



STUDY OF CONTAMINATION BY PARASITES IN LETTUCE (*LACTUCA SATIVA*) THE FREE FAIR AND THE MAIN ESTABLISHMENTS COMMERCIALS IN SANTANA DO IPANEMA – AL

*CONTAMINATION STUDY BY PARASITES IN LETTUCE (*LACTUCA SATIVA*) FROM THE OPEN MARKET AND MAIN COMMERCIAL ESTABLISHMENTS IN SANTANA FROM IPANEMA – AL*

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Summary

As it contains a considerable percentage of vitamins, minerals and dietary fiber, the intake of vegetables such as lettuce, especially *in natura* in salads it is very common, since healthy eating is increasingly encouraged and practiced, however, this uncooked intake makes vegetables an important vehicle for infection by various enteroparasites. This work aimed to analyze the occurrence of parasitic structures in lettuce from the street market and the main commercial establishments in Santana do Ipanema Alagoas. For this purpose, 23 samples were collected and subsequently sent to the Parasitology laboratory at the State University of Alagoas (UNEAL), to carry out the qualitative method of spontaneous sedimentation (HOFFMAN, PONS, JANER, 1934). In 35% of the samples analyzed, contamination by parasitic agents such as: *Strongyloides stercoralis*, *Giardia lamblia* and *Ancylostoma duodenale*. These results show that education combined with health is one of the main tools for reducing the risks of contamination, and can be worked on with the population, horticulturalists and in basic education. **Key words:** Enteroparasites; Vegetables; Infection.

Abstract

Because it has a considerable percentage of vitamins, minerals and dietary fiber, the intake of vegetables such as lettuce, especially *in natura* in salads, is very common, since healthy eating is increasingly encouraged and practiced, however this intake without cooking makes an important vehicle for infection by various enteroparasites. The objective of this work was to analyze the occurrence of parasitic structures in lettuce from the open market and the main commercial establishments of Santana do Ipanema Alagoas. For this, 23 samples were collected and later sent to the Parasitology Laboratory of the State University of Alagoas (UNEAL), to carry out the qualitative method of spontaneous sedimentation (HOFFMAN, PONS, JANER, 1934). In 35% of the samples analyzed, contamination by parasitic agents such as *Strongyloides stercoralis*, *Giardia lamblia* and *Ancylostoma duodenale* was found. These results show that education combined with health is one of the main tools for reducing the risk of contamination, and it can be worked with the population, horticulturalists and in basic education.

Keywords: Enteroparasites; Vegetables; Infection.

1. Introduction

According to Pinheiro, et al. (2010), one of the most important botanical families of leafy vegetables is Asteraceae, which includes lettuce (*Lactuca sativa*) in its different varieties of colors, leaves, shapes and sizes. Its use in human nutrition has occurred since 500 BC, originating from the Eastern Mediterranean, it is cultivated worldwide, being the most commercialized and consumed leafy vegetable in Brazil (SANTOS, 2014).

As it presents a considerable percentage of vitamins, minerals and dietary fiber, its intake mainly *in nature* in salads it is very common, since healthy eating is increasingly encouraged and practiced, however, this uncooked intake makes vegetables an important vehicle for infection by various enteroparasites (DUFLOTH et al. 2013).

Diseases that are transmitted by contaminated food are of great importance, in quantitative terms in nosological statistics, and tend to worsen in countries where basic sanitation is inefficient, and food health surveillance activities are not effective, thus people living in poverty or in extreme poverty, on the outskirts of cities they present greater vulnerability and susceptibility to being contaminated by parasitic diseases, as this is where there are favorable environments and conditions for transmission cycles to be always present, also taking into account the lack of knowledge about the importance of hygienic care practices (CASTRO, 2005).

According to data from the World Health Organization, enteroparasitoses have an extensive geographic distribution, which is reflected in the high rates of affected people, approximately 3.5 billion are infected by some type of helminth or protozoan that cause diseases in around 450 million, clinical manifestations vary and the degree of severity is closely associated with the parasitic load, which can range from intestinal obstruction, iron deficiency, malnutrition, to diarrhea and malabsorption of nutrients, thus constituting an important health problem public (CARDOSO et al. 2015; National Plan for Surveillance and Control of Enteroparasitosis, 2005).

However, even if there is a part of the population that is more vulnerable, the possibility of consuming contaminated lettuce is not limited to this part of the population, since from its origin in planting, to its final destination where consumers can purchase vegetables the possibilities of contamination are many, this

Therefore, it is extremely important to have knowledge of the correct ways to clean lettuce in order to reduce the risk of contamination (SANTOS, CABREIRA, 2011).

Washing with drinking water has a certain effectiveness, mainly eliminating macro-dirt, but the safer sanitization process requires the use of solutions that have proven efficiency against these types of pathogens, examples of which are sodium hypochlorite, which is distributed free of charge and is effective in eliminating microorganisms due to the oxidation it causes when it reacts with proteins present in the cell membrane, thus interfering with the transport of nutrients in the cells of pathogens, and the acetic acid found in alcohol vinegar which has bactericidal action since it has sporocidal properties, does not produce toxic residues, is low cost and easy to access, thus being considered an effective biocide (LIMA; SANTOS; WAUGHON; FIGUEIREDO, 2020).

Research that checks the levels of contamination of vegetables such as lettuce by enteroparasites or other pathogens are very relevant and must be carried out frequently, as with them it is possible to gain knowledge about the risks relating to food security in a region or locality, the In the same way, assessments of the knowledge of the population and horticulturists regarding hygienic habits are also important, making it possible to more easily and effectively develop public policies that seek to prevent parasitic diseases from becoming an even greater problem (LUDWING, FREI, FILHO, PAES, 1999).

Therefore, this work aimed to analyze the occurrence of parasitic structures in lettuce from the street market and main commercial establishments in Santana do Ipanema Alagoas, which is of great importance for public health, as it provides data on sanitary conditions, of vegetables consumed without cooking, such as lettuce, serving as a warning about the possible risks of infection by enteroparasites.

2 Theoretical Foundation

The Manual of Good Agricultural Practices in Lettuce Production (2014) reinforces the importance of food safety and addresses the microbiological risks that exist in the production of this vegetable. The recommendations address issues related to planting areas and the environment, such as soil health, care with biological waste and the existence of septic tanks, animal husbandry and possible contamination by leaching, infrastructure,

sanitary facilities so that people involved in the cultivation can clean themselves, as well as care with garbage collection and sanitary sewage, and another important guideline is with irrigation, advising that microbiological and parasitological analyzes of the water can periodically be carried out in order to know if contamination exceeds the levels allowed by legislation.

There are morphological characteristics in lettuces that make them more prone to contamination by microorganisms such as enteroparasites, the width of their leaves, the juxtaposition present, flexibility, as well as their compact structure mean that there is greater contact with the soil, favoring fixation of parasites and causing the need for greater care in hygiene processes (SCHEMES; SCHMES; RODRIGUES, 2015).

According to the National Plan for Surveillance and Control of Enteropathies (2005), enteroparasites cause a strong impact on public health, especially in developing countries and in regions that offer favorable conditions for the prevalence of parasites, reasons that make these pathogens one of the most frequently found in humans, and the recurrent finding of vegetables acting as an important source of infection shows that there are relevant aspects that result in this, the main ones being the form of cultivation, considering irrigation and the way the soil is fertilized, manipulation of vegetables both by horticulturists and also by marketers and other people who sell them, storage, and put an end to the way in which they are sanitized, or not, and how this process is carried out by those who will consume them.

Having a well-functioning and fully developed immunity as well as knowing and having hygienic habits are important factors to avoid possible enteroparasitic infections, therefore school-age children are the most vulnerable to being parasitized and developing more serious clinical manifestations, and physical development and academic performance can also be compromised, and even though over time there have been frequent discussions about this public health problem, there are no educational projects aimed at actions that aim to reduce students' risks of infections (MELO; FERRAZ, ALEIXO, 2010).

It is evident that the school has an extremely important social role, and the existence of Health Education in the school environment makes it possible to problematize enteroparasitosis and discussions on how this topic should be included in students' learning, being

It is important to highlight that teachers must be prepared to address the subject in question, because information and knowledge about enteroparasitosis, forms of contamination, and hygienic-sanitary measures can contribute to reducing this problem that does not receive much attention from public authorities (MONROE *et al.* 2013).

In this sense, public policies are decisive for the right to health to be ensured. Souza (2006) brings in his work several concepts that define what public policies are, which can then be a set of government actions with the aim of producing effects on certain problem in question, something that has an influence on the lives of citizens, or even analyzes and decisions that will make a difference and benefit the population, that is, when talking about intestinal parasites, it is known that there is a deficiency in actions that seek to minimize this problem in public health and the participation of the population has a strong contribution to changes that make measures to combat and/or control pathogens easier, so when talking about public policies it is necessary that the community is directly included in elaborations, participating in the creation of actions that will contribute to individual and collective health security.

The importance of parasitological diagnoses such as coproparasitological examinations provide important information, as they can identify which species of enteroparasites are present in the feces of infected people, mainly eggs and cysts of protozoa and helminth larvae, thus facilitating more specific diagnoses and treatments for infections, Furthermore, more effective prophylactic measures can be adopted since if analyzes are carried out on a considerable number of people and over a certain period of time, parasitological surveys can be obtained on the frequency of certain parasitic diseases in a location (MENEZES *et al.* 2013).

Knowing the various risks of contamination, it is in the fate of lettuces that there is the possibility of applying hygiene methods before ingestion, so that pathogenic microorganisms are eliminated or inactivated during these processes, thus ensuring that we can enjoy the nutritional qualities of this vegetable without our health is compromised. In studies by Oliveira (2005), lettuce sanitation protocols adopted in restaurants were verified, and the greatest reduction in aerobic mesophiles and total coliforms occurred through the use of sodium hypochlorite at 200ppm for 30 minutes, since the use of 2% alcohol vinegar, which is recommended, for 15 minutes,

showed lower effectiveness, its use in a higher concentration (20%) despite increasing its effectiveness showed that it causes damage to lettuce leaves.

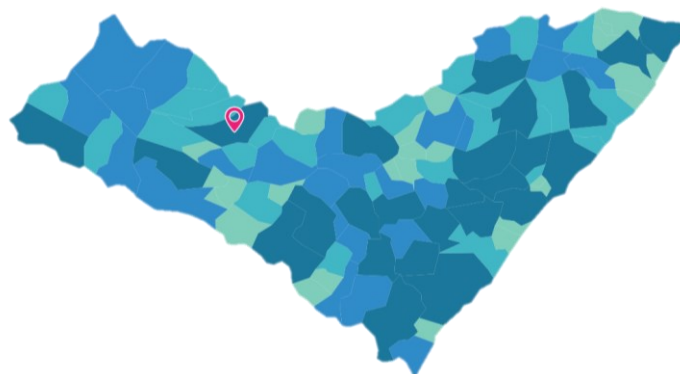
These results are in line with those found by Adami and Dutra (2011) who tested the use of vinegar as a sanitizer, comparing lettuces washed only in running water and others subjected to the use of vinegar, all samples showed high levels of contamination by total coliforms and feces, and although the results of use showed a reduction in contamination, this did not occur satisfactorily and was outside the standards defined by the National Health Surveillance Agency (ANVISA).

Jesus and Macedo (2014) artificially contaminated lettuce with parasite eggs *Toxocara canis* with the aim of evaluating the main sanitization methods, the use followed the guidelines of the manufacturer and the Ministry of Health, 4ml of sodium hypochlorite and 8ml of vinegar were used, the results showed once again that sodium hypochlorite has a greater effectiveness, in this case specifically in eliminating eggs from the *Toxocara canis*, There are several studies in the literature that verify and compare the performance of hygiene methods and it is seen that sodium hypochlorite is often the prophylactic method with the best results in eliminating microorganisms.

3 Methods

3.1 Study area

Figure 1 -Geographic location of Santana do Ipanema - AL



Source: <https://cidades.ibge.gov.br/brasil/al/santana-do-ipanema/panorama>

Santana do Ipanema is a municipality in the state of Alagoas, it is located in the backlands of Alagoas, 207 kilometers from the capital, according to a census carried out by the Brazilian Institute of Geography and Statistics (IBGE), 2018, its population was made up of 47,486 people, with a territorial area of 437,875 km², commerce, including street markets, are mainly responsible for driving the local economy, (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2018).

3.2 Data collection

In the present study, lettuce samples (*Lactuca sativa*), of the cressa and americana varieties, were collected at the street market and in the main commercial establishments in Santana do Ipanema (AL), thus seeking to note whether the level of contamination would vary according to the collection location, the way in which the samples were chosen was random, independent of weight and size, but the good quality of the samples was considered.

To obtain and transport the material to the laboratory, sterile plastic bags (properly closed and labeled) and recording sheets were used to note the origin, type of lettuce, among others. After that, they were sent to the parasitology laboratory at the State University of Alagoas (UNEAL), where the deteriorated leaves were defoliated and discarded, and the analyzes were carried out with the help of a clinical analysis technician.

Manually with the help of a flat brush Atlas n° 395, the vegetables were washed individually in a solution containing distilled water, to remove macro-dirt. Each cup received water from a sample, being labeled with a number, type of lettuce and collection location. The water resulting from washing underwent spontaneous sedimentation in conical cups for 24 hours.

Figure 2 -Spontaneous sedimentation in conical cups



Source:Authors, 2021.

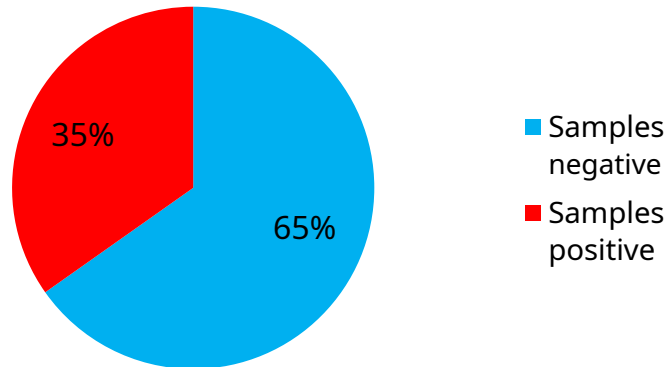
The product from washing was subjected to an adaptation of the qualitative method of spontaneous sedimentation, Hoffman, Pons, Janer, (1934), which basically consists of mixing feces with water, but in this case the water resulting from washing the lettuces was used, undergoing filtration through surgical gauze (or parasitofilter), placed on a small sieve and a cup, being kept at rest, forming a consistent sedimentation at the bottom of the cup, the reason for carrying out this method was due to its efficiency, low cost and practicality.

An aliquot of the sediment was pipetted onto a slide, smeared and observed under a microscope. This method detects the presence of eggs in the analyzed material, especially heavy ones, after staining with Lugol. Several variations are carried out in this technique in order to promote the visualization of more evolutionary forms. After 24 hours of sedimentation, protozoan cysts and helminth larvae can also be found more easily.

4 Results and Discussion

Of the 23 samples collected, eight of them were contaminated with one or more parasites, thus presenting a positivity shown in the following graph:

Figure 3 -Contamination of collected samples



Source: Authors 2021.

Strongyloides stercoralis was found in three of the five Supermarket samples A, in one of the five samples from Quitanda, in two of the eight samples from the street market, and in none of the five samples from Supermarket B, *Giardia lamblia* was found in two of the eight samples from the street market, and in none of the other samples from the other collection points, *Ancylostoma duodenale* was found in only two samples from the eight samples from the street market, as we can see in the tables below:

Table 1–Collection locations: Supermarket 1 and Supermarket 2, types of lettuce, quantities of positive samples, species and index of parasites and/or parasitic structures found.

Collect point		Collect point	
Supermarket A	Parasites found	Supermarket B	Parasites found
Sample 1	x	Sample 1	x
Sample 2	x	Sample 2	x
Sample 3	<i>Strongyloides stercoralis</i>	Sample 3	x
Sample 4	<i>Strongyloides stercoralis</i>	Sample 4	x
Sample 5	<i>Strongyloides stercoralis</i>	Sample 5	x
3 samples negative	60%	5 samples negative	x

x (negative sample).

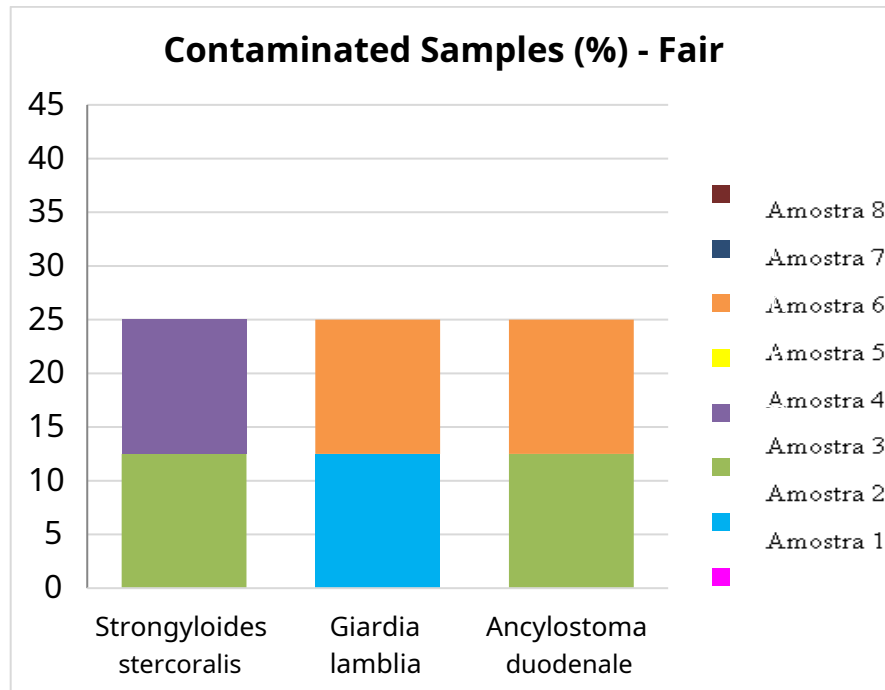
Table 2–Collection locations: Grocery store and street market, types of lettuce, sample quantities positive results, species and parasite index and/or parasite structure found.

Collect point		Collect point	
Greengrocer	Parasites found	Free market	Parasites found
Sample 1	x	Sample 1	x
Sample 2	x	Sample 2	<i>Giardia lamblia</i>
Sample 3	x	Sample 3	<i>Ancylostoma duodenale</i> + <i>Strongyloides stercoralis</i>
Sample 4	x	Sample 4	<i>Strongyloides stercoralis</i>
Sample 5	<i>Strongyloides stercoralis</i>	Sample 5	x
-		Sample 6	<i>Giardia lamblia</i> + <i>Ancylostoma duodenale</i>
-		Sample 7	x
-		Sample 8	x
1 positive sample	20%	4 samples positive	50%

- (absence of data), x (negative sample).

The graph below focuses on the street market, given the greater contamination among all collection points, demonstrating a positivity of more than one parasite per sample, reinforcing that hygienic-sanitary factors related to exposure, storage, handling and others, can be most worrying in street markets. Following the color scheme, it can be seen that *Ancylostoma duodenale* was found in **sample 3** (green) and **sample 6** (orange), *Giardia lamblia* in **sample 2** (blue) and **sample 6** (orange), and *Strongyloides stercoralis* in **sample 3** (green) and **sample 4** (lilac), all representing 25% positivity referring to 8 samples collected.

Figure 3:contamination of street market samples (%).



Source:authors 2021.

In general, *Strongyloides stercoralis*, was the most common parasite found with 60% of positive samples in Supermarket A, 25% in the street market and 20% in the grocery store, as was found by Constantin, Gelatti and Santos (2013), with 80% of positive results, and by Rocha, Mendes, Barbosa (2008), with 88.8% of samples collected in supermarkets and 100% of samples collected in street markets with high levels of contamination by this parasite, it is worth highlighting that the larvae found in this work, as well as in already mentioned, they were infective filarioid larvae. Infection by this helminth occurs through the penetration of larvae through the skin, that is, the handling of lettuce, by the population at the time of purchase, can be an important means by which infections occur, in the same way that traders who have contact with contaminated lettuce, and farmers in particular are at greater risk of being infected by larvae of this parasite, since these larvae also live in the soil.

Second, Rey (2010), *Giardia lamblia* It presents great genetic heterogeneity, despite its uniform morphology, with certain genotypes that infect both humans and other animals, such as dogs and cats. Thus, the existence of stray animals, both in the Santana do Ipanema open-air market and in the vegetable gardens, may correspond to a risk factor for

transmission of giardiasis, contaminating the food sold. This supports the idea that prophylaxis against these diseases also requires measures regarding animal health, given the zoonotic potential of some enteroparasites (ROSALES, MALHEIROS, 2017).

Positive samples for *Giardia*, represented a lower value, in a different context Luz et al. (2014), in the results of their work they found a high rate of contaminated samples, 80% of the samples were positive, it is worth highlighting that the number of samples analyzed directly influences the percentages of positivity, that is, the greater the number of samples collected for analysis, the more likely a greater number of positive samples will exist.

Other factors also influence these results, such as the climate where the study was carried out, which may be more or less conducive to the resistance and recurrence of parasites, and mainly the development of the city in relation to sanitary issues, such as basic sanitation, and educational character as knowledge on the part of the population regarding forms of contamination and how to avoid them.

The results of Constatin, Gelatti and Santos (2013) were higher, 30% of the samples were positive for *Ancylostoma duodenale*, and other parasites were also found, such as eggs of *Enterobius vermicularis* and *Hymenolepis nana*. The hypothesis of the use of animal manure may be an important element in the contamination of vegetables, for *Giardia* and other parasites, with the contamination of vegetables being common when they are irrigated, collected, transported, stored and at the points of sale, which may vary from place to place, and other factors already mentioned, such as the infrastructure of the cities where it was The study was carried out, taking into account hygienic-sanitary aspects, the way lettuce is grown and the population's level of knowledge about prophylaxis for parasitic diseases.

Hookworm is an infection transmitted by contact with contaminated soil, or by ingestion of larvae, and is caused by nematode parasites of the species *Necator americanus*. It is *Ancylostoma duodenale*. It is one of the most common forms of chronic infection in humans with an estimated 740 million cases, especially in rural areas and developing countries, according to the World Health Organization (WHO), (VALENTE, 2013).

Final considerations

This positivity reaffirms the risk and vulnerability that the population suffers from being contaminated with some kind of enteroparasite, since the attraction to the benefits that vegetables offer, such as lettuce, can result in pathologies arising from this consumption, mainly due to the fact lettuce is consumed without cooking, which makes it a frequent source of infection.

Education combined with health is one of the main tools for reducing the risks of contamination, and can be worked with the population that consumes and with horticulturists, addressing knowledge about enteroparasites, their pathologies, the different ways in which contaminations can occur, from its cultivation to its destination, and the correct and most effective ways to sanitize vegetables. Greater inclusion of parasitology focused on this issue in the basic education curriculum is also relevant, as well as encouraging research that checks the safety and contamination levels of foods that can act as sources of infection and other types of parasitological investigations, whether through universities, public authorities, or in partnerships between both.

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