

Analysis of the presence of enteroparasites in *Lactuca sativa* (LETTUCE) sold at fairs and supermarkets in the municipality of Grajaú-Maranhão

Análise da presença de enteroparásitos em *Lactuca sativa* (ALFACE) vendida em feiras e supermercados do município de Grajaú-Maranhão

Analisis de la presencia de enteroparásitos en *Lactuca sativa* (LECHUGA) vendida en ferias y supermercados del municipio de Grajaú-Maranhão

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Abstract

Among the vegetables, lettuce (*Lactuca sativa*) stands out as one of the most popular leafy vegetables and consumed fresh. Due to the lack of structural and hygiene conditions, the commercialization of raw products in open markets causes greater exposure to contaminating pathogens. Thus, this study aimed to analyze the presence of enteroparasites in lettuce sold in supermarkets and open markets in the municipality of Grajaú-MA. Samples were collected at two fair stands and four supermarket shelves. The lettuces were separated into leaves, washed manually with distilled water and 5 drops of Tween 80 and left to stand for 3 hours. The resulting liquid was stored in conical cups for sedimentation for 24 hours. The sediment was separated and analyzed under an optical microscope. All investigated samples presented some type of enteroparasites. The results obtained indicated that all samples studied contained some parasitic structure, *Entamoeba coli* and the nematode eggs, for example, were present in all samples. In addition

to protozoa, *Ascaris* and *Trichuris* eggs, fungi, yeasts, dirt and other types of single-celled life were found on the observed slides. The most common intestinal parasites were protozoa such as *Giardia lamblia* (63.3%) nematode egg (27.3%), and *Entamoeba coli*. (23.8%). As lettuce (*L. sativa*) is eaten raw, it is necessary to apply a health education program for farmers to start reducing contamination rates. And for consumers there is a need to wash the leaves correctly before consuming them. Using the recommended hygiene standards, they can avoid ingesting enteroparasites.

Keywords: Enteroparasites; Vegetables; *Lactuca sativa*; Free fairs e Supermarkets.

Resumo

Dentre as hortaliças, a alface (*Lactuca sativa*) destaca-se como um dos vegetais folhosos mais populares e de consumo in natura. Devido à falta de condições estruturais e de higiene, a comercialização de produtos crus em feiras livres ocasiona uma maior exposição aos patógenos contaminantes. Desta forma, este estudo objetivou analisar a presença de enteroparasitas em alfaces comercializadas em supermercados e feira livre no município de Grajaú-MA. Foram coletadas amostras em duas bancas de feiras e em quatro prateleiras de supermercados. As alfaces foram separadas em folhas, lavadas manualmente com água destilada e 5 gotas de Tween 80 e deixadas em repouso por 3 horas. O líquido resultante foi acondicionado em cílices cônicos para sedimentação por 24 horas. O sedimento foi separado e analisado em microscópio óptico. Todas as amostras investigadas apresentaram algum tipo de enteroparasitas. Os resultados obtidos indicaram que todas as amostras estudadas continham alguma estrutura parasitária, a *Entamoeba coli* e os ovos dos nematoïdes, por exemplo, estavam presentes em todas as amostras. Além de protozoários, foram encontrados ovos de *Ascaris* e de *Trichuris*, fungos, leveduras, sujidades e outros tipos de vida unicelulares nas lâminas observadas. Os parasitas intestinais mais encontrados foram protozoários como a *Giardia lamblia* (63,3 %) ovo de nematoide (27,3%), e *Entamoeba coli*. (23,8%). Como a alface (*L. sativa*) é consumida crua, é necessária a aplicação de algum programa de educação sanitária para que os agricultores comecem a reduzir os índices de contaminação. E para os consumidores há necessidade de fazer uma lavagem correta das folhas antes de consumi-las. Com uso dos padrões de higienização recomendados, poderão evitar a ingestão de enteroparasitas.

Palavras-chave: Enteroparasitas; Hortaliças; *Lactuca sativa*; Feiras livres e Supermercados.

Resumen

Entre las hortalizas, la lechuga (*Lactuca sativa*) destaca como una de las hortalizas de hoja más populares y de consumo en fresco. Debido a la falta de condiciones estructurales e higiénicas, la comercialización de materias primas en mercados abiertos provoca una mayor exposición a patógenos contaminantes. Por lo tanto, este estudio tuvo como objetivo analizar la presencia de parásitos intestinales en lechugas vendidas en supermercados y mercados abiertos en la ciudad de Grajaú-MA. Las muestras se recolectaron en dos puestos de mercado y cuatro estantes de supermercados. Las lechugas se separaron en hojas, se lavaron manualmente con agua destilada y 5 gotas de Tween 80 y se dejaron reposar por 3 horas. El líquido resultante se colocó en copas cónicas para su sedimentación durante 24 horas. El sedimento fue separado y analizado bajo un microscopio óptico. Todas las muestras investigadas presentaban algún tipo de parásitos intestinales. Los resultados obtenidos indicaron que todas las muestras estudiadas contenían alguna estructura parasitaria, *Entamoeba coli* y huevos de nematodos, por ejemplo, estaban presentes en todas las muestras. Además de protozoos, huevos de *Ascaris* y *Trichuris*, se encontraron hongos, levaduras, suciedad y otra vida unicelular en las láminas observadas. Los parásitos intestinales más frecuentes fueron protozoos como Giardia lamblia (63,3%), huevo de nematodo (27,3%) y *Entamoeba coli*. (23,8%). Como la lechuga (*Lactuca sativa*) se consume cruda, es necesario implementar un programa de educación sanitaria para que los agricultores comiencen a reducir los índices de contaminación. Y para los consumidores existe la necesidad de lavar correctamente las hojas antes de consumirlas. Con el uso de las normas de higiene recomendadas, pueden evitar la ingestión de enteroparásitos.

Palabras clave: Enteroparásitos; Verdura; *Lactuca sativa*; Ferias abiertas e Supermercados

1. Introduction

Intestinal parasitic infections are a serious public health problem that globally affects more than a quarter of the world's population (Jourdan et al., 2018; Li et al., 2020; Rocha et al., 2021). In Brazil, a high prevalence of intestinal parasites is common, especially in regions of low socioeconomic status, and is closely linked to the lack of basic sanitation, inadequate personal hygiene and lack of care in food handling. (de Andrade et al., 2010; Rodrigues et al., 2020)

Lettuce (*Lactuca sativa*) is a herbaceous plant belonging to the Asteraceae family, originating from wild species of the Asian region and southern Europe. In 4500 A. C. it was already known in ancient Egypt and arrived in Brazil in the sixteenth century through the Portuguese (Duque et al., 2014).

In countries like Brazil, lettuce has great prominence in the diet of the population, being the sixth most economically

important vegetable and the eighth in volume produced by large and small producers (Duque et al., 2014). However, the ideal cultivation of this leafy vegetable requires some care, as far as hygienic and sanitary aspects are concerned. Because, they are considered sources of transmission of parasites, bringing serious consequences to the health of consumers when not properly sanitized (Duque et al., 2014).

The main form of contamination of these vegetables occurs, mainly, through the water contaminated by faecal material of human origin used in the irrigation of gardens, or even, by contamination of the soil by the use of organic fertilizer with fecal waste or by the irrigation of these with water from streams or contaminated by sewage (Saraiva et al., 2005)

Enteroparasitosis are intestinal infections caused by parasites, which can be helminths or protozoa. It is a disease that affects all age groups, being more prevalent in children (Iasbik et al., 2018). Most parasitic diseases are clinically asymptomatic, however, the most common symptoms of parasite infection are diarrhea, anemia, malnutrition and low weight, being opportunistic infections, taking advantage of the physiological state of the individual (Streck; Salvador, 2018).

This vegetable can be grown by the conventional, organic and hydroponic method, the first two being planted directly in the soil, in this way they are more exposed to contamination. Among the vegetables, lettuce (*L. sativa*) stands out as one of the most popular leafy vegetables fresh consumption. (Han et al., 2018).

Among vegetables, lettuce (*L. sativa*) stands out as one of the most popular leafy vegetables and fresh consumption (Han et al., 2018). This vegetable can be grown by the conventional, organic and hydroponic method, the first two being planted directly in the soil in this way, they are more exposed to contamination. (Drissner; Zuercher, 2014).

The demand for lettuce by the population has grown a lot, since changes in eating habits are visible in the modern context. Thus, the growing consumption of the vegetable can be justified by the fact that it is a food of easy acquisition and acceptable flavor for different people and cultures (Shinohara et al., 2014).

As vegetables are easily treated because they contain larger contact surfaces, the leaves juxtaposed to the surface and to the reflections that favor the accumulation of residues. In addition, as they are cultivated directly in contact with the soil cultivation method, this cultivation method contributes to the functioning of microorganisms. (Arbos et al., 2010; Tefera et al., 2018).

Due to the lack of structural and hygienic conditions, the commercialization of raw products in open markets causes greater exposure to contaminating pathogens. This can compromise food quality and put consumers' health at risk. However, supermarkets also have a prevalence of contamination of vegetables by parasites. (Esteves; Figueirôa, 2009; do Nascimento; Alencar, 2014).

Parasitic infections are common in developing countries such as Brazil due to limited access to health services and lack of basic sanitation. In addition to the low socioeconomic, educational and cultural level of the population, which decisively influence the health conditions of the population (Lima et al., 2013; Hotez; Fujiwara, 2014).

According to Oliveira (2014), parasitism is defined as the interaction between two species, one of which is unable to survive and reproduce independently of the other, seeking two fundamental aspects: obtaining food and protection, leading to metabolic dependence on the part of the animal. of the parasite, and with the host as the only one harmed (Barçante, 2008).

Among the main intestinal parasites transmitted by products consumed in natura, the following can be cited as helminthiasis: ascariasis, taeniasis and hookworm, and as protozooses: giardiasis and amoebiasis (Gregório et al., 2012).

According to Borges, Marciano and Oliveira (2011), the most frequent intestinal parasites in Brazil are the helminths *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms, and protozoa, such as *entamoeba coli* and *Giardia lamblia*.

Commonly, ascariasis presents as asymptomatic, although in some individuals it may manifest abdominal pain, diarrhea, nausea and anorexia. In cases with a high infestation of worms, it may worsen to an intestinal obstruction. During the larval pulmonary cycle, pulmonary manifestations such as dry cough, chest pain and fever may appear (Brasil, 2004).

According to Schall (2008), as well as other intestinal parasites, trichuriasis may or may not have symptoms. It is more likely that only those infected with hundreds of worms will experience diarrhea, accompanied by mucus or blood, bloating, weight loss, and nausea. In pediatric patients, it is possible to see worms attached to the mucosa of part of the externalized rectum.

Entamoeba coli is a non-pathogenic protozoan of the genus amoeba, therefore it does not harm its host. These protozoa are found in humans in the large intestinal tract where they form a commensal relationship. They are often confused with *Escherichia coli* bacteria due to the similarity between the names and cysts according to Haidar and Jesus (2021).

The protozoan *Giardia lamblia* (also known as *Giardia duodenalis* or *Giardia intestinalis*). If symptoms appear, the most common are diarrhea, abdominal cramps, flatulence, nausea and vomiting, weight loss and fever in a few cases. In the acute phase, there is a spontaneous improvement of symptoms, while in the chronic phase, symptoms appear: loose stools, steatorrhea, weight loss, tiredness and depression (Ali; Hill, 2003).

Given the above, the relevance of this study lies in the fact that vegetables, especially lettuce (*L. sativa*), are widely consumed by the population, and may contain different evolutionary forms of parasites, serving as an important route of transmission of intestinal parasites that are harmful to human health. Therefore, this research aims to analyze the presence of intestinal parasites in samples of *L. sativa* lettuce sold at fairs and supermarkets in Grajaú, Maranhão.

2. Methodology

Lettuce samples were collected in street markets and supermarkets located in Canoeiro and Rodoviário neighborhoods in the municipality of Grajaú, Maranhão. Lettuce samples were randomly obtained from two market stalls and four supermarket shelves, characterized by (A, B, C, D, E, F). Those responsible were consulted in advance and, having ensured confidentiality, they verbally consented to carry out the research. Six fresh curly lettuce plants of different weights and sizes were collected.

The lettuce samples were packed in clean plastic bags provided by the trade or producer. Then, they were transferred to sterile and sealed bags, without contact with the hands of the volunteers participating in the purchase of the product. They were tagged, identified, and taken to the Laboratory of Chemistry and Biology of the Federal University of Maranhão – Campus Grajaú. From each sample, ten slides were obtained, totaling sixty slides that were visualized under an optical microscope for observation and identification of the intestinal parasites found.

Lettuce processing and analysis were performed according to the adapted technique of spontaneous sedimentation by Hoffman, (1934), in which each lettuce was manually defoliated using procedure gloves. The lettuces were separated into leaves and placed in sterilized glass containers with 1 liter of filtered water and 5 drops of Tween 80, washed manually and left to rest for three hours. The liquid resulting from this washing underwent a sedimentation process in conical cups for 24 hours. The sediment was removed with the aid of a pipette (Pasteur) stained with Lugol and analyzed under an optical microscope, using a 10x and 40x.

For data analysis, the statistical program Bioestat version 5.3 was used. To verify the difference between the results found in fairs and supermarkets and to analyze the differences found in the frequencies between helminths and protozoa, the t test was applied, considering $p < 0.05$ as statistically significant. To indicate the precision of these estimates, the 95% confidence interval (CI) was calculated.

3. Results and Discussion

The analyzes of lettuce (*L. sativa*) sold in supermarkets and street markets in Grajaú-MA showed a certain type of enteroparasites in all analyzed samples, as shown in Table 1 and Table 2. Of the lettuce samples sold in supermarkets and street markets in the city from Grajaú-MA that were collected and analyzed, more than 99% were contaminated. It was found 99.78% of the samples from free fairs and 99.70% of the samples from supermarkets showed parasitic contaminants.

Table 1. Evaluation of samples of vegetables sold in supermarkets and street markets in Grajaú, Maranhão, Brazil.

	A	B	C	D	E	F
<i>Balantidium</i>	-	-	-	+	+	+
<i>Blastocystis Hominis</i>	+	-	-	-	-	-
<i>Protozoan cyst</i>	+	-	-	-	-	-
<i>Entamoeba coli</i>	+	+	+	+	+	+
<i>Giardia lamblia</i>	+	+	+	-	+	+
<i>Hymenolepis diminuta</i>	-	-	+	+	+	+
Helminth larvae	+	-	-	-	-	-
Nematode larvae	-	-	+	-	-	+
Ascaris egg	+	-	-	-	-	+
Nematode egg	+	+	+	+	+	+
<i>Tricuris</i>	+	-	-	-	+	-
Contaminants *	+	+	+	+	+	+
TOTAL	185	41	123	35	54	42

+ Contaminated/- non-contaminated. *Algae, free-living larvae, insects, mites. Source: Authors.

The data in Table 1 makes an evaluation of samples of vegetables sold in supermarkets and street markets in Grajaú, Maranhão, Brazil. and how we can observe the amount of microorganisms present in the studied foods. Therefore, these data reinforce the need for adequate hygiene to leave the lettuces in conditions of consumption.

(Oliveira et al., 2012). Santos et., al (2009) carried out a parasitological evaluation of vegetables sold in supermarkets and street markets in the city of Salvador-BA. The results found corroborate with this study, presenting an index of contamination, where the vegetables acquired in open fairs present a superior value in relation to the samples acquired in supermarkets. The lower frequency of parasites found in lettuces in supermarkets may be a consequence of cleaning that provided an improvement in the quality of hygiene during planting (Santos et al., 2009). The production of collective food, whether on a small or large scale, then becomes a necessity for the population (Alves et al., 2013).

Table 2. Quantitative result of contamination by enteroparasites from analyzes of lettuce sold in supermarkets and street markets in Grajaú, Maranhão, Brazil.

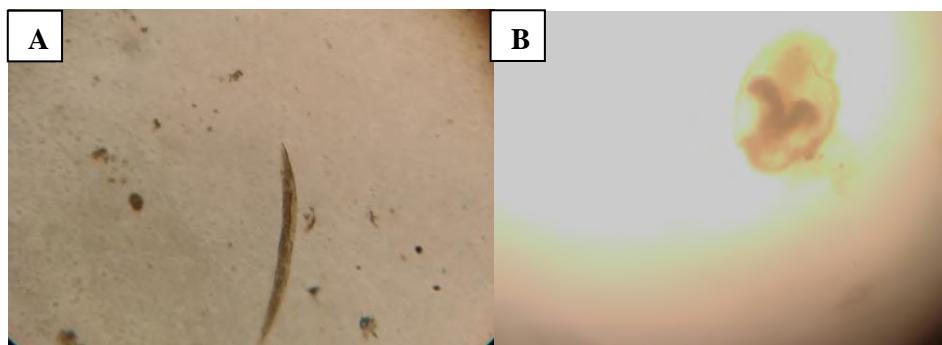
Sales Points	Lettuce TN*
Free fairs (A, C)	308
supermarkets (B, D, E, F)	172
Total	480

*TN (total number of protozoan cysts, eggs and helminth larvae). Source: Authors.

Table 2 shows the amount of contamination by enteroparasites from analyzes of lettuce sold in supermarkets and street markets in Grajaú, Maranhão. An important point to be observed in the table above is that the contamination in the samples sold in open markets has a higher number of contamination with 308 cysts found, and in the samples sold in the supermarket 109 cysts were identified.

It can be seen in Table 3 that *Giardia lamblia* presented the most expressive frequency (63.3%) followed by nematode egg (27.3%) and *Entamoeba coli* (23.8%). According to a study carried out in the interior of Ceará, the presence of pathogenic and non-genetic intestinal parasites was observed in the samples, with *Giardia lamblia* being prevalent in the population (14%) (Calegar, 2015). In the one carried out by Silva et al., evaluation in origin (2016) from supermarkets as more internal parasitic structures, were *Entamoeba coli* and *Giardia lamblia*. This is due to the lack of used basics and access to potable water. In addition to protozoa, helminths were found eggs of *Ascaris* and *Trichuris*, fungi, yeasts, dirt, and other types of unicellular life, in the observed blades of the vegetable. Figures 1A and 1B show *Giardia lamblia* cysts and nematode eggs found in the lettuce samples and in this study.

Figure 1. Images of the microorganisms found in the analyzed samples.



A: Giardia lamblia cyst B: Nematode larva. Source: Own authorship. Source: Authors.

Figure 1A shows cysts of *Giardia lamblia*, a protozoan capable of causing various diseases and Figure 1B shows nematode eggs, which can be ingested if food is not washed properly.

Entamoeba coli species and nematode eggs were found in all samples Mesquita et. al (1999) analyzing vegetables from Niterói and Rio de Janeiro, also found the presence of *Entamoeba coli* cysts in all their samples. He attributed the contamination of vegetables by feces of human origin. Well, this is a human intestinal protozoan, and probably its presence in food comes from failures in hygiene or through manipulation.

The results in Table 3 also show that the lack of hygiene and inadequate handling of vegetables in commercial outlets can act as a route of infection for consumers, because the presence of *Ascaris spp.* (7.14%) and *Trichuris* (1.1%) are fecal

contamination of human and/or animal origin. According to da Silva et. al (2014), the most frequent pathogenic intestinal parasites in the human body are helminths and protozoa, which in turn constitute a public health problem.

Table 3. Distribution of species of microorganisms found in the analysis of lettuce sold in supermarkets and street markets in Grajaú, Maranhão, Brazil.

Enteroparasitas	Free fairs (AF)	F%	Supermarkets (AF)	F%
<i>Balantidium</i>	-	-	21	12,2
<i>Blastocystis Hominis</i>	01	0,3	-	-
<i>Entamoeba coli</i>	43	13,9	41	23,8
<i>Cyst Giardia lamblia</i>	195	63,3	42	24,4
<i>Hymenolepis diminuta</i>	01	0,3	16	9,30
Helminth larvae	10	3,24	-	-
Nematode larvae	02	0,6	02	1,1
Ascaris egg	22	7,14	01	0,5
Nematode egg	33	10,7	47	27,3
<i>Tricuris</i>	01	0,3	02	1,1
	308	100%	172	100%
TOTAL	30,80 ^a (-11,89; 73,49)		17,20 ^b (3,29;31,10)	

*AF: Absolute Frequency. F% Relative frequency. The; b(p<0.05), CI (lower and upper confidence intervals). Source: Authors.

Table 3 shows the distribution of species found in lettuce samples sold in supermarkets and street markets in Grajaú. Therefore, it can be seen that the microorganism found in greater quantity was *Giardia lamblia* Cyst, especially those that are sold in open markets, thus showing that food handling at fairs is still precarious.

Several studies have shown in their results a high rate of contamination in lettuces by enteric parasites, such as protozoa and helminths. The wide flexible sheets, juxtaposed and compact structure present greater possibility of contamination. Because, during its cultivation, there is a greater contact with the polluted soil and water, consequently a greater adhesion of the parasitic evolutionary forms, giving greater resistance to the methods of hygiene (Mesquita et al., 1999; Falavigna et al., 2005; Santos e Merlini, 2010; Melo et al., 2011; Santarém et al., 2012; de Mesquita et al., 2015).

A study carried out in the city of Rio de Janeiro, in relation to the contamination of lettuce sold in open markets and restaurants, according to Pires et al. (2014) found that 70% of the samples submitted to analysis were contaminated by parasites, the result of which pointed to a greater number of contamination in in natura samples sold at retail, with the result of 90% of contamination by parasitic structures, while lettuce sold and 30% had parasite contamination from samples in restaurants.

Within this context, the importance of developing work stimulating awareness in food education, management and health of food and marketing is visible. The development of a critical posture as a consumer of these foods is also essential to achieve the production of safe foods, and fundamentally depends on investments in education. (Soares; Cantos, 2006).

The fact of contamination according to (Bekele et al., 2017), is justified by the high prevalence of contamination is related to the fact that the vegetable has multiple leaves, thus having a large contact surface, providing the fixation of the parasitic forms. In addition, the contact of this vegetable with the soil for a longer period of time, due to the form of cultivation, provides a greater chance of contamination.

According to Resolution nº 12, de 1978 of the National Commission of Norms and Standards for Food/ANVISA (BRASIL, 1978) which regulates the quality standards of vegetables, establish as unsuitable for consumption parasites those that present the presence of dirt, establishments and larvae. as samples in this study, all from supermarkets and tents of the free fair in Grajaú-MA, were unfeasible for human consumption, as they were at least monoparasitism.

Within this context, it is essential to carry out the hygiene of foods eaten raw in an appropriate place, washing the leaves of leafy vegetables, placing them in immersion in a hypochlorite solution for fifteen minutes. Immediately after rinsing and immersing in clean water or 2% vinegar for fifteen minutes. Then the residues are drained, and the supernatant is discarded (Silva JR., 2002). Thus, to reduce the probability of contamination, a viable alternative is to wash this vegetable with vinegar (ethanoic acid solution) or a small amount of sodium hypochlorite in a solution with water, being washed afterwards with potable water, these are methods that reduce the presence of contaminants in the vegetable (da Silva et al., 2016).

The use of the solutions mentioned above together with drinking water act effectively in the fight against enteroparasites, showing up as easily accessible options for adequate hygiene. The use of unfiltered tap water is not indicated for the correct washing of lettuces, as they are sources of intestinal parasites, favoring the infections caused by these agents. The exclusive use of tap water for washing vegetables is ineffective in removing enteroparasites (Al-binali, Ali M. et al., 2006), even because unfiltered water is a source of infection by intestinal parasites.

4. Final Considerations

In the present study it was found that the lettuce sold in the city of Grajaú-MA presented low hygienic standards due to the high percentage of parasitic contaminants. The most common intestinal parasites were protozoa such as *Giardia lamblia* (63.3%) and *Entamoeba coli* (23.8%), nematode eggs were also found (27.3%). As lettuce (*L. sativa*) is consumed raw, it is necessary to develop and implement programs about food, health education, cultivation and management of lettuce so that producers and consumers are aware of their role in reducing these contaminations through the quality consumption. It is worth emphasizing the need for action by the competent bodies in the process of raising awareness and involvement in the practice of public policies in the face of the identified problem.

The present research will serve as a guide for future studies, not only in loco, but in general with the objective of observing the rates of parasitic contamination and changes in the environment. Therefore, more research is needed on this topic to bring more information and awareness to the population.

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