

Contamination by intestinal parasites in
vegetables marketed in an area of
*Jequitinhonha Valley, Minas
Gerais, Brazil*

*Contaminação por parasitas intestinais
em hortaliças comercializadas em
uma área do Vale do Jequitinhonha,
Minas Gerais, Brasil*

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ABSTRACT

Objective

The present study aimed to evaluate the presence of helminthes and intestinal protozoa in vegetables commercialized in *Diamantina*, a municipality located at *Jequitinhonha Valley*, one of the poorest regions of the world.

Methods

A total of 108 specimens, including lettuce, green onion and rocket, were monthly collected from the most popular open street market, green grocery and supermarket of the municipality. The samples were processed by a concentration method and evaluated by light microscopy for parasitological identification.

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Results

The percentage of contamination was 50.9% (55/108), with predominance of nematode larvae (36.5%), cysts of *Entamoeba coli* (26.0%) and eggs of hookworms/*Strongyloides* spp. (12.9%). Lettuce showed greater contamination rate (61.1%) and samples from the open street market were more contaminated (77.8%). Information collected at each point of sale pointed the field cultivation as the critical step for such contaminations.

Conclusion

Vegetables marketed in *Diamantina* presents a wide variety of intestinal parasites, which may represent a potential risk to the health of consumers of fresh vegetables.

Keywords: Foodborne diseases. Helminthiasis. Parasites. Vegetables.

RESUMO

Objetivo

O presente estudo teve como objetivo avaliar a presença de helmintos e protozoários intestinais em hortaliças comercializadas em Diamantina, um município localizado no Vale do Jequitinhonha, uma das regiões mais pobres do mundo.

Métodos

Cento e oito exemplares, incluindo alface, cebolinha e rúcula, foram mensalmente coletados em uma feira livre, uma quitanda e um supermercado do município. As amostras foram processadas por um método de concentração e avaliadas por microscopia óptica para pesquisa de estruturas parasitárias.

Resultados

*O percentual global de contaminação foi de 50,9% (55/108), com predominância de larvas de nematódeos (36,5%), cistos de *Entamoeba coli* e ovos de ancilostomídeos/*Strongyloides* spp. (12,9%). A alface demonstrou a maior taxa de contaminação (61,1%) e as amostras da feira livre foram as mais contaminadas (77,8%). Informações coletadas em cada ponto de venda apontaram o cultivo em campo como a etapa crítica para a contaminação.*

Conclusão

Hortaliças comercializadas em Diamantina apresentam uma ampla variedade de parasitas intestinais, o que representa um risco potencial à saúde dos consumidores da área.

Palavras-chave: Doenças transmitidas por alimentos. Helmintíase. Parasitos. Verduras.

INTRODUCTION

It is well known that daily consumption of vegetables provides numerous benefits to human health with several implications for improving quality of life, once that they are sources of nutrients, help in the prevention of chronic diseases, weight reduction and maintenance, among other benefits¹. Thus, the World Health Organization (WHO) considers their consumption a priority for countries' nutrition, food and agricultural policies². Despite the great importance of a diet rich in this kind of food, the habit of consuming poorly washed raw vegetables could expose individuals to the risk of infection by several

microorganisms, such as intestinal parasites and commensals, since protozoan cysts and helminth eggs and/or larvae can be conveyed along with them³. The number of human infections and outbreaks associated with the consumption of raw vegetables contaminated with these foodborne pathogens has increased in the past decades, mainly in developing countries, where they represent a public health concern that is still underestimated^{4,5}.

Vegetable contamination with parasitic structures may occur at various stages of their production and commercial chain, in particular through the: sanitary conditions of field cultivation; use of fertilizers; quality of water used

for irrigation and washing; storage; transportation; exhibition and handling by infected individuals, as well as due to contact with flies, rats and domestic animals^{6,7}. However, parasitological contamination frequently occurs during the field cultivation of vegetables⁸, mainly due the use of contaminated water sources and organic fertilizers for irrigation and soil fertilization, respectively⁹.

Diamantina is a very important city of the *Jequitinhonha* Valley, one of the poorest regions of the world. Although the periodic long droughts are a relevant factor for the low performance of agriculture in the region, this activity is still responsible for 30% of regional gross domestic product¹⁰. In addition, due to the low socioeconomic indices, several public programs for the cultivation and commerce of vegetables based on familial agriculture have been implanted in rural areas of the region¹¹. However, the precarious sanitary conditions often found in these areas combined with the scarcity of water sources, may favor the use of water contaminated with fecal waste for agricultural purposes.

Although few studies have already reported that vegetables grown and marketed in some Brazilian regions present poor parasitological quality^{7,12}, in *Diamantina* city there are no data concerning the exposure of the population to vegetables contaminated with parasitic structures. Therefore, the present study aimed to qualitatively assess the presence of this kind of contaminant in vegetables commercialized in different retail establishments of such *Jequitinhonha* Valley locality.

METHODS

Study area

The municipality of *Diamantina* (18°9'S 43°22'W) is a reference center that occupies a total area of 3,891.654km² and has an estimated population of 47,803 inhabitants¹³. It is located in Southeastern Brazil at an altitude of 1296 m in

the Northeast of the State of *Minas Gerais* (Figure 1), more specifically in the "High *Jequitinhonha* Valley", a region characterized by the presence of the hydrographic basin of *Jequitinhonha* River and by a remarkable rate of poverty. The commerce of foodstuffs has great relevance in the local economy. Thus, in order to supply the internal and surrounding consumer market, the cultivation of vegetables is extensively practiced in the rural areas of the municipality.

Sample collection

A total of 108 specimens of fresh vegetables were analyzed, including lettuce (*Lactuca sativa* L.) (n=36), green onion (*Allium fistulosum* L.) (n=36) and rocket (*Eruca sativa* L.) (n=36). These vegetables were chosen because they are eaten raw, have high consumption by the local population, large volume of trade and cultivation in the municipality, availability at all times of the year, substantial contact with the ground and need for intensive fertilization and irrigation during cultivation. The sampling unit herein established was one head for lettuce and one sheaf for green onion and rocket, regardless of weight or size. All the samples were cultivated in traditional ways.

Sampling was carried out monthly, between February and July 2013, at the city's most popular retail markets, including an open street market, a supermarket and a green grocery. Thus,

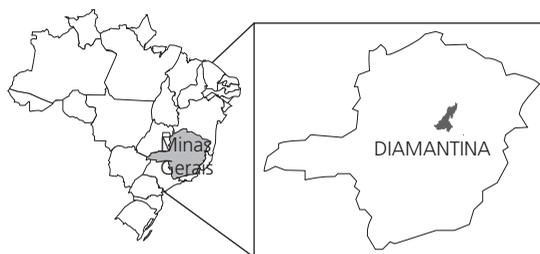


Figure 1. Location of study area. The location of the municipality of *Diamantina* in the Northeast of *Minas Gerais* State, Brazil.

two samples of each vegetable with approximate dimensions, good quality and preserved visual characteristics were randomly purchased and transported in sterile nylon bags for analysis at the *Laboratório de Doenças Parasitárias* of the *Universidade Federal dos Vales dos Jequitinhonha e Mucuri, Diamantina, Minas Gerais State, Brazil*.

In order to better understand the different contamination levels at the evaluated retail establishments, some information about the steps of irrigation, soil fertilization, washing, harvest, transport, handling and exhibition of the vegetables at the point of sale was obtained from the farmers and/or sellers at the time of sample collection

Detection of parasitic structures

The parasitological analyses were performed according to Oliveira & Germano¹⁴ with some modifications. Briefly, the samples were prepared by separating leaf by leaf, and any wilted, stained or damaged leaves were discarded. In a stainless tray, each leaf was washed using a flat paintbrush and a recently prepared detergent solution – 10mL of Tween 80 diluted in two liters of physiological saline solution (0,85% NaCl – sodium chloride – w/v). Each vegetable was washed with a maximum volume of 200mL of this solution.

Subsequently, leaves were discarded and the resulting liquid was filtered through gauze and collected in a sedimentation glass. The tray used was also washed with 50mL of the detergent solution and the resulting liquid was transferred to the same glass, which was covered with a Petri plate and left to settle for 24 hours at room temperature.

After that, the top liquid was discarded and the sediment with approximately 50mL of the remaining washing water was centrifuged at 855 x g for 2 minutes. The supernatant was carefully removed and a drop of the residue was transferred to a clean glass slide and mixed with

a 20µL of Lugol's Iodine solution. The preparation was examined systematically in triplicate under light microscopy for detection of parasitic structures using 10x and 40x objectives.

Statistical analysis

Statistical analyses were performed by Chi-square test in GraphPad Prism version® 5.00 for Windows (San Diego, California, United States of America) in order to compare the rate of parasitological contamination among different vegetables and different commercial establishments. The differences were considered significant at p -value ≤ 0.05 .

RESULTS

In the present study, 50.9% (55/108) of the fresh vegetable samples from different types of retail markets of *Diamantina* were positive for helminth eggs and/or larvae and protozoan cysts. Among them, 28.7% (31/108) were contaminated with only one structure type and 22.2% (24/108) with at least two species. The global contamination was similar among the three types of vegetable analyzed ($p=0,317$), with contamination rates detected of 61.1% (22/36) for lettuce, 44.4% (16/36) for green onion and 47.2% (17/36) for rocket (Table 1).

Helminth structures, *i.e.* eggs and/or larvae, corresponded to 68.2% of the findings in the samples analyzed, which was significantly higher than the presence of protozoan cysts (31.8%) ($p<0.001$). The species detected in the positive samples of each type of vegetable analyzed from each establishment evaluated are summarized in Table 2, where species with similar morphology are represented together. It can be noted that filariform and rhabditiform nematode larvae were the most frequent findings, followed by *Entamoeba coli* cysts and hookworm/*Strongyloides* spp. eggs.

Table 1. Vegetable samples positive for parasitic structures with mono and multiple contaminants sold in different retail establishments from *Diamantina*, Brazil, 2013.

Vegetable	Positive samples		One contaminant		Multiple contamination	
	n	%	n	%	n	%
Lettuce (n=36)	22	61.1	12	33.3	10	27.8
Green onion (n=36)	16	44.4	10	27.8	6	16.7
Rocket (n=36)	17	47.2	9	25.0	8	22.2
Total (n=108)	55	50.9	31	28.7	24	22.2

Table 2. Prevalence of intestinal protozoa and helminths in vegetable samples collected from the retail market of *Diamantina*, Brazil, 2013.

Intestinal protozoa or helminth	n (%) Positive											
	Open street market				Greengrocery				Supermarket			
	L n=12	GO n=12	R n=12	Total N=36	L n=12	GO n=12	R n=12	Total N=36	L n=12	GO n=12	R n=12	Total N=36
Nematode larvae	6 (50.0)	8 (66.7)	3 (25.0)	17 (47.2)	4 (33.3)	-	1 (8.3)	5 (13.9)	4 (33.3)	5 (41.7)	-	9 (25.0)
<i>Entamoeba coli</i>	4 (33.3)	4 (33.3)	3 (25.0)	11 (30.6)	5 (41.7)	1 (8.3)	-	6 (16.7)	2 (16.7)	2 (16.7)	1 (8.3)	5 (13.9)
Hookworm/ <i>Strongyloides</i> spp.	1(8.3)	3 (25.0)	4 (33.3)	8 (22.2)	-	-	-	-	-	2 (16.7)	1 (8.3)	3 (8.3)
<i>Hymenolepis nana</i>	2 (16.7)	1(8.3)	3 (25.0)	6 (16.7)	-	-	-	-	1 (8.3)	-	-	1 (2.8)
<i>Entamoeba histolytica/dispar</i>	-	-	-	-	-	-	-	-	-	-	3 (25.0)	3 (8.3)
<i>Fasciola hepatica</i>	-	-	-	-	-	-	-	-	1 (8.3)	-	2 (16.7)	3 (8.3)
<i>Ascaris</i> spp.	2 (16.7)	-	1 (8.3)	3 (8.3)	-	-	-	-	-	-	-	-
<i>Taenia/Echinococcus</i> spp.	-	-	-	-	-	-	-	-	-	2 (16.7)	1 (8.3)	3 (8.3)
<i>Endolimax nana</i>	-	-	1 (8.3)	1 (2.8)	-	-	-	-	-	-	-	-
<i>Giardia duodenalis</i>	-	-	1 (8.3)	1 (2.8)	-	-	-	-	-	-	-	-
Total	9 (75.0)	10 (83.3)	9 (75.0)	28 ^a (77.8)	7 (58.3)	1 (8.3)	1 (8.3)	9 ^b (25.0)	6 (50.0)	5 (41.7)	7 (58.3)	18 ^c (50.0)

Note: Different letters above total values represent significant differences ($p \leq 0.05$). The bold values represent the total of contaminated vegetables per retail market.

L: Lettuce; GO: Green Onion; R: Rocket.

Regarding the type of commercial establishment, parasitic contaminants were detected in 77.8% (28/36), 50.0% (18/36) and 25.0% (9/36) of the samples acquired in an open street market, a supermarket, and a greengrocery from *Diamantina*, respectively. Samples from the open street market were significantly more contaminated than other retail establishments ($p < 0,001$). Taking into account the type of vegetable, at the open street market, parasitological contamination was found in 83.3% of green onion (10/12), 75.0% of lettuce (9/12) and 75.0% (9/12) of rocket samples. At the greengrocery, lettuce was the most contaminated vegetable, with intestinal parasites

being detected in 58.3% (7/12) of the samples, followed by rocket (8,3%, 1/12) and green onion (8.3%, 1/12). It was detected contamination in 58.3% (7/12) of the rocket samples from the supermarket, followed by 50% of lettuce (6/12) and 41.7% of green onion samples (5/12) (Table 2).

Chart 1 summarizes information about the commercial and production chain of the vegetables obtained at each point of sale during sample collection. It can be noted that the main differences between the establishments are related to the irrigation, fertilization and washing of the vegetables, which represent steps associated with the cultivation stage in the field.

Chart 1. General information about some steps involved in the production and commercial chain of vegetables marketed in different retail markets of *Diamantina*, Brazil, 2013.

Stage	Open street market	Greengrocery	Supermarket
Irrigation source	Nearby stream	Artesian well	Not reported
Soil fertilization	Animal manure	Not reported	Not reported
Washing	Not carried out	Treated water before and during the exhibition	Treated water before and during the exhibition
Harvest	Carried out close to commercialization	Carried out close to commercialization	Not reported
Transport	Sealed boxes	Sealed boxes	Sealed boxes
Handling	Without hand washing or protection	Without hand washing or protection	Without hand washing or protection
Exhibition at point of sale	Vegetables out of plastic bags, exposed away from the floor on clean countertops and handled successively by several customers.	Only lettuce packaged in plastic bags, exposed away from the floor on clean countertops and handled successively by several customers.	Only lettuce packaged in plastic bags, exposed away from the floor on clean countertops and handled successively by several customers.

Note: The reports were obtained from the farmers and/or sellers during sample collection.

DISCUSSION

The data obtained from the current study suggested that vegetables marketed by the three most popular retail establishments of *Diamantina* are contaminated with a wide variety of intestinal parasites, which may represent a risk to the health of consumers of raw vegetables from the city. According to Brazilian legislation, vegetables for human consumption should not carry parasites and worms¹⁵. Thus, the samples here analyzed are considered to be of poor quality, since more than half of the specimens were positive for these contaminants.

Although high, the parasitological contamination found does not differ substantially from studies conducted in other areas of the country^{12,16} and abroad³. On the other hand, the present results contrast with the absence of contamination reported in vegetables sold in *Rio de Janeiro*, Brazil, where none of the analyzed samples showed such structures¹⁷. Abougrain *et al.*¹⁸ suggested that differences in prevalence rates between investigations are expected, probably due the origin of the vegetables and the consequential differences in cultivation, transport and storage of them.

The types of vegetables herein analyzed are widely produced and consumed in

Diamantina. Although the contamination rate found among them was statistically similar, a little variation was detected, which may be attributed to the anatomical differences of vegetable foliage. For example, lettuce presents broad leaves, with a large surface area and a compact structure that provides better fixation and permanence of parasitic structures¹⁹. In addition, the flexibility of its leaves can facilitate contact with the soil during cultivation and, consequently, with helminth structures probably found at this site¹². On the other hand, vegetables like green onions have cylindrical fine leaves, a smaller surface area and less contact with the ground, which can minimize the chances of contamination.

According to Falavigna *et al.*⁶, the remarkable presence of helminth rather than protozoan structures detected in present study is probably related to the practice of washing vegetables in the field and, subsequently, at the point of sale prior to commercialization. These washing steps may be more efficient in the removal of protozoa, since the structure and size of helminths may hinder their removal from the surface of vegetables. In current investigation, sellers from the majority of the establishments (supermarket and green grocery) reported the adoption of such practices.

The presence of different parasitic species demonstrates that the population of *Diamantina* is supposedly exposed to several debilitating diseases, such as hookworm infections, ascariasis, giardiasis and amebiasis. Indeed, a survey recently carried out in the city demonstrated that local children were infected with some of these pathogens²⁰. The varied contamination should be related to the fact that these kinds of parasites share many similarities in their life cycle, especially in relation to the elimination route of potentially contaminant structures, *i.e.* human and animal feces. In this context, the presence of animals and humans infected with these pathogens in rural areas of the region, as already described by Pereira *et al.*²¹, associated with precarious sanitary installations, could explain possible contamination of soil and water sources used for cultivation of the vegetables.

The demonstrated high frequency of filariform and rhabditiform nematode larvae supports this idea, since besides humans it is well known that some of these species can also parasitize several animals usually kept in rural areas of the municipality, such as pigs, cattle, dogs and cats²². Due to their morphological similarities and nature of the analyzed samples, it was not possible to identify the genus and species of larvae found. Nevertheless, attention should be drawn to the possible presence of hookworms and/or *Strongyloides stercoralis* filariform larvae on the samples, as they are capable of actively penetrating human skin or mucous membranes. This presents great clinical and epidemiological relevance, especially in malnourished or immunosuppressed individuals, which are vulnerable to severe forms of hookworm infections and strongyloidiasis, respectively²³.

Cysts of the commensal *E. coli* were also detected with high frequency by the present investigation. This elevated contamination corroborated the study conducted by Guilherme *et al.*²⁴ on vegetables marketed in *Maringá*, *Paraná*, Brazil, and is probably the result of the high viability of amebic cysts in the environment,

which is about 20 days in the presence of moisture and in the absence of direct sunlight²⁵. Despite being nonpathogenic, these findings represent a public health problem due to the fact that cysts are also eliminated with feces.

Regarding the different commercial establishments evaluated, parasitic structures were detected in all of them, but the samples from the open street market were significantly more contaminated. Brazilian open street markets are characterized by the sale of agricultural products grown in rural areas close to major centers, where farmers exhibit their products in tents and consumers move between. In *Diamantina*, the implanted public programs for the development of familial agriculture contribute to the importance of this kind of establishment to the local economy, being the most direct and lucrative way for the farmers to sell their agricultural products¹¹. Thus, the high percentage of contamination detected at the most frequented open street market of the city reinforces the considerable risk to which the local population is exposed.

The farmers from the open street market reported the use of animal manure and water from a nearby stream in field cultivation, along with the absence of washing vegetables before and during the exhibition. Taken together, these practices may explain the higher contamination detected at that establishment, since the quality of the water used is unknown, and it may receive untreated domestic sewage or be susceptible to fecal contamination by various mechanisms²⁶. In accordance with this supposition, studies carried out in Brazil have already demonstrated the presence of fecal contamination in river water sources used for the irrigation of crops across the country²⁷. Moreover, the use of animal manure as soil fertilizer is a well-known source of human pathogens that can survive there for a considerable time²⁸. Therefore, contaminant structures may be transferred to the produce and remain there for a long time, as previously demonstrated by Mootian *et al.*²⁹.

Transport, handling and exhibition at the point of sale can also influence the parasitological contamination of vegetables⁷. In current study, these steps probably were not critical enough to provide the contamination found, since similar and consistent information was reported at all the establishments. However, no farmers or merchants reported the use of disposable gloves or hand washing prior to handling vegetables. The failure of such practices associated with poor hygienic and sanitary habits may contribute to the contamination of vegetables if the handlers carry infections by intestinal parasites. This is more critical especially at small establishments, where it is common for the same trader to handle money and vegetables without washing their hands. As demonstrated by Saturnino *et al.*³⁰, paper money is an important route for transmission of intestinal parasites structures.

Although samples of water, soil or fertilizer used in the production of the vegetables were not analyzed, the present findings strongly suggest that contamination occurred during the field cultivation. Thus, an integrated chain of approaches, from the field to the consumer, must be performed in the region aiming to significantly reduce the level of contamination, ensure food safety and prevent its risk to public health⁷. Among these approaches, a thorough investigation of the production chain of these vegetables should be conducted, in order to ascertain the real critical points, alongside improving the hygienic and sanitary standards of the rural population, improving cultivation practices through practical education programs for the farmers, and periodic inspections by the local competent health and environmental authorities.

Additionally, the implementation of public awareness campaigns about the importance of properly washing and disinfecting raw vegetables before consumption is crucial. In this way, according to the Brazilian Ministry of Health, before being prepared and consumed, the vegetables should be washed with tap water followed by immersion in sodium hypochlorite

solution³¹. Indeed, recently Gomes Neto *et al.*³² showed that this compound is effective in reducing microorganism counts in leafy vegetables, even in high levels of contamination.

CONCLUSION

The findings indicated that vegetables marketed by the most popular retail establishments of *Diamantina* are contaminated with structures from a wide variety of intestinal protozoa and helminths, which may represent a risk to the health of consumers of raw vegetables from the area.

CONTRIBUTORS

JGG LUZ designed the study, participated on the collection and laboratory examination of the samples and wrote the paper. MV BARBOSA and SD RESENDE participated on the laboratory examination of the samples. AG CARVALHO participated on the collection of the samples and wrote the paper. JVL DIAS and HR MARTINS analyzed the data and revised the manuscript. HR MARTINS designed the study. All the authors read and approved the final version of the manuscript.

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