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# Parasitic contamination of lettuce: comparison between establishments located in areas with high and low economic values

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## ABSTRACT

**Introduction:** Diseases caused by contaminated food are a threat to public health. Lettuce (*Lactuca sativa*) is a widespread vegetable for human alimentation. Some systematic reviews showed parasitic contamination of lettuce in Brazil. **Objective:** To evaluate if the economic value of the places where lettuce is bought could be a determinant of parasitic contamination. **Methods:** We assessed the presence of parasites in the curly lettuces sold in the city of Passo Fundo, Brazil, and compared the frequency of contamination between markets and fruit stores, and between areas with high and low economic values. 130 samples of lettuce from 65 commercial establishments scattered throughout the city were washed with distilled water and analyzed under optical microscopy after 24 hours of spontaneous sedimentation method. **Results:** Parasitic forms were found in 80% of the samples, namely: ciliated cysts (probable *Balantioides coli*), larvae of nematode, *Ascaris lumbricoides,* cestode eggs, *Giardia lamblia*, *Hymenolepis nana*, *Entamoeba coli*, and *Fasciola hepatica*. Contamination by larvae of nematode was more frequent in fruit stores than in markets (35.9% vs. 19.7%; p=0.039). Areas with high and low economic values had the same frequency of contamination, but there was a trend to higher contamination by cestode eggs in areas with low economic values. **Conclusion:** This study was the first to show that the frequency of parasitic contamination is not different compared to establishments located in areas of high and low economic values, refuting any common sense that regions of the city with higher income are less exposed to parasitic contamination of food.

**Keywords:** lettuce; food contamination; parasites; Helminths; protozoan infections; income.

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## INTRODUCTION

Lettuce (*Lactuca sativa*) is a widespread vegetable for human alimentation, often eaten uncooked, as in salads. In Brazil, "garden," "crunch" or "curly" lettuce is the most common, comprising more than 50% of the consumption[1]. Thus, any health concern regarding lettuce, especially curly lettuce, may have a direct effect on the population.

Diseases caused by contaminated food are a constant threat to public health and a significant impediment to socioeconomic development worldwide[2]. The World Health Organization estimated that foodborne hazards caused 600 million foodborne illnesses and 420,000 deaths in 2010[2]. Uncooked food certainly brings a conspicuous risk to this setting.

Lettuce contamination by parasites can occur in several manners: cultivation in the soil, the use of water for irrigation, during harvesting, transportation, storage, and even during consumption in case of inadequate hygiene habits[3].

A couple of systematic reviews compiled the surveys about the parasitic contamination of lettuce in Brazil[4,5]. Most of the specimens were represented by curly lettuce. Contamination rates were reported up to 90%, with the presence of parasites with the potential to develop important diseases such as giardiasis, ascariasis, amoebiasis, and cysticercosis, among others, denoting the remarkable risk of eating lettuce without adequate hygienization[4,5].

The relation between low income and higher prevalence of parasitic infections due to inadequate sanitary conditions is long-lasting and remains patent in both rural and urban areas of South America[6,7].

However, if income is a determinant factor for the location where the people *live* and the consequent risks of acquiring intestinal parasitic infection[8], it is not known if the economic value of the places where the people *buy* the food could be such a determinant. This issue can be extended to the scenario related to parasitic contamination of lettuce in Brazil.

This study aimed to evaluate the presence of parasites in common curly lettuce (*Lactuca sativa*) sold in fruit stores and markets located in the city of Passo Fundo – RS and compare the percentages of such contamination between the diverse types of establishments and economic regions of the city.

## METHODS

### Study design and research location

This exploratory research was performed from July to September 2022 in Passo Fundo, a medium-sized city with 217,240 inhabitants located in the southernmost state of Brazil (Rio Grande do Sul). It was approved by the local ethics committee number 5,156,476.

The urban zone is divided into 92 tributary territories determined by the municipal government (Complementary Law 445, from May 14th, 2019) according to the economic values of properties, which considers the property area and the local facilities. This has enabled the classification of territories into two types for taxation: low economic value (from 17 to 110 municipal fiscal units) and high economic value (from 111 to 291 municipal fiscal units). This was assumed as an indirect inference of inhabitants' income from each territory.

### Sample planning

The Brazilian Federal Revenue Office maintains a public register with all commercial establishments in each city of the country. In the middle of 2022, 145 fruit stores and 93 markets/supermarkets were registered in Passo Fundo. The method described by Agranonik and Hirakata for a known population size we applied to determine the necessary number of establishments[9]. A sample composed of 32 fruit stores and 33 markets/supermarkets was considered the minimum necessary. After defining the types of establishments, they were classified according to the economic areas where they were located. Finally, the establishments were randomly included in a way to cover most areas scattered through the city, but respecting a balanced division according to the economic areas.

### Obtainment, processing, and analysis of lettuce specimens

Two specimens of curly lettuce (*Lactuca sativa*) from conventional cultivation were collected from each commercial establishment through a purchasing process. The samples were packed in sterile plastic bags properly sealed and immediately sent for preparation and analysis in the Parasitology and Microscopy laboratories of the Universidade de Passo Fundo (UPF).

For sample preparation, specimens were placed in polyethylene plastic bags (excluding roots and dry/damaged leaves). 250 mL of distilled water and four drops of neutral detergent were added into the bags, followed by manual agitation for 5 minutes to release parasitic structures. After that, the wash water was transferred directly to a sedimentation glass and left undisturbed for 24 hours to allow the sedimentation of parasitic structures according to the method of spontaneous sedimentation originally described by Hoffman et al.[10].

After this period, a sediment aliquot of approximately 50 µL was collected for each sample unit using a Pasteur pipette to prepare two slides, one of them stained with Lugol. Both were analyzed under optical microscopy (Olympus CH20) with 10x and 40x objectives. For the identification of parasitic forms, specialized parasitology manuals[11,12] and Ponce-Gordo & García-Rodríguez[13] were employed, as well as the bench aids for the diagnosis of intestinal parasites prepared by the Pan American Health Organization[14]. A sole researcher performed this analysis. Samples were qualitatively classified as contaminated or not, and no quantitative measure of parasitic structures for each specimen was undertaken.

### Statistical analysis

The numbers of contaminated samples were compared between fruit stores and markets/supermarkets as well as between areas of high and low economic values with the Mann-Whitney U test because of asymmetric distribution. Analysis was performed R Core Team (2021). The p-value for significance was established as 0.05.

## RESULTS

At the end of the sampling process, 65 establishments (each one with two specimens of lettuce) were included (32 fruit stores and 33 markets/supermarkets), 33 of them in territories with high economic value (16 fruit stores and 17 markets/supermarkets) and 32 in areas of low economic value (16 fruit stores and 16 markets/supermarkets). Figure 1 depicts the geographical location of the establishments included in a way that covers the whole city area.

Most lettuce specimens were contaminated by protozoa and/or helminths (Table 1). The presence of ciliated cysts, the protozoan *Balantioides coli* stood out with the highest prevalence, followed by nematode larvae. The latter were the only ones that exhibited a significantly higher prevalence of contamination in fruit stores compared with markets/supermarkets. No other difference emerged from the analysis.

Figure 2 shows the number of contaminated specimens of curly lettuce among the 130 samples obtained from the 65 establishments and the comparisons between the areas with high and low economic values with the Mann-Whitney U test. No significant difference was found, although there was a trend (p=0.071) toward higher contamination by cestode eggs among samples obtained from establishments in areas with low economic value.

## DISCUSSION

There is compelling evidence that parasitic contamination is quite common in lettuce, as well as in other fresh vegetables[4,5,15-17] and our survey was not different from the literature. The relative profile of frequency of each protozoan and helminth is variable throughout the studies, but the main parasites that we found are among those described in other cities from Brazil.

A couple of studies reported an initial attempt to compare the frequency of parasitic structures in vegetables commercialized in markets with that from fruit stores, with methodological paucity, inconsistent results, and without a statistical analysis[15-17].

In this setting, our survey offered a systematic approach to the establishments from the city of Passo Fundo and delivered the results regarding similar frequencies of parasitic contamination both in markets/supermarkets and fruit stores/fairs, except for larvae of nematode, which was less common in markets. The main sources for parasitic contamination of vegetables are lack of adequate basic sanitation systems and care in the production, cultivation, and handling[3]. There is no center for the distribution of vegetables and fruits in Passo Fundo. Consequently, all fruit stores and markets obtain such products from several small or medium-sized producers scattered in the North of Rio Grande

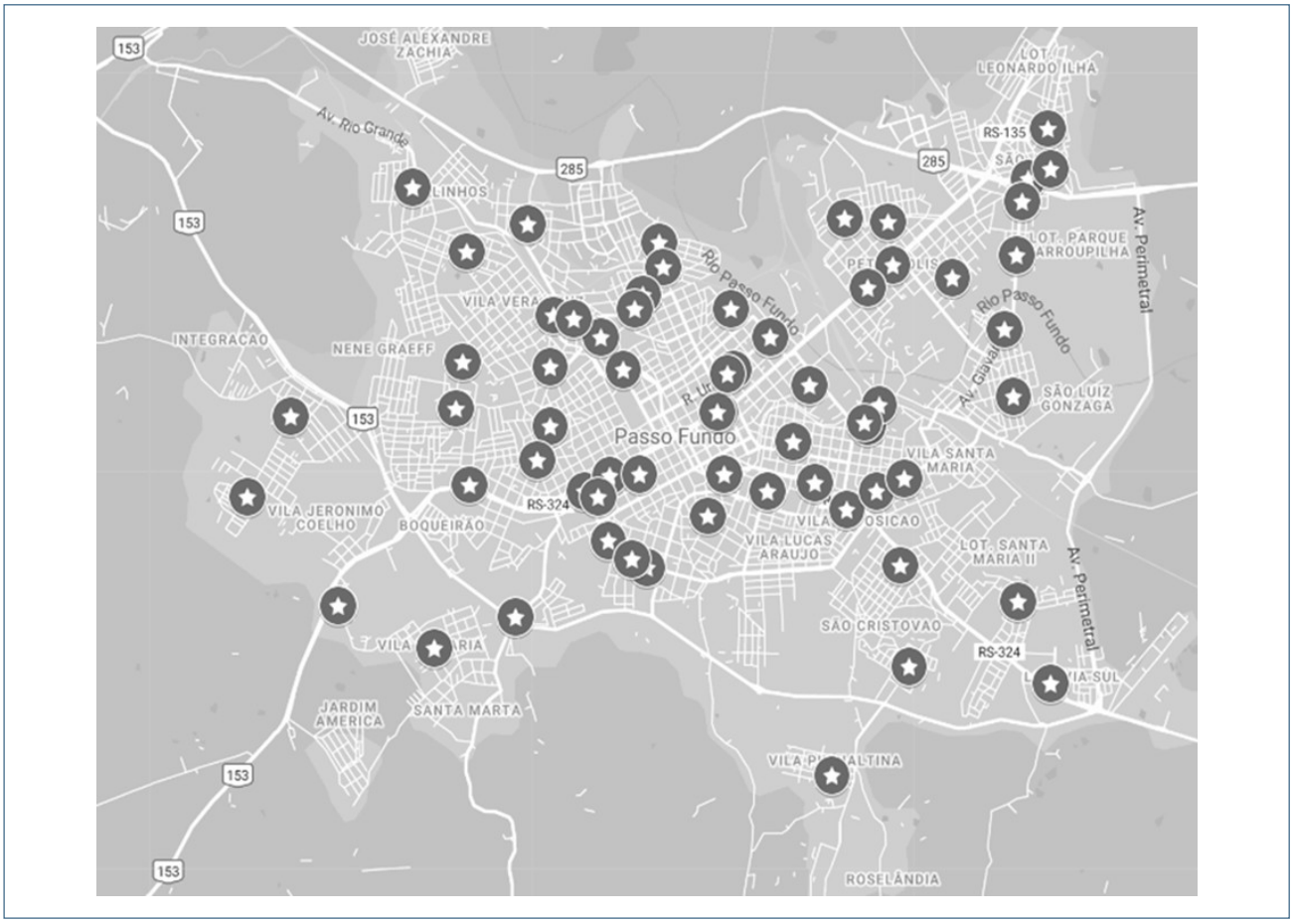


**Figure 1:** Distribution of establishments included in the survey in the urban area of Passo Fundo (Source: Google Earth, 2022).

**Table 1:** Contaminated specimens of curly lettuce according to the type of establishment.

| | Total | | Fruit stores | | Markets | | p-value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | |
| Analyzed samples | 130 | 100 | 64 | 100 | 66 | 100 | - |
| Contaminated samples | 104 | 80.0 | 51 | 79.7 | 53 | 80.3 | 0.172 |
| Ciliated cysts | 87 | 66.9 | 46 | 71.9 | 41 | 62.1 | 0.240 |
| Nematode larvae | 36 | 27.7 | 23 | 35.9 | 13 | 19.7 | 0.039* |
| *Ascaris lumbricoides* | 30 | 23.1 | 15 | 23.4 | 15 | 22.7 | 0.924 |
| Cestode eggs | 22 | 16.9 | 12 | 18.8 | 10 | 15.2 | 0.588 |
| *Giardia lamblia* | 2 | 1.5 | 1 | 1.6 | 1 | 1.5 | 0.982 |
| *Hymenolepis nana* | 2 | 1.5 | 1 | 1.6 | 1 | 1.5 | 0.982 |
| *Entamoeba coli* | 2 | 1.5 | 2 | 3.1 | 0 | 0 | 0.160 |
| *Fasciola hepatica* | 3 | 2.3 | 1 | 1.6 | 2 | 3.0 | 0.578 |

Comparisons with the Mann-Whitney U test. *Significant difference.

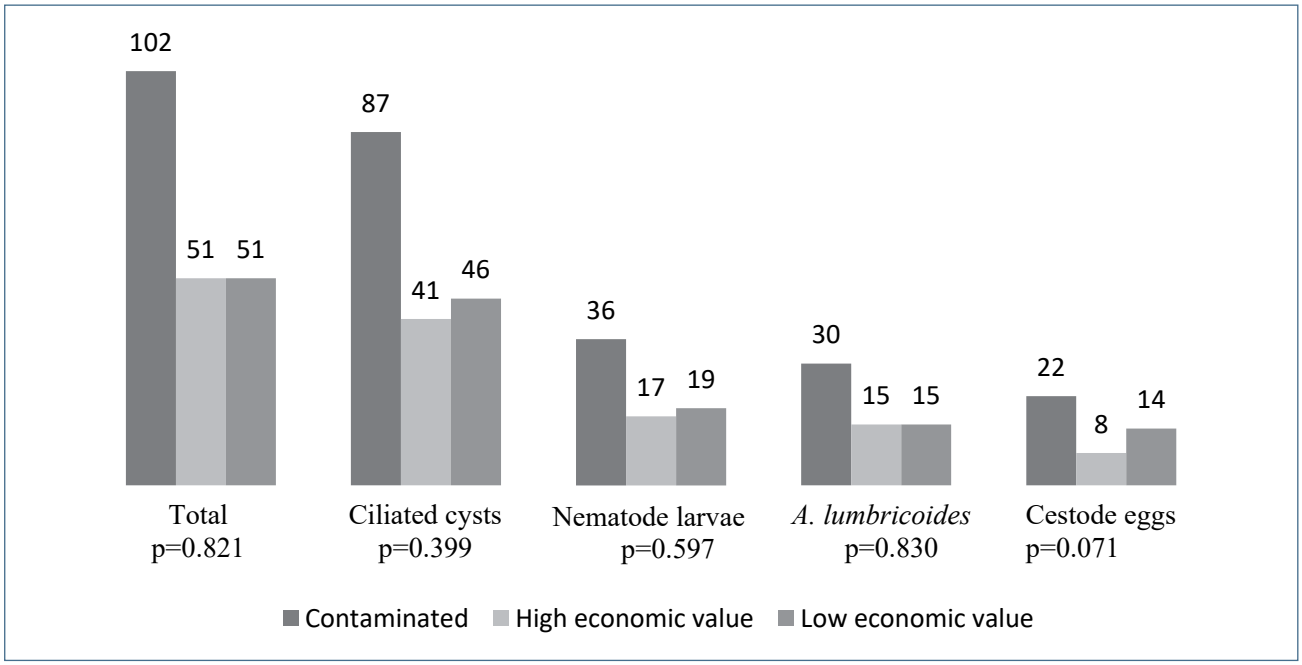


**Figure 2:** Number of contaminated specimens of curly lettuce among the 130 samples obtained from the 65 establishments and comparisons between the areas with high and low economic values with the Mann-Whitney U test.

do Sul. Thus, it is difficult to indicate a reason for such a difference because we could not assess all the possible steps of contamination (soil of cultivation, water for irrigation, harvesting, storing, transportation, and place of exposition). This could be recognized as a limitation of our study, but we point out that our purpose was to make a correlation between the frequency of contamination with the various places of purchase, and not the other steps of the productive chain.

The main finding of our survey was that the frequency of parasitic contamination was not different compared to establishments located in areas of high economic values with those located in areas of low economic values. In other words, it is an illusion to believe that an establishment located in a city territory thought to be more sophisticated offers more hygienic food. At least this is the case with curly lettuce in the city of Passo Fundo. Despite the establishments' economic values, an indirect inference of the income of people who buy in these places, all regions of the city offer curly lettuce with a similar frequency of parasitic contamination to the population.

The only exception to the similarity above was a trend toward higher contamination with cestode eggs among samples obtained from establishments in areas with low economic value. The presence of such eggs may indicate the contamination of soil, water, or even handlers with human feces, a step necessary for the development of neurocysticercosis. We hypothesize that these areas with low economic value may be more prone to contamination due to the lack of sewage treatment in the suburbs of Passo Fundo, a city located in a region where neurocysticercosis is a worthy cause of severe neurological disease[18]. Nevertheless, this trend should be investigated in a larger study, since the non-significance may be a limitation of our sample size.

Other limitations of this survey must be cited. We did not perform a quantitative approach to parasitic contamination because this would render a huge effort with limited feasibility and still an elevated risk of inaccuracy. Other types of lettuce, vegetables, and even fruits were not evaluated, but we decided to include only curly lettuce because of its large consumption by the population. Finally, other promising types of methodological analysis beyond optical microscopy could enhance the knowledge about the characteristics of the contamination and distinguish species among the nematode larvae and cestode eggs that were found in lettuce[19,20].

In conclusion, this was the first study to show that the frequency of parasitic contamination is not different compared to establishments located in areas of high and low economic values, refuting any common sense that regions of the city with higher income are less exposed to parasitic contamination of food. This underscores the importance of adequate hygienization of food before consumption independently from the place where it is purchased. Further studies are needed to determine the sources of contamination and propose measures for prevention.

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