ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 12, Issue, 07, pp. 57196-57199, July, 2022
https://doi.org/10.37118/ijdr.24777.07.2022

RESEARCH ARTICLE OPEN ACCESS

EVALUATION OF INTESTINAL PARASITES PRESENT IN VEGETABLES SOLD IN THE CITY OF IMPERATRIZ, MARANHÃO, BRAZIL

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ARTICLE INFO

Article History:
Received 12th April, 2022
Received in revised form
19th May, 2022
Accepted 10th June, 2022
Published online 25th July, 2022

Key Words:
Enteroparasitosis, Sanitarypractices, Lactucasativa, Brassicaoleracea.

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ABSTRACT

Worldwide, diarrheal diseases caused by helminths and protozoa represent a public health problem. Despite the benefits that vegetables and fruits have as non-pharmacological prophylaxis against diseases, they are also sources of transmission of pathogens of fecal origin. The carelessness in planting and handling vegetables brings harm to human health if there is not adequate hygiene. Thus, the study aimed to analyze the presence of helminths and protozoa in leafy vegetables lettuce (*Lactucasativa*) and cauliflower (*Brassica oleracea*) sold in supermarkets and street markets in the city of Imperatriz, Maranhão, Brazil. A total of 30 samples were analyzed by the sedimentation method, all of which were collected in different months and at the same locations. Thus, the most common parasites in cauliflower and lettuce leaves were *Balantidium coli*, *Strongyloidesstercoralis* and *Ascarislumbricoides*. In addition, it was highlighted that all 30 samples were positive, and parasites such as *Hymenolepisdiminuta, Trichuristrichiura*, *Enterobiusvermicularis*, *Entamoebahistolystica* and *Giardialamblia* were also found. Based on the study, it is suggested that direct actions by specific government agencies are necessary for education and sanitary inspection, as errors in the production and commercialization of these foods represent a risk to the population.

Citation: ***Edislan da Silva Lima, Osvaldo Gomes Pereira Junior, Ediane da Silva Lima, Edilson Vitoriano Lima et al.*** "Evaluation of intestinal parasites present in vegetables sold in the city of imperatriz, maranhão, brazil*", International Journal of Development Research*, 12, (07), 57196-57199.

INTRODUCTION

Food contamination by parasites such as helminths and protozoa is a reality around the world, representing a significant public health problem. Even with the low mortality rate, contamination by intestinal parasites still an important causes of malabsorption and gastrointestinal disorders. In this scenario, it is estimated that more than 1.7 billion cases of diarrhea are reported, many of which are caused by these parasites (Li, *et al*., 2020). In this way, infection with intestinal parasites can present severe symptoms such as dehydration, vomiting, abdominal pain and diarrhea. In general, these parasites are classified as diarrheal diseases and may present different symptoms depending on the level of parasitic infection (Celestino, *et al*., 2021). According to the World Health Organization (WHO), it is emphasized that it is important to distribute antiparasitics against helminths and intestinal protozoa to the population risk, in order to control the number of infections. Cases of parasitosis are common mainly in developing and underdeveloped countries, mainly linked to health issues (Li, *et al*., 2020).

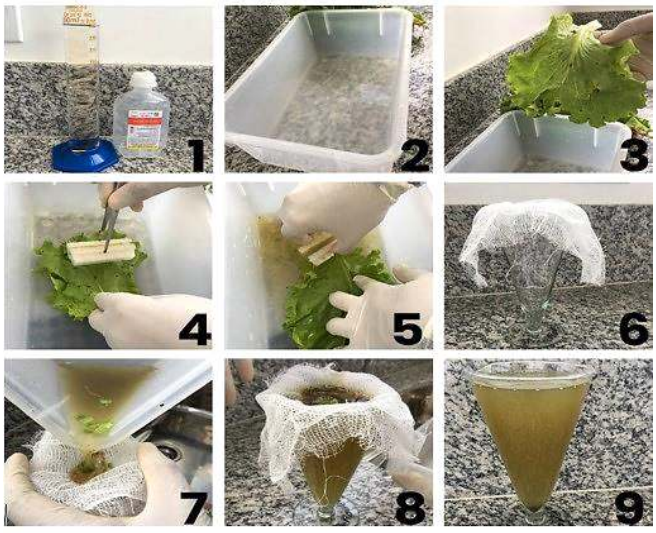
Although there are plans and actions to control and prevent parasitic infections, they are still not fully effective. One of the examples of this fact is represented by the contamination of food in the plantations, manipulation and distribution of the same (Khan, *et al*., 2021). Leaf vegetables are a widely used means of nutrition, due to their high availability and low cost for consumption. However, they also represent an easy means for the transmission of intestinal parasites to humans due to the ingestion of these contaminated foods (Rocha, *et al*., 2021). In these foods, several parasites can be found in their infectious form, such as *Entamoebahistolytica*, *Giardialamblia*, *Cryptosporidiumspp*, *Cyclosporacayenensis*, *Toxoplasma gondii*, *Balantioidescoli*, *Cystoisosporabelli*, *Blastocystissp* and *Enterocytozoonbieneusi* (Li, *et al*., 2020). Worldwide, epidemiological studies for the prevention and treatment of parasitic diseases are often interrupted due to the high cost of collecting data and testing water and food samples from people residing in the most unhealthy places (Sobral, *et al*, 2018). Therefore, there is no exact proportion of the size of the problem, added to the fact that many of these diseases are underreported and neglected (Celestino, *et al*., 2021).

The prevalence of infections caused by intestinal parasites is frequent in children aged 0 to 12 years. One of the possible explanations for the data is related to the lack of hygiene and sanitation where they live, as well as low economic conditions. In addition, added to the lack of guidance and little monitoring by government agencies, it is estimated that for this age group there are about 200 million infected worldwide (Silva, *et al*., 2021). Based on the above, the research aimed to identify the presence of helminths and intestinal protozoa in vegetables sold in the city of Imperatriz, Maranhão, Brazil.

MATERIAL AND METHODS

Sample collection: For the research, lettuce (*Lactucasativa*) and cauliflower (*Brassicaoleracea*) were used as sample units to identify the quality of vegetables at the points of sale where they were purchased, separating them by neighborhood and origin (street markets or supermarket). Thus, 3 collections were carried out at each selected location, in different months, to control the representativeness of the samples, resulting in 15 lettuce samples and 15 cauliflower samples (n total of 30 samples). The vegetables were purchased at street markets and supermarkets in the Bacuri, Mercadinho and TrêsPoderes neighborhoods of the city of Imperatriz between November 2021 and February 2022. The samples were analyzed in the clinical school of the University CEUMA *campus* of Imperatriz - MA, in the clinical analysis laboratory, in the parasitology sector.

Sample preparation for analysis: For the processing of the samples, the technique described by Rocha *et al*. (2021) was used with some modifications. In general, the samples were defoliated so that the leaves could be washed separately. A brush was used for mechanical removal of parasites during washing with saline solution (250 mL for each vegetable) in a container with a capacity of up to 2 L. After the process, the post-wash water was reserved for further parasitological analysis, as shown in Figure 1.



Fonte: *Elaborado pelo autor.*

Figure 1. Procedure for washing foliar vegetables: A beaker (250mL) was used to measure sodium chloride (1). Container with a capacity of 2L was used for washing the material (2). Washing the sheets separately (3, 4, 5). Preparation of the cup with the gauzes to strain the washing material (6, 7, 8). Filtered material settling (9)

Parasitological analysis: In order to identify the parasites present in vegetables, the Hoffman method was adopted. Thus, after preparing the material, the samples were placed in parasitological goblets for 2 hours for sedimentation, as shown in Figure 2. Two slides of each sample were prepared, stained with Lugol to improve visualization under the optical microscope. Subsequently, the intestinal parasites were identified, being recorded through photographs for each positive sample.

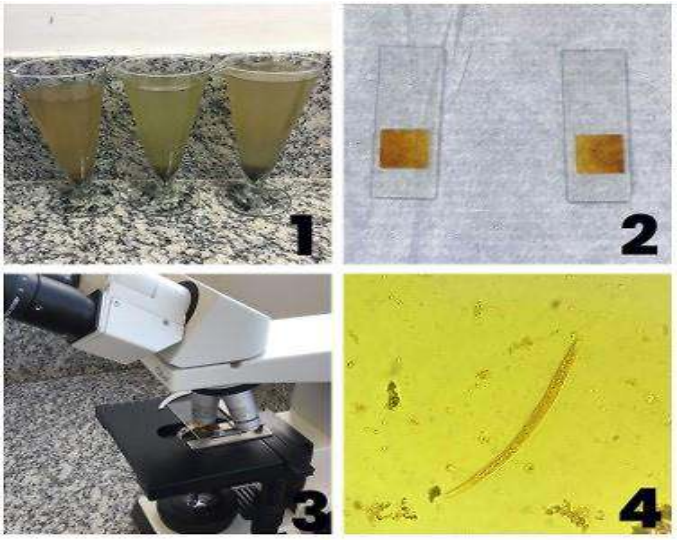


Figure 2. Sedimentation of the parasites and assembly of the slides

Data analysis: After identifying the positive samples and the respective parasites found in them, the results were tabulated and analyzed in Microsoft Excel and GraphPad Prism 6.0 programs.

RESULTS

Among the 30 samples of leaf vegetables acquired in the city of Imperatriz, the presence of human intestinal parasites was identified in all of them. As shown in Figure 3A, in the first sample of lettuce acquired in 5 different points of Imperatriz, being anstreet market and supermarkets, was observed the presence of *Balantidium coli* (5; 29%), *Strongyloides stercoralis* (5; 29%), *Ascarislumbricoides* (4; 24%), *Entamoebahistolystica* (2; 12%) and *Giardia lamblia* (1; 6%).

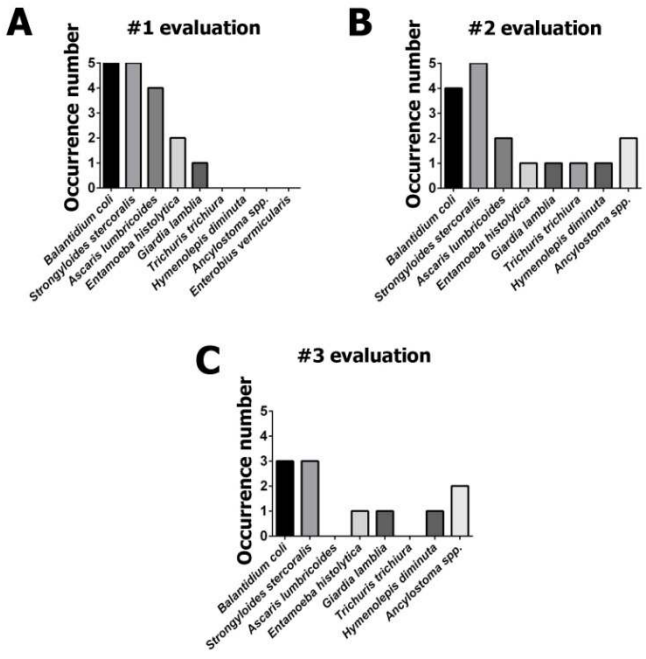


Figure 3. Enteroparasites found on lettuce leaves (*Lactuca sativa*). Parasites present in (A) first (N=5), (B) second (N=5) and (C) third (N=5) evaluation of lettuces acquired in 5 markets in the city of Imperatriz, Maranhão

Similarly, Figure 3B shows the percentage of enteroparasites on the lettuce leaf from the same 5 points, collected 1 month after the first evaluation. Thus, it was observed that in the second evaluation a greater variety of helminths and protozoa, as *Strongyloides stercoralis* (5; 29%), *Balantidium coli* (4; 23%), *Ancylostoma spp.* (2; 12%),

Ascaris lumbricoides (2; 12%), *Hymenolepisdiminuta* (1; 6%), *Giardia lamblia* (1; 6%), *Trichuris trichiura* (1; 6%) and *Entamoeba histolystica* (1; 6%). Finally, in the third evaluation (Figure 3C) of lettuce leaves, a lower rate of parasitic forms was observed: *Balantidium coli* (3; 27,5%), *Strongyloides stercoralis* (3; 27,5%), *Ancylostoma spp.* (2; 18 %), *Giardia lamblia*(1; 9%), *Hymenolepisdiminuta* (1; 9%) *Entamoeba histolytica* (1; 9%). In turn, the cauliflower samples showed a lower number of parasite diversity, compared to that found in lettuce. As shown in Figure 4A, the first cauliflower sampling showed *Balantidum coli* (4; 36.5%), *Strongyloides stercoralis* (4; 36.5%), *Ancylostomaspp* (1; 9%), *Ascaris lumbricoides* (1; 9 %) and *Entamoeba histolystica* (1; 9%).

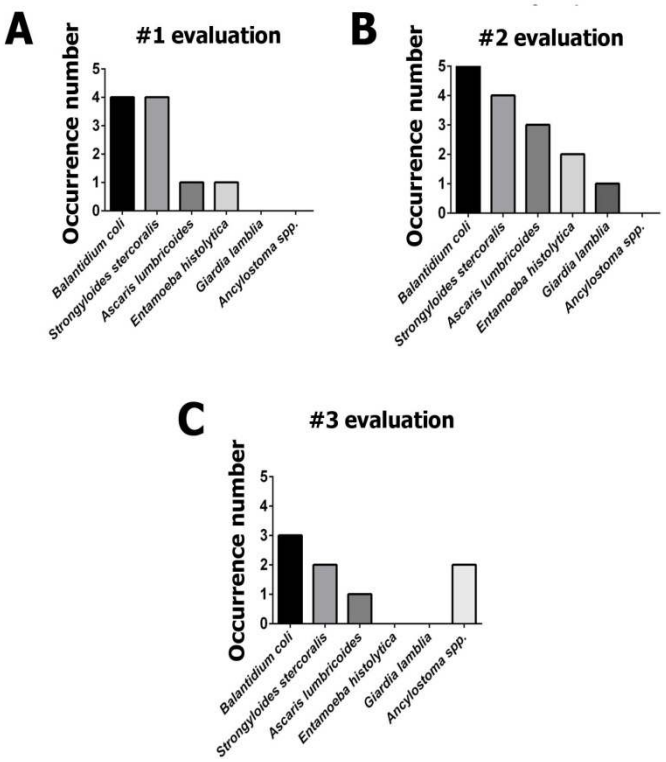


Figure 4. Enteroparasites found in cauliflower (*Brassica oleracea*) leaves. Parasites present in (A) first (N=5), (B) second (N=5) and (C) third (N=5) evaluation of cauliflower acquired in 5 markets in the city of Imperatriz, Maranhão

The second cauliflower sample showed slightly different percentages from the first evaluation (Figure 4B). Thus, was identified the presence of *Balantidium coli* (5; 33%), Strongyloides stercoralis (4; 27%), *Ascaris lumbricoides* (3; 20%), *Entamoeba histolystica* (2; 13%) and *Giardia lamblia* (1; 7). %). At the end, the third sample of cabbage (Figure 4C) identified the parasites *Balantidium coli* (3; 37.5%), *Strongyloides stercoralis* (2; 25%), *Ancylostomaspp*(2; 25%), *Ascaris lumbricoides* (1 ; 12.5%). As shown in figures 3 and 4, another important point was the presence of polyparasitism, highlighting *Strongyloidesstercoralis* and *Balantidiumcoli* with a constant frequency in the analyzed samples, they were found present in 100% of the samples. This result is important because it corroborates the findings in the literature, which demonstrate the high prevalence of infections by these parasites in the world (Castiñeira and Martins, 2006). In the same way, disease-causing parasites were found in animals such as *Dipylidium caninum* and *Trypanoxyurisspp*, and, to a lesser extent, free-living parasites (not shown in the results). According to Figure 5, it is observed that the most frequent parasites found in cauliflower and lettuce leaves were*Balantidium coli*, *Strongyloides stercoralis* and *Ascaris lumbricoides*. Followed by those mentioned, the intestinal protozoa that cause diarrheal syndromes in children, elderly and frail adults were also frequently identified.Similar to the present study, according to Celestino *et al.* (2021), *Giardia lamblia*, *Entamoeba coli* and *Ascaris lumbricoides* had the highest rate of contamination in Brazil.

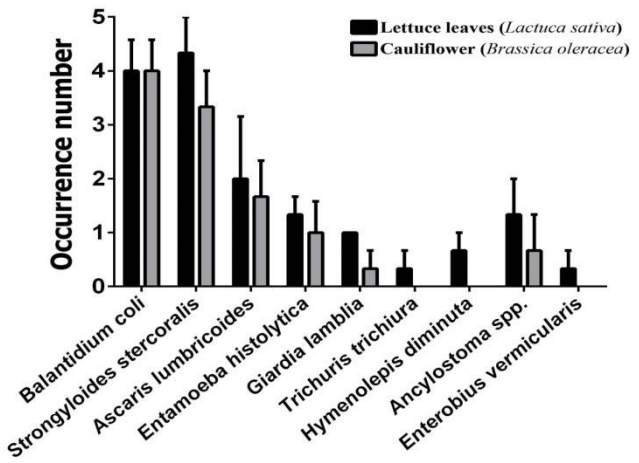


Figure 5. Comparison between enteroparasites found in cauliflower (*Brassica oleracea*) and lettuce (*Lactuca sativa*) leaves sold in Imperatriz, Maranhão. N=15 lettuces; N= 15 cauliflowers

DISCUSSION

Brazil has high rates of intestinal parasites, but their real prevalence in numbers still unknown because they are not included in the notifiable diseases. Thus, brazilians studies report that the prevalence of infections by human intestinal pathogens as 2 to 36%. However, this number can be much higher when a specific age group is selected, such as school-age children, reaching 70% (Teixeira, *et al*., 2020). Helminths belong to the group of free-living or parasitic metazoans, and humans are the definitive host of several species. They can be further divided according to the place of the evolutionary cycle, being the geohelminths those that occur in part in the soil, and the biohelminths that pass through more than one host besides humans. The first group includes egg-infecting worms, such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostomaspp*, *Necator americanus*, *Strongyloides stercoralis*, *Enterobius vermicularis*. In turn, examplesofbiohelminths are *Schitosoma mansoni*, *Taeniasp*, *Hymenolepis nana* (Melo, *et al*., 2020). In addition to the parasites mentioned above, intestinal protozoa such as *Giardia lamblia* and *Entamoeba histolytica* represent important causes of diarrhea in children and adults (Li, *et al*., 2020). In view of the helminths and protozoa described, it is known that the prevalence of these infections is more frequent in children aged 0 to 12 years. In addition, it is also highlighted that situations of vulnerability, such as lack of hygiene, sanitation, economic condition, access to food in good condition, lack of guidance and little monitoring by government agencies are relevant points for the lack of control of these parasites (Silva, *et al*., 2021). In the cases presented in this study, there is a possibility of contamination of these foods, ranging from errors in their production, conservation and handling. If the consumer does not clean them correctly before consumption, contamination may occur. As for contamination in production, it is noteworthy that the parasites of the geohelminths group are present in the soil, hence the need for constant control and evaluation of the place where vegetables are planted and vermifuges are used. In turn, during conservation, a contaminated food can be reserved with another uncontaminated one, triggering the spread of parasites. Finally, like *Ancylostomaspp*, when improperly handled and sanitized, the parasite can contaminate through skin penetration without necessarily ingesting it (Punsawad, *et al*., 2019). Thus, one of the possible causes associated with the spread of enteroparasites is also due to the lack of health professionals voted for epidemiological research actions, as there is still underreporting of cases. It is also noteworthy that the failure to carry out routine consultations for the evaluation of intestinal parasites together with self-medication can negatively influence this underreporting scenario (Lago, *et al*., 2020). Knowledge of adequate techniques for cleaning leaf vegetables before consumption is of great importance in order to reduce the number of infected. The use of 1% sodium hypochlorite in the cleaning of these leafy vegetables has

been shown to be effective in combating these pathogens (Silva, *et al*., 2020). In addition, leaf vegetables need to be washed in running water, manually rubbing leaf by leaf, as eggs and cysts can be eliminated by mechanical friction (Lima, *et al*., 2020). It is known that the immune response of the human body plays an important role in recognizing and fighting the organism with pathogenic potential. Such protection occurs through innate and adaptive immunity. The first is represented by the skin, mucous membranes, neutrophils, macrophages, dendritic and antigen-presenting cells, for example. While the adaptive is presented by T and B lymphocytes (Silva, *et al*., 2020; Cardoso, *et al*., 2021). Thus, infections by intestinal parasites can still happen opportunistically, where the parasites take advantage of the compromise of the innate and adaptive immune system. In these cases, the clinical presentation of the disease is usually more severe, so that the individual develops severe disease in a rapid and often lethal manner. In this scenario, patients with Acquired Immunodeficiency Syndrome (AIDS) belong to the risk group for many of these parasitoses, due to their low CD4 T cell count (Paes, *et al*., 2020). In addition, infections by intestinal parasites interfere with the absorption of nutrients, which can also result in intestinal bleeding and intestinal obstructions. The signs and symptoms resulting from this process are decreased absorption of iron, folate and B12vitamin, in addition to chronic blood loss, abscess formation and rectal prolapse (Rocha, *et al*., 2021). Among the ways to eliminate the aforementioned parasites, there are benzimidazole drugs such as albendazole, mebendazole, thiabendazole and cambendazole, in addition to nitroimidazole compounds such as metronidazole and tinidazole. The benzimidazoles promote the inhibition of the parasitic fumarate reductase enzyme, thus blocking the uptake of glucose and amino acid by the helminth. In turn, nitroimidazole compounds inhibit the proliferation of parasites by interrupting DNA replication (Silva, ***et al***., 2019). According to the Ministry of Health, in Brazil, antiparasitic drugs should be indicated based on their dosage, time of use, place of residence, age and level of infection that the patient is. It is necessary to monitor their use by a health professional, as the adverse effects of these drugs must be evaluated to avoid possible, ensuring greater effectiveness in the treatment (Silva, ***et al***., 2020). In order to guarantee of safety and quality in the food distributed in Brazil, the National Health Surveillance Agency (ANVISA) has as one of its duties the inspection and monitoring of the procedures provided in the production, handling and distribution of food. In its principle, it supervises and determines standards for Good Manufacturing Practices with the help of the Technical Regulation of Standard Operating Procedures nº 275 of 2002 in order to reduce the risks to the health of consumers of these foods (Manhique, 2020).

CONCLUSION

The foliar vegetables acquired in supermarkets and open markets under study showed high rates of contamination by helminths, especially those that contained remnants of soil used in the plantation. This contamination may have occurred in the planting, transport or handling of these vegetables. Therefore, consumption without adequate hygiene becomes a source of mass contamination due to the large consumption of these foods on a daily basis. The present study reports the vulnerability in inspection during the production and marketing of these foods, the lack of information makes the ingestion of these intestinal parasites frequent and exposes a need in education programs for society.

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