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PARASITOLOGICAL RISK ASSOCIATED WITH THE CONSUMPTION OF LETTUCE (*LACTUCA SATIVA*) SOLD AT OPEN-AIR MARKETS IN THE CITY OF BOM JESUS DO ITABAPOANA, NORTHWEST REGION OF THE PROVINCE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT

Lettuce (*Lactuca sativa*) is a leafy vegetable recognized as a significant vehicle for neglected parasitic diseases, primarily due to its common consumption in raw form and its exposure to inadequate agricultural and handling practices. This study aimed to assess the presence of enteroparasites in non-hydroponic lettuce samples sold at open-air markets, small retail outlets, and by informal vendors in the city of Bom Jesus do Itabapoana, Rio de Janeiro State, Brazil. Thirty-five samples were collected during the first half of April 2024 and analyzed using the spontaneous sedimentation technique in water, followed by microscopic examination for the detection of eggs, cysts, larvae, and other parasitic elements. Results revealed that 77.14% (26/35) of the samples were contaminated: 46.15% exclusively with protozoa, 26.92% exclusively with helminths, and 26.92% with both groups concurrently. Identified parasites included *Cryptosporidium* spp. (11.43%), *Giardia* spp. (20%), *Ascaris lumbricoides*, *Trichuris* spp., and *Balantidium coli*, as well as potentially zoonotic species such as *Toxocara* spp. Additionally, free-living mites were detected in 28.57% of the samples, indicating shortcomings in post-harvest hygiene procedures. The observed contamination rate substantially exceeds the global average and closely resembles patterns reported in certain regions of the Province of Espírito Santo, suggesting the influence of local factors, including the use of untreated water for irrigation, inadequate sanitation infrastructure, and substandard handling practices. The presence of parasites previously identified in fecal samples from local children and in the waters of the Itabapoana River supports the hypothesis of an ongoing cycle of environmental and foodborne contamination. These findings highlight a significant public health risk and underscore the urgent need for integrated policies encompassing improved sanitation, monitoring of agricultural water quality, training of producers, and public health education campaigns targeting consumers.

KEYWORDS: Food safety; Lettuce; Enteroparasites; Foodborn parasitoses; Bom Jesus do Itabapoana; Public Health.

INTRODUCTION

In recent years, the paradigm of food safety has shifted from a narrow focus on the mere absence of chemical or bacterial contaminants toward a broader understanding of biological hazards present in fresh produce, particularly leafy vegetables. Within this evolving framework, lettuce (*Lactuca sativa*), commonly consumed raw and often perceived as a symbol of a healthy diet, has emerged as a key factor in the eco-epidemiology of neglected parasitic diseases.[1,2,3,4]

Unlike contamination by chemical agents (as pesticides) or bacterial foodborne pathogens, whose effects are typically acute and clinically apparent, parasites transmitted via lettuce often cause subclinical or chronic infections, complicating their detection and leading to an underestimation of their disease burden.[5,6,7] The environmental persistence of resistant forms of protozoa and helminths, combined with rising consumer demand for minimally processed and ready-to-eat produce, creates optimal conditions for the silent dissemination of these agents. Moreover, methods for detecting bacterial contamination are more standardized and widely implemented than those for parasitic agents, which frequently require specialized techniques and expertise, such as sedimentation and flotation protocols.[8,9,10] Consequently, the traditional emphasis on bacterial infections, coupled with challenges in epidemiological tracing and the technical specificity of parasitological diagnostics, directly contributes to the underreporting and underestimation of parasitic contamination in vegetables. According to the Food and Agriculture Organization of the United Nations[11], approximately 30% of foodborne pathogens globally are parasitic in nature.

Studies indicate an increasing trend in foodborne illnesses associated with the consumption of raw vegetables since the early 21st century.[12,13] In developing countries such as Brazil, intestinal parasitoses resulting from the ingestion of contaminated raw vegetables constitute a significant public health concern.[14,15,16,17] Contributing factors include inadequate sanitation infrastructure, the use of untreated wastewater for irrigation, insufficient post-harvest washing of produce, ineffective public health policies for surveillance and control of parasitic diseases, and socioeconomic conditions that limit access to health education.[17,18,19,20]

Against this backdrop, understanding the parasitological contamination profiles of raw-consumed vegetables is crucial for designing more effective public health policies and targeted sanitary education strategies aimed at mitigating contamination risks. This study therefore aims to conduct a parasitological analysis to assess the presence of enteroparasites in non-hydroponic lettuce (*Lactuca sativa*) samples sold at open-air markets, small retail establishments, and by informal vendors in the city of Bom Jesus do Itabapoana, Northwest of the Province of Rio de Janeiro, Brazil.

MATERIAL AND METHODS

A set of 35 lettuce samples were collected during the first half of April 2024 from various local vendors and producers. A convenience sampling strategy was employed, with samples randomly selected among the lettuce units available at local points of sale. Vendors were interviewed regarding the origin of their produce to minimize the risk of collecting multiple samples from the same grower. Only lettuce heads containing at least five leaves were selected.

Each sample was placed directly at the point of sale into sterile plastic bags, labeled for identification, and stored in insulated coolers under refrigerated conditions. Samples were transported to the Laboratory of Research in Infectious and Parasitic Diseases at UniFAMESC and processed on the same day of collection. Prior to washing, visibly stained, damaged leaves, and roots were discarded. Each lettuce head was then manually separated into individual leaves, placed in sterile, disposable plastic containers, sliced, and homogenized.

Leaf surfaces were washed using clean, filtered water. To obtain the sediment derived from the vegetable washings, approximately 200 mL of distilled water was added to each collection bag containing the homogenized sample. The mixture was vigorously agitated and mechanically extracted, and the resulting suspension was transferred to a sedimentation cup and allowed to settle for four hours. This procedure was performed individually for each sample, with each sedimentation cup clearly labeled to maintain sample traceability.

After the four-hour sedimentation period, a 1 mL aliquot of the sediment was collected using a graduated pipette. Approximately 0.1 mL of this sediment was transferred onto a microscope slide, and one drop (~0.05 mL) of 1% Lugol's iodine solution was added to enhance the visualization of protozoan cyst nuclei. Slides were then coverslipped and examined under an optical microscope using 10×, 20×, and 40× objectives for the detection of helminth eggs, protozoan cysts, larvae, and other enteroparasitic elements. The spontaneous sedimentation technique in water was employed for sediment preparation.

For the detection of *Cryptosporidium* spp. and *Isospora* spp. oocysts, the wash water from each lettuce sample was also subjected to four hours of sedimentation. Smears prepared from the resulting sediment were stained using the modified Ziehl–Neelsen method. After air-drying at room temperature, the stained slides were examined under an oil immersion objective (100×) with standard immersion oil (refractive index ≈ 1.515) to optimize resolution and facilitate the identification of acid-fast oocysts.

For *Giardia* spp. cyst detection, approximately 0.1 mL of sediment was placed directly onto a microscope slide, homogenized, and mixed with one drop of 1% Lugol's iodine solution to improve visualization of cyst morphology and internal structures. A coverslip was applied, and slides were immediately examined under the 40× objective of an optical microscope for the microscopic identification of *Giardia* spp.

RESULTS

Among the 35 lettuce samples examined, 26 (77.14%) tested positive for parasitic elements. Of these positive samples, 12 (46.15%) were contaminated exclusively by protozoa, 7 (26.92%) exclusively by helminths, and 7 (26.92%) harbored both protozoa and helminths concurrently. Additionally, free-living mites were detected in 10 samples.

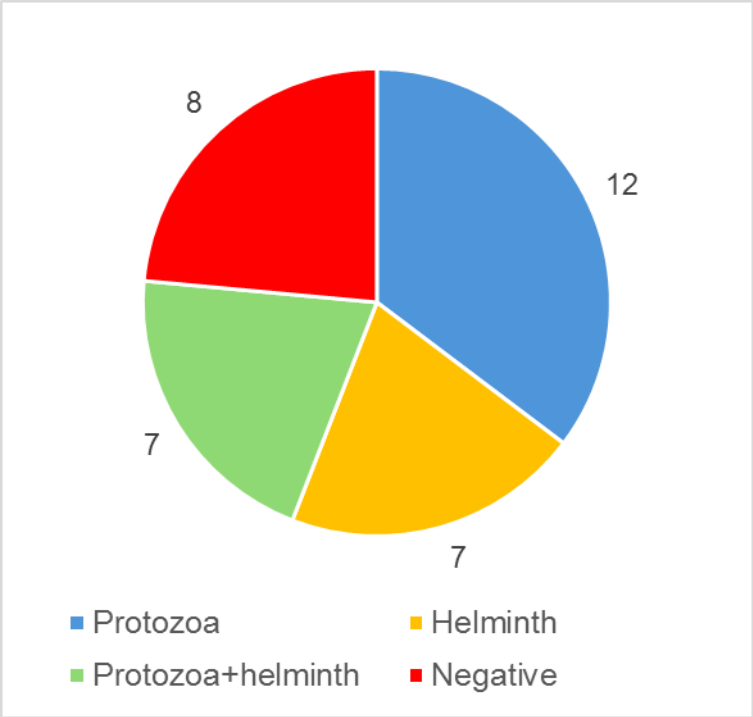

Figure 1: Distribution of Results According to Parasitic Elements Detected in Lettuce Samples Sold in Bom Jesus do Itabapoana.

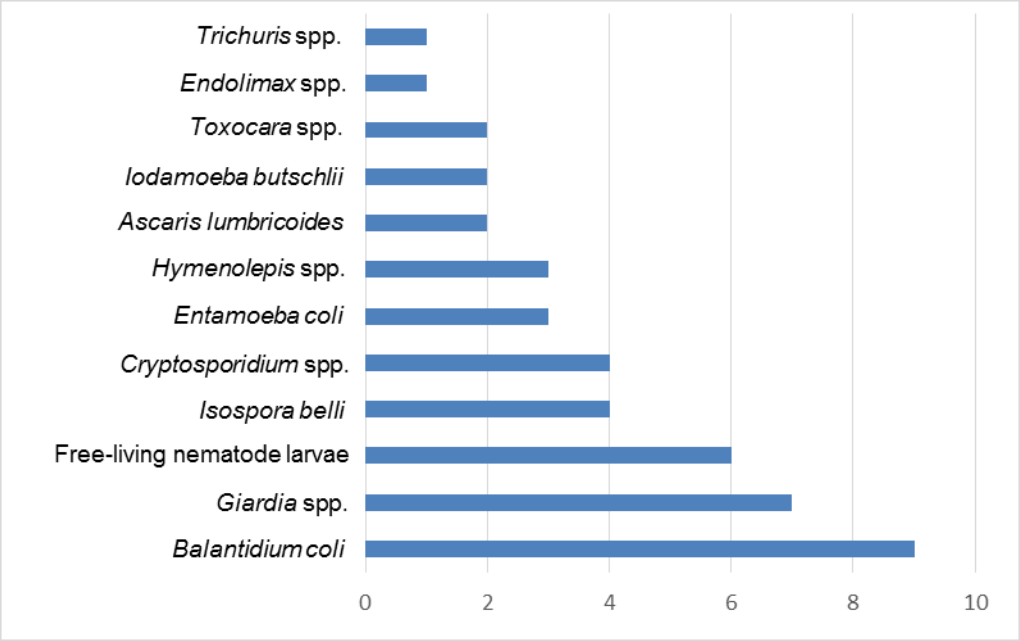

Figure 2: Incidence of Parasitic Elements Detected in Lettuce Samples Sold in Bom Jesus do Itabapoana.

DISCUSSION

Lettuce is widely regarded as the leafy vegetable most frequently contaminated with parasitic elements. A systematic review of studies published between 2001 and 2021 reported a global average contamination rate of 20.43% in lettuce samples[14], substantially lower than the 77.11% contamination rate observed in non-hydroponic lettuce sold in Bom Jesus do Itabapoana. Indeed, contamination levels in Brazil, particularly in non-hydroponic lettuce, are notably high compared to other countries, with numerous studies documenting significant parasitic loads across different regions.

In Manaus, Province of Amazonas, 100% of lettuce samples collected from open markets and street fairs were contaminated, with *Hymenolepis* spp. and *Entamoeba* spp. being the most prevalent parasites.[21] Similarly, in Rio Branco, Province of Acre, 96.2% of lettuce samples were contaminated, primarily by *Ascaris lumbricoides*, which was detected in 42.3% of samples.[22] In Belém, Province of Pará, 89% of samples tested positive for parasitic contamination, with street-market produce showing higher levels of pathogenic parasites than supermarket-sourced lettuce.[23] In Santana do Ipanema, Province of Alagoas, 35% of samples were contaminated, with *Strongyloides stercoralis* and *Ancylostoma duodenale* frequently identified.[24] In Grajaú, Province of Maranhão, all lettuce samples were contaminated, predominantly with *Giardia lamblia* and *Entamoeba coli*.[25] In Itabuna, Province of Bahia, 73% of samples were positive, with *Ancylostoma* spp., *Strongyloides* spp., and *Entamoeba coli* as the main contaminants.[26]

Given that Bom Jesus do Itabapoana lies on the border between the Provinces of Rio de Janeiro and Espírito Santo, it is pertinent to consider regional contamination patterns. Previous studies in both states have documented high levels of parasitic contamination in locally sold lettuce. In Nova Iguaçu, Province of Rio de Janeiro, 21% of samples tested positive for enteroparasites, with *Ascaris* spp., *Giardia lamblia*, and *Entamoeba coli* being the most common.[27] In the Baixada Fluminense region, a metropolitan area adjacent to the city of Rio de Janeiro, contamination was detected in 25% of samples, primarily involving *Balantidium coli* and *Ascaris lumbricoides*.[28] In contrast, studies in the Province of Espírito Santo have reported markedly higher rates: in Jaguaré, 83.3% of lettuce samples were contaminated with parasites including Ancylostomatidae, *Balantidium coli*, *Isospora belli*, *Ascaris lumbricoides*, and *Entamoeba* spp., a pattern attributed to inadequate agricultural, transport, and post-harvest handling practices.[29] In Conceição da Barra, contamination reached 100%, with *Entamoeba coli*, *Entamoeba histolytica/dispar*, and Ancylostomatidae identified.[30] In Nova Venécia, 46% of samples were positive, with *Strongyloides* spp. and *Balantidium coli* as the most prevalent agents.[31]

The parasitological profile and high contamination frequency observed in Bom Jesus do Itabapoana align more closely with findings from the Province of Espírito Santo than with those reported for Rio de Janeiro or other Brazilian regions. This similarity is evident both in the diversity of detected parasites, particularly the high prevalence of pathogenic protozoa such as *Cryptosporidium* spp., *Giardia* spp., and *Balantidium coli*, as well as geohelminths like *Ascaris lumbricoides* and *Trichuris* spp., and in the presence of zoonotic agents such as *Toxocara* spp. (and possibly *Balantidium coli*). Moreover, the frequency with which these agents were found in marketed lettuce suggests the influence of shared local factors, including deficient sanitation infrastructure, the use of untreated water for irrigation, and inadequate post-harvest handling, conditions more characteristic of the epidemiological setting in the Espírito Santo small cities than of more urbanized or structured contexts in the studied areas of Rio de Janeiro.

Although enteroparasitoses are recognized as highly prevalent in clinical practice within local health facilities in Bom Jesus do Itabapoana[32], no prior scientific study had investigated parasitic contamination in vegetables in this city. Notably, among children under five years of age attending a local school, *Cryptosporidium* spp. was detected in 17.91% of stool samples.[33] Furthermore, oocysts of *Cryptosporidium* spp. and cysts of *Giardia* spp. have been identified in the waters of the Itabapoana River[34], suggesting that irrigation with contaminated river water likely plays a key role in the parasitic contamination of locally grown lettuce. In the present study, *Cryptosporidium* spp. and *Giardia* spp. were detected in 11.43% and 20% of lettuce samples, respectively: rates consistent with those observed in pediatric epidemiological surveys and in river water analyses. This concordance implies that, beyond waterborne transmission, the consumption of inadequately washed raw lettuce represents a significant route of exposure to these protozoan pathogens.

A prior investigation into bacterial indicators in lettuce sold in Bom Jesus do Itabapoana already revealed the presence of fecal coliforms[35], signaling human fecal contamination. The parasitological findings of the current study corroborate this conclusion while expanding the understanding of contamination sources by also revealing zoonotic and environmental origins. Specifically, the identification of zoonotic parasites and the high frequency (17.14%) of geohelminths, whose eggs originate from contaminated soil, underscore the multifactorial nature of contamination in this setting.

Free-living mites were detected in 10 of the 35 analyzed samples (28.57%), reinforcing the hypothesis of inadequate post-harvest hygiene. Although these mites are not pathogenic to humans, their presence serves as a reliable indicator of insufficient cleaning practices. Of particular note, mites were found in two samples that tested negative for enteroparasites, suggesting that the absence of pathogenic parasites does not necessarily reflect proper hygiene during processing. Collectively, these findings highlight the critical need for the rigorous implementation of good agricultural and handling practices throughout the entire production chain of fresh, ready-to-eat leafy vegetables.

CONCLUSIONS

The findings of this study reveal a high rate of parasitic contamination in lettuce samples sold in Bom Jesus do Itabapoana, with 77.14% of samples testing positive for enteroparasites, including protozoa and helminths of clinical and epidemiological significance, whose public health implications for consumers should not be

overlooked. The contamination rate observed in Bom Jesus do Itabapoana significantly exceeds the global average reported in the scientific literature and closely aligns with the elevated rates documented in various regions of the neighboring Province of Espírito Santo. This pattern suggests the influence of shared local factors, such as the use of untreated water for irrigation, inadequate sanitation infrastructure, and improper post-harvest handling practices.

The detection of pathogenic agents, including *Cryptosporidium* spp., *Giardia* spp., *Ascaris lumbricoides*, and *Balantidium coli*, some of which have previously been identified in the waters of the Itabapoana River and in fecal samples from local children, supports the hypothesis of an ongoing environmental contamination cycle. In this cycle, lettuce acts as a foodborne vector for neglected parasitic diseases, while the irrigation of crops with river water contaminated by feces from infected humans and animals perpetuates the transmission and local incidence of these parasitoses.

The presence of free-living mites in nearly one-third of the samples (28.57%) indicates widespread deficiencies in post-harvest hygiene, even in samples that tested negative for human-pathogenic parasites. This finding underscores that the absence of detectable pathogens does not necessarily reflect adherence to adequate cleaning protocols.

These results should inform and strengthen public health policies aimed at improving sanitation, enforcing rigorous monitoring of agricultural water quality, providing training for farmers and vendors in good agricultural and handling practices, and implementing targeted educational strategies for consumers of fresh produce in the municipality. Such integrated measures are essential to mitigate the risk of foodborne parasitic infections and to safeguard public health in this region

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