Medicina Veterinária (UFRPE) ISSN 2675-3618

Parasites in vegetables used for human consumption: a risk for public health

[*Parasitos em vegetais utilizados no consumo humano: um risco para a saúde pública*]

"Scientific Article/Artigo Científico"

Guilherme Mota Maciel Rego **Barros**[1], Lucia Oliveira **Macedo**[2*], Tatiene Rossana Móta **Silva**[2], Gílcia Aparecida **Carvalho**[1], Rafael Antonio Nascimento **Ramos**[1]

[1]Universidade Federal do Agreste de Pernambuco, Garanhuns-PE, Brasil.
[2]Universidade Federal Rural de Pernambuco, Recife-PE, Brasil.
[*]Corresponding author/Autora para correspondência: E-mail: luciamacedo162@gmail.com

Abstract

Vegetables are pivotal food source to the human beings, representing the source of key nutrients, which in turn the occurrence of chronic diseases. Nonetheless, the consumption of poorly washed or *in nature* vegetables may expose individuals to the risk of infection by parasites. The aim of this study was to assess the contamination by parasites of medical and veterinary concern in raw vegetables used for human consumption. Fresh vegetables [i.e., lettuces (n = 50), carrots (n = 50) and beet (n = 50)] were acquired in different market places across the municipality of Garanhuns (northeastern Brazil) and the presence of parasites assessed by a sedimentation technique. Parasites were detected in 44.7% (67/150) of the evaluated samples. This study provides important data on the occurrence of zoonotic agents in vegetables. Therefore, strict hygienic measures should be adopted to reduce the risk for vegetable contamination leading to a reduction of human infection by pathogens of medical importance.

Keywords: food-safety; foodborne diseases; helminths; protozoan.

Resumo

Os vegetais são essenciais para os seres humanos, representando uma importante fonte de nutrientes e reduzindo a ocorrência de doenças crônicas. No entanto, uma dieta importante com esse tipo de alimento pode expor indivíduos a infecções por parasitos, especialmente quando os vegetais são mal higienizados ou consumidos *in natura*. O objetivo desse estudo foi avaliar a contaminação por parasitos de interesse médico-veterinário em vegetais crus utilizados para consumo humano. Vegetais frescos [alfaces (n = 50), cenouras (n = 50) e beterraba (n = 50)] foram adquiridos em diferentes mercados privados do município de Garanhuns (nordeste do Brasil) e a presença de parasitos foi avaliada por uma técnica de sedimentação. Parasitos foram detectados em 44,7% (67/150) das amostras examinadas. Este estudo fornece dados importantes sobre a ocorrência de agentes com potencial zoonótico. Portanto, devem ser adotadas medidas de higiene rigorosas para reduzir o risco de contaminação vegetal, levando à redução de infecções em humanos por patógenos de importância médica.

Palavras-chave: segurança alimentar; doenças transmitidas por alimentos; helmintos; protozoários.

Introduction

Vegetables are pivotal food source to the human beings, representing the source of important nutrients (e.g., vitamins, amino acids) that are involved in the reduction of the occurrence of chronic diseases, as well as maintenance of body weight (Jaime and Monteiro, 2005). The consumption of poorly washed raw vegetables can expose individuals to viral, bacterial and parasitic infections (Jaime and Monteiro, 2005; Luz et al., 2017). Among the most common parasites detected in vegetables, helminths (e.g., *Toxocara* spp., *Angiostrongylus* spp.) and protozoa (e.g., *Giardia duodenalis*, *Cryptosporidium* spp.) have a great

Received 05 June 2020. Accepted 07 April 2021.
DOI: https://doi.org/10.26605/medvet-v15n2-4398

importance (Tefera et al., 2014). In general, food-borne infections cause abdominal pain, bloating and diarrhoea (Garcia et al., 2018).

Previous studies have demonstrated that sociodemographic characteristics, personal hygiene, basic sanitation and health promotion are important factors involved in the transmission of parasites by vegetables (Kiani et al., 2016; Bekele et al., 2017). In fact, this contamination may occur from cultivation to commercialization, since various factors such as use of low quality organic fertilizers, aspect of water for irrigation, inadequate storage and transportation, and handling may be considered risk factors (Falavigna et al., 2005; Takayanagui et al., 2006). The number outbreaks associated with the consumption of contaminated raw vegetables is most likely underestimated due to major failures in the diagnostic and notification system, and with cases hard to be documented (Eraky et al., 2014).

According to World Health Organization (WHO, 2019), the presence of up to one nematode egg per litter of irrigation water is considered acceptable; however, this cannot be appropriate in favourable conditions that allow the nematode eggs to survive (e.g., low mean temperatures). In this case, the threshold should be reduced to less 0.1 egg for litter (Blumenthal et al., 2000). In this context, the parasite detection in vegetables is a major public health issue (Luz et al., 2017). Therefore, the aim of this study was to assess the contamination by parasites of medical and veterinary concern in raw vegetables used for human consumption.

Materials and Methods

Study area and sampling

Samples were obtained in different market places located in the urban area of the municipality of Garanhuns (8°53′25″S and 36°29′34″W), Pernambuco, northeastern Brazil. This region is located at a height of 900 m above sea level and has an average temperature of 22°C, with a maximum in January (24°C) and minimum in June (19°C). The average relative humidity is 90%. Climatic information was obtained from the Pernambuco Water and Climate Agency (*Agência Pernambucana de Águas e Clima, APAC*).

All fresh vegetables [i.e., lettuces (n = 50), carrots (n = 50) and beets (n = 50)] were produced in conventional farming and commercialized in different market places. The vegetables were randomly selected by convenience (Reis, 2003). Each sample was individually stored in plastic bags and kept in isothermal boxes at 8ºC until laboratory processing.

Laboratorial procedures

Vegetables were assessed through a sedimentation technique. Briefly, samples were individually washed with distilled water (250 mL) in a plastic container with an aid of brushes, which were sanitized after each procedure in sodium hypochlorite. Washing liquid was filtered through sterile gauze in a sedimentation glass. After 24h, the upper layer was discarded and 30 mL of the wash liquid was transferred to plastic tubes and centrifuged (200 × *g* for 1 minute) (Abougrain et al., 2010). Subsequently, the supernatant was carefully removed and 100 µL of the pellet was transferred to five glass slides, mixed withLugol's Iodine solution and analysed under optical microscope with 100x and 400x magnification.

Data analysis

The chi-square test (x^2) was used to compare the positivity among vegetables. The significance level was set up at 5%. All analyses were performed using the statistical software BioEstat version 5.0 (Ayres et al., 2007).

Results

Out of all samples analysed 44.7% (67/150) scored positive for at least one immature form of parasites (eggs, larvae, oocyst and/or protozoan cysts). The overall results according to single and mixed contamination are summarized on Table 1.

Table 1. Frequency of contaminated vegetables by parasites commercialized in the municipality of Garanhuns, Pernambuco state, northeastern Brazil.

Contamination	Lettuce % (n)	Carrot % (n)	Beet % (n)
Simple	42 (21)	24 (12)	22 (11)
Double	20 (10)	-	6 (3)
Triple	18 (9)	-	-
Fourfold	2 (1)	-	-
Total	82 (41)	24 (12)	28 (14)

The occurrence of each parasite according to the vegetable is described on Table 2.

Difference statistically significant was observed when compared the positivity between lettuces and carrots ($x^2 = 31.4733$; $p < 0.05$), and lettuces and beets ($x^2 = 29.4545$; $p < 0.05$).

Table 2. Immature forms of gastrointestinal parasites detected in vegetables commercialized in the municipality of Garanhuns, Pernambuco state, northeastern Brazil

Helminth/protozoan	Lettuce % (n)	Carrot % (n)	Beet % (n)	Total % (n)
Ascarididae (eggs)	30 (15)	10 (5)	8 (4)	16.0 (24)
Trichuridae (eggs)	2 (1)	-	-	0.6 (1)
Rhabditidae (larvae)	18 (9)	-	8 (4)	8.6 (13)
Oxyuridae (eggs)	2 (1)	6 (3)	6 (3)	4.6 (7)
Trichostrongylidae (eggs)	2 (1)	-	-	0.6 (1)
Ancylostomatidae (eggs)	14 (7)	6 (3)	8 (4)	9.3 (14)
Anoplocephalidae (eggs)	2 (1)	-	-	0.6 (1)
Endolimax (cysts)	8 (4)	-	-	2.6 (4)
Entamoeba (cysts)	46 (23)	-	-	15.3 (23)
Giardia (cysts)	8 (4)	-	-	2.6 (4)
Unidentified coccidian (oocysts)	12 (6)	2 (1)	4 (2)	4.0 (6)

Discussion

This study demonstrated that vegetables commercialized in the municipality of Garanhuns show a high rate of contamination by parasites of public health concern. The overall positivity (44.7%; 67/150) herein observed indicates the lack of hygiene conditions during the cultivation and/or handling of these vegetables. Similarly, studies have reported frequencies ranging from 6.2% (Mesquita et al., 1999; Gabre and Shakir, 2016) to 61.1% (Luz et al., 2017). In particular, lettuce contamination (82.0%) was very close to that found in a previous study (79.0%) in the metropolitan region of Recife (Ramos et al., 2019). It is known that these variations most likely are related to the hygienic conditions presented in each area of sampling and to the method used to recovery the parasites. The vegetables assessed are widely used *in natura*, especially in fresh salads. In general, lettuces were more contaminated than carrots and beets. This variation may be due to the fact that lettuce has irregular surfaces, which facilitates the fixation of parasites in this vegetable when compared to those with smooth surface (Tefera et al., 2014).

The parasitic structures observed were viable and were morphologically identifiable, through visualization by optical microscopy. Parasites of the families Ascarididae (16.0%) and Ancylostomatidae (9.3%) were the most common nematodes retrieved. It is important to note that the climatic conditions of the area of study allow the development of these parasites (Gamboa, 2005; Tchakounté et al., 2018). This finding is worthy of note since representants of these families may cause Visceral and Cutaneous Larva migrans, respectively (Sharma et al., 2015). The presence of larvae of the Trichostrongylidae family may be related to the presence of animal faeces contaminating the environment, which may be associated with the use of low-quality fertilizers or the circulation of animals in the area of cultivation. Similarly, parasites forms belonging to the Rhabditidae family were observed in 8.6% of samples analysed. This family includes *Strongyloides stercoralis*, important causative agent of disease in humans (Schär et al., 2013; CDC, 2019).

Amongst the protozoa detected, those of the genus *Entamoeba* (15.3%) were the most abundant. Vegetables and water have been considered important source of contamination to human infection. Clinically, diarrhoea, weight loss, vomiting, bloating and abdominal cramps feature these infections; however asymptomatic cases may also occur (Feng and Xiao, 2011; Rafael et al., 2017). The predomination of immature forms of helminths over protozoa may be related to the washing process. It has been demonstrated that the mechanical washing is more efficient to remove cysts/oocysts of protozoan than eggs of helminths (Luz et al., 2017).

Findings of this study are pivotal to demonstrate the absence of hygiene conditions and the risk that the consumption of these contaminated vegetables represents for the public health. It is difficult to state when the contamination occurred, especially because information about the conditions of storage and water used during the cultivation are absent. However, it is known that failures may occur during the whole process (from cultivation to commercialization) allowing the contamination by parasites of medical and veterinary concern (Luz et al., 2017; Tefera et al., 2018). Due the difficulty to control the vegetal contamination, it is important to highlight that vegetables must be always sanitized before the consumption. Raw vegetables are important routes

of transmission of intestinal parasites and have been considered cause of foodborne outbreaks throughout the world (Bekele and Shumbej, 2019). It is believed that these infections may affect about 550 million people and cause until 230.000 deaths annually (WHO, 2019).

This study provides important data on contamination by zoonotic agents in vegetables (lettuces, carrots and beets) commercialized in the municipality of Garanhuns. Therefore, it is extremely important to adopt hygienic measures through health education of producers and consumers aiming to reduce the vegetable contamination, and consequently the risk of human infection by these pathogens.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

Authors would like thank Alessio Giannelli (Poulpharm BVBA, Belgium) for his suggestions on the manuscript. This article is based on a research project developed by the first author at the Federal Rural University of Pernambuco, with support from a fellowship from *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE).

References

Abougrain, A.K.; Nahaisi, M.H.; Madi, N.S.; Saied, M.M.; Ghenghesh, K.S. Parasitological contamination in salad vegetables in Tripoli-Libya. **Food Control**, 21: 760-762, 2010.

Ayres, M.; Ayres Júnior, M.; Ayres, D.L.; Santos, A.S. **BioEstat 5.0: Aplicações estatísticas nas áreas das ciências biológicas e médicas.** 5th ed. Belém: Sociedade Civil Mamirauá. CNPq, 2007.

Bekele, F.; Tefera, T.; Biresaw, G.; Yohannes, T. Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. **Infectious Diseases of Poverty**, 6: 1-7, 2017.

Bekele, F.; Shumbej, T. Fruit and vegetable contamination with medically important helminths and protozoans in Tarcha town, Dawuro zone, South West Ethiopia. **Research and Reports in Tropical Medicine**, 10: 19-23, 2019.

Blumenthal, U.J.; Mara, D.D.; Peasey, A.; Ruiz-Palacios, G.; Stott, R. Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. **Bull World Health Organ**, 78: 1104-1116, 2000.

CDC. Center for Disease Control and Prevention. **Global Health, Division of Parasitic Diseases and Malaria**. 2019 Available in: <https://www.cdc.gov/dpdx/strongyloidiasis/index.html>. Accessed on: 02 oct. 2019.

Eraky, M.A.; Rashed, S.M.; Nasr, M.E.S.; El-Hamshary, A.M.S.; El-Ghannam, A.S. Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. **Journal of Parasitology Research**, 1-7, 2014.

Falavigna, L.M.; Freitas, C.B.R.; Melo, G.C.; Nishi, L.; Araújo, S.M.; Falavigna-Guilherme, A.L. Quality of green vegetables marketed in the northwest of Paraná, Brazil. **Parasitología Latino Americana**, 60: 144-149, 2005.

Feng, Y.; Xiao, L. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. **Clinical Microbiology Reviews**, 24: 110-40, 2011.

Gabre, R.M.; Shakir, A. Prevalence of some human enteroparasites in commonly consumed raw vegetables in Tabuk, Saudi Arabia. **Journal of Food Protection**, 79: 655-658, 2016.

Gamboa, M. I. Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions. **Journal of Helminthology**, 79(4): 327-331, 2005.

Garcia, L.S.; Arrowood, M.; Kokoskin, E.; Paltridge, G.P.; Pillai, D.R.; Procop, G.W.; Ryan, N.; Shimizu, R.Y.; Visvesvara, G. Laboratory diagnosis of parasites from the gastrointestinal tract. **Clinical Microbiology Reviews**, 31: 1-81, 2018.

Jaime, P.C.; Monteiro, C.A. Fruit and vegetable intake by Brazilian adults, 2003. **Cadernos de Saúde Pública**, 21: 19-24, 2005.

Kiani, H.; Haghighi, A.; Rostami, A.; Azargashb, E.; Seyyed Tabae, S.J.; Solgi, A.; Zebardast, N. Prevalence, risk factors and symptoms associated to intestinal parasite infections among patients with gastrointestinal disorders in Nahavand, western Iran. **Revista do Instituto de Medicina Tropical de São Paulo**, 58: 1-7, 2016.

Luz, J.G.G.; Barbosa, M.V.; Carvalho, A.G.; Resende, S.D.; Dias, J.V.L.; Martins, H.R. Contamination by intestinal parasites in vegetables marketed in an area of Jequitinhonha

Valley, Minas Gerais, Brazil. **Revista de Nutrição**, 30(1): 127-136, 2017.

Mesquita, V.C.L.; Serra, C.M.; Bastos, O.M.; Uchôa, C. The enteroparasitic contamination of commercial vegetables in the cities of Niterói and Rio de Janeiro, Brazil. **Revista da Sociedade Brasileira de Medicina Tropical**, 32: 363-366, 1999.

Rafael, K.; Marchioro, A.A.; Colli, C.M.; Tiyo, B.T.; Evangelista, F.F.; Bezagio, R.C.; Falavigna-Guilherme, A.L. Genotyping of *Giardia duodenalis* in vegetables cultivated with organic and chemical fertilizer from street markets and community vegetable gardens in a region of Southern Brazil. **Transactions of the Royal Society of Tropical Medicine Hygiene**, 111: 540-545, 2017.

Ramos, I.C.N.; Ramos, R.A.N.; Giannelli, A.; Lima, V.F.S.; Cringoli, G.; Rinaldi, L.; Carvalho, G.A.; Alves, L.C. An additional asset for the FLOTAC technique: detection of gastrointestinal parasites in vegetables. **Acta Parasitologica**, 64: 423-425, 2019.

Reis, J.C. **Estatística aplicada à pesquisa em ciência veterinária**. 1ª ed. Olinda: Luci Artes Gráficas, 2003. 651p.

Schär F.; Trostdorf U.; Giardina F.; Khieu, Muth S.; Marti H.; Vounatsou P.; Odermatt P. *Strongyloides stercoralis*: Global distribution and risk factors. **PLOS Neglected Tropical Disease**, 7: 1-17, 2013.

Sharma, R.; Singh, B.B.; Gill, J.P. *Larva migrans* in India: veterinary and public health perspectives. **Journal of Parasitic Diseases**, 39: 604-612, 2015.

Tchakounté, B.N.; Nkouayep, V.R.; Poné, J.W. Soil contamination rate, prevalence, intensity of infection of geohelminths and associated risk factors among residents in Bazou (West Cameroon). **Ethiopian Journal of Health Sciences**, 18: 63-72 2018.

Takayanagui, O.M.; Capuano, D.M.; Oliveira, C.A.; Bergamini, A.M.; Okino, M.H.; Castro Silva, A.A.; Oliveira, M.A.; Ribeiro, E.G.; Takayanagui, A.M. Analysis of the vegetable productive chain in Ribeirão Preto, SP. **Revista da Sociedade Brasileira de Medicina Tropical**, 39: 224-226, 2006.

Tefera, T.; Biruksew, A.; Mekonnem, Z.; Eshetu, T. Parasitic contamination of fruits and vegetables collected from selected local markets of Jimma Town, Southwest Ethiopia. **International Scholarly Research Notices**, 2014: 1-7, 2014.

Tefera, T.; Tysnes, K.R.; Utaaker, K.S.; Robertson, L.J. Parasite contamination of berries: Risk, occurrence, and approaches for mitigation. **Food and Waterborne Parasitology**, 10: 23-38, 2018.

WHO. World Health Organization. **Food safety**. 2019. Available in: <https://www.who.int/en/news-room/fact-sheets/detail/food-safety>. Accessed on: 04 jul. 2019.