SAÚDE E AMBIENTE

ISSN IMPRESSO 2316-3313

E - ISSN 2316-3798

DOI - 10.17564/2316-3798.2019v7n2p9-16

CONTAMINATION OF LETTUCE BY PARASITES IN MUNICIPALITIES IN NORTHWESTERN RIO GRANDE DO SUL, BRAZIL

CONTAMINAÇÃO DE ALFACES POR PARASITAS EM MUNICÍPIOS DA REGIÃO NOROESTE DO RIO GRANDE DO SUL, BRASIL

CONTAMINACIÓN DE LAS LECHUGAS POR LOS PARÁSITOS EN MUNICIPIOS DE LA REGIÓN NOROESTE DEL RIO GRANDE DEL SUR, BRAZIL

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ABSTRACT

Lettuce, one of the vegetables most consumed by people, can serve as a vehicle for various parasites, such as protozoa and helminth worms. The present study aimed to characterize the contamination of lettuce sold in Rio Grande do Sul northwestern, Brazil by parasitic forms potentially infectious to humans. Between August 2015 and July 2016, 320 lettuce samples from eight municipalities in northwestern Rio Grande do Sul were analyzed using the spontaneous sedimentation and centrifugal-flotation methods. Material obtained with these methods was observed under a microscope and the parasites present were identified. Seventy-one samples (22.9%) had at least one parasite group. The following parasites were found: Ancylostomatidae (8.75%), *Toxocara* sp. (6.87%), *Trichuris* sp. (6.25%), *Toxoplasma gondii* (2.19%), *Ascaris* sp. (1.25%), *Entamoeba* sp. (0.94%), *Giardia* sp. (0.94%), *Balantidium* sp. (0.62%) and *Isospora* sp. (0.62%). Lettuce sold in the municipalities sampled showed relatively low parasite contamination. However, Brazilian law indicates that only parasite-free vegetables are of adequate quality for consumption. Thus, there is a need for greater sanitary care to avoid fecal contamination of this type of food.

KEYWORDS

Enteroparasites. Vegetables. Parasitology.

RESUMO

A alface, uma das hortaliças mais consumidas pelas pessoas, pode servir como veículo de diversos parasitas, como protozoários e vermes helmintos. O presente estudo teve como objetivo caracterizar a contaminação de alfaces comercializadas na região Noroeste do Rio Grande do Sul por formas parasitárias potencialmente infectantes aos humanos. Entre agosto de 2015 a julho de 2016 foram analisadas 320 amostras de alfaces de oito municípios da região Noroeste do Rio Grande do Sul pelos métodos de sedimentação espontânea e de centrífugo-flutuação. O material proveniente desses métodos foi observado em microscópio e os parasitas presentes foram identificados. Setenta e uma amostras (22,9%) apresentaram pelo menos um grupo de parasita. Foram encontrados os seguintes parasitas: Ancylostomatidae (8,75%), *Toxocara* sp. (6,87%), *Trichuris* sp. (6,25%), *Toxoplasma gondii* (2,19%), *Ascaris* sp. (1,25%), *Entamoeba* sp. (0,94%), *Giardia* sp. (0,94%), *Balantidium* sp. (0,62%) e *Isospora* sp. (0,62%). As alfaces comercializadas em municípios da região Noroeste do Rio Grande do Sul apresentaram uma contaminação relativamente baixa por parasitas. No entanto, a legislação brasileira indica que apenas verduras livres de parasitas apresentam padrão adequado para o consumo. Dessa forma, existe a necessidade de um maior cuidado sanitário a fim de se evitar a contaminação fecal desse tipo de alimento.

PALAVRAS-CHAVE

Enteroparasitas. Hortaliças. Parasitologia.

RESUMEN

La lechuga, una de las hortalizas más consumidas por las personas, puede servir como vehículo de diversos parásitos, como los protozoos y gusanos helmintos. El presente estudio tiene como objetivo caracterizar la contaminación de las lechugas comercializadas en la región Noroeste del Rio Grande del Sur, por forma de parásitos potencialmente infecciosos a los seres humanos. Entre los meses de agosto de 2015 hasta julio de 2016, han sido analizadas 320 muestras de lechugas de ocho municipios de la región Noroeste del Rio Grande del Sur, por los métodos de sedimentación espontánea y de centrífugo-fluctuación. En el material proveniente de estos métodos ha sido observado en microscopio y los parásitos presentes fueron identificados. Setenta y una muestras (22,9%) presentaron, por lo menos, un grupo de parásitos. Han sido encontrados los siguientes parásitos: Ancylostomatidae (8,75%), *Toxocara* sp. (6,87%), *Trichuris* sp. (6,25%), *Toxoplasma gondii* (2,19%), *Ascaris* sp. (1,25%), *Entamoeba* sp. (0,94%), *Giardia* sp. (0,94%), *Balantidium* sp. (0,62%) e *Isospora* sp. (0,62%). Las lechugas comercializadas en los municipios de la región Noroeste del Rio Grande del Sur presentan una contaminación relativamente baja por los parásitos. Sin embargo, la legislación brasileña señala que solamente verduras libres de los parásitos presiéntanse adecuadas para el consumo. De este modo, hay la necesidad de cuidado sanitario con la finalidad de evitar la contaminación fecal de esto tipo de alimento.

PALABRAS CLAVES

Enteroparásitos. Hortalizas. Parasitología.

1 INTRODUCTION

Lettuce (*Lactuca sativa*), one of the most consumed vegetables in the world, has a nutritional composition with appreciable amounts of vitamins, folate, iron and fiber, besides having low caloric levels. Its leaves are also sources of compounds that reduce inflammation and blood glucose and cholesterol levels (KIM *et al.*, 2016).

In the last years, the consumption of this vegetable increased in Brazil with the production of new lettuce cultivars. In a study by Souza and coworkers (2008), in municipalities in the central region of the state of Rio Grande do Sul, more than 70% of the people interviewed stated that they ate lettuce daily. Despite the increase in the area of hydroponics in the last decade, most of the lettuce consumed by Brazilians is produced in the traditional system, where the crops are in direct contact with the soil (SALA; COSTA, 2012).

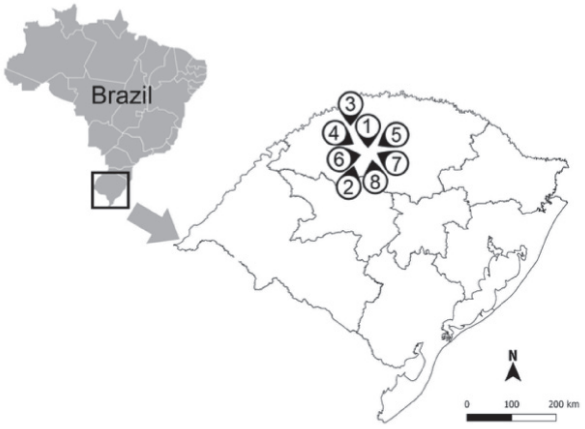
Lettuce is usually eaten raw in a salad and is therefore a food that if not properly sanitized, can serve as a vehicle for pathogens to be carried to humans, for example, bacteria and protists and animals such as helminth worms (GOMES-NETO *et al.*, 2012). The contamination of these vegetables can occur from irrigation with contaminated water (THURSTON-ENRIQUEZ *et al.*, 2002), by the use of contaminated organic fertilizers (KLAPEC; BORECKA, 2012) or by poor hygienic conditions of the people who handle them this food at points of sale and at restaurants (ABERA *et al.*, 2010).

In Brazil, several studies have shown that lettuce that is sold at markets, often by farmers themselves, such as those sold in large market chains, shows substantial contamination rates for intestinal parasites (RAMOS *et al.*, 2014; NOMURA *et al.*, 2015). The northwest region of Rio Grande do Sul is characterized by the strong presence of family farms, with one of the production activities being vegetable crops, such as lettuce. Thus, the present study aimed to characterize the contamination of lettuce sold in northwestern Rio Grande do Sul by parasitic forms potentially infectious to humans.

2 MATERIALS AND METHODS

The lettuce samples from traditional crops were purchased in supermarkets or free markets or directly from farmers, according to availability, in the municipalities of Ajuricaba, Augusto Pestana, Bozano, Catuípe, Condor, Ijuí, Panambi and Pejuçara, all located in the northwest region of Rio Grande do Sul, Brazil (Figure 1). Ten samples were obtained quarterly in each of the municipalities between August 2015 and July 2016, totaling 320 samples in this period. The sample unit established for this study was a head of lettuce, regardless of its weight, size or variety.

Figure 1 – Municipalities in the northwest of Rio Grande do Sul where lettuces were collected between August 2015 and July 2016 (1. Ajuricaba; 2. Augusto Pestana; 3. Bozano; 4. Catuípe; 5. Condor; 6. Ijuí; 7. Panambi; 8. Pejuçara)



Source: authors

In the laboratory, the samples had their leaves individualized, and the stem and deteriorated leaves were discarded. The selected leaves were then washed with a size 12 paintbrush along with 100 mL of sterile distilled water, which was sprayed onto the material using a nozzle. After this initial procedure, the leaves of each sample were placed in plastic bags

containing 100 mL of sterile distilled water and vigorously shaken for one minute.

The material from these washing procedures was filtered in funnels with the aid of a gauze folded eight times according to the procedure described by Oliveira and Germano (1992). The product obtained from this filtration was then divided into two sub-samples, one being processed by the spontaneous sedimentation method (modified Lutz method) and the other processed by the centrifugal-flotation method with concentrated sucrose solution (modified Sheather method) (QUADROS *et al.*, 2008). Subsequently, the material was analyzed under a light microscope at 100X and 400X magnification, after the prepared slides were stained with Lugol's solution and mounted. The parasites were identified based on the morphology of their developing forms such as cysts, trophozoites, eggs and larvae (REY, 2002; NEVES, 2011).

3 RESULTS AND DISCUSSION

Of the 320 samples, 71 (22.9%) had at least one enteroparasite group. Of these, 53 (74.6%) were monoparasitized and 18 (25.4%) were polyparasitized. Nine groups of parasites, six protozoa and three helminths were identified. The occurrence of all the taxa found in this study is widely recorded in the scientific literature (DARYANI *et al.*, 2008; MESQUITA *et al.*, 2015; LUZ *et al.*, 2017).

The presence of potentially pathogenic enteroparasites in humans in almost 23% of the analyzed samples indicated that the lettuce produced in northwestern Rio Grande do Sul, when compared to other regions of the country, show good sanitary quality. Similar studies carried out in the states of Paraná, Santa Catarina, São Paulo and Minas Gerais showed higher than 50% contamination rates for this type of vegetable (FREITAS *et al.*, 2004; SOARES; CANTOS, 2006; GREGÓRIO *et al.*, 2012; LUZ *et al.*, 2017).

All parasite rates found were less than 10%, and Ancylostomatidae was the most frequent (Table 1). Lima and coworkers (2018a) observed a similar frequency as in the present study of Ancylostomatidae contamination in lettuce sold in Aracaju in the state of Sergipe. However, this dominance of Ancylostomatidae should be interpreted with caution, since there are several species of parasites within this family that are indistinguishable when identified with the methods used in the present study.

Table 1 – Parasites found in lettuce samples from municipalities in northwestern Rio Grande do Sul, Brazil, between August 2015 and July 2016

Parasitas	No.	%
Ancylostomatidae	28	8.75
Toxocara sp.	22	6.87
Trichuris sp.	20	6.25
Toxoplasma gondii	7	2.19
Ascaris sp.	4	1.25
Entamoeba sp.	3	0.94
Giardia sp.	3	0.94
Balantidium sp.	2	0.62
Isospora sp.	2	0.62

Source: research data

The presence of developing forms of intestinal parasites that affect domestic animals in the samples of this study indicated that fecal contamination by domestic animals may represent a health risk, even if small, for consumers of this type of vegetable. An example of this is the presence of eggs of *Toxocara* sp., whose species *T. canis* and *T. cati* parasitize, dogs and cats respectively. Eggs of these parasites, when accidentally ingested, especially by children, may cause a syndrome known as visceral larva migrans, which has diverse clinical manifestations including chronic weakness, sleep disorders and behavioral to allergic manifestations (CARVALHO; ROCHA, 2014). Another example is *Giardia* sp. This group includes parasites of birds,

amphibians, mammals and reptiles, and *G. lamblia* is of great importance to public health, where it is also reported in domestic dogs (FANTINATTI *et al*., 2018). Traub and coworkers (2002) observed in faecal samples of dogs the presence of *Ascaris* sp., *Trichuris* sp., *Entamoeba* sp. and *Isospora belli***.** All these parasites can infect humans and were recorded in the samples analyzed in this study.

The contamination of lettuces by *Balantidium* sp. has already been recorded by Lima and coworkers (2018b). The infective forms of this parasite are commonly found in feces of infected pigs (NEVES, 2011). This reinforces the hypothesis of lettuce contamination by domestic animal feces.

The occurrence of *Toxoplasma gondii* oocysts in the present study should be emphasized because, although the level of contamination is low, i.e., only 2.19%, this parasite is one of the most widespread in human and animal populations, and severe forms of toxoplasmosis can be observed in neonates with congenital infections and immunocompromised individuals. Marchioro and coworkers (2016) described rates of *T. gondii* contamination in lettuce sold in the state of Paraná that were similar to those observed in the present study. In addition, they found that the management and the way of production of these vegetables (organic, hydroponic or traditional) seemed to have no great influence on contamination by *T. gondii*. Eating raw vegetables and fruits contaminated with oocysts of this parasite represents one of the most important infection pathways (PEREIRA *et al*., 2010).

Among the municipalities analyzed, Condor showed the highest rates of positive samples (27.4%), followed by Pejuçara (16.5%), Ajuricaba (14.3%), Catuípe (12.1%), Panambi 9%), Ijuí (8.8%), Bozano (7.7%) and Augusto Pestana (3.3%). With regard to the richness of taxa, Pejuçara showed positive samples for seven groups, followed by Ajuricaba and Condor, with five, Catuípe and Ijuí with four, Bozano and Panambi with three, and Augusto Pestana with only two (Table 2). In a study conducted by Nagel and coworkers (2017) also in northwestern Rio Grande do Sul, it was observed that 59.3% of school-age children were contaminated with enteroparasites or commensal agents, indicating that enteroparasitoses are a potential public health problem in this region.

Table 2 – Groups of parasites found in lettuce in eight sampled municipalities in northwestern Rio Grande do Sul, Brazil, between August 2015 and July 2016

Parasitas/ Municípios	**Ajuricaba**	**Augusto Pestana**	**Bozano**	**Catuípe**	**Condor**	**Ijuí**	**Panambi**	**Pejuçara**
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Ancylostomatidae	6 (46.1)	1 (33.3)	4 (57.1)	3 (27.3)	5 (20.0)	0 (0.0)	4 (44.4)	5 (33.4)
Toxocara sp.	2 (15.4)	2 (66.7)	2 (28.6)	2 (18.2)	5 (20.0)	5 (62.5)	2 (22.2)	2 (13.3)
Trichuris sp.	0 (0.0)	0 (0.0)	0 (0.0)	5 (45.4)	11 (44.0)	0 (0.0)	3 (33.3)	1 (6.7)
Toxoplasma gondii	2 (1.,4)	0 (0.0)	1 (14.3)	0 (0.0)	2 (8.0)	0 (0.0)	0 (0.0)	2 (13.3)
Ascaris sp.	2 (15.4)	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)
Entamoeba sp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	2 (13.3)
Giardia sp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)	1 (12.5)	0 (0.0)	0 (0.0)

Parasitas/ Municípios	Ajuricaba	Augusto Pestana	Bozano	Catuípe	Condor	Ijuí	Panambi	Pejuçara
Balantidium sp	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Isospora sp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)
Total	13 (100.0)	3 (100.0)	7 (100.0)	11 (100.0)	25 (100.0)	8 (100.0)	9 (100.0)	15 (100.0)

Source: research data

4 CONCLUSION

Lettuce sold in municipalities in the northwest region of Rio Grande do Sul showed relatively low parasite contamination. However, the Brazilian legislation states that only parasite-free vegetables are of adequate quality for consumption. Thus, there is a need for greater sanitary care to avoid fecal contamination of this type of food.

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Recebido em: **14 de Agosto de 2018**
Avaliado em: **1 de Setembro de 2018**
Aceito em: **3 de Outubro de 2018**

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