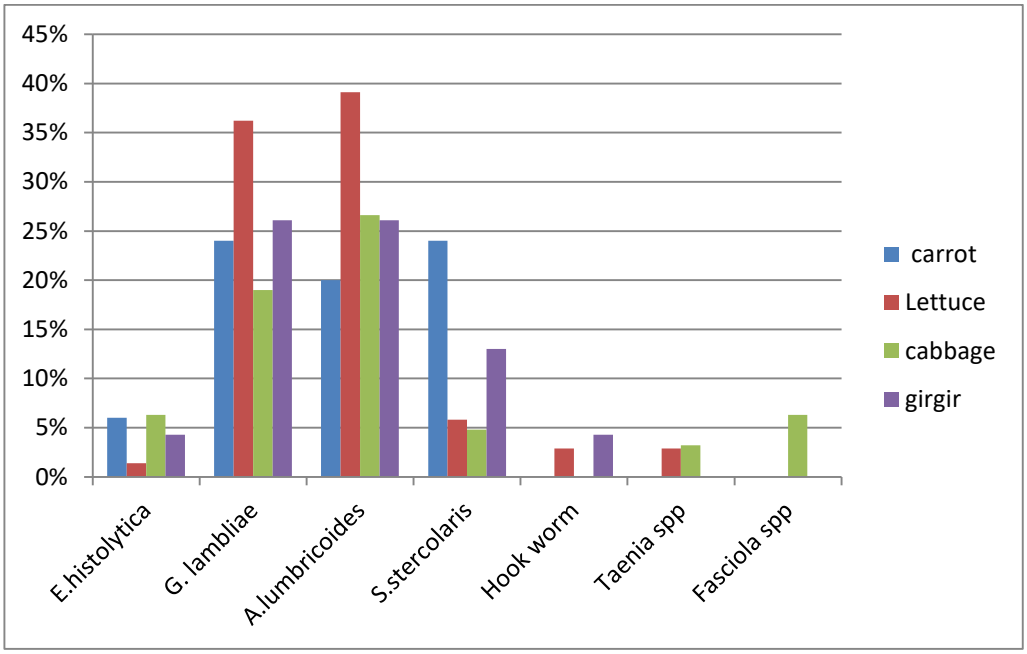

Figure 4: Single, Double and Quadruple Contamination

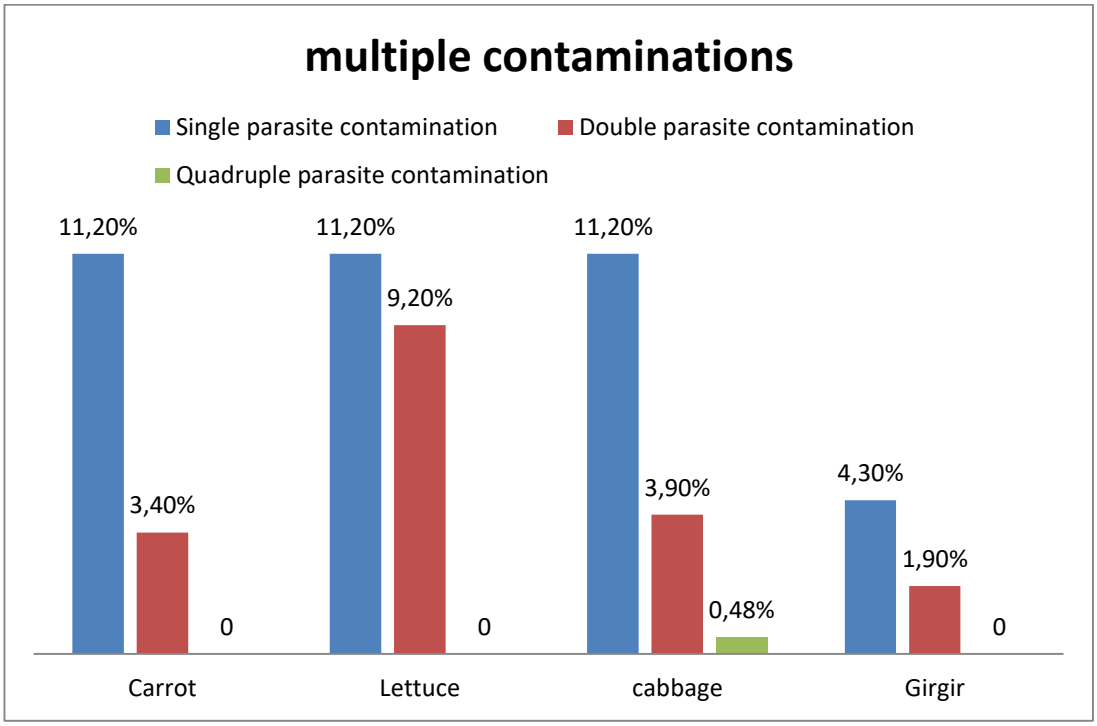

Figure 5: Comparison of parasites frequencies detected in farm fields and market places in terms of percentages

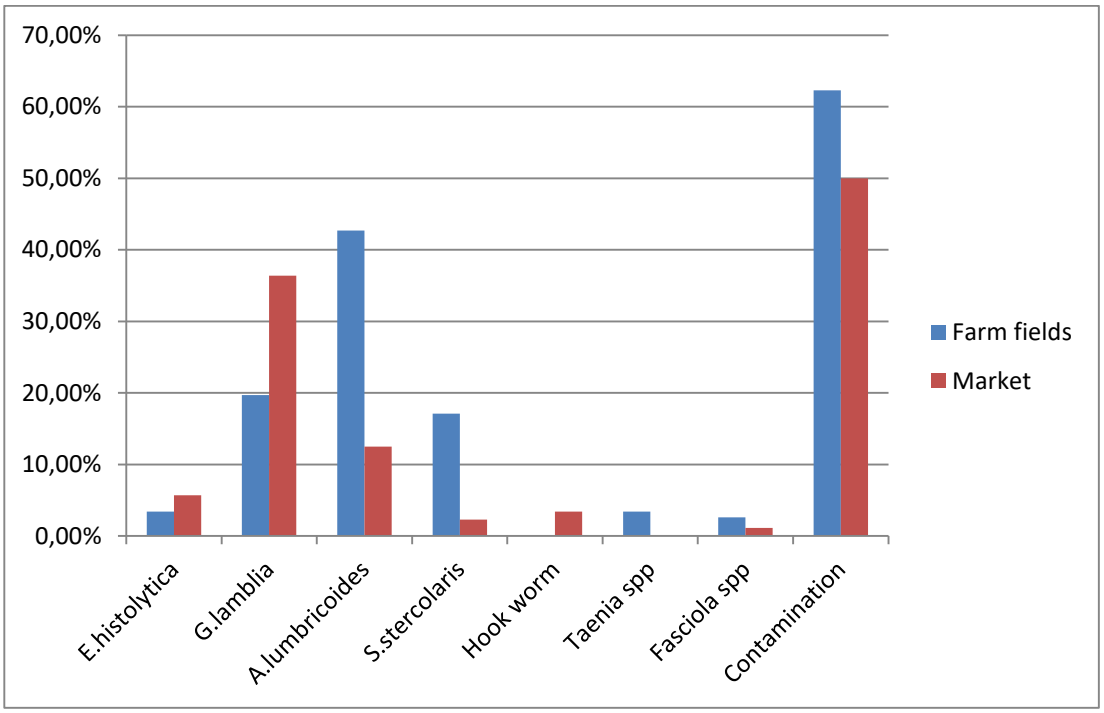

Figure 6: *Strongyloide Stercoralis* filariform larvae and *Ascaris lumbricoides* ova detected from farm fields and Market vegetable samples