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# OCCURRENCE OF PARASITIC STRUCTURES IN LETTUCE (Lactuca sativa) SAMPLES COMERCIALIZED IN THE OPEN MARKET OF SÃO MAMEDE, PARAIBA

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**ABSTRACT**

The consumption of raw vegetables can bring risks of food contamination by eating parasitic structures. Due to the lack of sanitary inspection in the marketing of vegetable sites, this study aimed to evaluate the occurrence of parasitic structures in lettuce samples (Lactuca sativa) traded on the open market of São Mamede - PB. It was randomly collected 25 samples of lettuce of all sales points located on the open market. The results showed a 100% positivity infection, and in all riding poly samples. The cysts, oocysts, and protozoa trophozoite presented prevalence (45.54%), and eggs and larvae of helminths (54.46%). Predominant species Entamoeba coli (80.0%); Giardia lamblia (52.0%); Ascaris lumbricoides (40.0%); Taenia spp. (32.0%) and Trichuris trichiura (32.0%) and a marked incidence of nematode larvae (72.0%). The inspection at the point of sale and implementation of socio-educational campaigns are essential to raise awareness of food producers and handlers on hygiene aspects.

## 1. INTRODUCTION

People socioeconomically disadvantaged, low level of education, with little access to information, housing, sanitation and inadequate hygiene constitute the population at risk, with more vulnerability to parasitic diseases.

Intestinal parasites are diseases caused by parasites. Its etiological agents are protozoa and/or Helminths (NEVES et al., 2010). They are present all over the world, committing mainly the poorest population in the developing countries (QUADROS et al., 2008).
The socioeconomic conditions of the population and the tropical climate of the country are favorable factors for the spread of disease (SOARES; CORNERS 2006) Brazil has a high prevalence of some protozoa, making these pathogens a serious public health problem (OLIVEIRA; GERMANO, 1992).

According to the World Health Organization (WHO, 1997), among the pathogens most commonly found in humans are enteroparasites. They can trigger some types of diseases that cause physical and mental discomfort to the hosts, associated with economic damages (FRANÇA; BONNAS; SILVA, 2014).

Contamination can occur by ingestion of contaminated water and/or food causing anemia, poor absorption of nutrients, diarrhea, weight loss, decreased ability to learn and work, and can cause delayed growth in children (QUADROS et al., 2008).

Studies on the parasitological profile of vegetables in different localities of the country have revealed alarming levels of contamination by parasitic structures present in these vegetables, classifying them as an important source of infection and spread of diseases. When they are contaminated, vegetables can present protozoan cysts and oocytes, larvae and helminth eggs, as well as pathogenic microorganisms, such as bacteria (BARUFFALDI et al., 1984).

Among the vegetables with the highest contamination rates, lettuce stands out. According to França; Bonnas; Silva (2014), they can present precarious hygienic conditions with regard to the whole productive chain, being able to have contamination in any stage.

The lettuce (Lactuca sativa) belongs to the family Asteraceae, is one of the vegetables most appreciated by Brazilians who seek healthy eating habits, possessing innumerable nutritional properties, acting as a source of vitamins, minerals and fibers, besides being low in calories and has a pleasant flavor, and is therefore suitable for all types of diets (RAMOS et al., 2014)

Man has developed the habit of consuming them raw, because when cooked some nutrients are lost due to temperature, and if they are not well-sanitized the lettuces can bring serious damages to the health of those who consume them, thus, it becomes dangerous to consume raw food, therefore, may present infectious agents of fecal origin and serve as a source of infection (ADAMI; DUTRA, 2011; PERES JUNIOR; GONTIJO; SILVA, 2012).

While there are many risk factors, which make them more likely lettuces are: the quality of water used for irrigation, fertilizers from fecal waste of human or animal origin, contaminated soil, inadequate handling and transport, measures that contribute to there of parasitic structures in plants (TAYAYANAGUE et al., 2001).

Among the various care that helps in the production of healthy vegetables, it is recommended to raise awareness as a great ally. Therefore, preventive measures such as basic sanitation, good quality water for irrigation and hygiene practices by producers, consumers and food handlers, are important care for the control of risk factors.

Studies on the prevalence of parasitic structures in vegetables are of great importance for local public health, since they serve as a prophylactic measure (COSTA, 2012).

Given the above, the objective of this study was to analyze the occurrence of parasitic structures in samples of lettuce (Lactuca sativa) traded on the open market of São Mamede, Paraíba.

## 2. MATERIAL AND METHODS

### 2.1 Search Location Characterization

São Mamede is a Brazilian municipality belonging to the state of Paraíba, is located in the region of Western Seridó in the center of

the state, in the mesorregion of Borborema and microregion of Patos. The municipality (Figure 1) extends through a territorial area of approximately 530,728 km, in the last sense its population was of approximately 7,748 inhabitants with a demographic density of 14,6 hab./km„ its climate is tropical semi-arid with summer rains and low rainfall (IBGE, 2010).

**Figure 1.** Map of the state of Paraíba, evidencing the city of São Mamede (Source: IBGE, 2010).

### 2.1 Collection and preparation of samples

For five weeks between the months of February and March 2016, weekly collections were held on Saturdays in the morning at 05 points of sale located in the open market of São Mamede, Paraíba, removing one sample unit of each bank, totaling 05 samples per week.

All the lettuces collected belong to the curly variety and were chosen at random, regardless of weight or size, and were stored in plastic bags of first use, duly closed, identified (Figure 3) and kept under refrigeration until the analyzes carried out in the laboratory of the Federal University of Campina Grande (UFCG) - Patos Campus.

**Figure 3.** Lettuces properly sealed and identified before the beginning of the analyzes (Source: Authors, 2016).

In the laboratory, the technique used was the spontaneous sedimentation method described by Hoffman; Pons; Janer (1934), where some arrangements and some modifications were made, adapting it to this work.

During the laboratory procedure, disposable gloves were used and all materials used were sanitized with distilled water. At first, the vegetables underwent a previous analysis, in order to get an idea about the physical quality of the material. The samples were individually defoliated and observed in an electronic magnifying glass; in this stage, it was possible to visualize nematode larvae, small insects and mites, structures that were present in many samples.

For washing and sedimentation were weighed 50 g of each sample on an analytical balance. These were cut into small pieces, transferred to conical chalices, each containing 200 mL of sodium chloride (NaCl 0.9%), and distilled water, leaving the samples submerged in this solution. After being homogenized, they were capped with plastic film and stood for 24 hours.

After this time, the material was filtered, using a nylon cloth covered by surgical gauze in four folds. Subsequently, the plant material was discarded, leaving only the liquid coming from the filtration of each sample. These were homogenized and transferred to test tubes, each containing 5 ml of the liquid of interest, all made in triplicate. The tubes were centrifuged at 3000 RPM for 5 minutes. After this procedure, the supernatant liquid was discarded leaving only the sediment of interest remaining. The sequences of the methodological steps are illustrated in Figure 4.

**Figure 4**. Methodological steps, carried out in the laboratory (Source: Authors, 2016).

With the help of graduated glass pipettes (5 mL), one drop of the resulting pellet of each sample was transferred to its respective glass slide, each from a test tube. These were covered with coverslip and analyzed under the optical microscope, in the 10x and 40x objective, making at the end of the research, a total of 75 slides, from 75 tubes and 25 samples. The results of the microscopic observations were analyzed using Microsoft Excel 2013 software.

## 3. RESULTS AND DISCUSSIONS

It was evidenced that all the samples were positive for the presence of some type of parasitic structure, all the slides observed presented more than one distinct structure, characterizing poly parasitism. The parasitic structures found in samples of lettuce collected at the open market of São Mamede, and the individual frequency are described in (Table 1).

**Table 1.** Absolute frequency and general percentage of the presence of the parasites evidenced in lettuce samples from the open market of São Mamede, PB.

| Parasites | Number of samples (+) | Frequency of samples(+) (%) |
|---|---|---|
| Cysts of Entamoeba coli | 20 | 80,0 |
| Larvae of nematódeos | 18 | 72,0 |
| Cysts of Giardia lamblia | 13 | 52,0 |
| Eggs of Ascaris lumbricoides | 10 | 40,0 |

| | | |
|---|---|---|
| Eggs of Taenia spp. | 8 | 32,0 |
| Eggs of Trichuris trichiura | 8 | 32,0 |
| Eggs of Dicrocoelium sp. | 7 | 25,0 |
| Cysts of Entamoeba histolytica | 6 | 24,0 |
| Cysts of Balantidium coli | 5 | 20,0 |
| Cysts of Endolimax nana | 3 | 12,0 |
| Eggs of Schistosoma mansoni | 3 | 12,0 |
| Eggs of Ancylostoma spp. | 2 | 8,0 |
| Eggs of Hymenolepis sp. | 2 | 8,0 |
| Eggs of Strongyloides stercorales | 2 | 8,0 |
| Oocysts of Cryptosporidium sp. | 2 | 8,0 |
| Oocysts of Isospora sp. | 1 | 4,0 |
| Eggs of Moniezia sp. | 1 | 4,0 |
| Trophozoite of Balantidium coli | 1 | 4,0 |

Source: Authors, 2016.

The most frequent parasite was E. coli (80.0%), which is a commensal microorganism considered to be non-pathogenic to humans. However, it is present in most studies on the quality of vegetables. Relevant fact, therefore, reflects the precariousness of sanitary conditions. For Baruffaldi et al., (1984), manure, stagnant water, mud and sewage, are favorable sites for the development of these microorganisms.

There was a great diversity of species in relation to the number of samples analyzed, besides, the occurrence of poly parasitism proves the poor quality of the vegetables coming from the open market of São Mamede. These results were similar to those obtained by Terto; Oliveira; Lima (2014), which showed 100% poly parasitism in samples sold at the open market in Serra Talhada-PE.

Of the 25 samples collected, E. coli cysts were present in 80.0% (20/25) of them. Other structures presented a high percentage of presence, such as: nematode larvae - 72.0% (18/25), G. lamblia cysts - 52.0% (13/25), A. lumbricoides eggs - 40.0 (10/25), T. trichiura and taenia spp. eggs presented the same percentage, both with - 32.0% (8/25), eggs of Dicrocoelium sp. - 28.0% (7/25), and E. histolytica cysts - 24.0% (6/25), as shown in Figure 5.

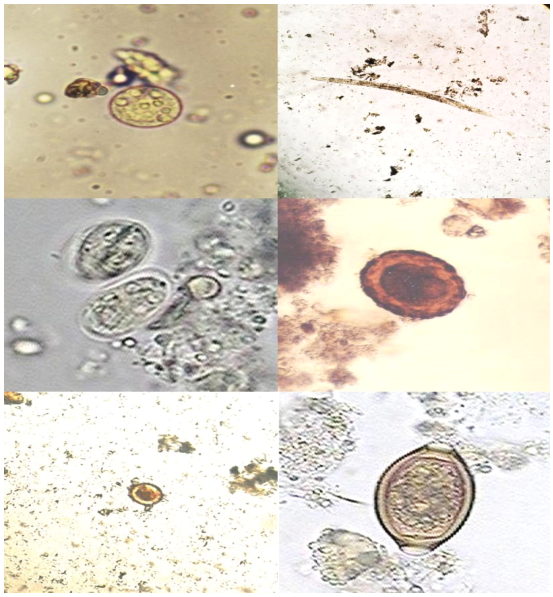

**Figure 5.** Parasitic structures with higher representativity in the analyzed lettuce, in descending order. E. coli cysts; nematode larvae; Cysts of G. lamblia; Egg of A. lumbricoides; Egg of Taenia spp .; Egg of T. trichiura (Source: http://www.ufrgs.br/parasite/siteantigo/alfabe.htm, Authors, 2016).

Through the morphology, the identification of contaminant structures was performed, however, this technique encounters difficulties in the differentiation of cysts, eggs and larvae of different parasitic species of man and animals, mainly in the differentiation of some free-living helminths (BARUFFALDI et al., 1984).

In addition to potentially parasitic structures, there were presence of fungi, caterpillars and arthropods, including some insects and mites (Figure 6), although the lettuces presented a green and healthy appearance, characteristics that attract the attention of consumers.

**Figure 6.** Some soils found in lettuces, such as: Spore fungus; Mite; Beetle and Ant (Source: Authors, 2016).

Terto; Oliveira; Lima (2014), believe that arthropods can act as a means of propagating diseases, acting as vectors that carry parasites to vegetables. In lettuces traded in Lavras - MG, the authors observed that arthropods were present in 34.2% of the samples, and emphasize the precarious hygienic conditions in these vegetables.

With respect to protozoa, the species E. coli - 80.0%, G. lamblia - 52.0%, and E. histolytica - 24.0%, were the most frequent. Similar results were obtained by Souto (2005) when he analyzed lettuce grown in Lagoa Seca - PB, differing as the order of the species of the genus Entamoeba, which showed the following percentage: E. histolytica (31.46%), followed by G. lamblia (23.59%) and E. coli (20.22%).

For Nolla; Cantos (2005), these parasites may indicate that there was contamination mainly through the hands of the manipulators and/or the water that was used for irrigation.

Besides Souto's research (2005), other authors such as Santos (2012), Oliveira; Germano (1992), and Osaki et al. (2010), showed a higher prevalence of the genus Entamoeba in their studies, carried out in the respective cities: Campina Grande - PB; Sao Paulo-SP; and Guarapuava - PR, agreeing with the results obtained in São Mamede - PB.

Although not considered pathogenic to humans, the high constancy of E. coli in most of the analyzed studies should be treated with significant relevance, since both E. coli and G. lamblia are parasites that serve as bioindicators of fecal contamination of animal and/or human origin (OLIVEIRA; GERMANO, 1992).

Infections with E. histolytica can be serious because this parasite is considered pathogenic to man and has a high incidence since contamination will mainly occur through water, food and dirty hands or even dust which is released from contaminated soils

(NEVES et al., 2010).

Other parasitic forms of protozoa were also recorded less frequently, and no less important than the others, among them cysts and trophozoites of B. coli, which have the pigs as the main hosts; oocysts of Cryptosporidium sp. parasites of domestic animals; cysts of E. nana parasitic on humans, but considered harmless; and oocyst of Isospora sp. found mainly in dogs, cats, cattle and pigs.

The presence of these microorganisms point to the low hygienic quality of the plants, since they are species of medical interest, since they can affect the health of man and animals.

Helminths were more prevalent in relation to protozoa; among the three major percentages are nematode larvae - 72.0%, A. lumbricoides eggs - 40.0%, Taenia spp. and T. trichiura, which presented the same percentage, both with - 32.0%.

It is observed that the nematode larvae were the most frequent among the helminths, agreeing with the results obtained by Terto; Oliveira; Lima (2009), which reported that 66.66% of larvae for lettuces from the open market, according to the authors, this kind of parasitic structure indicates the presence of contamination by fecal waste from the human and/or animal.

The large number of A. lumbricoides eggs found in this study indicates the absence of hygienic conditions during lettuce production. Since the eggs contained in the feces spread easily through the environment, either by dirty hands, fertilization with human feces or by habit of defecating on the ground, a common practice among rural dwellers (REY, 2011).

The presence of Taenia spp. eggs reinforces hygienic precariousness and indicates fecal waste contamination, caused mainly by the use of untreated water, since sewage effluents contain viable parasite forms, in rainy periods these can be taken to rivers, wells, and small reservoirs, waters that will later be used in the irrigation of the gardens, besides, the presence of these eggs shows the risks of contracting human cysticercosis, if they are T. solium (REY, 2011; SILVA, et al. 1995).

In places that are deprived of basic sanitation, sewage is "open", and faeces can reach water reservoirs, which can be used by man for domestic use and irrigation of crops (MORAES; LEITE; GOULART, 2008).

In the dry season horticulturalists need to intensify the irrigation of the gardens for the cultivation of their plantations and because the water resources are scarce and limited, many use untreated water from small reservoirs. The vegetables irrigated with these waters constitute sources of dissemination of enteroparasites.

The possibility of contamination occurred in the open market itself should not be discarded, as some marketers expose the vegetables without any hygienic care, under bags, wooden crates and in balaios, where the vegetables are exposed in the open air. Coutinho et al. (2005), believes that vegetables comercialized at the open market are susceptible to contact with insects and microorganisms present in the environment.

For Esteves; Figueiroa (2009), these conditions facilitate that resistant forms of some parasites that are there, come to contaminate the vegetables. Some helminths have eggs that are resistant to environmental conditions, such as eggs of A. lumbricoides, T. trichiura, and Taenia spp., which were highly representative in the current research.

Among the contaminants found in S. Mamede lettuce, they were observed many species of parasites of humans and animals. However, there were two helminths of veterinary medical interest that were not evidenced in previous research, they are: Dicrocoelium sp. and Moniezia sp. The presence of these parasites can be attributed to the use of animal manure used in gardening.
Among the less common helminths are: Moniezia sp, found in a sample, Ancylostoma spp, Hymenolepis sp.; S. stercorales, found in two samples each species; and S. mansoni eggs in three samples. However, Dicrocoelium sp. had a considerable representativeness, appearing in seven samples. Although at a low level, the presence of these parasites is relevant to public health, because their importance lies in the risks of disease transmission, since all can pose risks to the health of humans or animals.

Many researches on the study of the contamination by parasites in vegetables were carried out, among them: França; Bonnas; Silva (2014); Coutinho et al. (2014); Pires et al. (2014), report high levels of contamination, which indicate the need for special attention by health surveillance agencies.

The high percentage of parasite contamination evidenced in the present study, corroborates with the results obtained by França; Bonnas; Silva (2014), presenting a greater diversity of parasites. When carrying out studies on the quality of lettuce from open markets, they found that of the 96 collected samples all contained some type of enteroparasitic form, a percentage equivalent to 100% of contamination.

When analyzing lettuce from different commercial establishments, in Lavras - MG, Guimarães et al. (2003) also found that all samples (n = 120) contained some possibly contaminant structure. In São Miguel do Oeste, Landivar; Vidigal (2015), obtained a great variety of parasitic types, resembling the data obtained in São Mamede. For them, lettuces can carry different types of parasitic structures.

According to Guimarães et al. (2003), studies carried out in Brazil on the quality of vegetables, evidenced a high degree of fecal contamination, it is believed that the water used for irrigation is directly related to the contamination of the vegetable gardens.

For Coelho et al. (2001), besides the water quality, the type of fertilizer used and the way they are transported and handled, are among the several factors that influence the contamination.

Oliveira; Germano (1992), when studying different types of vegetables (lettuces, curly and smooth varieties, watercress, and endive) concluded that the physical structure of each plant type influences the degree of contamination of these plants.

Shinohara et al. (2014) observed that the veins present in lettuce leaves facilitate the permanence of microorganisms and reinforces that they can remain in the vegetables if a correct hygiene is not carried out.

Lettuces, because they have flexible leaves, may have direct contact with the soil during cultivation, and because they are imbricated, juxtaposed with irregular and curly surfaces, facilitate soil permanence (SILVA; ANDRADE; STANFORD, 2005).

In some samples analyzed in this research, the presence of sand particles and manure residues from animal feces used in fertilization was notorious. The high degree of soiling may be related to faults during the washing process performed by the producers after harvest (COUTINHO et al., 2015).

The National Health Surveillance Agency (ANVISA) establishes that when analyzed microscopically, vegetables should be free of soil, parasites and larvae (BRASIL, 1978).

In this way, the sources of infection are broad, including contamination of the soil with fecal material, use of fertilizer from animal or human feces, use of contaminated water for irrigation to wash the vegetables, the way are transported and conditioned at the point of sale, by the hands of manipulators and even by the contact with vector animals (COELHO et al., 2001; QUADROS et al., 2008).

In view of the seriousness of the facts reported in the scientific literature, it is noticed that the papers seek to expose the severity of the problem, as well as the importance of evaluating the frequency

of parasitic structures in lettuces, since such procedures can be considered as control measures.

The laboratory analysis of parasite structures in lettuce is necessary because it allows to monitor and to know the hygienic-sanitary conditions involved in the production process of the vegetables, besides identifying the risks of contamination of the consumers, it makes possible the provision of data to the health organs public health surveillance and sanitation so that they can develop efficient means of control (SILVA et al., 2005).

However, it is important that the population is informed about how to proceed with the hygiene of vegetables. According to Oliveira; Germano (1992), disinfection of vegetables before consumption should be done, so that there is a lower probability of some type of food contamination occurring, since simple washing does not reduce the chances of contamination by cysts.

It is necessary to use products that may reduce the risk of infection, sanitizers such as sodium hypochlorite 100-200 ppm (mg/mL) for fifteen minutes, are effective to kill the infectious agents present, followed by rinsing in treated water before consumption (JÚNIOR, 1996 Apud OSAKI, et al., 2010).

Another way would be to immerse the leaves in warm water at 60ş for ten minutes, it is an easy technique and considered effective to eliminate protozoal cysts and helminth eggs. However, although it is an effective method, it is not usually frequent, because, due to temperature, some nutritional properties can be lost, since lettuce leaves are totally sensitive to heat (OLIVEIRA; GERMANO, 1992).

## 4. CONCLUSIONS

The evaluation of the parasitological contamination of lettuces was efficient, since it allowed to know the high frequency and diversity of parasitic structures present in these vegetables, with poly parasitism evidenced in all the observations, and prevalence of helminths, being: E. coli, larvae of nematodes, G. lamblia, A. lumbricoides, Taenia spp. and T. trichiura, the most frequent contaminants. Data showing the low hygienic conditions of lettuce and indicate possible failures throughout the production chain, as well as in the control and monitoring by local health surveillance.

Therefore, according to current Brazilian legislation, the lettuces commercialized at the open market of São Mamede - PB are of poor quality due to their low sanitary and hygienic standards, reinforcing the need for strict inspection at points of sale, production and adoption of socio-educational measures with producers and food handlers.

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