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Enteroparasites in vegetables marketed in an ancient Brazilian city

Enteroparásitos en verduras comercializadas en una antigua ciudad de Brasil

Fabiana Mendonça Ambrozim, Josiane Pezzin, Débora Barreto Teresa Gradella and Marco Antônio Andrade de Souza

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FA: Pharmaceutical Chemist. Laboratório de Parasitologia e Hematologia, Universidade Federal do Espírito Santo. São Mateus, Brazil. *fabianavni@msn.com*
JP: Pharmaceutical Chemist. Secretaria do Estado da Saúde do Espírito Santo, Farmácia Cidadã Estadual de São Mateus. São Mateus, Brazil. *josianepezzin@gmail.com*
DG: Pharmaceutical Chemist. Professor. Departamento de Ciências da Saúde, Universidade Federal do Espírito Santo. São Mateus, Brazil. *teresadb14@gmail.com*
MS: Pharmaceutical Chemist. Professor. Departamento de Ciências da Saúde, Universidade Federal do Espírito Santo. São Mateus, Brasil. *marco.souza@ufes.br*

ABSTRACT

Objective To assess contamination by enteroparasites in raw vegetables consumed in the city of São Mateus, Espírito Santo, Brazil.
Material and Methods Samples of lettuce *(Lactuca sativa)* and parsley *(Petrosolium sativum)* were collected from six popular establishments, such as supermarkets and street markets. The obtained material was analyzed, after washing, by the spontaneous sedimentation method in the Clinical Analysis Laboratory of the Federal University of Espírito Santo, São Mateus Campus.
Results From a total of 120 analyzed samples, 71 (59 %) had one or more parasitic contaminants. Lettuce samples had a contamination rate of 78.3 %, and parsley samples of 40 %.
Conclusions The results show that the vegetables consumed in the city had protozoa, helminths and arthropods, which points to the need for implementing hygienic-sanitary measures in production, handling and transportation of these vegetables.

Key Words: Parasitic diseases; vegetables; food contamination *(source: MeSH, NLM).*

RESUMEN

Objetivo Evaluar la contaminación por enteroparásitos de verduras consumidas crudas, vendidas en la ciudad de São Mateus, Espírito Santo, Brasil.
Materiales y Métodos Se recolectaron muestras de lechuga *(Lactuca sativa)* y perejil *(Petrosolium sativum)* de seis establecimientos populares, como supermercados y mercados callejeros y se analizó el material obtenido, luego del lavado, mediante el método de sedimentación espontánea en el Laboratorio de Análisis Clínicos de la Universidad Federal de Espírito Santo, campus de São Mateus.
Resultados De un total de 120 muestras analizadas, 71 (59 %) tenían uno o más contaminantes parasitarios. Las muestras de lechuga tenían una tasa de contaminación del 78,3 % y muestras de perejil, 40 %.
Conclusión Los resultados muestran que las verduras consumidas en la ciudad tenían protozoos, helmintos y artrópodos, lo que indica la necesidad de adoptar medidas higiénico-sanitarias en la producción, manejo y transporte de estos vegetales.

Palabras Clave: Enfermedades parasitarias; verduras; contaminación de alimentos *(fuente: DeCS BIREME).*

Disease transmission and maintenance among humans is the result of interactive processes between the agent, the environment and the human host, known as the epidemiological triad (1). An agent is a factor whose presence is essential for the onset of the disease; a host organism is susceptible to infection by an agent, and the environment is the set of factors that interact with the agent. In this context, ente-

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roparasites appear as a serious public health issue, which has a wide geographical distribution (1-3).

Human contamination by intestinal parasites occurs in both rural and urban areas of developing countries, mainly due to poor sanitary, hygiene and food conditions (4-7). Food security has gained growing importance around the world, given the high frequency and severity of Foodborne Diseases (FBD), which represent a major public health concern (8-10).

In general, the transmission of enteroparasites occurs orally, through the ingestion of water or food contaminated by parasitic structures, being more prevalent in areas where hygienic-sanitary conditions are not adequate, especially because of the poor treatment of water and sewage (11,12).

The consumption of fresh vegetables underscores the importance of monitoring these foods, since intestinal parasites are often found in both rural and urban areas of Brazil due to poor sanitary conditions (13). Controlling the presence of enteroparasites in vegetables is, therefore, of great interest to public health, since it provides data for health surveillance on the hygienic condition of these products and allows the retrospective control of the conditions in which they are produced (14).

With this in mind, lettuce (*Lactuca sativa*), which is grown in all regions of the country and is very accessible to all economic classes (15), and parsley (*Petrosolium sativum*), a condiment used to flavor various dishes, stand out as some of the most common products of the Brazilian cuisine (16). These vegetables are recommended by doctors and nutritionists as an essential part of a healthy diet due to their vitamin, mineral and dietary fiber content (17).

Many parasites may be present as contaminants in vegetables and, depending on their characteristics, they may or may not pose a threat to the health of consumers of this type of food without proper hygiene. Said parasites include: *Giardia lamblia*, *Entamoeba histolytica/dispar*, *Hymenolepis nana*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Fasciola hepatica*, *Enterobius vermicularis*, *Trichuris trichiura*, hookworms and, not least important, commensal parasites, such as *Entamoeba coli* and *Endolimax nana*, great indicators of the hygiene conditions of the population (18).

Therefore, considering the importance of monitoring the incidence of enteroparasites in lettuce and parsley sold in São Mateus, the assessment of the quality of these vegetables offered to the population was proposed. Data provided to the Municipal Sanitary Surveillance on the hygienic-sanitary conditions of these products will contribute to improve the quality of the vegetables consumed by the population.

MATERIAL AND METHODS

Study area

This study was conducted in the municipality of São Mateus, northern region of the state of Espírito Santo, 220km from the capital, Vitória (19). According to the Instituto Brasileiro de Geografia e Estatística (IBGE by its acronym in Portuguese) (20), the town has an estimated population of 124 575 people.

Sample Collection

A sample unit was defined as 50g of leaves obtained from a "head" of crisp lettuce (*Lactuca sativa*) (11), and 8g of leaves obtained from a "bunch" of parsley (*Petrosolium sativum*), regardless of the weight or size of the "head" or "bunch". A total of 120 samples were analyzed.

Each sample unit was selected randomly, as they were accessible to consumers in the stores where they were purchased.

Collection procedure

All samples (120) were collected from six popular commercial establishments in São Mateus, referred to in this study as 1, 2, 3, 4, 5 and 6. Samples 1 and 6 were characterized as street markets and the remaining as supermarkets. 10 lettuce samples and 10 parsley samples were collected from each establishment.

In both supermarkets and street markets, the samples, once selected, were immediately wrapped (individually) in first-use plastic containers and sent to the Clinical Analysis Laboratory of the Federal University of Espírito Santo, São Mateus Campus, for processing and analysis.

Sample Analysis

In the laboratory, about 50g of leaves suitable for consumption were extracted from each lettuce "head" (11), disregarding burnt and damaged leaves, as well as the stem. For parsley, 8g of leaves from each "bunch" were extracted, also disregarding the parts inappropriate for consumption.

After defoliation and weighing, the sample unit was transferred to a plastic tube, where it was washed with a volume of distilled water of 200 mL for parsley and 400 mL for lettuce. During washing, the leaves were rubbed with the fingers, using latex gloves, and then suspended and agitated so that the liquid present on the surface was drained as deep as possible into the tube. After the procedure, the leaves were discarded and, for each new sample, a new pair of gloves was used (5).

Subsequently, the solutions were filtered to a 200-mL conical cup, through surgical gauze folded in four folds. After filtration, the liquid was kept at rest for a period of

24 hours, according to the spontaneous sedimentation method (21). Subsequently, the obtained precipitate was collected and transferred to 50-mL centrifuge tubes and then centrifuged at 4 000 rpm for one minute. After centrifugation, the obtained precipitate was collected by depositing a drop on a glass slide. It was stained with lugol; the use of cover slips was optional. At least three slides of each sample were examined under a light microscope with objective lenses of 10x and/or 40x.

Statistical Analysis

The chi-square test was performed for categorical variables using the GraphPad InStat 3.05 software. The significance level was $p<0.05$.

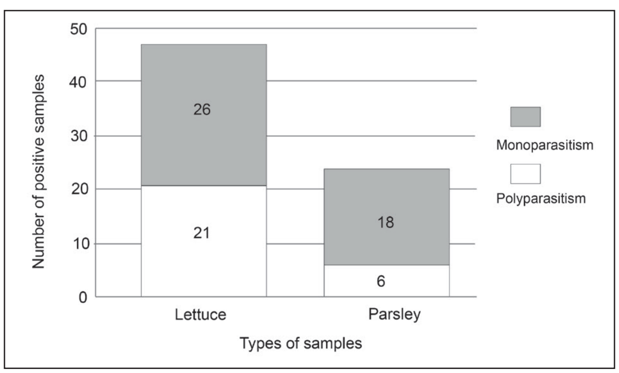
RESULTS

From a total of 120 analyzed samples, 71 (59 %) had one or more parasitic contaminants, and 49 (41 %) were negative. From this total, 60 samples corresponded to lettuce, and 47 (78.3 %) were positive. Among the 47 positive samples, 26 (43.3 %) had polyparasitism (Figure 1).

In relation to the 60 parsley samples, 24 (40 %) showed positive results. From the total positive samples, 6 (10 %) had more than one parasite per sample (Figure 1).

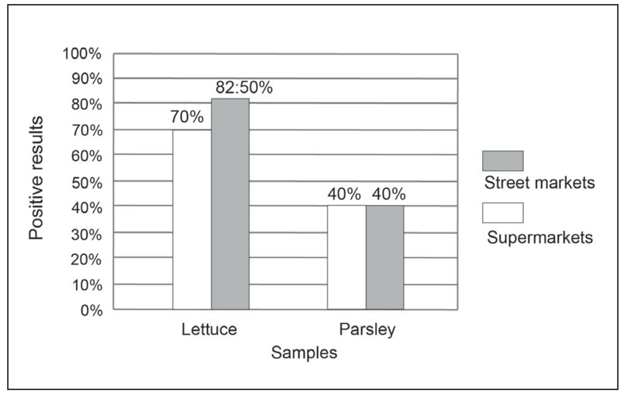
There was no statistically significant difference between the results of the presence of parasites in lettuce and parsley. The contamination rate was higher in lettuce than in parsley ($p<0.0001$), as well as polyparasitism ($p=0.0295$).

Figure 1. Parasitism frequency in lettuce and parsley samples from the São Mateus municipality, Espírito Santo State, Brazil



When collection facilities were evaluated, it was observed that, in street markets, 14 (70 %) of 20 lettuce samples and 8 (40 %) of 20 parsley samples were positive. On the other hand, lettuce and parsley samples from supermarkets showed 33 (82.5 %) and 16 (40 %) positive samples, respectively, from a total of 40 analyzed samples (Figure 2). There was no significant difference between establishments and lettuce and parsley samples ($p=0.9726$).

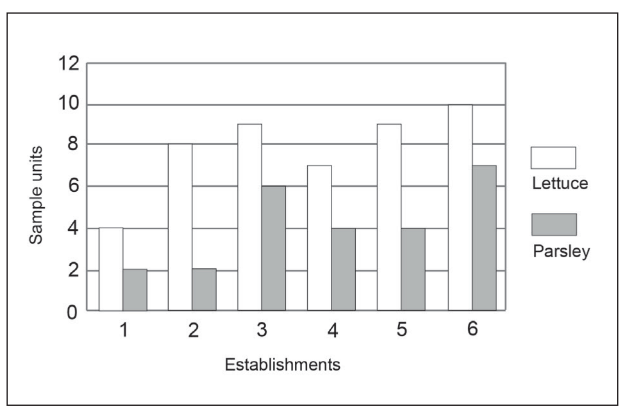
Figure 2. Frequency of parasitism in lettuce and parsley samples by type of commercial establishments from the São Mateus municipality, Espírito Santo State, Brazil



When the results obtained in each of the evaluated establishments (Figure 3) were compared, it was observed that establishment 6 (street market) showed the highest positive rates for both parsley and lettuce. The second establishment with the largest number of positive samples for both vegetables was number 3 (supermarket), followed by number 5 which, despite having the same number of contaminated parsley samples as establishment 4, presented a higher total contamination due to the large amount of lettuce samples with positive results. Establishment 2 showed a frequency of positive results four times higher for lettuce when compared to parsley, and establishment 1 (street market) had the lowest contamination rates. Although the number of contaminated parsley samples was the same as in establishment 2; positive results for lettuce were two times lower.

When considering each establishment separately, a statistically significant difference was found in relation to positive or negative samples ($p=0.0073$). It was observed that establishment 1 had the lowest frequency of contaminated samples when compared to other establishments.

Figure 3. Number of contaminated samples of lettuce and parsley from commercial establishments in the municipality of São Mateus, Espírito Santo State, Brazil



Regarding parasitic contaminants in lettuce samples, it was observed that *Ancylostomatidae* larvae (61.7 %) were the most frequent, followed by *F. hepatica* eggs (41.7 %) and mites (11.7 %). Other parasites were also found in lower amounts (Table 1).

Table 1. Relationship between parasites found in lettuce (*Lactuca sativa*) samples, number of positive samples and frequency of contamination in the city of São Mateus, ES

Parasites	Nº of positive samples	%
Ancylostomatidae	37	61.7
Ascaris lumbricoides	1	5.0
Dipylidium caninum	1	1.7
Enterobius vermicularis	1	1.7
Fasciola hepatica	25	41.7
Hymenolepis nana	1	1.7
Oxyurida	3	5.0
Strongyloides stercoralis	1	3.3
Toxocara cati	1	3.3
Toxocara vitulorum	1	1.7
Trichuris sp.	2	1.7
Balantidium coli	1	1.7
Entamoeba coli	3	5.0
Entamoeba histolytica/dispar	1	1.7
Giardia lamblia	1	1.7
Mites	7	11.7

For parsley samples, nine different parasitic structures were identified and parasites of the family *Ancylostomatidae* were the most frequent (35 %), followed by mites and *F. hepatica* eggs, both 5 % positive. The other structures had a frequency of 3.3 % and 1.7 % (Table 2).

Table 2. Correlation between parasites found in parsley (*Petrosolium sativum*) samples, number of positive samples and frequency of contamination in the city of São Mateus, ES

Parasites	No. of positive samples	%
Ancylostomatidae	21	35.0
Ascaris sp.	1	1.7
Fasciola hepatica	3	5.0
Hymenolepis sp.	1	1.7
Oxyurida	1	1.7
Strongylida	2	3.3
Cystoisospora sp.	1	1.7
Entamoeba coli	1	1.7
Mites	3	5.0

DISCUSSION

Parasitic infection rates in a given region are frequently deemed as indicators of socioeconomic development. In order to attempt to identify possible means of transmission and frequency of enteroparasites in vegetables consumed by the population of the municipality of São Mateus, a central city of the northern region of the state, 120 samples were analyzed (60 lettuce "heads" and 60 parsley "bunches"), which yielded 78.3 % and 43.3 % positive results, respectively. These results are similar to those found in studies conducted in supermarkets and street markets of Recife, where the index of positive samples reflects the importance of the transmission of parasitosis to people through food (5).

Similarly, Vollkopf *et al.* (2) studied the occurrence of enteroparasites in lettuce sold in Porto Murtinho-MS, and obtained positive results for 91.5 % of the samples, which suggests poor sanitary conditions in the trade of that food. The same can be observed with the results obtained in a study conducted in the city of Cuiabá (22), where 66.7 % of crisp lettuce samples had some kind of parasitological contamination.

Furthermore, Guilherme *et al.* (23) and Mesquita *et al.* (24) studied vegetables in a street market from a producer in Maringá, Paraná, and supermarkets, street markets and grocery stores in the cities of Niterói and Rio de Janeiro. They obtained contamination rates in lettuce of 23.2 % and 3.9 %, respectively, which is considerably better when compared to the results observed in São Mateus, probably due to better growing conditions and manipulation.

When comparing rates of vegetable contamination, it was observed that the index of positive samples for parasites and parasitic species in lettuce in São Mateus was higher than for parsley, as reported in studies conducted in the states of Paraná and São Paulo (14,23). Since lettuce has large leaves with a much higher contact surface than parsley, its physical structure can interfere with contamination rates. (14) Another factor to be borne in mind is that parsley is considered a repellent plant and can ward off insects and other animals, including nematode larvae (25).

In relation to contamination rates of samples from supermarkets and street markets, there was no significant difference among establishments (p=0.9726). Nevertheless, studies conducted in supermarkets of Cuiabá, Mato Grosso state (22), reported that the contamination process of vegetables probably occurs due to poor hygienic-sanitary conditions during the production chain and marketing. The same was observed by Takayanagui *et al.* (26), who reported that the contamination of vegetables may occur in the vegetable garden itself, as a result of inadequate irrigation water or fertilizers, during transport or handling at points of sale, since successive handling increases the chances of contamination.

An enteroparasites frequency of 78.3 % in lettuce and 43.3 % in parsley in São Mateus is worrisome, taking into account the observed parasitic species, as well as their amount. Among the parasites observed in this study, four helminth eggs of the genus *Ascaris* stand out, one of which was identified as *A. lumbricoides*. It is known that the eggs of this parasite have a great capacity of adherence to surfaces (27) and, therefore, it is possible to admit the possibility that more eggs, which were not removed during the washing process, could be found in the vegetables.

Other enteroparasites found in the study were *Ancylostomatidae*, whose contamination levels in the samples reached 61.7 %. It is noteworthy that most of the findings were represented by larvae, and that hookworm infection in humans occurs only when L_3 larvae (infective or filarioid) penetrate actively through the skin, or passively through the mouth (18). Although the larvae found were not differentiated in relation to their stage, the fact that they are present in large amounts and frequenty still alive suggests that ingestion, or even the simple handling of these vegetables, can lead to contamination of the host and to the development of hookworm disease (18).

It is also important to note that *F. hepatica* eggs, although not directly infectious to man since the transmission of fascioliasis occurs through the ingestion of water and vegetables contaminated with metacercariae (18), show contamination by ruminant feces, probably used in fertilization (14). Similarly, *Toxocara* sp. eggs in lettuce samples indicate contamination of vegetables with feces of canids or felids, which can result in manifestations of visceral Larva migrans syndrome, including severe pulmonary, ocular and neurological complications (26).

Other species of helminths and protozoa of clinical importance included *G. lamblia*, *E. histolytica/E. dispar*, *Tricuris* sp. and *E. vermicularis*, which are the parasites that most frequently cause human contamination. Individuals with immune disorders, children and the elderly are most at risk for parasitic and opportunistic diseases, such as *Giardia* sp. and *Entamoeba* sp (28).

The consumption of fresh vegetables enables the exposure of a large portion of the population to transmissible forms of parasites. As a result, to avoid contamination, proper hygiene of vegetables is the first step to reduce the transmission of intestinal parasites (28,29). In consequence, it is recommended to soak the vegetables for 15 minutes in water containing hypochlorite (1 tablespoon for 1 liter of water) before they are cut (30).

From a hygienic-sanitary point of view, the results of this study may indicate lack of knowledge on the subject by the communities (31), as well as inadequate practices in cultivation, transportation, storage or trade of vegetables, as observed by Oliveira & Pinto (32), who reported that food contamination is caused mainly by poor water quality, lack of control of pests and vectors, lack of adequacy and/or precariousness of production facilities, transportation and marketing, in addition to inadequate cleaning of equipment, utensils and food handlers themselves.

Thus, considering that the results of this study showed that the vegetables lettuce (*Lactuca sativa*) and parsley (*Petrosolium sativum*) sold in supermarkets and street markets in the city of São Mateus had high levels of contamination by parasites, a more effective monitoring by the competent authorities is expected in order to ensure quality in the food that is offered to the population ♣

Conflict of interest: None.

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