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Assessment of Parasitic Contamination of Lettuce and Cabbages Sold in Selected Markets in Maputo City, Mozambique

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Abstract

Background: In Mozambique, vegetables are the main source of nutrients and they are consumed raw or cooked. Their consumption, especially in their raw form, might be a source of contamination, which may take place during production and/or transportation as well as in the markets. In this study we aimed to assess the profile and frequency of parasites in lettuces and cabbages sold in some selected markets in Maputo city, the capital of Mozambique.

Methods: A cross sectional study was conducted between February and March 2018, in 10 selected markets from Maputo City. A total of 100 combined samples of lettuces and cabbages were analyzed using a spontaneous sedimentation method. Data such as gender and age of the vendors and about handling of vegetables at the point of sale were also collected.

Results: Out of the samples, 84 (84%) were contaminated with parasites (86% of lettuces and 82% of cabbages). The analyses performed revealed the presence of *E. coli* (66%), *S. stercoralis* (40%), *E. histolytica* (20%), *A. duodenalis* (6%), *S. haematobium* (4%), *A. lumbricoides* (2%), *S. mansoni* (2%) and *T. trichiura* (1%). Two markets, Janete and Fajardo showed contamination in all samples. A Chi square analysis revealed a significant association between the contaminated cabbages and the market where they were obtained. The majority of vendors (57%) do not wash their hands before handling vegetables. Most of the vendors had tables to place vegetables (72%) and those tables are cleaned before display (86%). None of the differences observed in each risk factor studied were statistically significant.

Conclusion: The present study highlights that contamination of raw vegetables with pathogenic parasites in markets might represent a vector for transmission of intestinal and water- borne parasites to consumers. Future studies should investigate the extent of vegetable contamination

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Conflict of Interest
The authors declare that they have no conflict of interest.

at each stage of the supply chain from the farm to consumers, as well as its relationship to human parasitic infection and its consequences. Good hygienic practices of farmers, vendors and consumers should be enforced to break the contamination chain. Whenever possible, hydroponic cultivation should be encouraged to avoid the influence of highly contaminated soils.

Keywords

Lactuca sativa; Brassica oleracea; Parasitic Contamination of Vegetables; Intestinal Parasites; Water-Borne Parasites; Mozambique

Introduction

Food and water-borne outbreaks of parasitic diseases in humans due to consumption of raw, improperly washed vegetables and fruits have been increasingly reported worldwide in recent decades and result in high morbidity and a negative impact on public health [1,2].

It is estimated that 3.5 billion people are infected annually and of those, 450 million are colonized with intestinal parasites with an estimated 200,000 deaths per year [3]. Globally, the rates of contamination of vegetables by the most common parasites are *Ascaris lumbricoides* (1.9% – 21%), *Entamoeba histolytica/dispar* (30% – 42%), *Enterobius vermicularis* (6% – 18%), *Giardia intestinalis* (3% −19%), *Trichuris trichiura* (4% – 14%) and *Cryptosporidium* spp. (2% – 8%) [4–6]. Protozoan contaminations account for 1.9% – 9.3% worldwide [6].

Moreover, studies conducted in settings such as Ethiopia, Sudan, Ghana, Nigeria, Iran, Poland, and the Philippines, found that consumption of contaminated raw and/or improperly washed vegetables or fruits was the main route for acquisition of parasitic infection in humans [2,7,8]. Contamination can occur at different stages due to poor hygienic practices, planting in contaminated soil, use of human feces, sewage and animal manure for fertilization, and during the harvesting, storage and transportation to markets as well as from rising and sprinkling vegetables with contaminated water as a way to keep them fresh [2,4,9].

Mozambique is a tropical country located in Southeast Africa, with a population of 28.8 million and adult literacy rate of 60.7%. The minority (44%) of its population lives in urban and suburban areas, and only 48.7% have access to safe drinking water and 54,2% do not have latrines in their homes [10]. Maputo City, the capital of Mozambique has approximately 1.12 million inhabitants, half of whom reside in unplanned settlements, resulting in a high population density and poor access to basic safe drinking water (86%) and sewage drainage (89%) [10,11]. This conditions contribute to the disease profile which is dominated by infectious and neglected tropical diseases, including intestinal parasites (53%), schistosomiasis (47%), diarrheal infection and respiratory infections (52%) malaria (55.2%) and chronic malnutrition (43%) [12–15].

However, in Mozambique, there is a relative paucity of data about parasitic contamination of raw vegetables and fruits sold in the markets, including from Maputo City, as well as risk factors associated with contamination and their relationship to parasitic transmission to humans.

A study of vegetables collected from two farmers located in regions surrounding Maputo City, namely the Infulene Valley and Laulane market, which sought to assess the level of vegetable contamination and the most frequent intestinal parasites in vegetables and in water, revealed an overall contamination of 57.2% of samples. The parasites most commonly detected were *Strongyloides stercoralis* (28%), *Ancylostoma* spp. (19.5%) and *A. lumbricoides* (5%). In the same study, the analysis of water revealed contamination with *G. lamblia* (40.3%), followed by *S. stercoralis* (27.7%) and *A. lumbricoides* (10.2%) [7]. Further, another study aimed at assessing the risk to public health of the supply chain for horticultural products in Maputo City from farmers-vendors to consumers, found that the majority of vendors used water from boreholes or artisan sources and, of those who washed their products (53.3%), only 7.5% used tap water for washing vegetables [2].

Studies conducted in some sub-Saharan African countries, including Mozambique, to determine the prevalence and profile of parasitic infection in humans, found numerous parasitic species, with those most frequently detected being: *A. lumbricoides* (5.2% – 56%), *T. trichiura* (12.9% – 38%), *G. intestinalis* (3.2% – 52.2%), *Entamoeba coli* (7.7% – 34.9%), *S. mansoni* (10.9%), *S. haematobium* (54.9%), *Strongyloides stercoralis* (14% – 48%), *Ancylostoma* spp. (25%) and *Necator* spp. (15%) [14,16–19].

Due to the endemicity of parasitic infections in Maputo and the surrounding region, we hypothesized that raw vegetables and fruits sold in the markets may be highly contaminated by eggs, cysts or larva of helminths and protozoa and thus constitute an important source of parasitic infection for the citizens of Maputo.

Hence, in this pilot study, we aimed to assess the profile and frequency of parasitic contamination in lettuces (*Lactuca sativa)* and cabbages (*Brassica oleracea*) sold in some selected markets from Maputo City as well as risk factors related to these contaminations.

Based on the results of this study, we provide baseline information about the profile and the frequency of parasitic contamination of these leafy vegetables along with risk factors associated with contamination and propose future studies that focus on the entire supply chain (from farmer to vendors and consumers) as well as recommendations to eliminate or decrease this contamination.

Materials and Methods

Study area

We conducted a cross sectional study between February 2018 to March 2018 in selected markets from Maputo City to determine the level of parasitic contamination of *Lactuca sativa* and *Brassica oleracea*. Maputo city is served by 63 formal markets distributed across both urban and suburban areas [20]. Most of them have poor sanitation conditions such as proliferation of insects, trash scattered on the floor, water ponds, toilets without basic conditions or no toilets at all, and no water plumbing system [2].

Vegetables sold in these markets come from local wholesale markets or local farmers, like those in the Vale do Infulene and Zonas Verdes. Many of farmers do not have irrigation system or treated water for irrigation and the manure used for fertilization is not treated. In addition, some of these farmers (including those in the Vale do Infulene) use a combination of irrigated water and water from the sewage system to irrigate their horticulture [2,7,21].

Purpose of the Study

For the purpose of our study, we selected the ten most frequented and popular markets in Maputo city, namely, the markets of Xipamanine, Malanga, Xiquelene, Central, Adelina, Fajardo, Janete, Povo, Compone and Zimpeto.

Data collection

The study was approved by the Maputo City Council. Prior to the study we met with the supervisors of each market to inform them about the purpose of the study and to seek their collaboration. We also informed the vendors about the aims and purpose of the study to seek their consent to participate in the study, having informed them that all data to be collected were for study purposes only and that their identity would be kept confidential.

We used a pre-tested semi-structured questionnaire to ask the vendors about socio-demographic data that might be associated with parasitic contamination of *Lactuca sativa* and *Brassica oleracea*, such as status of the produce (washed before display or not), source of water used for washing and educational level of the vendors. A total of 100 vendors participated in the questionnaire. In addition, information such as the means of display of the produce, cleaning of the station before display and handling of the vegetables at the point of sale were recorded by observation.

Sample collection and laboratory screening

In each selected market, we randomly purchased five heads each of *Lactuca sativa* and *Brassica oleracea* and placed them in individual plastic bags, encoded with a unique number and date of collection and immediately transported them in a container at 4°C to the Eduardo Mondlane University, Parasitology Laboratory, Faculty of Medicine, for further processing on the same day.

For parasitological analysis, we used the spontaneous sedimentation method of Montanher with some modifications [22]. Basically, we stripped 200g of the leaves from each sample of either *Lactuca sativa* or *Brassica oleracea* and immersed them in 0.9% sodium chloride solution with 5% Tween® 20, for 15 minutes and then removed the leave. The resulting solution was filtered and allowed to stand for sedimentation at room temperature for 24 hours. Then the sediment was gently agitated using a spatula to redistribute any parasites. Finally, a drop of the sediment was placed into a slide and another drop of lugol was added, prior to placing a cover slip to the slide. For examination under the light microscope we used the microscope 350-Optika® using 10^x and 40^x objectives. Two independent observers read the slides and a third blinded examination was made when discordant results were obtained.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 21.0. Armonk, NY: IBM Corp. Descriptive analysis of the data was performed. Then, to verify any association between the factors studied and contamination of either *Lactuca sativa* or *Brassica oleracea*, the chi squared test was used. We also calculated the OR and 95% confidence interval. The significance level was set at 5%.

Results and Discussion

Socio and demographic data of vendors

Table 1 summarizes the vendor's socio-demographic data and observations made in markets regarding their hygiene practices for the handling of vegetables. We interviewed in total 100 vendors. The majority of vendors were female, aged over 18 years and had a primary school education. With regards to hygiene practices, we found that the majority of vendors (57%) did not wash their hands before handling vegetables, but washed the vegetables before displaying them (83%). Further, they did not change the water after washing each head of lettuce or cabbage. With regard to the placement of vegetables for sale, we found that the majority of vendors (72%) had tables on which to place them and these tables (86%) were cleaned before vegetable display them.

Table 2 summarizes results of parasitological analysis of vegetables from each of the ten markets and according to each vegetable studied. Vegetables purchased from the Janete, Fajardo and Xipamanine markets showed contamination in all samples. A bivariate analysis revealed a significant association between contaminated cabbages and the market from which they were obtained ($p < 0.05$) although this association did not hold for lettuces. The variability of the prevalence of contamination between the markets studied might be due to exposure to the different environmental conditions of the markets, such whether toilets and running water were present, different sources of vegetables, and variations in the hygienic practices of the vendors when handling vegetables. All of these could contribute to vegetable contamination, as found in other similar studies in Mozambique, Ethiopia and Nigeria [2,4,7,9,21].

Profile and prevalence of detected parasites according to the type of vegetable

Figure 2 illustrates the profile of parasites identified in either lettuce or cabbages. In total we identified up to eight species of parasites in all vegetables studied. Overall, we found that 84% (84/100) of vegetables were contaminated with at least one parasite species. The most frequently found parasites were *Entamoeba coli* 66% (66/100), *S. stercoralis* 40/100 (40%) and *E. histolytica* 20/100 (20%). The prevalence of *S. stercoralis* (64%) and *E. histolytica* (22%) was higher in *Lactuca sativa* while for *Brassica oleracea*, the prevalence of *E. coli cysts* (72%) was higher than in *Lactuca sativa* (60%). No *Giardia intestinalis* cysts were observed in these samples although the prevalence of this parasite in humans as well as in water varies from 1.3% to 40.3% in Maputo city [7,16]. These results should be viewed in the context of similar studies performed in Mozambique and other countries such as Nigeria, Ghana, Sudan and Ethiopia where similar parasitic species and prevalences were found in contaminated vegetables [1,4,5,7,9,23]. The similarities found between the parasite profiles previously reported in humans and found here on the vegetables (*Lactuca sativa* and *Brassica oleracea*), support the hypothesis that contaminated vegetables may play an important role as a source of human contamination [4–7,14,16–18].

Frequency distribution of parasitological contamination of vegetables from selected markets in Maputo city

Table 3 summarize the overall frequency of contamination of the samples studied and for each sample group. We found that contamination rates of the studied vegetables decreased according to the number of parasite species identified, with a range of 38% of samples harboring one species to 1% of samples harboring for four species of parasite. However, when analyzing our data according to each vegetable species and for multiparasitic contamination, we found that *Lactuca sativa* had a higher prevalence (51.2%) compared to *Brassica oleracea* (34.2%) for two parasite species and 18.6% vs 2.4% for three parasite species. Only one sample of *Brassica oleracea* was contaminated with 4 parasite species.

Analysis of the frequency of parasitic contamination for each group of vegetables showed that *Brassica oleracea* had a higher frequency of contamination (61%) by one parasite species compared to *Lactuca sativa* (30.2%), but tended to have less contamination with multiple parasite species compared to lettuce. This could possibly be attributed to the development and growth process of the former vegetable, since cabbages enclose their leaves, which possibly decreases the chances of contamination by cysts, eggs and parasite larvae.

In addition, the variation in contamination rates of the vegetables may be in part due to their particular shapes and surfaces. Green leafy vegetables such as lettuce and cabbage have uneven surfaces that likely facilitate more ready adhesion of parasitic forms compared to other vegetables, either at the farm or when washed with contaminated water. More importantly, like many other settings, lettuce is more likely to be consumed raw than cabbage. Further, the leaves of cabbages are slightly thicker and smoother than those of lettuces, which are rugose, so it is likely that microorganisms adhere more readily to lettuce than to cabbage leaves, as observed in other studies in Mozambique and in countries such as Ethiopia and Sudan [3,7,9,23].

Mixed contamination

Table 4 summarizes the frequency of mixed contamination with all identified parasites and for each vegetable species. In total we had 15 different parasitic combinations, with *E. coli* present in 12 of them. *E. coli* (28.5%), *S. stercoralis* (10.7%) and *E. histolytica* (6%) were most frequently identified as single parasite contaminants. As multiple contaminants, *E. coli* plus *S. stercoralis* (25%) and *E. coli* plus *E. histolytica* (8.3%) were the most frequently observed. Though *E. coli* is not pathogenic for humans, it is a strong indicator of fecal contamination [24,25]. Our findings correspond to these of other studies in vegetables and in humans where it is frequent to find mixed infections, as all these parasites share the same route of contamination (i.e. fecal/oral for transmission to humans) as well as environmental conditions such as contaminated soils and/or, water, plus the use of manure for fertilization [16,21]. In addition, a study by Muchimbane in 2010 that sought to assess the impacts of "in situ" sanitation systems on subterranean waters in one of Maputo city's neighborhoods found that groundwater and food were contaminated with fecal coliforms. Possibly, the contamination of ground water occurs throughout all places were vegetables are grown, as they are all places that share the same demographic and social profile.

Bivariate analysis of parasitism in the studied vegetables according to some selected variables

Table 5 summarizes factors that might be associated with parasitic contamination of vegetables, such as gender, age, education, hand washing before handling vegetables, washing of vegetables before display, and the means of display as well as whether this was cleaned before displaying the vegetables.

None of these factors was significantly associated with the presence of any vegetable contamination. However, we noted that those who clean station before display vegetables were more likely to have their vegetables contaminated.

It is possible that the water used to sprinkle vegetables and clean the tables was already contaminated with parasitic forms. Further, vegetables are traded close to cloudy and stagnant waters and garbage and are exposed to insects and high temperatures. Thus, it would not be surprising to find that the displaying stations were contaminated before displaying the vegetables. Most of the markets in Maputo city have suboptimal sanitary conditions, lacking running water at least some hours or all day and, also with deficient toilets and sewage disposal. Several authors [7,21,25] also argue that the high frequency of vegetable contamination results from the use of fecal manure from man and/or animals due to poor sanitary conditions, which is a reality in the Maputo city farmers and markets [2,7].

However, it is difficult to know in which extent the contamination found in the vegetables studied was due to poor hygienic conditions of the markets, poor hygienic practices of the vendors or if the vegetables were already contaminated in the farms or during transportation and storage. Therefore, the implementation of hygienic practices at every step between production and consumption should be a target as suggested by other authors as a way to eliminate contamination [6,25].

As noted previously, in some farms and ground water (Vale do Infulene, Zonas Verdes) where vegetables are cultivated there are notable poor cultivation practices, as they use untreated manure for fertilization, and sewage drainage system water for irrigation. The use of water contaminated with urban sewage (from Infulene's water treatment plant) for agricultural irrigation in the study area probably causes most of the contamination with intestinal parasites as previously suggested from previous studies [2,7,21]. In addition, the fact that in the study area, lettuce is most likely to be consumed raw may in part contribute to increase the risk of acquiring intestinal parasites.

It is important to note that our study had some limitations including the number and variety of vegetables studied and the parasitological methods that would not be expected to detect certain parasites such as coccidia oocysts that require modified Zielh Neelsen staining for detection. It is possible, therefore, that our findings underestimated the presence of parasites in the vegetable samples studied or more broadly in market vegetables. Other risk factors that contribute to food contamination were not included in observations and questionnaire, thus some correlations cannot be inferred.

Conclusion

The present study revealed high levels of parasitic contamination of *Lactuca sativa* and *Brassica oleracea* sold in selected markets of Maputo city. The contamination observed could have occurred during the cultivation, transportation and/or storage and handling of the vegetables in the markets, i.e. at each stage of the production chain and commercialization.

Most probably other raw vegetables handled and sold in the same context through the country and region have similar degrees of contamination and are also important players in the transmission of intestinal parasites to consumers.

Future studies should investigate the extent of vegetable contamination at each stage of the supply chain from the farm to consumers, as well as its relationship to human parasitic infection and the consequences of these infections in either immunocompetent or immunocompromised individuals.

Good hygienic practices of farmers, vendors and consumers should be enforced to break the contamination chain. Whenever possible, hydroponic cultivation (using clean water sources) should be encouraged to avoid the influence of highly contaminated soils.

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Figure 1:

Illustrates all vegetables chain production from farmers to the consumers. a) Vale do Infulene drainage system alongside with vegetables cultivation; b) vehicle with human excretes to be thrown in the Infulene drainage system; c) irrigation of vegetables with drainage system water from Infulene Vale; d)Transportation of vegetables to the markets in open vehicle and displayed on the ground; e) vegetables displayed on the floor and sprinkled with dirty water to keep them fresh; f) vegetables displayed on raffia bag placed directly on the floor; g) in door market where vegetables are displayed on the clean tables; h) vegetables displayed on tables in open market and exposed to dusty; i) vegetables displayed on a tables and floors under poor sanitation conditions around and on the road.

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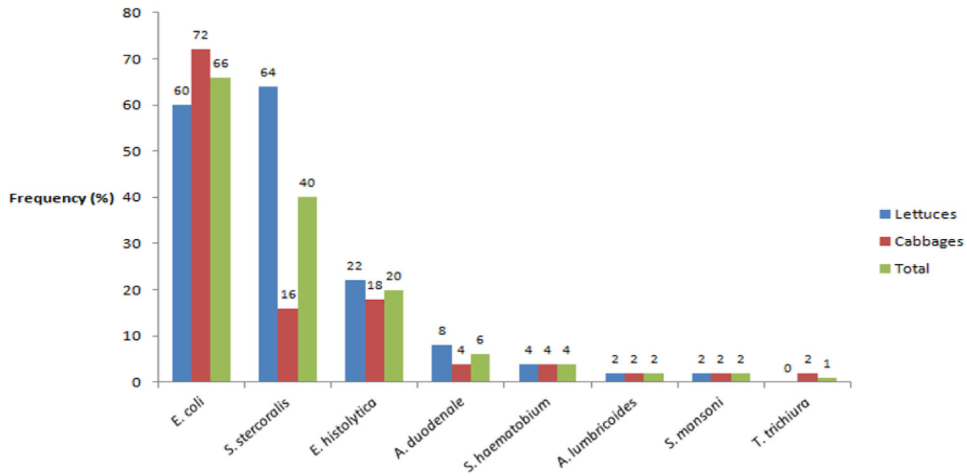

Figure 2:
Profile and frequency of parasites identified in the study.

Table 1:

Socio demographic data of vendors and observations made in markets regarding handling of vegetables.

Variable		Total n = 100 (%)
Gender of the vendors	Female	93 (93)
	Male	7 (7)
Age of the vendors	< 18	9 (9)
	> 18	91 (91)
Education of the vendors	No formal education	15 (15)
	Primary education	68 (68)
	Secondary education	17 (17)
Wash hands before handling	Yes	43 (43)
	No	57 (57)
Vegetables washed before display	Yes	83 (83)
	No	17 (17)
Means of display	On a table	72 (72)
	On the ground	25 (25)
	Other	3 (3)
Cleans station before display	Yes	86 (86)
	No	14 (14)

Table 2:

Frequency and distribution of parasitological contamination of vegetables in selected markets in Maputo city.

Market	Results of parasitological analysis			
	Lettuces	Cabbages		
	Number Positive (%)	p value	Positive (%)	p value
Janete	5 (100)	0.079	5 (100)	0.036
Compone	5 (100)		4 (80)	
Xipamanine	5 (100)		5 (100)	
Povo	5 (100)		4 (80)	
Zimpeto	5 (100)		4 (80)	
Adelina	3 (60)		5 (100)	
Central	4 (80)		4 (80)	
Xiquelene	1 (20)		4 (80)	
Fajardo	5 (100)		5 (100)	
Malanga	5 (100)		1 (20)	

Table 3:

Summarize the overall frequency of contamination of the samples studied and for each sample group.

Type of vegetable	Number examined	Number positive (%)	Number of parasite species detected			
			One (%)	Two (%)	Three (%)	Four (%)
Lactuca sativa	50	43 (86)	13 (30.2)	22 (51.2)	8 (18.6)	0
Brassica oleracea	50	41 (82)	25 (61)	14 (34.2)	1 (2.4)	1 (2.4)
Total	100	84 (84)	38 (38)	36 (36)	9 (9)	1 (1)

Table 4:

Summarizes the frequency of mixed contamination with all identified parasites and for each vegetable species.

Multiparasitism	Parasite species	*Lactuca sativa*	Brassica oleracea	Total
		n = 50 (%)	n = 50 (%)	n = 100 (%)
	Contaminated by at least one parasite	43(86)	41(82)	84(84%)
One Parasite	*E. coli*	4 (9.3)	20 (49)	24 (28.5)
	S. stercoralis	7 (16.3)	2 (4.9)	9 (10.7)
	E. histolytica	2 (4.7)	3 (7.3)	5 (6)
	E. coli + S. stercoralis	16 (37.2)	5 (12.2)	21 (25)
Two parasites	*E. coli + E. histolytica*	2 (4.7)	5 (12.2)	7 (8.3)
	E. coli + S. haematobium	0	1 (2.4)	1 (1.2)
	E. coli + A. duodenale	1 (2.3)	1 (2.4)	2 (2.4)
	E. coli + T trichiura	0	1 (2.4)	1 (1.2)
	E. coli + A. lumbricoides	0	1 (2.4)	1 (1.2)
	S. stercoralis + E. histolytica	2 (4.7)	0	2 (2.4)
	S. stercoralis + A. duodenale	1 (2.3)	0	1 (1.2)
Three parasites	*E. coli + S. stercoralis + E. histolytica*	3 (7)	0	3 (3.5)
	E. coli + S. stercoralis + A. duodenale	1 (2.3)	1 (2.4)	2 (2.4)
	E. coli + S. stercoralis + S. haematobium	1 (2.3)	0	1 (1.2)
	E. coli + E. histolytica+S. haematobium	1 (2.3)	0	1 (1.2)
	E.coli + E. histolytica + S. mansoni	1 (2.3)	0	1 (1.2)
	S. stercoralis + A. duodenale + A. Lumbricoides	1 (2.3)	0	1 (1.2)
Four parasites	*E. coli + E. histolytica + S. mansoni+ S. haematobium*	0	1 (2.4)	1 (1.2)

Table 5:

Bivariate analysis of parasitism in lettuces and cabbages according to some selected variables.

Variable		Results of parasitism in lettuces and cabbages according to some selected variables							
	Lettuces				Cabbages				
	Positive (%)	Total	Odds ratio	p value	Positive (%)	Total	Odds ratio	p value	
Gender	Female	38 (88.4)	45	0.8 (0.7–0.9)	0.4	40 (97.6)	48	0.2 (0.01–3.5)	0.2
	Male	5 (11.6)	5			1 (2.4)	2		
Age	< 18	4 (9.3)	7	0.6 (0.1–6.5)	0.7	4 (9.8)	9	0.8 (0.7–0.9)	0.3
	> 18	39 (90.7)	43			37 (90.2)	41		
Education	No formal education	5 (11.6)	7	3.0 (0.5–20.1)	0.1	7 (17.1)	8	0.6 (0.1–5.7)	0.6
	Primary school	28 (65.1)	33			29 (70.7)	35		
	Secondary school	10 (23.3)	10			5 (12.2)	7		
Wash hands before handling vegetables	Yes	21 (48.8)	23	0.4 (0.1–2.4)	0.3	17 (41.5)	20	0.7 (0.2–3.2)	0.7
	No	22 (51.2)	27			24 (58.5)	30		
Vegetables washed before display	Yes	37 (86)	42	0.4 (0.1–2.6)	0.3	34 (82.9)	41	0.7 (0.1–4.2)	0.7
	No	6 (14)	8			7 (17.1)	9		
Means of display	On the top of tables	33 (76.7)	36		0.2	30 (73.2)	36		0.8
	On the floor	6 (13.9)	12			10 (24.4)	13		
	Other	2 (9.4)	2			1 (2.4)	1		
Cleans station before display	Yes	38 (88.4)	43	0.3 (0.1–2.2)	0.2	35 (85.4)	43	1.4 (1.4–13.0)	0.8
	No	5 (11.6)	7			6 (14.6)	7		