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A COMPARATIVE SURVEY ON THE PREVALENCE OF PARASITE ELEMENTS IN FRESH VEGETABLES AND READY-TO-EAT SALADS

Tihana Marček*, Sarah Čorluka, Martina Gložinić, Eleonora Jažić, Pamela Radman, Mia Sučić, Maja Ižaković, Ines Banjari

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia

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Summary

While highly nutritional, raw vegetables pose a significant health risk of food-borne diseases. Along with their popularity among the consumers, the number of food recalls for ready-to-eat salads around the world is constantly increasing. The study focused on the prevalence of parasite elements in vegetables selected randomly from two fresh food markets and two retail supermarkets from the area of Osijek city between March and June 2018. Four types of vegetables: lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* var. *capitata*), kale (*Brassica oleracea* var. *acephala*) and spinach (*Spinacia oleracea* L.), and ready-to-eat salads (combinations of carrot, different types of lettuce, cabbage, and beetroot) have been analysed. Total of 36 samples from fresh food markets and 17 ready-to-eat salads were analysed by light microscopy. Parasite elements were detected in 3.77% of all tested samples. Total of 33.96% of lettuce and ready-to-eat salads samples contained undefined cysts. The highest increase in detected number of parasitic elements was observed in June. All other types of vegetables analysed were free from parasitic elements. Our research confirms that ready-to-eat salads pose significant health concern for the consumer. More promotional activities are needed to increase consumer’s awareness of the potential risks related to consumption of ready-to-eat salads.

Keywords: ready-to-eat salads, fresh vegetables, parasite elements, fresh food markets, retail supermarkets

Introduction

Vegetables are important source of a number of minerals, vitamins, insoluble fibres and many bioactive compounds. Especially potent are dark green leafy vegetables like cabbage, kale, spinach and lettuce varieties. Intake of vegetables (and fruits) is linked to lower risk of cardiovascular diseases, type 2 diabetes, colorectal cancer and many other health conditions which are the lead causes of mortality and morbidity today in the world (Slavin and Lloyd, 2012). Insufficient intake of fruit and vegetables is estimated to cause around 14% of gastrointestinal cancer deaths, about 11% of ischaemic heart disease deaths and about 9% of stroke deaths worldwide (WHO, 2009).

Many types of vegetables are consumed raw, without any thermal processing and as such pose a potential health risk for the consumer (FAO, 2008). One of the main concern is parasitic contamination (Daryani et al., 2008; Erdogrul and Sener, 2005). The hygienic-quality of vegetables depends not only on chemical, but also on biological safety, which includes parasites and bacterial abundance. Special emphasis should be put on ready-to-eat salads and easy-to-prepare products which become more popular among the consumers due to lifestyle changes due to progress of the society (Caradonna et al., 2017). The main sources of vegetable parasite contamination are irrigation water contaminated with sewage, soil contamination, post-harvest handling and low hygienic preparation practice related with home or food service (Beuchat, 2002; Simões et al., 2001; Barjaktarović-Labović et al., 2018). From the aspect of ready-to-eat salads, some types of vegetables, especially lettuce, poses a high risk contamination during production and harvesting. Large surface area and gaps on leaf surface such stomata or veins may keep the parasite elements, cysts or eggs, during washing increasing the contamination level (Robertson, 2017). Foodborne contamination of vegetables caused by parasites has been reported in many countries (Abougrain et al., 2010; Amorós et al., 2010). However, there are limited number of studies related with contamination of ready-to-eat salads by parasites on global level.

Parasites are obligate organisms which cannot multiply without the presence of host organism so for that reason the parasite food control practice is more difficult than bacteriological safety programs (Paniker, 2018). Cysts and spores are infective stages of parasitic protozoa, i.e. resting, nonfeeding and resistant life-forms of protozoa bounded by hard cell wall which protect them from unpleasant environment (Corliss, 1994). In addition, cysts and spores are important for the dispersal of parasites and is critical for their population. After ingestion, cysts undergo the excessive excitation process usually in

*Corresponding author: tihana.marcek@ptfos.hr

digestive tract, releasing the mobile stage of parasite, sporozoites, which adhere on surface of the intestine (Paniker, 2018). Definitive host releases a huge amount of sporulated cysts by faeces, which may contaminate the environment. Presence of intestinal parasite can trigger the development of different disorders like diarrheal disease (Beyene and Tasew, 2014), megaloblastic anaemia (Vuylsteke et al., 2004), skin allergies (Giacometti et al., 2003; Mehta et al., 2002) and duodenal, gastric or peptic ulcers (Obiajuru and Adogu, 2013).

Climate changes has great impact on the whole ecosystem (IPCC, 2014). The impact of climate on parasite occurrence is mostly seen thought temporal shifts in parasite transition routes which together with the local ecosystem properties may result in radical perturbations in host-parasite interaction (Kutz et al., 2005). For example, temperature oscillations can affect the development and reproduction of definitive parasites ectothermic hosts (fish, crabs or clams) or indirect host organisms (e.g. snails) endangering their health status and persistence in ecosystems (van Dijk et al., 2010). These climate-related changes in parasite dissemination eventually lead to human and animal disease (Mas-Coma et al., 2009).

The aim of this research was to assess the health safety observed through parasitic contamination of vegetables that are most commonly consumed raw, including ready-to-eat salads from the area of Osijek. To our best knowledge, this is the first report of parasitic contamination of ready-to-eat salads in Croatia.

Materials and methods

A total of 36 samples of fresh seasonal vegetables were collected from two fresh food markets and two retail supermarkets, while 17 ready-to-eat salads were purchased in two retail supermarkets. All samples were collected between March and June 2018 from the area of Osijek city. The following vegetables were tested: 15 samples of lettuce (*Lactuca sativa* L.), 6 samples of cabbage (*Brassica oleracea* var. *capitata*), 13 samples of kale (*Brassica oleracea* var. *acephala*) and 2 samples of spinach (*Spinacia oleracea* L.). Ready-to-eat salads were mixtures of carrot, different types of lettuce, cabbage, and beetroot.

The fresh vegetables were chopped into small pieces and 150-200 g of each vegetable was weighted and used for the analysis. In the case of ready-to-eat salads, the cutting procedure was skipped. Samples was stored in polyethylene bags, rinsed in physiological saline solution (0.85% NaCl) (200 mL) with addition of few drops of Tween 80 and agitated vigorously (manually) for about two minutes. Liquid obtained was left to sediment for 24 hours. Sediment was taken (10 mL), centrifuged at 1500 rpm for 3 min and supernatant was discarded (El Said Said, 2012). Smears were prepared with 50 μL of sediment and stained with 50 μL of Lugol iodine solution. For each sample, three slides were made. Parasite elements were detected in sample under a light microscope (Optika B-159) using x10 and x40 objectives. Photographs and measurements of objects were made with Dino-Eye microscope eye-piece camera and the matching software (Dino-Lite Pro). The data for the precipitation from March to June 2018 was obtained from Croatian Metrological and Hydrological Service (DHMZ).

Statistical analysis was conducted with STATISTICA 13.4 (Stat Soft Inc., USA). Chi-square test was used to calculate significance, and where appropriate, McNeimar test was also used. The value of $P<0.05$ was considered statistically significant.

Results and discussion

The prevalence of protozoa cysts was 3.77% in all tested samples (Fig. 1). The prevalence of cysts in infected samples was 33.96% (Table 1).

Fig. 1. Unidentified protozoan cysts detected in fresh vegetables under objective magnification x10 and x40, respectively

Table 1. Prevalence of cysts in contaminated samples

Type of vegetable sample	Ready to eat	Lettuce	No.	%No.
Parasite element	17	1	18	33,96

No.: Total number of cysts detected in analysed samples
% No.: Percentage of contamination

Out of all samples taken from two local markets only lettuce samples were positive to cysts (6.7% of contamination) while kale, cabbage and spinach were negative (Table 2). The highest contamination of lettuce can be partially explained with rough surface of the leaves and open-shape of shoot which can be easily reached by cysts in the case of faecal route contamination or watering with contaminated water. Lettuce has tender, soft leaves, sensitive to vigorous washing compared to kale or cabbage which increases the probability to keep cysts more attached to the surface. Contrarily, closed-shoot shape morphology, seen in cabbage, decreases the probability of parasitic contamination (Damen et al., 2007). The connection between morphology of vegetables and parasite prevalence was observed for lettuce (45%) and rocket (*Eruca sativa* L.) (46.7%) (El Said Said, 2012). Additionally, lettuce was the most commonly contaminated vegetable in other studies. The rate of contamination was 40% (Damen et al., 2007), 45.5% (Eraky et al., 2014) and 96% (Abougrain et al., 2010). Another reason may be frequent watering of the plant to maintain freshness, therefore increasing consumer's interest into buying and consuming it.

Table 2. Prevalence of cysts in different vegetables from two fresh food markets

Sample	No.	No.C.	% No.
Cabbage	6	0	0
Spinach	2	0	0
Kale	13	0	0
Lettuce	15	1	6.67
Total No.:	36	1	2,78

No.: Number of examined samples
No. C.: Number of contaminated samples
% No.: Percentage of contamination
Total No.: Total number of examined and contaminated samples and percentage of contamination

We found that ready-to-eat salads have significantly higher rate of contamination than other vegetable samples (x^2 =0.027). Poor food handling and processing, and low hygienic practice can explain these findings. According to Little and Gillespe (2008) poor hygiene practice of salad vegetables was the major cause of health problems. Furthermore, the food handling from farm to market may also be one of the possible route of parasite contamination in these products (Kwabena et al., 2014). The protozoan parasite contamination of ready-to-eat salads was also recorded across Europe. In Italy, mixed packaged salads contained the oo/cysts of *Giardia duodenalis*, *Cyclospora cayatanensis*, *Toxoplasma gondii* and *Criptosporidium* sp. (Caradonna et al., 2017). In the same study 4.2% of salads had one protozoan species while in 0.6% of samples two parasite elements were detected. Contamination of fresh salads with parasite cysts was found in Turkey (Erdogrul and Sener, 2005), Poland (Lass et al., 2012), Spain (Amoros et al., 2010) and Norway (Robertson and Gjerde, 2001).

Comparing the contamination prevalence during time, in June the number of protozoan cysts was significantly higher than in May (x^2 = 0.006), April (x^2 = 0.02) or March ($p<0.001$), respectively (Table 3). Higher contamination of packaged salads with *T. gondii* was noticed in Summer and for *G. dudendalis* greater occurrence was in Spring and Autumn (Caradonna et al., 2017). El Said Said (2012) reported higher parasitic contamination in Spring and Summer than in Autumn or Winter. We also found that leafy vegetables have significantly higher probability for contamination in June in comparison to March ($p=0.041$, McNeimar test). While we found no correlation with the rainfall for respective months for the study area, it is visible that highest rainfall was measured in June (Table 3). We presume that increased precipitation facilitated the dispersal of parasites, therefore increasing the number of contaminated samples. The connection between increased rainfall and parasite transmission was reported in numerous studies (Hunter, 2003; Patz et al., 2000; Sterk et al., 2013).

Table 3. Prevalence of cysts in vegetable samples and ready-to-eat salads from March to June 2018

Month	Precipitation (mm)	No. C.	Total No.
March	83	0	19
April	21	0	14
May	26.7	1	13
June	126.8	15	7

No. C.: Number of cysts detected in analysed samples
Total No.: Total number of examined and contaminated samples and percentage of contamination

Conclusions

Our data is the first report of parasite presence in vegetables from retail markets and fresh food markets from the area of Osijek. Consumption of tested samples of vegetables can be potentially hazardous, and of special concern are ready-to-eat salads because the customers are instructed that no washing prior consumption is needed (written on the package). More promotional activities by the local health providers are needed to increase consumer awareness of potential health-related hazards of consumption of uncooked vegetables.

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