Incidence of Zoonotic Parasites In Egyptian Raw Vegetable Salads

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Abstract: Feeding on Vegetables which probably contaminated order to pre or post-harvesting activities through soil, animals or wastage water contacts, consider as non-neglected source for several zoonotic parasites .So, this study describes the possible parasites contamination in raw vegetables salads, directly reflect public health hazard. A total of 527 salad samples of seven varied types as; 94 sample of Onion, 93 Cucumbers, 88 dill & Parsley, 87 Tomatoes, 73 Lettuce, 51 cabbages and 41 carrots were collected from different types of restaurants within two Egyptian governorates (Mnia and Assiut). Samples were directly washed each by Tris-buffer-saline (TBS), followed by concentration of the extract by centrifugation, staining of the different stages by modified Zeihl–Neelsen stain centrifuged and the sediments were fast microscopic examined for parasites. The overall incidence of parasite contamination in each type of vegetable were; 7.4% in *Onion*, 22.6% in *Cucumbers*, 19.3% in *Dill & Parsley*, 13.8% in *Tomatoes*, 8.2 % in *Lettuce*, 15.7% in *Cabbages* and 34.5% in *Carrots*. Also, five types of parasites were detected by 16.1% in the total examined samples. Also, the overall incidence of each parasite reference to total vegetable samples were; *Ascarislumbricoides* (3.2%), *Cryptosporidium parvum* (1.7%), *Entamoeba spp. cysts* (3.4%), *Giardia lamblia* (2.8%), and *Toxoplasma gondii* (4.9%).The study validates some zoonotic parasites of public health worry in vegetables salad. The results alarm must be connected to the Egyptian health authorities for fast adjustment the public health educational progressions, and modified sterilization or radiation techniques for raw vegetable.

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1. Introduction

Vegetables containing fiber, vitamins and minerals are essential source for health fitness. But, it's an important mode of parasites diffusion and has been shown to be an important source for food borne outbreaks in both developed and wealthy countries. (Pires, et al. 2012). The higher request for vegetables is the motiveof increased reports of zoonotic parasitosis in recent years (Vazquez et al. 1997). Humans can be infectedvia vegetables which polluted pre or post-harvesting, connect harmfully effects on people's health especially in non-industrialized countries. However, extra changing of vegetables eating habits, production systems, increased awareness and better diagnostic tools are some of the main drivers affecting the emergence or re-emergence of these parasitic diseases(Northrop-Clewes& Shaw; 2000). Vegetables possibly contaminated through pets contact and other people. Also, thru rodents, insects and improper treated waste water associates (Simoes M, et al. 2001 and Mahvi AH & Kia EB. 2006).

The immune-compromised individuals cannot repel parasite infection, and they involving digestive disturbances such as dysentery, diarrhea, obstruction and anemia (Srikanth R & Naik D. 2004). Also, some parasites could be invade internal organs, discharging toxins and tissue-destroying enzymes, probable stimulate abortion, arthritis, asthma, degenerative muscle diseases, ovarian cysts, cutaneous ulcers, dermatitis, and more(Robertson & Gjerde, 2001).

The purpose of the current study was to judge the pollution degree and the public health hazards of zoonotic parasites in consumed vegetables salad from Egyptian restaurant.

2. Methodology

Sample collection

A total of 527 raw and ready to eat vegetable samples were collected once weekly of seven varied types as; (*n*=94) Onion, (*n*=93) Cucumbers, (*n*=88) dill & Parsley, (*n*=87) Tomatoes, (*n*=73) Lettuce, (*n*=51) cabbages and (*n*=41) carrots. All vegetable samples were picked randomly from the commercial different types of Egyptian restaurants within two Egyptian governorates (Mnia and Assiut). It were transported in sterile plastic bags to lab. (Zoonotic Disease Department-National Research Center, Egypt). The study covered a period from 11/ 2014 to 1/ 2016.

Table, 1; the numbers and percentages of the isolated parasite stages in relation to various vegetable types.

Parasite types	The examined vegetables .NO/ (%)							
	Onion	Cucumbers	Dill & Parsley	Tomatoes	Lettuce	Cabbages	Carrots	Total
NO of samples	94	93	88	87	73	51	41	527
Ascarislumbricoides eggs.	2(2.1)	6(6.5)	4(4.5)	2(2.3)	1(1.4)	0	2(4.9)	17(3.2)
Cryptosporidium spp. oocysts.	1(1.5)	3(3.2)	3(3.4)	1(1.1)	0	0	1(2.4)	9(1.7)
Entamoebaspp. cysts	1(1.5)	4(4.3)	5(5.7)	3(3.4)	0	2(3.9)	3(7.3)	18(3.4)
Giardia lamblia cysts.	1(1.5)	5(5.4)	0	2(2.3)	2(2.7)	2(3.9)	3(7.3)	15(2.8)
Toxoplasma gondii oocysts.	2(2.1)	3(3.2)	5(5.7)	4(4.6)	3(41)	4(7.8)	5(12.2)	26(4.9)
Total/ (%)	7(7.4)	21(22.6)	17(19.3)	12(13.8)	6(8.2)	8(15.7)	14(34.5)	85(16.1)

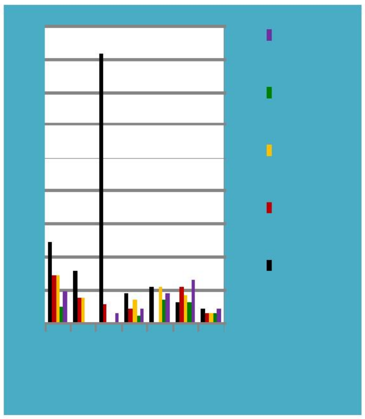

Fig. 1; The incidence of each parasite reference to each vegetable type

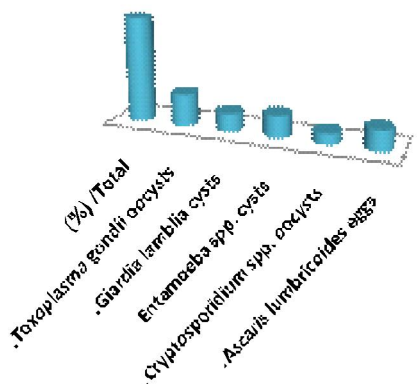

FIG. 2: The total percentages of each parasite reference to the total vegetable samples

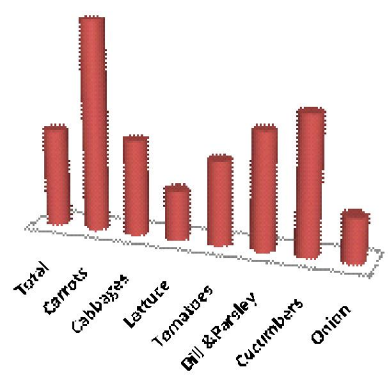

FIG. 3; The total percentages of each vegetable type reference to the total isolated parasites

Sample preparation & parasites recognition

According to Opara KN & Udoidung NI (2002), we were recycled TBS for the extraction. Each vegetable sample wasvisible to Tris-buffer-saline (TBS) (20 mMtris base, 0.5 mM sodium chloride, 0.2% tween 20 and double-distilled water), were used as washing solutions. Portions of about 100 gm of each vegetable sample were assorted together and were soaked in 150 ml of the extraction solution, with the aid of a mechanical shaker; the beaker was shaken at 150 rpm for 30 minutes. The washing solution (TBS), was poured via a sterile gauze into a sedimentation flask and left to residue for 60 minutes. The supernatant was decanted and the sediment was relocated into a 15 ml conical centrifuge, and centrifuged at 1500 rpm for 5 minutes. The supernatant was decanted, a few drops of 10% formal-saline (10ml formalin in 90 ml of 9% normal saline), was added and the mixture was spread on at least 3 slides and inspected for parasites. A drop of the sediment was put on the slide. A clean cover slip was gently placed to avoid air bubbles., a drop of the sediment was mixed with a drop of Lugol's Iodine solution and examined as before , Simple and iodine smears were used for detection of parasitic eggs, cysts and larva. The process was analytically recurrent until the end of the mixture in each test tube. Eggs, cysts and oocysts of parasites found under the light microscope were identified. Staining of sediment smear was performed by Modified Zeihl–Neelsen14 and modified trichrome15 to detect protozoal parasitic (oo) cysts. The preparation was tested under a light microscope using x10 and x100 objectives (Garcia LS. 2007). Infective stages of parasites were recognized according to Downes and Ito (2001).

3. Results

The results are summarized in (Tables 1 and Figs. 1, 2 and 3). The incidence of the total parasite contamination reference to each type of vegetable were; 7.4% in *Onion*, 22.6% in *Cucumbers*, 19.3% in *Dill & Parsley*, 13.8% in *Tomatoes*, 8.2 % in *Lettuce*, 15.7% in *Cabbages* and 34.5% in *Carrots* (Table 1 & figure 1 & 2). The overall percent of parasites detected were 16.1% in the total examined samples (Table 1). Also, the results encountered five types of parasites during the study; and the total isolation of each parasite reference to total vegetables were; *Ascarislumbricoides* (3.2%), *Cryptosporidium parvum* (1.7%), *Entamoeba spp. Cysts* (3.4%), *Giardia lamblia* (2.8%), and *Toxoplasma gondii* (4.9%) (Table 1 & figure, 2). Carrots and Cucumbers were detected the most parasites contaminated vegetables (34.5 & 22.6) respectively. While sporulated oocysts of *Toxoplasma gondii* and cysts of *Endameba histolytica* were the most prevalent isolated parasites (Table 1 & figure 3).

4. Discussion

The present study is on target zoonosis, evaluates the public health hazards via direct Egyptian consuming vegetable salad through the detected of overall1 6.1% of contaminated vegetable samples with varied pollution percentages sequence to the different examined vegetable types. The isolation of parasite infective stages from vegetable salad that are fully prepared and ready to eat is a good indicator of sapro-zoonotic hazards via improper washing. This authorize that tap water doesn't completely eliminate parasites. Ali et al., (2006) were settling our impression, and practical that 7.8% of parasites still after the use of tap water. Also, we observed that washed *Carrots, Cucumbers and Dill & Parsley were* recorded higher parasitic contamination than the other vegetable types. This may be due to it cultivated in soil contact than others. Also, other factors are stimulating vegetables contamination, where leafy vegetables likely exposed to parasites via dust than the roots ones. Also, irregularun even surface facilitate parasite connection and survival. *Carrot, Cucumbers* and leafy vegetables as *lettuce* and *parsley* have uneven surfaces and makes parasitic eggs, cysts and oocysts more tight attached (Kniel.et al., 2002). This concept approve the pre-harvesting soil pollution, order to inadequate treated water used for irrigation.

In the present study the values of contaminated percentages were lower than the former recorded by Doaa, (2012); presented a significant higher level of vegetables pollution (31.7%) in Alexandria-Egypt. probably attributed to unwashed vegetables used in his study. Also, several factors may contribute to such differences include, geographical location, type and number of examined samples, methods used for parasites isolation and the post-harvesting handling and transmission methods. So, varied level of parasites contamination were detected in many studies; one in Ghana the overall parasitic contamination of the vegetables as 36% (Amoah et al. 2006). In Nigeria was also 36% (Damen . et al., 2007). Ettehad et al., (2008) reported, slightly lower level of contamination of consumed native garden vegetables with intestinal parasites (29%) in Ardabil city, Iran. Higher rate of contamination of vegetables was detected in wholesale and retail markets in Tripoli, Libya, the study done by Abougraina.et al., 2010. This study identified 58% positive samples for intestinal parasites (Abougraina .et al., 2010).Inspection of vegetable samples in Kenya by Nyarango RM.et al.,(2008) also publicized higher rate of contamination (75.9%).In contrasts, other study in Saudi Arabia in 2006 shown that the highest number of contaminated samples was found in green onions (28%) and the lowest contamination was in leek (13%). The prevalence of parasites in other vegetables in the same study was 17% in each of lettuce and water cress (Al-Binali .et al., 2006).

The sporulated oocysts of *Toxoplasma gondii* and the cysts of *Endameba spp*were the most prevalent isolated parasitic stages (4.9 &3.4 %%) respectively. The isolation of *T. gondii oocysts* confirm habitat of stray shedder kittens in the vegetables cultivated zone, the oocysts were sporulated in soil and pollutes vegetables via dusting during pre-or post-harvesting dynamics. Also, possibly indirectly maximize human infection through meat tissue cysts of herbivores animals (Hassanain *et al.,* 2011). The opportunistic *T. gondii* protozoan signifies varies complicated pregnancy induces abortion, fetus malformation and latent brain cyst (Sibley et al., 2009). The dormant bradyzoitespersist viable for the rest of the host survives and probable reverted to acute infectionsequence to pregnancy estradiol hormonal sift (Elfadaly et al., 2012) or in diabetic personals (Hassanain et al., 2014). In addition to anti-inflammatory corticosteroids therapy (Elfadaly et al., 2015). *Entamoebaspp*cysts could be survive outside hosts in water, soils, and on vegetables, especially under moist conditions. Its isolation (Table 1 & figure 3) approves fecal contamination through farms used insufficient treated waste water or unutilized human or animal fertilizers (Srikanth R & Naik D. 2004).

Ascarislumbricoides is the largest and the most common human roundworm, it growto a length of up to 35 cm, responsible for human ascariasis (Harhay et al., 2010). The fertilized egg become infectious and resist some chemicals (Murray et al., 2005). So, the egg can persist in soil for 10 years or more (Piper R, 2007). Ascaris ova are sticky and can tight adhere to vegetables (Kagei, 1983). In the present study *A. lumbricoides* was frequently encountered in different vegetable types (Table 1 & figure 3). The results were agree with somereports who found *A. lumbricoides* as the most common parasite in vegetables (Mesquita etal,1999 and Simoes M, et al. 2001) , in contrast to Robertson & Gjerde , 2001, who did not encounter Ascaris at all. The varied human and environmental factors might explain this difference.

Ingestion of *Cryptosporidium*sporulated oocysts is a water-borne zoonosis,responsible for the prolonged diarrhea in both immune-competent and immune-compromised hosts, It is resistant to all practical levels of chlorination, surviving for 24hrs at 1000 mg/L free chlorine (Deng et al, 2004). Oocyst of Cryptosporidium is not often looked in routine microbiology laboratories. Its presence in our study is acall for increased vigilance by microbiologists and clinicians in our region. Its isolation (Table 1 & figure 3), confirm human or animal fecal contamination.

Giardia lamblia is a flagellated protozoan parasite, the infection can occur through ingestion of dormant microbial cysts in contaminated water and vegetables. The *Giardia* cysts found within surface water, it of great sapro-zoonotic concern, suggested that dogs, cats, cattle, sheep and humans are reservoirs (Robertson LJ, Gjerde B; 2000).

The wide variation in reported frequencies of parasitic contamination in vegetables doesn't relates to the level of environmental contamination only, but also to the accurate diagnostic methods (Akujobi, et al; 2005). However, Robertson and Gjerde (2000) found that the use of immune-magnetic separation and identification by immunofluorescence, appreciably improved the yield for *Giardia* and *Cryptosporidium*. Similarly, Knieland Jenkins (2005) found that the use of polyclonal sera specific to the recombinant viral capsid protein (rCPV40) in a dot blotted hybridization assay to detect *Cryptosporidium oocysts* from green onions and was superior to other methods. Also, the wide variation of results in developing countries was related to the nonexistent systems for routine diagnosis and monitoring or reporting for many of the food-borne pathogens.

In conclusion; raw vegetable salads are connecting zoonotic parasites of public health impact, different chemical or radiation methods for vegetables disinfection or sterilization should be developed. Further researches and inspections should be done concerning viability bio-assays of parasite stages in raw vegetable.

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