BIOLOGIA (PAKISTAN)
June, 2018, 64 (1), 61-67

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Raw vegetables as a risk factor for parasitic infections in District Mardan, Pakistan

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ABSTRACT

Assessment of parasitic contamination of raw vegetables in District Mardan, Khyber Pakhtunkhwa was carried out by detection of parasitic eggs, cysts and larvae. Seven vegetables including cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*), mint (*Mentha spicata*), chili (*Capsicum frutescens*), cucumber (*Cucumis sativus*) and corriander (*Coriandrum sativum*) were assessed using standard methods. Overall parasitic contamination of 30.7% was observed. Highest contamination was detected in Mardan (36%) followed by Katlang (30%) while the lowest contamination was observed in Takhat bhai (26%). Hookworm (32.6%) was the most common contaminant found followed by *Ascaris lumbricoides* (21.7%) and *Trichostrongylus spp*. (10.87%) and the least detected parasite was *Schistosoma* (1.08%). Among vegetables, lettuce and mint showed highest parasitic contamination i.e., 42.85% followed by corriander 40.9%, while the lowest contamination was in carrot 9.52%. The results highlighted the potential of unwashed raw vegetables in transmission of intestinal parasites to human and emphasize the need for proper washings of vegetables before they are consumed.

Key words: Raw vegetables, contamination, parasites, helminthes, Mardan, Pakistan

INTRODUCTION

Human diet includes vegetables as essential part due to their healthy nutritional value while their regular consumption is associated with a reduced risk of many fatal diseases like cardiovascular diseases, stroke and certain cancers (Van Duyn & Pivonka, 2000).

The nutritional content of vegetables varies considerably. Many vegetables are rich sources of small proportion of protein, fat and a relatively high proportion of vitamins and provitamins. Some beneficial fibers like; non-starch polysaccharides are also found in vegetables which offers protection from conditions like haemorrhoids, colon cancer, chronic constipation, rectal fissures and diverticulosis and lowers the risk of heart disease by reducing blood cholesterol levels. Phytochemicals are found which are known to reduce the risk of several diseases like bacterial, fungal, viral and cancer (Kalia & Gupta, 2006; Silvia *et al.*, 2007; Alade & Adewuyi, 2013).

There is a common practice of consumption of raw vegetables and salads, as they retain natural flavor and preserve heat labile nutrients, but on the dark side they are also a good mode of transmission of several infectious diseases due to their complex surface and porosity, which unfortunately facilitate pathogen attachment and survival (El Said, 2012).

Vegetables can get contaminated through different parasitic stages which if not properly washed, can transmit the parasites and other microorganisms to the human, and can be a potential source of expanding infection in parasites life cycle (Siyadatpanah *et al.,* 2013). Thus, vegetables that are consumed raw, without prior peeling or washing, present a serious health risk.

Parasites that can cause human infections through ingestion of contaminated vegetables include: amoebas, flagellates, coccidians and ciliates (protozoa); digenean trematoda, tapeworms and soil transmitted nematodes (helminths). It is known that infection with these parasites can cause

Author's Contribution: A.W.Q., Supervised research and wrote up thesis; **S.A., C**onducted research work, wrote thesis and contributed in data analysis and research article drafting.
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malnourishment and stunted growth in humans. The reasons include reduced food intake, or an increase in nutrient depletion due to the infection itself (Stephenson *et al.*, 2000). An increase in the number of cases of food-borne illnesses linked to consuming fresh vegetables has been reported in the recent years particularly in developing countries (Olyaei & Hajivandi, 2013).

Pakistan is one of the developing countries that suffer from a wide range of parasitic infections in humans. Among them soil transmitted parasites have a significant impact on public health all over the country. Moreover in Pakistan there is no or less preventing measure taken by the public health for these parasitic infection and above all the people are merely educated about these diseases.

There is no study conducted in District Mardan to evaluate the level of contamination of vegetables with parasites of medical and zoonotic importance. If target is to control the intestinal parasitic diseases, it is not enough to depend only on the chemotherapeutic intervention of identified cases, but need the concentrated effort to decrease and remove the possible sources of infection. Therefore, this study is designed to determine the level of parasitic contamination of vegetables commonly eaten raw or undercooked.

MATERIALS AND METHODS

Study Area

The study was conducted in District Mardan from October 2014 to April 2015**.** Raw vegetables were selected from major vegetable selling markets and road side vender sellers of the three tehsils Mardan, Takht Bhai and Katlang.

Sample Collection

300 samples of raw vegetables including cabbage *(Brassica oleracea*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*), mint (*Mentha spicata*), chili (*Capsicum frutescens*), cucumber (*Cucumis sativus*) and coriander (*Coriandrum sativum*) were randomly collected from local markets of study areas. These samples were properly collected in labeled sterile bags and carried to the laboratory to analyze for parasitological contaminations.

Sample Analysis

The vegetables were analyzed for the presence of parasites forms (cysts, eggs and larvae) following the techniques described by Abougrain *et al.* (2009) and Uga *et al.* (2009): Vegetable samples were cut into small pieces and thoroughly rinsed in physiological saline solution (0.85% NaCl). This solution was allowed to stand overnight for settling down of the eggs/cysts/larvae. After sedimentation, the top layer of the washing saline was carefully discarded leaving 5ml behind. The 5ml sediments were centrifuged at 2000 rpm for 15minute, the supernatant was discarded and the sediment was carefully collected. Parasites present in the sediment were identified according to the descriptions provided by Soulsby (1982).

Data was analyzed by SPSS version 20, using Chi-square test. P-value <0.05 was considered statistically significant.

RESULTS

Out of 300 samples of the examined vegetables, 92 (30.7%) were positive for different parasitic contamination.

Area Wise Distribution

Highest numbers of vegetable samples positive for parasitic contamination were detected in Mardan city (36%) followed by Katlang (30%), while lowest numbers of positive samples were found in Takht Bhai (26%) as shown in Table 1. Statistically there was non-significance difference in prevalence of parasitic contamination of raw vegetables in study areas of District Mardan (P> 0.05).

Prevalence of Parasites

A total of 115 parasites were recovered from vegetables, out of which 8 were protozoa, 84 were nematodes and 7 were trematodes while 16 were cestodes. The parasite recovered were included cysts of *Entamoeba spp. Giardia spp.* eggs of *Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Fasciola spp*., *Schistosoma spp.*, *Taenia spp.*, *Hymenolepis nana, Toxocara spp.* and larvae of *Trichostrongylus spp*., hookworm (Fig. 1). The most prevalent parasite was hook worm i.e., 30 were positive out of 92 contaminated samples and least common

Schistosoma spp. i.e., only 1 sample was positive (Fig. 1).

Vegetable Samples Contamination

Analysis of vegetables revealed that lettuce *(L. sativa)* and mint *(M. spicata*) had highest parasitic contamination i.e., 42.85% followed by coriander *(C. sativum)* 40.9%, cabbage (*B. oleracea)* 33.33%, cucumber *(C. sativus)* 27.27%, green chili (*C. frutescens)* 18.18%, while the least contaminated was carrot (*D. carota*) 9.52% (Fig. 2). Chi-square test showed overall significant difference ($P<0.05$) in contamination of vegetables.

Assessment of parasitic contamination of vegetables also shown that one vegetable sample might be contaminated by more than one parasite at the same time. Single parasitic contamination was common in lettuce while poly parasitic contaminations were high in coriander (Fig. 3).

DISCUSSION

Raw vegetable consumption is common all over the world as they are healthy and provide nutrients in natural form. In present study overall parasitic contamination of vegetables was very high (30.7%). Many studies have been conducted in different parts of the world for the parasitic contamination of vegetables and reported similar findings. EI Said (2012) reported intestinal parasites in 31.7% from Alexandria & Eraky *et al.* (2014) detected in 29.60% of fresh vegetables consumed in Benha, Egypt. Alahabbal (2015) in Alqalamoun region, Syria indicated 31.38% while Fallah *et al.* (2012) from Shahrekord, Iran, Tomass & Kidane (2012) from Tigray, Ethiopia and Shahnazi *et al.* (2009) from Qazvin reported 32.6%, 32.41% and 35.3% contamination, respectively. The high rate of parasitic contamination detected might be due to poor hygienic and sanitary conditions in these areas. Also due to the poor transport, packing of these vegetables and irrigation with contaminated water. Moreover poor washing techniques and other conditions of cultivation are equally responsible for the contamination. (Orlandi *et al.,* 2002).

Results of present study showed overall non-significant ($P>0.05$) variations in the parasitic contamination on vegetables in three different areas of district Mardan. This may be due to similar local climatic conditions and type of water used for irrigation. While 4-10% difference in contamination may be attributed to public related hygienic practices and sanitary facilities in these areas (Eraky *et al.,* 2014).

Twelve genera of parasites including protozoa and helminthes were isolated from vegetables during current examination. Among nematode parasites, hookworm was the most common contaminant followed by *Ascaris lumbricoides* and *Trichostrongylus spp.* was found next to *Ascaris*. Many workers reported presence of these nematodes in vegetables. Idahosa (2011) & Elom *et al.* (2012) reported 28.2% and 23.8% contamination of hook worm in vegetables, respectively. El Said (2012) from Alexandria & Dias *et al.* (2014) from India determine 20.30% and 23.0% contamination by *A. lumbricoides*, respectively, while Al Shawa & Mwafy (2007) detected in 20% from Gaza, Palestine. Shafa-ul-haq *et al.* (2014) recovered 8.9% of *Trichostrongylus spp.* indicating vegetables are good source of transmission for these parasites. *E. vermicularis* was also found 10.87% in positive samples. Al Shawa & Mwafy (2007) and Alhabbal (2015) also reported 2.5% and 5.83% samples of vegetables having *E. vermicularis* in Ghaza and Syria, respectively. In this work 8.7% contamination of vegetables with *Toxocara spp.* was also reported. The similar findings were also noticed by others. Dias *et al*. (2014) found 7.14 % *Toxocara* eggs in fresh leafy vegetables in Mangalore (India). Adanir & Tasci (2013) also found *Toxocara spp*. Egg in 10% of pepper mint and 8.33% of spinach in Turkey. Hajjami *et al.* (2013) detected 8.6% of vegetable samples contaminated by eggs of *Toxocara* in Morocco. *Trichuris trichiura* was present in 6.52% of all positive samples of vegetables. These results are supported by Olyaei & Hajivandi (2013), who isolated 6.77% *T. trichiura* from market and farm vegetables in Iran. Uga *et al.* (2009) also observed 8% of *Trichuri*s in vegetables in Vietnam.

In present survey some cestodes were also observed. 10.87% of vegetables were contaminated with *Taenia spp.* eggs and 6.52% of *Hymenolepis nana* eggs. Abougrain *et al.* (2010) from Libya reported 22.0% of vegetables contaminated with eggs of *Taenia spp.* Moreover *Taenia* was also

found in 9.2% raw vegetable in a study carried out by Falah *et al.* (2012) in Iran while Ebrahimzadeh *et al.* (2013) reported less (5.1%) prevalence of *H. nana* on vegetables consumed in Zahedan, Iran. The difference in contamination may be due to geographical differences in study areas.

Among trematodes 6.52% of *Fasciola spp.* was examined in this study, which is in close accordance with finding of Shafa-ul-haq *et al.* (2014) who isolated 5.1% *Fasciola* eggs from vegetables in Lahore, Pakistan. Curtale *et al.* (2003) from Egypt also reported the presence of *Fasciola spp* ova in most types of vegetables, even if samples of watercress were not present and thus responsible for its transmission.

Protozoan parasite included *Giardia* spp. and *Entamoeba* spp. which were found on 4.35% of vegetable observed. Erdogrul & Sener (2005) found *Giardia* cysts in 5.5% of fresh vegetables (lettuce, parsley, cress, and spinach). Moreover, Ebrahimzadeh *et al.* (2013) from Iran and Duedu *et al*. (2014) from Accra, Ghana also reported that 5% and 4% of raw vegetables were contaminated with *Entamoeba spp.*, respectively. These findings are also in accordance with other studies in Amol, Iran by Siyadatpanah *et al.* (2013) who reported *Entamoeba spp.* in 4.3% vegetables.

Analysis of vegetables indicated that lettuce and mint were the highly contaminated vegetables followed by coriander, then cabbage, cucumber, chili and carrot. Shafa-ul-haq *et al.* (2014) also reported high parasitic contamination (48.0%) of lettuce, 44% of cabbage and 42% of mint (podina) and 16% of chili from Lahore, Pakistan. Lettuce and cabbage were contaminated significantly more frequently than those of tomato in the same study. Olyaei & Hajivandi (2013) also detected contamination in 60.0% of lettuce samples, and 26.7% in mint in Iran. High Prevalence of parasitic contamination of lettuce is due to is broad leaves which make it more prone to be contaminants as compare to small surfaced vegetables (Larkin *et al.*, 1978). It could be due to degree of contamination is directly proportional to the surface and shape of the vegetables. Cabbage, lettuce and other green leafy vegetables had also uneven surfaces that make parasitic eggs, cysts and larvae stick to their surface more easily, when washed with contaminated water either in the farm or market (Avcioglu *et al,* 2011 & Kozn *et al.*, 2005). Furthermore, the other high contaminated vegetable was coriander. These observations are supported by Hajjami *et al.* (2013). The highest parasitic contamination of coriander and mint can be supported by the fact that these vegetables grows near ground level where this herb are in direct contact with contaminated soil and water, also their overlapping leaves protect parasitic 'eggs from sunlight, dryness and wind (Shafa-ul-haq *et al.,* 2014).

The least number of parasites were found on cucumber and chili in current study. Uga *et al.* (2009) also reported that fruit vegetables (cucumber, chili, tomato and eggplant etc.) were least contaminated as compared with leafy and root vegetables. This may be due to smooth skin of these vegetables decreases the percentage of parasitic attachment and augments the elimination of parasite eggs when washed.

In addition, differences among present results and other similar studies may be due to several factors such as geographical location, number and type of vegetables observed, techniques used for detection of the parasites, source of water used for irrigation and post harvesting handling methods of such vegetables. Different laboratory procedures may also contribute to detection of different parasites since some method can either sediment or float the parasites.

The present study may have important implications for worldwide food safety and stress the potential of these types of vegetables in threatening public health by transmission of parasites to humans in District Mardan, Khyber Pakhtunkhwa, Pakistan. The results highlight the potential of raw vegetables either unwashed or washed with contaminated water, in transmission of intestinal parasites to human and emphasize the need for proper handling of vegetables before they are consumed or cooked.

Table 1: Over all parasitic contamination of vegetables in District Mardan

Areas	Number of vegetables collected	Number of Contaminated vegetables	Prevalence (%)
Mardan	100	36	36
Takht Bhai	100	26	26
Katlang	100	30	30
Overall	**300**	**92**	**30.7**

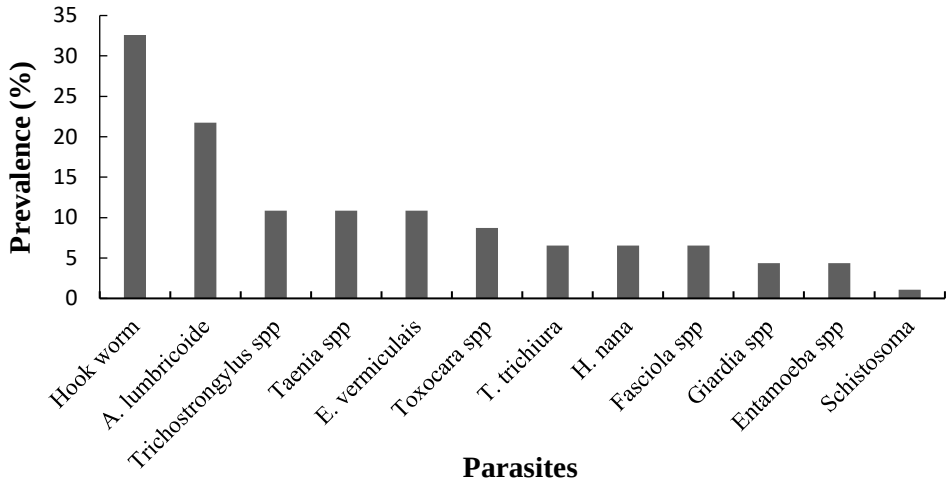

Fig. 1: Overall prevalence of different parasites on vegetables

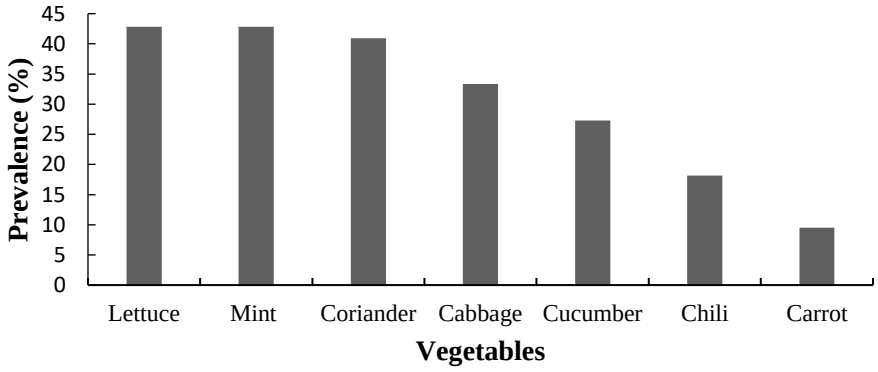

Fig. 2: Overall parasitic contamination of different types of vegetables

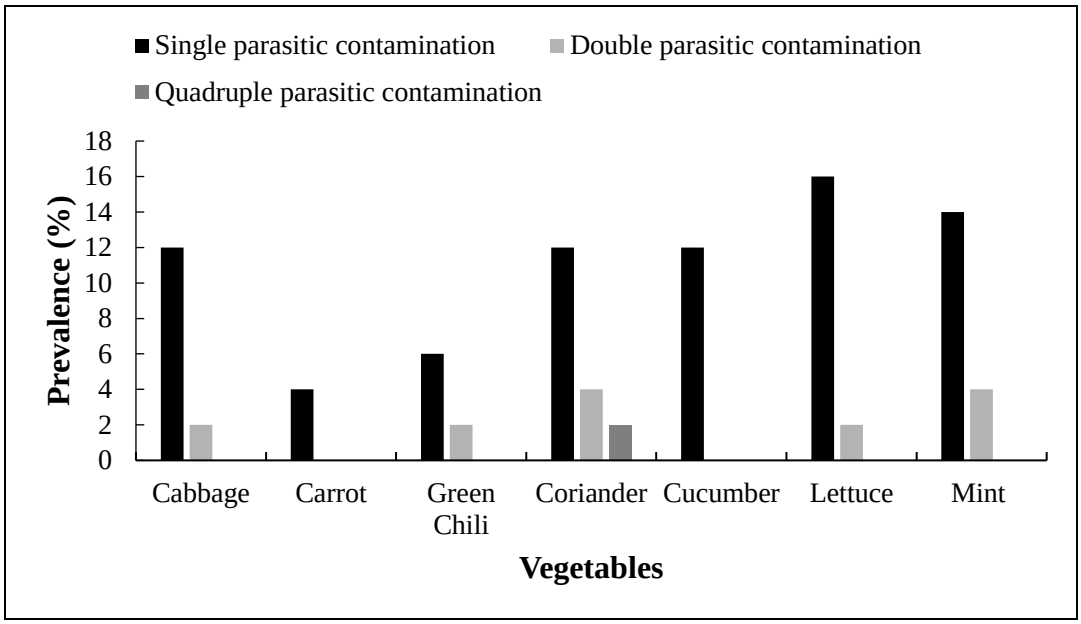

Fig. 3: Single and poly parasitic contamination on vegetables

REFERENCES

Abougrain, A. K., Nahaisi, N. H., Madi, N. S., Saied, M. M. & Ghenghesh, K. S., 2010. Parasitological contamination in salad vegetables in Tripoli-Libya. *Fd. Contrl*., **21(5)**: 760-762.

Adanir, R. & Tasci, F., 2013. Prevalence of helminth eggs in raw vegetables consumed in Burdur, Turkey. *Fd. Contrl.*, **31(2)**:482-484.

Al Shawa, R. M. & Mwafy, S. N., 2007. The enteroparasitic contamination of commercial vegetables in Gaza Governorates. *J. Infect. Dev. Countr.*, **1(1)**: 62-66.

Alade, G. O., Alade, T. O. & Adewuyi, I. K., 2013. Prevalence of Intestinal Parasites in Vegetables Sold in Ilorin, Nigeria. *Am. Eurasian J. Agric. Environ. Sci*., **13(9)**: 1275-1282.

Alhabbal, T. A., 2015. The Prevalence of Parasitic Contamination on Common Sold Vegetables in Alqalamoun Region. *Int. J. Pharm. Sci. Rev. Res.*, **30(1)**: 94-97.

Avcioglu, H., Soykan, E. & Tarakci, U., 2011. Control of helminth contamination of raw vegetables by washing. *Vector Borne Zoonotic Dis.*, **11(2)**: 189-191.

Curtale, F., Mas-Coma, S., Wahab E.I., Hassanein, Y.A., Barduagni, P., Pezzotti, P. & Savioli, L., 2003. Clinical signs and household characteristics associated with human fascioliasis among rural population in Egypt: A case-control study. *Parasitologia*, **45(1):**5-11.

Dias, D. M., George, M. & Dias, A., 2014. How safe are vegetables: Parasitological contamination of fresh leafy vegetables in Mangalore. *Int. J. App. Bio. Pharm. Technol.*, **5**: 80-82.

Duedu, K. O., Yarnie, E. A., Tetteh-Quarcoo, P. B., Attah, S. K., Donkor, E. S. & Ayeh-Kumi, P. F., 2014. A comparative survey of the prevalence of human parasites found in fresh vegetables sold in supermarkets and open-aired markets in Accra, Ghana. *Bio. Med Central Res. Notes*, **7**: 836.

Ebrahimzadeh, A., Jamshidi, A. & Mohammadi, S., 2013. The Parasitic Contamination of Raw Vegetables Consumed in Zahedan, Iran. *Health Scope Int. J.*, **1(4)**: 205-209.

EL Said, S., 2012. Detection of parasites in commonly consumed raw vegetables. *Alexandria J. Med.,* **48**: 345–352.

Elom, M. O., Eze, U. A., Nworie, A., & Akpotomi, I.O., 2012. Prevalence of geohelminths on edible fruits and vegetables cultivated in 'rural villages of Ebonyi State, South East Nigeria. *Am. J. Fd. Nutr*., **2(3)**: 58-64.

Eraky, A. M., Rashed, M. S., Nasr, E. M., El-Hamshary, S. M. A. & El-Ghannam, S. A., 2014. Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. *J. Parasitol. Res.,* **2014**: 1-7

Erdogrul, O. R. & Sener, H., 2005. The contamination of various fruit and vegetable with *Enterobius vermicularis*, *Ascaris* eggs, *Entamoeba histolytica* cysts and *Giardia lamblia* cysts. *Fd. Contrl*., **16**: 557-560.

Fallah, A. A., Pirali-Kheirabadi, K., Shirvani, F. & Saei-Dehkordi, S., 2012. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Fd. Contrl*., **25(2)**: 617-620.

Hajjami, K., Ennaji, M. M., Amdiouni, H., Fouad, S. & Cohen, N., 2013. Parasitic Contamination on Fresh Vegetable Consumed in Casablanca City (Morocco) and Risk for Consumer. *Int. J. Sci. Technol.,* **2(7)**: 543-549.

Idahosa, O. T. 2011. Parasitic Contamination of Fresh Vegetables Sold in Jos Markets. *Global J. Med. Res.,* **11(1)**: 21-25.

Kalia, A. & Gupta, R., 2006. *Fruit Microbiology*. In: Hui, Y.H.J., Cano, M.P., Gusek, W., Sidhu, J.W. and Sinha, N.K. Hand book of Fruit and Fruit processing. 1st Ed., Blackwell Publishing. 3-28 pp.

Kozan, Gonenc, B., Arimehmetoglu, O. & Aycicek, H., 2005. Prevalence of helminth eggs on raw vegetables used for salad. *Fd. Contrl.,* **16**: 239-242.

Larkin, E. P., Tierney, J. T., Lovett, J., Van Donsel, D., Francis, D.W. & Jackson, G. J., 1978. Land application of sewage wastes: potential l for contamination of foodstuffs and agricultural soils by viruses, bacterial pathogens and parasites. *In: State of knowledge inland treatment of wastewater* (ed. H. L. Mc Kim). US Army Corps of Engineers, Crrel, Hanover, NH. 215–223 pp.

Olyaei, A. & Hajivandi, L., 2013. Parasitological Contamination of Markets and Farms in Vegetables Consumed in Southern Iran. *Global Veterinaria*, **10(3)**: 327-331.

Orlandi, P. A., Chu, D. M. T., Bier, J.W. & Jackson, G. J., 2002. Parasites and the food supply. *Fd. Technol.*, **56**: 72–81.

Shafa-ul-Haq, Maqbool, A., Khan, U. J., Yasmin, G. & Sultana, R., 2014. Parasitic contamination of vegetables eaten raw in Lahore. *Pak. J. Zool.*, **46**: 1303-1309.

Shahnazi, M., Sharifi, M., Kalantari, Z., Heidari, M. A & Agamirkarimi, N., 2009. The study of consumed vegetable parasitic infections in Qazvin. *J. Qazvin Univ. Med. Sci.*, **12(4)**: 83.

Silvia, R. D. S., Sylvia, E. F. V., Dariene, C. P., Ahie, M. J. & Gertrudes, C., 2007. Microbiological quality of minimally processed vegetables sold in Porto Alegre, Brazil. *Brazilian J. Microbiol.*, **38**: 594-598.

Siyadatpanah, A, Tabatabaei, F, EmamiZeydi, A, Spotin, A, Fallah-Omrani, V, Assadi, M, Moradi, S, Rostami, A, Memari, F. & Hajialiani, F., 2013. Parasitic Contamination of Raw Vegetables in Amol, North of Iran. *Arch. Clin. Infect. Dis*., **8(2)**:15983.

Soulsby, E. J. L., 1982. *Helminths, Arthropods and Protozoa of Domesticated Animals*. 7th ed. Bailliere Tindall, London.

Stephenson, L. S., Latham, M. C. & Ottesen, E.A., 2000. Malnutrition and parasitic helminth infections. *Parasitol.,* **121**: 23-38.

Tomass, Z. & Kidane, D., 2012. Parasitological contamination of wastewater irrigated and raw manure fertilized vegetables in Mekelle city and its suburb, Tigray, Ethiopia. *Momona Ethiopian J. Sci.,* **4(1**): 77-89.

Uga, S., Hoa, N., Noda, S., Moji, K., Cong, L., Aoki, Y., Rai, S.K. & Fujimaki, Y., 2009. Parasite eggs contamination of vegetables from a suburban market in Hanoi, Vietnam. *Nepal Med. Coll. J*., **11(2**):75-78.

van Duyn, M. & Pivonka, E., 2000. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional, selected literature. *J. Am. Diet. Assoc.*, **100(12**): 1511–1521.