

Prevalence of enteroparasites in vegetables marketed in the city of Jaguaré, Espírito Santo, Brazil

Prevalencia de enteroparásitos en hortalizas comercializadas en el municipio de Jaguaré, Espírito Santo, Brasil

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Received 13th November 2018 / Send for modification 16th December 2019 / Accepted 26th February 2020

ABSTRACT

Objective To monitor the frequency of enteroparasites in lettuce (*Lactuca sativa*) and parsley (*Petroselinum sativum*) samples, marketed in commercial buildings in the municipality of Jaguaré, Espírito Santo, Brazil.

Materials and Methods Between August 2016 and July 2017, five different popular commercial buildings (markets, supermarkets and fairs) in the municipality of Jaguaré, considered the main local vegetable suppliers were selected. Throughout the research, 30 vegetable samples were randomly selected for parasitological analysis in 3 replicates for each commercial building under study. The obtained samples were analyzed by the modified spontaneous sedimentation method at the Laboratory of Parasitology and Hematology of Universidade Federal do Espírito Santo.

Results Of the 30 samples analyzed, 83.3% were positive for parasitic contamination and the most common parasites were Hookworm, *Balantidium coli*, *Isospora belli*, *Ascaris* sp. and *Entamoeba* sp.

Conclusions The results indicate a high degree of contamination of the vegetables, probably related to the precariousness of production, transportation and handling, making it necessary to adopt measures that ensure the best quality of food and, consequently, better population health.

Key Words: Parasitology; vegetables; food contamination; public health (source: MeSH, NLM).

RESUMEN

Objetivo Monitorear la frecuencia de enteroparásitos en muestras de lechuga (*Lactuca sativa*) y perejil (*Petroselinum sativum*), comercializadas en edificios comerciales en el municipio de Jaguaré, Espírito Santo, Brasil.

Materiales y Métodos Entre agosto de 2016 y julio de 2017, se seleccionaron cinco diferentes edificios comerciales populares (mercados, supermercados y ferias) en el municipio de Jaguaré, considerados los principales proveedores locales de vegetales. A lo largo de la investigación, se seleccionaron al azar 30 muestras de vegetales para el análisis parasitológico en 3 repeticiones para cada edificio comercial en estudio. Las muestras obtenidas fueron analizadas por el método modificado de sedimentación espontánea en el Laboratorio de Parasitología y Hematología de la Universidade Federal del Espírito Santo.

Resultados De las 30 muestras analizadas 83.3% fueron positivas para la contaminación parasitaria y los parásitos más comunes fueron *Anquilostoma*, *Balantidium coli*, *Isospora belli*, *Ascaris* sp. y *Entamoeba* sp.

Conclusiones Los resultados indican un alto grado de contaminación de las hortalizas, probablemente relacionado con la precariedad de la producción, el transporte y la manipulación, por lo que es necesario adoptar medidas que aseguren la mejor calidad

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de los alimentos y, en consecuencia, una mejor salud de la población.

Palabras Clave: Parasitología; verduras; contaminación de alimentos; salud pública (*fuentes: DeCS, BIREME*).

Food safety issues are becoming increasingly important worldwide, mainly due to the high frequency and severity of Foodborne Diseases (DTA), which are a serious public health problem (1-3).

Intestinal parasites are part of this group of diseases, as well as food infections caused by bacteria, viruses, among others (2). The transmission of enteroparasites most frequently occurs in a fecal-oral manner, where the individual ingests food or water contaminated with parasitic species, commonly eggs or cysts (4,5).

Brazil is a developing country with a population whose basic living and sanitation conditions are still unsatisfactory or even non-existent, besides a favorable climate for the spread of parasitic diseases (6-8).

Studies conducted by Nomura et al. (9) show that the individuals most affected by enteroparasites are those with low income, living in rural environments. Furthermore, the lack of knowledge about basic hygiene conditions and carelessness during food preparation and handling are important factors in the recidivism of these parasitic diseases (9,10).

Vegetables, like fruits, have been included in the diet of many individuals, mainly due to their nutritional attributes, simple preparation and low cost, and the consumption of these foods fresh has increased in recent decades with a growing population search for healthier eating habits (9,11).

In this context, lettuce (*Lactuca sativa*) stands out, since it is easily accessible to the population, cultivated in all regions of Brazil, widely used in salads, as well as parsley (*Petroselinum sativum*), an aromatic seasoning that is highly appreciated in the Brazilian cuisine. Both are recommended by doctors and nutritionists for having vitamins and dietary fiber that are important for body maintenance (12).

Despite these attractive factors, the consumption of these raw or minimally processed foods is associated with parasitic disease outbreaks caused by helminths and protozoa (11,13,14).

The main sources of contamination are directly related to the water used for irrigation, contaminated with human fecal waste, and to the soil contaminated by the use of organic fertilizers with fecal material, transportation in inadequate conditions, and poor sanitation of enteroparasite-infected handlers, frequently asymptomatic (11,13,14).

Research on enteroparasites carried out in Teresina (PI), Salvador (BA) and Pelotas (RS) showed that samples of vegetables can be contaminated mainly by Hookworm,

Ascaris lumbricoides, *Balantidium coli*, *Entamoeba coli*, *Entamoeba histolytica/E. dispar*, *Endolimax nana*, *Iodamoeba butschilii*, *Hymenolepis nana*, *Toxocara* sp. and *Trichuris trichiura*, and lead consumers of these foods to develop intestinal diseases (15-17).

Intestinal parasitic diseases are difficult to diagnose, since they are frequently asymptomatic, preventing adequate treatment or the adoption of prophylactic measures that could prevent a possible reinfection (5,10).

In general, the main symptoms presented during a parasitic infection are anorexia, irritability, sleep disorders, nausea, vomiting, abdominal pain and diarrhea. More serious cases occur according to a greater parasite load of the individual, their immunological and nutritional status (18).

In this context, in order to contribute to an improvement in the perception of hygienic-sanitary conditions and the quality of products consumed by the population, this study aims to monitor the frequency of enteroparasites in vegetables *L. sativa* and *P. sativum* marketed in the city of Jaguaré, Espírito Santo, Brazil.

MATERIALS AND METHODS

Study area

The municipality of Jaguaré is located in the far north of the state of Espírito Santo, and currently occupies an area of 659,751 km². According to the Census conducted by the Brazilian Institute of Geography and Statistics (IBGE) in 2010, the municipality had a population of 24,678 inhabitants, and may be considered small, when compared to the total population of Espírito Santo (3,514,952 inhabitants) (19).

Sample collection

Between August 2016 and July 2017, the vegetables were collected in five different popular commercial buildings (markets, supermarkets and fairs) in the municipality of Jaguaré, considered the main local vegetable suppliers.

Throughout the research, 30 vegetable samples were randomly selected for parasitological analysis, in 3 replicates for each commercial building under study.

The selected vegetables were lettuce (*L. sativa*) and parsley (*P. sativum*), considering the head as the sample unit for lettuce and the package for parsley, regardless of the weight and size they had (9).

The selected samples were isolated in individual plastic bags, identified and sent to the Laboratory of Parasitology

and Hematology of Centro Universitário Norte do Espírito Santo (CEUNES), Universidade Federal do Espírito Santo, to be properly analyzed.

Processing and analysis of vegetables

The samples were processed according to the spontaneous sedimentation method (20) with some modifications, and the samples were submitted to two washing processes. In the first, by rinsing, 250 mL of distilled water were added to the plastic bag containing the sample, shaking it manually for 30 seconds, while in the second process, by defoliation, each leaf was carefully rubbed in a plastic container with 250 mL of distilled water (9,21,22).

The washing solution from each of the two processes was filtered into a conical cup using a surgical gauze folded into four, resting for 24 hours. After this period, this solution was centrifuged at 9000 rpm for 3 minutes, and the sediment was collected in test tubes (21,22).

The parasitic forms were identified using light microscopy, in which the sediment was deposited on a glass slide, covered by a coverslip, and stained with lugol solution. At least two slides were examined per test tube for each vegetable sample, using 10x and 40x lenses (23).

RESULTS

During the study, 25 (83.3%) of the 30 samples of vegetables analyzed during the 12-month period were positive for contamination with some parasitic species. It should be observed that 61.5% of the contaminated lettuce

samples had more than two parasitic species (polyparasitism), while 38.5% of these samples were equivalent to those contaminated with only one parasitic species (monoparasitism). These values were similar for parsley samples, in which polyparasitism was observed in 66.7% of the samples and monoparasitism in 33.3% (Table 1).

Table 1. Degree of parasitism in lettuce and parsley samples marketed in the municipality of Jaguaré, Espírito Santo, Brazil, 2017

Samples	Degree of parasitism (%)	
	Monoparasitism	Polyparasitism
Lettuce	38.5	61.5
Parsley	33.3	66.7

Regarding the sampled buildings, vegetables from supermarkets presented a contamination index of 79.2%, while vegetables collected in fairs showed 100% contamination with parasitic species (Table 2).

Table 2. Frequency of positive vegetable samples from supermarkets and fairs in Jaguaré, Espírito Santo, Brazil, 2017

Buildings	Analyzed samples (number)	Positive samples (number)	Positive samples (%)
Supermarkets	24	19	79.2
Fairs	6	6	100
Total	30	25	83.3

As for the distribution of parasites in the municipality of Jaguaré, it was observed that all five buildings in the study had lettuce and parsley samples positive for some parasitic species (Figure 1).

Figure 1. Commercial buildings in the municipality of Jaguaré, Espírito Santo, Brazil, participating in the study, 2017



Collection point 1. S18° 54' 49.82"/O40° 4' 41.47"; Collection point 2. S18° 54' 19.57"/O40° 4' 55.37"; Collection point 3. S18° 54' 28.07"/O40° 4' 32.31"; Collection point 4. S18° 54' 22.03"/O40° 4' 20.10"; Collection point 5. S18° 54' 27.14"/O40° 4' 5.83". Source: Google Earth, 2018.

The parasites with the highest frequency in parsley samples in the parasitological analyses were Hookworm (60.0%), *Isospora belli* (33.3%) and *B. coli* (33.3%) (Table 3).

Table 3. Frequency of enteroparasites in parsley (*Petroselinum sativum*) samples from commercial buildings in the municipality of Jaguaré, Espírito Santo, Brazil, 2017

Parasitic species	Frequency(%)
<i>Toxocara</i> sp.	6.7
<i>Entamoeba</i> sp.	13.3
<i>Ascaris</i> sp.	13.3
<i>Balantidium coli</i>	33.3
<i>Isospora belli</i>	33.3
Hookworm	60
<i>Entamoeba coli</i>	65

In relation to lettuce samples, the most frequent parasites were Hookworm (66.7%), *B. coli* (46.7%), *Ascaris* sp. (20.0%) and *Entamoeba* sp. (20.0%) (Table 4).

Table 4. Frequency of enteroparasites and commensals in lettuce (*Lactuca sativa*) samples from commercial buildings in the municipality of Jaguaré, Espírito Santo, Brazil, 2017

Parasitic species	Frequency (%)
<i>Paramecium</i> sp.	6.7
<i>Giardia</i> sp.	6.7
<i>Isospora belli</i>	13.3
<i>Entamoeba</i> sp.	20.0
<i>Ascaris</i> sp.	20.0
<i>Balantidium coli</i>	46.7
Hookworm	66.7

DISCUSSION

The consumption of fruits and vegetables, especially fresh, provides important health benefits, with direct implications in life quality due to their nutritional attributes. However, the ingestion of these raw or minimally processed foods is directly linked to parasitic disease outbreaks, since these vegetables can be grown in areas contaminated with fecal waste or irrigated with polluted waters (6,9,11).

The results presented in the analyses carried out in Jaguaré showed 83.3% positive samples for some parasitic species, indicating a higher percentage, when compared to parasitological analyses carried out in Teresina, Piauí, and in the city of Rio de Janeiro which showed, respectively, 34.1% and 36.4% positive samples. However, they are considered inferior to the value found in samples collected in Campina Grande, Paraíba, where 100% were contaminated (14,17,24).

The variability in positive indices is probably related to the great territorial extension and its diversity regarding climatic and environmental characteristics, which may favor the occurrence of parasitic contamination in vegetables and fruits (25).

When the degree of polyparasitism was evaluated, a similar result was observed for lettuce and parsley (61.5% and 66.7%, respectively). Alves *et al.* (25) indicated 66.7% positive samples for the analyzed lettuce of supermarkets in the city of Cuiabá, Mato Grosso. Polyparasitism was the predominant type of contamination in the evaluated samples (70%).

Based on these data, the high parasitic contamination rates of the samples collected in Jaguaré, as well as the majority of them having presented multiple contamination, may be directly related to inadequate practices during the planting process, where soil and irrigation water may be contaminated by fecal waste. In addition, transport and storage under inadequate conditions, besides poor food sanitation before it is available to consumers, can directly influence its quality (11,13,14,25).

Freitas *et al.* (26) confirmed in their studies that samples of vegetables from supermarkets and fairs in the municipality of Campo Mourão, Paraná, had a percentage of contamination of 56% and 58.7%, respectively, while the samples collected in the municipality of Jaguaré showed much higher values (79.2% for supermarkets and 100% for fairs). These results may indicate that planting is carried out under inadequate conditions, possibly with contamination of soil or irrigation water with human or animal fecal waste. However, although the contamination rate is higher in fairs, the contamination index in vegetables from supermarkets is also worrisome, once they demonstrate that the hygiene practices for vegetables are frequently neglected by producers and distributors, as reported by Fernandes *et al.* (27).

The parasitic species most frequently found during the parasitological analyses of vegetables sold in Jaguaré were Hookworm, *B. coli*, *I. belli*, *Ascaris* sp. and *Entamoeba* sp. These parasites were also found in studies conducted in Londrina, Teresina, Rio de Janeiro and the Federal District, showing that environmental and climatic conditions throughout the Brazilian territory favor the spread of enteroparasites, in the same way that socioeconomic characteristics and hygienic-sanitary conditions influence these processes (9,17,28,29).

The main clinical manifestations of Hookworm and *Ascaris* sp. in the host, involve diarrhea, anemia, nutritional deficiency, intestinal obstruction and, in more severe conditions, can lead to death (17,23,30); the high incidence of *Ascaris* sp. (20%) in lettuce samples may be related to the morphology of the peel, which allows better adhesion to the plant foliage (26,31).

In general, *B. coli* is a commensal protozoa of pig intestinal lumen that can parasitize humans when a lesion occurs in the mucosa of the colon and the cecum, occurring

a secondary infection, since it is not able to penetrate alone the intact mucosa. Its presence frequently indicates contamination of food and soil fertilized with swine, ruminant and human fecal waste (9,23).

Human isosporosis is more frequent in hot regions where hygiene conditions are poor. Its infection occurs through the ingestion of food or water contaminated with sporulated *I. belli* oocysts, whose symptoms include fever, diarrhea, abdominal cramps and vomiting. More severe cases of this pathology commonly occur in children and individuals with some immunodeficiency, and may cause chronic diarrhea, leading to dehydration, weight loss and even hospitalization (23).

Not less important, the presence of *E. coli* in parsley samples, despite being a commensal, is considered an indicative factor of parasitic contamination with fecal waste, and its diagnosis is essential in order to propagate prophylactic measures to avoid other contaminations, for example, by pathogenic amoeba, such as *E. histolytica* (9,27,32). In addition, the observed *Toxocara* sp. eggs suggest the presence of dogs and cats, definitive hosts of the disease, in regions close to planting and in water sources used for irrigation (23,33).

The results obtained in Jaguaré indicate a high degree of contamination of vegetables and are probably related to the precariousness of hygiene care, which directly influences the quality of these products. According to the National Sanitary Surveillance Agency (34), any type of food must be free from foreign matter indicating health risks such as dirt, insects and parasites, so that they are suitable for consumption. In this context, vegetables supplied in the municipality are in disagreement with the current legislation, have an important role in the dissemination of enteroparasites, given the high contamination rates observed during the parasitological analyses and may pose great risks to the health of the local population.

Therefore, in order to avoid parasitic disease outbreaks it is necessary to improve hygiene and sanitation during food production, processing, transportation and final handling as well as instructions to the population about prophylactic measures and the treatment of these diseases, emphasizing the need for Sanitary Surveillance in the inspection processes of vegetable gardens and food distributors, in order to ensure greater safety and food quality for the population ♥

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