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A PRELIMINARY ALGERIAN STUDY OF CONTAMINATION ASSESSMENT FOR PATHOGENIC INTESTINAL PARASITE IN FRESH VEGETABLE

Ziane Mohammed[1,2], Berroukeche Farid[3,4], Ben Braïek Olfa[5], Lachlache Nesrine[1], and Khoualef Touraya[1]

[1] Centre universitaire de Ain Témouchent, route de Sidi bel abbès, N°101, Ain Témouchent 46000, Algérie

[2] Laboratoire de Microbiologie Appliquée à l'Agroalimentaire, au Biomédical et à l'Environnement (LAMAABE), Faculté des SNV/STU, Université de Tlemcen, 13000 Tlemcen, Algérie

[3] Faculty of Medicine, University of TAHRI Mohammed., B.P 417 Kenadsa road, Bechar 08000, Algeria

[4] Laboratory of Physiology, Pathophysiology, and Biochemistry of Nutrition, Department of Biology, Faculty of Nature and Life Sciences, Earth and Universe, University of Tlemcen 13000, Algeria

[5] Laboratory of Transmissible Diseases and Biologically Active Substances (LR99ES27), Faculty of Pharmacy, University of Monastir, Tunis

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ABSTRACT

Fresh vegetables are frequently used to prepare fresh salad dishes widely consumed in the world. They are known to be the main source of pathogenic intestinal parasites. In this context, the present work aimed to (a) detect the pathogenic intestinal parasites (PIP) in washed fresh vegetables, and (b) assess the exposure to PIP in the city of Ain Témouchent (Algeria). In fact, 26 samples belonging to six groups of fresh vegetables (cabbages, lettuces, leeks, radishes, peppers and tomatoes) were collected, washed (similarly to domestic wash with or without vinegar addition), then examined for PIP contamination. The results indicated that 90% of the examined vegetable samples were contaminated with intestinal parasites showing higher prevalence in lettuce (70.85%) while the lowest contamination (23.52%) was reported in pepper. In addition, *Cryptosperidium* (72%) has been found to be the most dominant parasite in all vegetables while *Toxocara* (8%) was less present. These finding are a preliminary report to conduct a risk assessment of PIP linked to vegetable feeding.

Keywords: intestinal parasites, fresh vegetables, salads, contamination, diseases, Ain Témouchent.

INTRODUCTION

Salad based on fresh and fresh-cut vegetables is a widely consumed dish in Algeria and is becoming more popular in family's menus. It is mainly consumed due to its nutritional values and its dietary significance as salad is mostly containing fresh-cut vegetable ingredients like lettuce, chicory, cress, tomatoes, olives, maize…etc. Moreover, the fresh and fresh-cut vegetables are known to prevent widespread diseases like cardiovascular diseases and cancer (2005). The WHO (2005) recommends a minimum of 400 g of fruits and vegetables per day. Unfortunately, clear data about Algerian consumption statistics regarding fresh and/or fresh-cut (especially salad) does not exist.

In industrial countries, the commercialized fresh vegetables are pretreated before being exposed to consumers in order to improve both of sensory and microbiological qualities of the products. The treatments consist of cleaning, washing, selecting, steaming (or heating) and packaging (Siddiqui *et al.,* 2011). None of these process steps effectively eliminate human pathogens. In developing countries, such as Algeria, fresh vegetables do not undergo any minimal processing, and are sold at formal and informal markets. Vegetables used for salads are mostly not heated and are thus more risky than cooked vegetables (100°C).

In Algeria, fresh vegetables are only examined for *E. coli* contamination according to the JORAD n°39 of 2017, but parasitological contamination is not investigated, even though the latter is often more linked to this kind of food. In fact, the consumption of fresh vegetables is the most important way of intestinal parasites transmission, which is generally considered as a major concern of public health. Protozoan cysts, worm eggs and larvae survive and develop in moist soil and environments of farm vegetables. The initial contamination of vegetables frequently occurs during their field cultivation due to two major reasons: (a) the soil is the most contaminated medium with autochthonous, feces of animals, animal fertilizer…etc, and (b) non-potable water and water dams are highly loaded with several microorganisms. Mishandling of vegetables seems to be frequently related to the sale of cheap products with a low quality, and consumers are not aware about these risks. In fact, salads prepared with such vegetables could contain hazardous parasites involved in gastroenteritis diseases.

Several countries notified food poisoning outbreaks via salad (based on fresh and fresh-cut vegetables) consumption contaminated by parasites. Among these countries, we could cite for example, the USA, England and France (AFSSA, 2002). Regarding Algeria, no clear data on food poisoning or parasitological disease outbreaks assigned with salad dishes exist until now. Therefore, studies based on parasitological contamination of fresh vegetables have not been extensively performed. In this line, this study was undertaken to evaluate the occurrence of intestinal parasites in fresh vegetables sold in Algeria and detect the most common parasitic contaminants.

Table 1 : Data contamination of examined vegetable collected from Ain Temouchent city.

Vegetable	Samples number (N)	Parasite contamination %	% of intestinal parasites (IP) detected vs all examined samples								
			No pathogenic	Pathogenic intestinal parasites							
			Entamoeba spp.	Giardia spp.	Cryptosporidium ssp.	Cyclospora spp.	Toxoplasma gondii	Balantidium coli	Helminthes eggs	Hymenolepis nana	Fasciola spp.
			16	20	48	36	36		28	12	12
Leeks	3	29.41	4	4	12	6					
Cabbages	2	58.82	4			16	4		6		6
Radishes	3	29.41	4	4		4		8	4		
Peppers	3	23.52			12				9		8
Tomatoes	4	52.95	12	8		3		12	12		8
Prevalence(Ps)											
in examined samples (%)	//	1	40	36	72	59	40	20	59	12	34

MATERIAL AND METHODS

Sample collection

A total of 25 samples belonging to six groups of fresh vegetables were collected from different formal and informal markets of Ain Témouchent city (position on google maps 35.309391, -1.132110) in Algeria (Table 1). The samples were purchased randomly during one month and then transported to the laboratory in sterile bags at 4°C.

Processing

The vegetables were processed according to the domestic practice used traditionally by the consumers. Firstly, the non consumed parts were removed from all of the collected vegetables. Then, they were examined for parasites' presence before rinsing according to the consumer's procedure and after it. The consumer's rinsing procedure at Ain Témouchent city consists of washing the fresh vegetable by the water for three times, while only the lettuce was rinsed according two methods: (a) flushing three times with tap water, and (b) addition of 5 mL of vinegar to the rinse water (100 mL of distilled water) and waiting for 20 minutes. As for the non rinsing vegetables, the samples were soaked in 100 mL of distilled water in a plastic container for 15 minutes.

For each patterns, the final volume was centrifuged at 1500 rpm for 10 minutes. The pellet was harvested then examined under a light microscope at 10X and 40X magnification, then at 100X for the qualitative detection of different parasites forms (helminthes eggs and protozoan or oocyst cysts). The eggs/cysts were identified based on morphological characteristics as described by Soulsby (1982).

Statistical analysis and simulation

The Excel software was used to draw the IP prevalence and its distribution. However, ANOVA analysis was used to assess the effect between the different vegetable types and the contaminated genus.

RESULTS AND DISCUSSION

The results revealed the presence of intestinal parasites (IP) in 90% of the 26 analyzed samples. The high prevalence of IP was detected in lettuce (70.85%) followed by cabbage (58.82%), while these parasites occurred less in pepper (23.52%) (Table 1). The parasitic contamination is not dependent on the vegetable category. It was characterized by the predominance of *Cryptosporidium* spp. oocysts (72%) then *Cyclospora* spp. (59.5%) and helminthes' eggs (59.5%) followed by *Entamoeba spp.*, *Toxoplasma gondi* and *Fasciola spp.* (each at 40%), then *Giardia spp.* (39%), *Balantidium coli* (20%) and *Hymenolepis nana* (12%). The identified parasites were classified into two groups: pathogenic and non pathogenic intestinal parasites basing on Ezatpour *et al.,* (2013). Therefore, one genus of non pathogenic intestinal parasites was detected as *Entamoeba coli* in 30% of analyzed samples. Likewise, *Entamoeba histolitica* was present in 15% of samples. It was observed only in cabbages and tomatoes.

Regarding the pathogenic parasites, 7 genus and 4 helminthes eggs have been detected in all examined samples. *Giardia spp.* (36%) was detected in lettuce (20%) followed by tomatoes (8%), while the lowest prevalence was observed for radishes and leeks (4%). Otherwise, *Cryptosporidium* ssp. (72%) was widespread in 48% lettuce followed by peppers and leeks at 12%. Indeed, it has been shown that *Cyclospora spp.* contaminated 88% of the examined samples especially lettuce samples (36%) while the radishes were the lowest parasitic contaminated vegetable (4%). Furthermore, the data indicated that *Toxoplasma gondii* was present in 40% of samples with lettuce at 36% and cabbages at 4 %. Thereby, low contamination (12%) was detected for *H. nana* only in lettuce. Also a low prevalence (40%) of *Fasciola spp.* was reported in lettuce (12%), cabbages (12%), peppers (8%) and tomatoes (8%). Similarly, another low prevalence was observed for *Batantidium coli* (20%) in radishes and tomatoes at 8% and 12% respectively.

Other pathogenic intestinal parasites such as *Ascaris lumbricoides* eggs (24%) and *Enterobuis vermicularis* (12%) were more common in lettuce. Additionally, *Toxocara* spp. was shown to contaminate radishes with a low percentage of 8%.

Discussion

Vegetables represent a source of transmission of several pathogenic and non pathogenic intestinal parasites. In this study, the contamination (90%) was higher than those previously shown in the vegetables' market; 31.2% (25/80), 50% (48/96), 31.7% (95/300), 52.7% (286/550) and 42.6 (115/270) as reported by Asadpour *et al.,* (2016), Bekele and Shumbej (2019), Daryani *et al.,* (2008), El Said Said (2012), and Ezatpour *et al.,* (2013), respectively. According to the vegetable type, as in the current study, El Said Said (2012) reported high contamination levels in lettuce samples (73.3%) and the lowest contamination levels were found in peppers (23%). Concerning radishes contamination, our results were similar to those (5.3% and 3.6%) shown by Ezatpour *et al.,* (2013) and Rahmati-Najarkolaei *et al.,* (2015), respectively.

In this line, this contamination variability may be due to (a) the fact that shrubby plants are close to the soil which predisposes them to helminthes parasites contamination during irrigation (Amaechi *et al.,* 2016), (b) treatment and washing methods *(*Adamu *et al.,* 2012; Fallah *et al.,* 2016*)* and (c) environment conditions, especially high humidity, warm and ambient temperature, which increase parasite life span (Omrani *et al.,* 2015).

The results revealed that there is no depending relationship between the parasites genus and the type of vegetable. At this level, it is difficult to assess parasite concentration per sample because of the absence of a McMaster chamber. For this reason, the results obtained in this study are qualitative (presence or absence). According to parasite identification, our results regarding the *Entamoeba coli* contamination are in agreement with those reported (11.3%) by Ezatpour *et al.,* (2013). However, the Iranian study undertaken by Fallah *et al.,* (2012) indicated their presence in only 9.2% of the vegetables. Although, *Entamoeba coli* is a non-pathogenic intestinal protozoan, but it is a good marker of vegetable contamination with human feces (e.g., sewage) which enhances the probable presence of other pathogenic and non-pathogenic parasites on vegetables (Daryani *et al.,* 2008; Fallah *et al.,* 2012; Rahmati *et al.,* 2014). Likewise, *Entamoeba histolitica* was present in 8% of cabbages and tomatoes. These results are in accordance with those shown by Afzan *et al.,* (2017), Leon *et al.,* (1992), Ogunleye *et al.,* (2010), and Da Silva *et al.,* (2014). These authors suggested that the contamination with *Entamoeba histolitica* may be due to an unsuitable agricultural practices during cultivation, in which vegetables were grown in direct contact with soil and water already contaminated with human and animal fecal matters. This parasite is assigned with amoebiasis (amoebic dysentery). In regards to pathogenic intestinal parasites, the vegetables examined in this study were found to be more contaminated compared to those evaluated (10%) by Bishop and Yohanna (2018) and less contaminated in comparison with those (31.6%) of Al-Megrin (2010). This variability could probably be explained by the harvesting period, seasonal temperature and method of the harvest, the number of samples, the location and the type of water used for irrigation.

Cryptosporidium oocysts prevalence is close to those shown by El Said Said (2012), Amaros *et al.,* (2010) who found that these oocytes' parasites were detected at 52% in lettuce irrigated by wastewater. El Said Said (2012) and Fallah *et al.,* (2016) showed that *Cryptosporidium* is mainly associated with the quality of water used for irrigation. They also found that the incidence of *Cryptosporidium* contamination is significantly higher in vegetables irrigated with untreated wastewater than those irrigated with groundwater. According to Adamu *et al.,* (2012), Afzan *et al.,* (2017), El Said Said (2012), leafy green vegetables, such as lettuce, provide a large and irregular surface which allows parasitic eggs and cysts to easily attach on its leaves making it as the vegetable the most susceptible to parasitic contamination. However, chili peppers and leeks are recognized as vegetables with smooth surfaces which reduce the rate of parasitic attachment. Accordingly, these vegetables were demonstrated to have the lowest incidence of parasites contamination in the present study.

The results of *Cyclospora* spp. contamination are similar to those found by El Said Said (2012) reporting 38.3% and 8.3% contamination levels for lettuce and leeks, respectively. These values are also in accordance with those of a German study which reported that lettuce, imported from southern of Europe, prepared and spiced with fresh green leafy herbs, was the only food associated with *Cyclospora* (El Said Said, 2012). Furthermore, other vegetables contaminated with *Cyclospora* have been detected such as tomatoes (3%), cabbages (16%), leeks (8%) and radishes (4%).

T. gondii is the causative agent of toxoplasmosis, one of the most prevalent parasitic infections that especially humans (Tenter *et al.,* 2000). Congenital toxoplasmosis is a particular concern related to *T. gondii* infection which can be especially serious for the fetus if the mother is seronegative; when the mother acquires the primary infection during the pregnancy (Umoh *et al.,* 2001). In Algeria, 49 cases of congenital toxoplasmosis were diagnosed during the last 16 years (2002–2017) (Theel *et al.,* 2012).

Balantidium coli is a ciliated protozoan parasite of the large intestine which infects approximately 1% of the world's population (Theel *et al.,* 2012). In this study, it was detected in tomatoes and radishes at relatively low prevalence of 8%, which is similar to the results (10-13%) reported by Kudah *et al.,* (2018). Balantidiasis is caused by ingesting infective cysts from food and water already contaminated by feces. Mostly asymptomatic, in some cases it can cause diarrhea and abdominal pains.

The eggs of these nematodes are known to be resistant to harsh soil and environmental conditions for months (Afzan *et al.,* 2017). Another study of Rahmati *et al.,* (2017) showed a high prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* on all sold vegetables kept on nicked ground. Indeed, this contamination can occur by various ways: dried feces conveyed by the air, contaminated washing water or irrigation water, and transport. Additionally, lettuce is the most contaminated fresh vegetable by these parasites, probably due to its large and flexible leaves as well as its compact structure. These features ensure better fixation of lettuce in the soil during cultivation which facilitate its contact with helminthes probably present there (Luz *et al.,* 2017). In contrast, other vegetables like chili peppers and radishes contain a smooth peel that prevents the parasite and helminthes eggs from their fixation into its surfaces. Otherwise, it is important to denote that the presence of helminthes transmissible from soil is a marker of socio-economic status, as well as poor environmental and sanitation practices (Amaechi *et al.,* 2016). *A. lumbricoides* contamination has been found at different prevalences. A similar study showed that *A. lumbricoides* was soil-transmitted helminthes (52%). These data in regards to the high contamination levels by *A. lumbricoides* could mainly be explained by the fact that the eggs of these nematodes are considered to be able to withstand harsh soil and environmental conditions for several months. Furthermore, the predominance of these parasites could also be attributed to favorable climatic conditions, such as high temperature and humidity and rainy season (Atay *et al.,* 2001). On the other hand, Fallah *et al.,* (2016) stated that the outer surface of *A. lumbricoides* has a layer of mucopolysaccharides which allows them to possess a strong adhesion on different surfaces making their remove very difficult and incomplete by an improper washing application.

In this study, vegetables were less contaminated by *H. nana*, as reported (2.4%) also by El said (2012). Oliveira *et al.,* (2011) suggested that the main source of this parasitic contamination type is anthropogenic disturbance in humans, animals and irrigation water.

Similar to this study, Bishop and Yohanna (2018) found a contamination of 20% and 0% of lettuce and cabbage respectively by *Fasciola*. According to Umoh *et al.,* (2001), the rate of food contamination depends on sanitation in terms of environments and sanitary habits of people living there. The persistence of these parasites suggests high levels of contamination.

CONCLUSION

The obtained results indicated the potential risk of different parasites found in raw vegetables involved in digestive problems and / or gastrointestinal disorders. Indeed, a range of parasites (example: *Cryptosporidium* spp.) have been detected in the fresh vegetables used to prepare salad dishes. Among these parasites detected, some are not pathogenic but are indicators of fecal contamination, while other parasites are known to be involved in gastrointestinal disorders. In the light of these results, several suggestions and recommendations can be proposed:

- Production: (a) improvement and control of agricultural areas for vegetables (soil, irrigation water, fertilizer, etc.), installation of preventive measures to treat the irrigation and washing water and a carefully choice of fertilizers, (b) an awareness program should be undertaken for farmers. It is also important to respect the good agricultural practices and the hygiene principles;

- Marketing: preventive measures (e.g. bleaching, closed surface, etc.) should be taken for products exposition in order to ensure their safety as well as their microbial and sensory qualities;

- Consumers' habits: consumers should be aware about the human parasitism and health consequences after consuming contaminated raw vegetables with an emphasized focus on pathogenic parasites and their sanitary risks in order to adopt healthier cleaning procedures (for example washing fresh vegetables with vinegar added to water several times before serving). Therefore, by following these precious advices and recommendations, parasites contamination will mainly be reduced in raw vegetables markets.

As perspectives, surveys at the level of vegetable production farms will be considered on the cultivation method and the water used for irrigation and washing. Finally, the acquisition of new approaches for estimation of different forms of parasites may be eventually established.

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