



Presence of parasitic structures in lettuces served in self-service restaurants of São Miguel do Oeste, Santa Catarina State, Brazil

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ABSTRACT. Consuming raw food like lettuce may carry protozoa cysts and helminth eggs, causing parasitic infections. Therefore, the parasitological analysis of this type of food it's important for public health because it provides data on the hygiene conditions in which these vegetables were produced, transported, processed and handled until the ingestion. This study aimed to evaluate the occurrence of parasitic structures in samples of lettuce (*Lactuca sativa*) sold in self-service restaurants in São Miguel do Oeste, Santa Catarina State, Brazil. The samples were randomly collected in 20 locations in duplicate, prepared according to the method of Hoffman, Pons, and Janer (1934) and analyzed with an optical light microscopy. The parasitic structures found were Cysts *Balantidium coli*, *Giardia spp.* and *Entamoeba spp.*, *Ascaris lumbricoides* eggs and grubs of *Strongyloides stercoralis*. These results demonstrate that the lettuce served at most self-service restaurants evaluated is unfit for intake and indicates the need to implement hygienic and educational measures to minimize contamination and ensure the safety of the ingestion of this vegetable.

Keywords: protozoa; helminths; contamination; food; vegetables.

Presença de estruturas parasitárias em alfaces servidas em restaurantes self-service de São Miguel do Oeste, SC, Brasil

RESUMO. Alimentos consumidos crus como a alface (*Lactuca sativa*), podem carrear cistos de protozoários e ovos de helmintos causando infecções parasitárias. Assim, a análise parasitológica deste tipo de alimento é importante para a saúde pública, pois fornece dados sobre as condições de higiene em que esses vegetais foram produzidos, transportados, processados e manipulados até o consumo. O objetivo deste estudo foi avaliar a ocorrência de estruturas parasitárias em amostras de alface servidas em restaurantes *self-service* em São Miguel do Oeste, SC. As amostras foram colhidas aleatoriamente em 20 estabelecimentos em duplicata, preparadas de acordo com o método de Hoffman et al. (1934) e analisadas sob microscopia óptica de luz. As estruturas parasitárias encontradas foram Cistos *Balantidium coli*, *Giardia spp.* e *Entamoeba spp.*, ovos de *Ascaris lumbricoides* e larvas de *Strongyloides stercoralis*. Esses resultados demonstram que a alface servida na maioria dos restaurantes self-service avaliados é imprópria para consumo e indica a necessidade de implementar medidas higiênicas e educativas para minimizar a contaminação e garantir a segurança do consumo deste vegetal.

Palavras-chave: protozoários; helmintos; contaminação; comida; vegetais.

Introduction

The parasitic infections caused by protozoa and helminths are the cause of intestinal affections that occur with a wide range of clinical manifestations and comorbidities. The symptoms caused by these infections are well diversified, and may vary from specific to nonspecific, and may cause more aggressive manifestations, which will depend on what kind of parasite was responsible for infecting the organism, as well as the parasite load. Common symptoms and signs of enteroparasitosis may include anemia, diarrhea, weight loss, malabsorption of nutrients, decreased learning and work ability,

and reduced growth rate in children (Laudanna, Zeitune, & Silva, 2007; Andrade, Leite, Rodrigues, & Cesca, 2010).

Parasitic diseases are responsible for causing high morbidity rates in the international context, estimating that approximately 56 million people are parasitized in more than 70 countries, generating in 2011 about 600,000 treatments, leading to death over 2 million people (World Health Organization [Who], 2011). In this context, parasitic infections are a public health problem mainly in places where there are no well-established hygienic-sanitary conditions.

Brazil is a developing country, inhabited by many people with unfavorable economic status, showing a high incidence of parasitic intestinal pathologies. The transmission of these diseases occurs mainly through the ingestion of water or food contaminated by infective forms of these parasites (Freitas, Kwiatkowski, Nunes, Simonelli, & Sangioni, 2004). Lettuce (*Lactuca sativa*) is the most widely consumed vegetable in Brazil. Recommended as part of the daily diet due to its content in vitamins, minerals and dietary fiber, it has low caloric content, high nutritional value and tranquilizing properties. This vegetable presents in its composition substances with antioxidant activities, such as carotenoids, vitamins C and flavonoids (Silva, Andrade, & Stamford, 2005). However, because it's consumed raw, the lettuce can carry infective forms of protozoa cysts, worms and helminths eggs (Takayanagui et al., 2001; Soares & Cantos, 2005).

Parasitological contamination of vegetables can occur in several stages of the productive cycle ranging from planting, fertilization, irrigation, harvesting, distribution and handling during preparation for ingestion. Contamination is related to the absence or ineffective sanitary education of the people who manipulate and prepare the food (Esteves & Figueirôa, 2009).

Hygienic control of vegetables represents a challenge due to factors such as globalization in food distribution, expansion in commercialized food services, emergence of new large-scale production methods, and the increase in consumption of these food by the population (Falavigna et al., 2005).

The research of parasites in vegetables is of immense importance for Public Health, since it allows to identify the conditions in which vegetables were cultivated, handled and marketed (Oliveira & Germano, 1992), serving to promote educational and surveillance measures. That's why this study aimed to conduct a parasitological analysis of the lettuce samples served in self - service restaurants in the city of São Miguel do Oeste, Santa Catarina State, Brazil.

Material and methods

Study design

Lettuce samples were collected in 20 self-service restaurants in the city of São Miguel do Oeste, from March to April 2016. Two different samples of lettuce ready for ingestion were obtained in each restaurant, making a total of 40 samples.

The samples were collected in aluminum pots, offered by the restaurant and used by the customers

to transport food. These were identified according to their origin and sent to the parasitology laboratory of *Universidade do Oeste de Santa Catarina* (Unoesc), Campus of São Miguel do Oeste, Santa Catarina State. The sample unit was established as an amount of 100 g. Each sample was placed in a clean plastic pot individually and then washed in 200 mL of distilled water, using the friction of the fingers protected by sterile latex gloves against the surface of the samples.

After each washing, the liquid obtained was filtered through a gauze folded in four as a conical cup and stood for 24 hours to sediment as the spontaneous sedimentation method by Hoffman et al. (1934).

Microscopic analysis

After the sedimentation, the supernatant liquid from the conical cup was discarded, then were prepared two microscope slides for sample, using the sediment. The samples were stained with lugol and the microscopy analysis was performed with an increase of 100 and 400 x.

Analysis of results

Data were analyzed with the help of Microsoft Excel 2016® software, using descriptive statistics.

Results and discussion

A number of 224 parasite structures were measure in 38 positive samples among the 40 samples tested, resulting in a contamination index of 95% of samples (Table 1). Restaurant 20 showed higher amount of parasites structures. The parasite structures identified were: *Balantidium coli*, *Giardia spp.* and *Entamoeba spp.* cysts; *Ascaris lumbricoides* eggs and *Strongyloides stercoralis* worms (Figure 1).

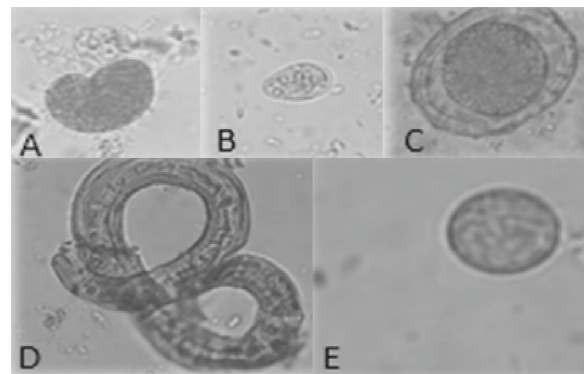


Figure 1. Parasitic structures visualized in lettuce served in restaurants in the city of São Miguel do Oeste, Santa Catarina State, Brazil. Parasitic structures visualized in lettuce samples (400 x magnification). Source: photographed by the Vidigal and Landivar (2016). In A – *Balantidium coli*; B – *Giardia spp.*; C – *Ascaris lumbricoides* egg; D – *S. stercoralis* worm; E – *Entamoeba spp.*

Restaurant 7 presented the greatest variety of parasitic structures. In samples from two establishments, three species of parasites were found. In nine restaurants, at least two parasites were identified. Samples from six restaurants presented only a parasitic structure, this being *Balantidium coli*. Only the samples provided from restaurant 2 and 8 were negative for parasitic structures. *Balantidium coli* was the structure with the highest incidence, present in 81.5% (31/38) of the total samples. The occurrence of *Giardia spp.*, *Ascaris lumbricoides*, *Entamoeba spp.* and worms of *S. stercoralis* and were respectively 23.7 (9/38), 23.7 (9/38), 10.5 (4/38) and 2.6% (1/38) respectively.

Table 1. Number of parasite structures per sampling unit, found in lettuce served in restaurants in the city of São Miguel do Oeste, Santa Catarina, Brazil.

Restaurant	<i>Balantidium coli</i>		<i>Giardia spp.</i>		<i>Ascaris lumbricoides</i>		<i>Entamoeba spp.</i>		Worm of <i>S. stercoralis</i>	
	Sample		Sample		Sample		Sample		Sample	
	I	II	I	II	I	II	I	II	I	II
1	3	4	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	5	2	1	-	-	-	-	-	-	-
4	2	-	1	1	-	-	-	-	-	-
5	-	1	-	-	-	-	-	-	-	-
6	4	2	1	-	1	-	-	-	-	-
7	4	7	1	-	-	1	1	-	-	1
8	-	-	-	-	-	-	-	-	-	-
9	15	18	-	-	-	-	-	-	-	-
10	3	5	-	-	4	-	-	-	-	-
11	2	7	-	-	1	-	-	-	-	-
12	2	-	-	-	1	-	-	-	-	-
13	4	8	-	-	-	-	1	-	-	-
14	2	2	-	2	-	-	3	5	-	-
15	1	1	1	-	-	-	-	-	-	-
16	3	7	1	-	-	-	-	-	-	-
17	2	3	-	-	-	-	-	-	-	-
18	2	3	-	-	1	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-
20	22	45	-	-	-	-	-	-	-	-

The percentage of samples contaminated in this study (95%) is higher than related by Silva and Gotijo (2012) and Soares and Cantos (2005), in similar studies performed in the cities of Gurupi, Tocantins State, Brazil, and Florianópolis, Santa Catarina State, Brazil, respectively.

The most abundant parasitic structure found in our samples was *Balantidium coli*, the occurrence of cysts of this protozoan suggests contamination by dejects of swine breeding, since the protozoan is present in swine faeces (Silva & Gotijo, 2012). *Balantidium coli* is the causative agent of balantidiosis, a zoonosis with fecal-oral contamination route. The parasite lives in the intestine of its host producing generally asymptomatic infections. In some cases, it may invade the submucosa of the cecum and colon, causing dysentery and even intestinal perforation. The disease occurs worldwide, but is more frequent in countries or regions where health services

(education and sanitary engineering) are insufficient (Bermudez & Cimerman, 2009)

Among the analyzed samples, 23.7% (9/38) were positive for *Ascaris lumbricoides*. The egg intake of this parasite directly impacts the health of the consumer. Man is a host of *A. lumbricoides* which can be transmitted through the ingestion of contaminated food or water (Neves, 2012). The percentage of samples contaminated by *Ascaris lumbricoides* eggs here evidenced is higher than reported in the work of Eraky, Rashed, Nasr, El-Hamshary, & El-Grannam (2014), who analyzed vegetables commercialized and consumed fresh in Benha, Egypt. In that study, 0.6% of the samples were positive for these helminths. According to Santana et al. (2006), the occurrence of this parasite suggests contamination by human faeces.

The occurrence of *Giardia spp.* cysts represents possible contamination of the fertilizer used in the cultivation of lettuce by human or animal waste, since these protozoa are parasites of various species (Osaki, Moura, Zulpo, & Calderon, 2010). If viable, these protozoa can cause diarrhea and steatorrhea, due to malabsorption syndrome (Neves, 2012). The contamination of samples by *Giardia spp.* evidenced in the present study was lower to that found by Simões et al. (2001), who analyzed 88 samples of supermarkets in the city of Lavras, Minas Gerais State, and obtained a frequency of 12.12% of contamination by *Giardia* cysts.

Cysts of *Entamoeba spp.* were identified in 10.5% (4/38) of the analyzed samples. In study performed in the State of Lara, Venezuela by Traviezo-Valles, Dávila, Rodríguez, Perdomo, and Pérez (2004), this parasite was present in 5% of samples obtained at supermarkets. The contamination by cysts of *Entamoeba spp.* may be correlated with the absence of basic sanitation, as occurs in many regions of Brazil (Silva, Marzochi, Camillo-Coura, Messias, & Marques, 1995) and indicates the possible contamination of the water used in the irrigation by human or animal faeces. Furthermore, the contamination can be caused by poor hand hygiene of the people who handle the food at some stage of production chain (Silva et al., 2005).

In this research paper, 2.6% (1/38), of the samples were positive for *S. stercoralis*. This nemathelminth can survive in the form of free life or as parasite. The parasite has a worldwide distribution, with a preference for tropical and temperate weather. The amount reported here was lower than found by Rocha, Mendes, and Barbosa (2008) (19.2%), who evaluated samples obtained in supermarkets in the city of Recife. Guilherme et al., (1999) in Maringá, Paraná State, obtained free-

trade samples, where 16.6% were contaminated by these worms. *S. stercoralis* can penetrate the skin, so handling these vegetables without gloves during stages of collection or preparation can trigger a parasitism. The occurrence of these helminths may come from human and animal fecal material, which may be transmitted by irrigation water or by the use of organic fertilizers (Neves, 2012).

The main cause of parasite distribution within a population is associated with the precariousness of basic sanitation. The absence of sewerage system causes contamination of rivers which in turn promotes the contamination of vegetables during the irrigation process (Takayanagui et al., 2001). Water can also be contaminated in the distribution network (Erdoğan & Şener, 2005), so it is important that the irrigation water is treated even if it comes through the network.

A crucial step to ensure food security of vegetables is the mechanical washing. According to Abougrain, Nahaisi, Madi, Saied, and Ghenghesh (2010), the friction of the fingers downwards on the surfaces of the plants, using treated tap water, can considerably reduce the visible dirt and possible worms and eggs of parasites. After washing is recommended a sanitization step. The sanitization before the consumption minimizes the risks of contamination and transmission of enteroparasites through vegetables (Silva et al., 2005). Adami and Dutra (2011) quote that a treatment with superior quality running water can reduce the microbial load of plants by up to 90%, but, to maintain the contamination at safe levels it's essential to apply a sanitization step. Sanitizers, in addition to being essential, at the appropriate dosages are toxicologically safe for the consumers.

The most widely used sanitizer is hypochlorite. Another crucial step is mechanical washing: according to Abougrain et al. (2010), the friction of the fingers downwards on the surfaces of the plants, using treated water, can considerably reduce the visible dirt and possible worms and eggs of parasites.

Conclusion

The results presented here indicate that most of the samples evaluated are not adequate for consumption. Since contamination can occur at any of the several stages of the productive scale, we call attention to necessity of adopting measures that guarantee the safety of ingestion of this kind of food. In addition to the intensification of sanitary inspection of food consumed raw, it emphasizes the importance of a proper sewage network, reducing environmental pollution, improvement of crops

methods and educational measures to food handlers in order to minimize the transmission of enteroparasitics.

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