



## ORIGINAL ARTICLE

# Occurrence of enteroparasites in stallholders and their role as disseminators in Vitória, Espírito Santo, Brazil

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#### Keywords

#### Abstract

Food Parasitology; Parasitic Diseases; Market Sanitation; Helminths; Protozoan Infections; Health Education

Introduction: The enteroparasitosis are among the diseases transmitted by food due to its improper handling. Studies that evaluate the parasites in food handlers professionals may shed light on the matter, as well as aid in the control of their dissemination. **Objective:** Assess the occurrence of enteroparasites in stallholders and characterize their importance in the dissemination of enteroparasitosis to customers. Methods: The samples were composed of biological material collected from stallholders in the municipality of Vitória, state of Espírito Santo, Brazil. The material from the stallholders' nail beds and hand palms were obtained through swab and analyzed by the modified Mello and collaborators method. Three stool samples were obtained and then analyzed by the Hoffmann, Pons and Janer method. Results: The helminths and protozoans with the highest prevalence in feces were: Blastocystis sp. (67.0%), Ascaris lumbricoides (40.0%), hookworms (40.0%), Giardia lamblia (20.0%), and Schistosoma mansoni (13.3%). Regarding the eggs and cysts found on the hands, they are: A. lumbricoides (33.3%), hookworms (16.7%), Rodentolepis nana (16.7%), and G. lamblia (16.7%). The results indicate that food handlers still represent a risk factor in the transmission of enteroparasites to the population. Conclusion: The

poor hygienic control, from food cultivation to commercialization, represents one of the main reasons for the dissemination of pathogens. Thus, in order to reduce the prevalence and transmission of these enteroparasites in markets, an intervention is fundamental through educative-sanitary measures.

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#### Introduction

Foodborne diseases (FDs) represent a major public health issue and negatively reflect on the health of populations and on the economic development of countries, leading to labor incapacity and costs with treatments and hospitalization.<sup>1,2</sup> In Brazil, between 1999 and 2004, 3 million hospitalizations due to FDs were recorded, with an average of more than 500 thousand cases and 8 thousand deaths per year.<sup>3</sup>

According to the Atlanta Center for Disease Control and Prevention (CDC), most of the FDs are linked to the inadequate handling of food. Therefore, food handlers play an important role in the transmission, partly due to poor personal hygiene and domestic habits, besides a deficiency in hygiene and environmental control.<sup>4,5</sup>

Some intestinal parasitosis can be found among FDs. In Brazil, despite of the decline of enteroparasites in the last decades, they still prevail in a high rate where the socioeconomic status of the population is low, being a cause of relevant health aggravation.<sup>5-7</sup>

Several scientists made evident the transmission of helminths and protozoans to humans through the ingestion of fruits and vegetables consumed raw, from cultivated areas contaminated by fecal matter.<sup>8-10</sup> Even asymptomatic carriers may contaminate food while handling it.<sup>11</sup> However, despite being a relevant issue, there seems to be little research that characterizes the occurrence of enteroparasites in food handlers and their importance in dissemination in a given environment.

Given the latter, this study aims at evaluating the occurrence of enteroparasite eggs and cysts in the hands and feces of stallholders in order to highlight the importance these professionals play in the transmission of entereoparasites to their clients. For this study, it was chosen as a model an open-air market, located in a wealthy district in the city of Vitória, state of Espírito Santo, Brazil.

## Method

General considerations:

The samples of the present study are composed of socioeconomic data, habits, and biological material from in natura food handlers who work in an open-air market located in a wealthy district in the municipality of Vitória, state of Espírito Santo, Brazil. All market professionals have been informed of the project and invited to take part of it, being the following its required criteria to join it: be over 18 years old; work with in natura food handling; accept the research terms by signing the Informed Consent Form (ICF). The stallholders who accepted to take part of the study responded to a socioeconomic and habit questionnaire. Afterwards, nail beds and hand palm biological material were collected, besides stool samples.

Socioeconomic data and hygiene habits:

The stallholders responded to a socioeconomic questionnaire containing the following items: age, gender, marital status, ethnicity, hometown, municipality of residence, household type and

characteristics, area where they live, occupation, workload, level of education, access to means of information, family income, type of agriculture, basic sanitation, and hygiene habits.

## Collection of biological material:

Nail bed material was collected from all fingers of each stallholder, as well as the material from their hand palms. The method utilized was the swab technique rinsed in a fixative solution of acetic acid, sodium acetate, distilled water and formalin (SAF). During the collection, the material was promptly packaged in the same substance in a test tube. This solution also acts in the preservation of the parasites' infecting forms.

For the stool test, the stallholders were told to collect three samples, in consecutive weeks, in a dischargeable recipient, utilizing the built-in collection instrument, containing a 10% formalin solution for conservation. After each week of collection, the recipients were taken to laboratory for analysis.

# Sample analysis:

The material collected from nail beds and hand palms was processed following the Mello, Souza- $Jr^{12}$  modified method, which consists in the vigorous agitation and later centrifugation of the sample for 10 minutes, under a speed of 3,000 rpm. After that, samples were analyzed in a microscope. It has been determined that the duration and speed of centrifugation be higher than the ones found in the literature (1,500 rpm for 2 min), since in the event of lower ones, litter was still present on the sides of the centrifuged tubes, which would affect the sediment quality analysis.

The stool samples were processed according to the Hoffman, Pons,<sup>13</sup> method, which consists in dissolving feces in water, filtering them through an appropriate gauze pad, and after allowing them to sediment in a conical graduate for 24 hours, and pipetting the accumulated deposit in the bottom. The use of the conical graduate

provides a shorter fashion in sample sedimentation, increases parasite concentration for a microscopical analysis, minimizes risks of contamination, reduces odor and optimizes the workplace. In addition to the latter, this method can be easily carried out, provides a low-cost option, has a great sensibility in detecting helminths and protozoans, using conserved or fresh stool samples.<sup>14</sup>

At least 5 slides were produced and analyzed for each stool sample. In the negative cases of the first 5 slides, more 5 slides were produced afterward. The same criterion was adopted in the analyses of the nail bed. It was dedicated a minimum of 30 minutes of analysis per slide.

## Statistical analyses:

The Fisher's exact test was utilized to verify association between gender and the parasitic disease. By applying the Logistic Regression Analysis, it has been verified the relationship between the occurrence of a determined parasite with the level of education, income and access to information. The statistical analyses were carried out with the aid of the Statistical Package for the Social Sciences (SPSS) software. version 20. Results were considered relevant when the value of *p* was lower than 0.05.

#### Ethics:

This project has been approved by the Ethics and Research Committee of the High School of Sciences of Santa Casa de Misericórdia in Vitória (EMESCAM) and has been registered under the protocol number 095/2010. The results were handed individually and under absolute in confidentiality to each subject, who have been referred when necessary to the Service of Infectious Diseases of the Santa Casa de Misericórdia in Vitória, or directed to seek a physician of their trust. The biological material has been disposed of in accordance with the protocol of the Health Service Residue Management Program, following the Board of Trustee's Resolution no.

306/2004 by the Brazilian Health Surveillance Agency (ANVISA), implemented in EMESCAM's Parasitology laboratory.

#### Results

Out of the 15 participants, 11 were male and 4 female, ranging from 18 to 60 years of age, residing in the municipalities of Iconha and Santa Maria de Jetibá, in the state of Espírito Santo. With regards to level of education, 46.7% partially attended the elementary school and 40.0% fully completed the latter. Concerning family revenue, there has been a prevalence of up to USD 591.00 (73.3%). The sewage system and water supply of more than 90% of the participants are made through a septic tank and without a piping arrangement, respectively (Table 1).

Table 1 - Results of the socioenomic questionnaire distributed to the stallholders

Epidemiologic Aspects	no.	%
Age		
18-30 years old	04	26.7%
30-45 years old	04	26.7%
45-60 years old	07	46.7%
Gender		
Male	11	73.3%
Female	04	46.7%
City of Residence		
Santa Maria de Jetibá, ES	11	73.3%
Iconha, ES	04	26.7%
Level of Education		
Unfinished primary school	07	46.7%
Complete primary school	06	40.0%
Unfinished high school	01	6.7%
Complete high school	01	6.7%
Family income (number of minimum wages)		
<1	01	6.7%
1-2	05	33.3%
2-3	05	33.3%
3-5	02	13.3%
5-10	02	13.3%
Basic sanitation		
Sanitary sewage system		
General sewage system	01	6.7%
Septic tank	14	93.3%
Means of water supply		
General system with internal piping	01	6.7%
General system with internal piping, artesian well	02	13.3%
Well or river source without internal piping	12	80.0%

Source: author. Note: translated.

Based on the participants' habit questionnaire, more than 90% of the stallholders affirmed they wash their hands before and after meals, before handling food and after using the toilet. All stallholders affirmed handling both cash and food while commercializing them, although only 13.3% claimed to wash their hands between these two processes — 53.4% of the stallholders wear jewelry or ornaments while working (Table 2).

Table 2 -	Results on	the hygiene	habits o	distributed	to the	stallholders
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Hygiene habits	no.	%
Do you wash your hands before meals?		
Yes	15	100.0%
No		
Do you wash your hands after meals?		
Yes	14	93.3%
No	01	6.7%
Do you wash your hands after going to the toilet?		
Yes	14	93.3%
No	8778	
Sometimes	01	6.7%
Do you wash your hands before handling food?		
Yes	14	93.3%
No	01	6.7%
Do you wear hand jewelry or ornaments while working at the	open-air market?	
Yes	08	53.4%
No	07	46.6%
Does the person who handle food also handle cash?		
Yes	15	100.0%
No		1. <del></del>
Do you wash your hands between these operations?		
Yes	02	13.3%
No	13	86.7%

Source: author. Note: translated.

Also out of the 15 participants, stool tests showed that 11 individuals were positive for at least one parasite (73.3%). In these samples, eight species (parasites and commensals) were identified (Table 3 and Figures 1 and 2).

#### Table 3 - Prevalence of species present in the stallholders' stool tests

Species	Group	Pathogenic	Prevalence (%)
Blastocystis sp.	Protozoan	Variable	66.7
Ascaris lumbricoides	Helminth	Yes	40.0
Ancilostomídeo	Helminth	Yes	40.0
Schistosoma mansoni	Helminth	Yes	13.3
Rodentolepis nana	Helminth	Yes	13.3
Giardia lamblia	Protozoan	Yes	20.0
Entamoeba histolytica	Protozoan	Yes	6.7
Balantidium coli	Protozoan	Yes	6.7
Endolimax nana	Protozoan	No	13.3
lodameba bütschilii	Protozoan	No	6.7
Entamoeba poleck	Protozoan	No	6.7

Source: author. Note: translated.

Figure 1: Eggs found in stool samples: (a) hookworm, (b) Ascaris lumbricoides, (c) Rodentolepis nana, (d) Schistosoma mansoni.



Source: author. Note: translated.



Figure 2: Prevalence of species found in the stallholders' stool tests

Source: author. Note: translated.

The species with the greatest prevalence were *Blastocystis sp.* Brumpt, 1912 (66.7%), *Ascaris lumbricoides* Linnaeus, 1758 (40%), hookworms (40.0%), *Giardia lamblia* Kofoid and Christiansen, 1915 (20%), and *Schistosoma mansoni* Sambon, 1907 (13.3%). Other species recorded in a lower prevalence were *Rodentolepis nana* Siebold, 1852, *Entamoeba histolytica* Schaudinn, 1903, and *Balantidium coli* Stein, 1863. Multiple parasitosis was observed in nine stallholders (60%), ranging from individuals with two and up to five parasites. *A. lumbricoides* was present in all multiple-parasite affected individuals, and hookworms and *G. lamblia* in half of them.

Biological material was analyzed from the hands of 6 stallholders, and 2 of them (33.3%) presented cysts/eggs of the following parasites: Ascaris lumbricoides (33.3%); hookworms (16.7%); R. nana (16.7%); and G. lamblia (16.6%). In one of them A. lumbricoides and G. lamblia was found in the hand, but not in the stool sample. In the other subject, Α. *lumbricoides*, hookworm and *R*, *nana* was found both in the hands and in the stool sample, besides Blastocystis sp. and G. *lamblia* (Table 4 and Figure 3).

Table 4 - Prevalence of species present in the test of the stallholders' hand palms and nail beds

Species	Hand	Nail beds	Prevalence (%)	
Ascaris lumbricoides	Yes	Yes	33.3	
Hookworm	Yes Yes	Yes	16.7 16.7	
Rodentolepis nana		No		
Giardia lamblia	No	Yes	16.7	

Fonte:



Figure 3: Prevalence of the species found in the stallholders' hand tests

Source: author. Note: translated.

It has not been observed a significant statistical association ( $p \ge 0.05$ ) between the stallholders' gender and any species, nor a relationship between the occurrence of a determined species and level of education, income, and access to information.

#### Discussion

In Brazil, the intestinal enteroparasitosis are widely spread and present high rates of

occurrence. In a multicentric study carried out in schools of several Brazilian states, 55% of students were diagnosed with some kind of parasitosis.<sup>15,16</sup> Estimates show that Brazil is still among the countries with the highest rates of A. lumbricoides, being the infection detected in around 40% of the population.<sup>17</sup> In a study administered with food handlers from the state of Paraná, Takizawa, Falamina<sup>9</sup> have found an enteroparasite prevalence of 38.2%. The stallholder prevalence of the enteroparasites identified in this study was higher than 70%, value alarmingly higher than the ones found in human populations, especially in those populations whose prevalence usually tends to be higher, as in schoolchildren. Such prevalence of infection per species whether they be parasitic or commensal whose cycle contains the elimination of infecting forms in stool, makes room for one to argue about in which conditions the stallholders live.

The species found in the stool samples that presented the highest prevalence rate were Blastocystis sp. (66.7%), A. lumbricoides (40.0%) and hookworms (40.0%). Some authors argue over the pathogenicity of Blastocystis sp., which varies according to some factors, such as its genotype and hosts. Furthermore, there is some evidence suggesting that some strains of *Blastocystis* may be part of the human intestine flora or even act in favor of the development of intestinal inflammatory diseases.<sup>18</sup> The high frequency of A. lumbricoides is usually expected in groups exposed to inadequate conditions, due to its elevated biotic potential and great egg viability, factors which contribute to making this parasite the most common in the world, with an estimated prevalence of 25%.<sup>17,19</sup> The ideal conditions for its existence can be observed in places where the sewage system infrastructure is of low quality. The presence of open-air sewage fluids and the lack of sanitary facilities also facilitate the infection by other parasites, such as hookworms (7). Eggs of S. mansoni have also been found (13.3%), and according to the Ministry of Health, the state of Espírito Santo is among the seven Brazilian states with the highest prevalence rates of this parasite.<sup>20</sup>

A poor sewage system, along with a bad sanitary education, are risk factors for the prevalence of the enteroparasitosis found in human populations.<sup>21</sup> More than 90% of the professionals' interviewed households count with a sanitary sewage system connected to a septic tank, which facilitates the contamination of the water utilized for consumption and for the irrigation of the products commercialized in the markets. The water supply of these professionals is mostly performed through wells or river sources without an internal piping system, becoming a conducive environment for the infection of the own food handler while utilizing this water without proper treatment. Studies have shown that there is inverse relationship between an the prevalence and the quality of the sanitary conditions of the environment, as well as the adopted practices.<sup>22</sup>

The low level of education found in the present study — approximately 90% of the subjects interviewed completed up to the \_\_\_\_ represents primary school an aggravating factor for the high prevalence of enteroparasitosis. Education, culture, and eating habits are factors that determinedly influence the exposure to infection.<sup>23</sup> Therefore, health education represents one of the most important interventions for improving the life quality of communities.<sup>24,25</sup>

All stallholders have affirmed washing their hands before meals, and more than 90% have affirmed doing the same after meals, after using the toilet and before handling food. However, given the reported and observed characteristics during the study, with regards to the household water supply, the aspect of the hands and the conditions of the market, we believe that the high rates of hand washing reported may have been biased data. Still in our study, all stallholders handled cash, and more than half of them wore ornaments and jewelry while working. Wearing ornaments and jewelry, as well as handling objects — such as coins and bills — are risk factors for the dissemination of infecting agents.<sup>26</sup>

## Conclusion

The results obtained show that in natura food handlers in open-air markets play an important role in the dissemination of enteroparasitosis to the population. As a general picture, the food handlers analyzed in the present study have contributed to several aspects in the dissemination and increase of the infection prevalence by enteroparasitosis. The recorded high prevalence of infection by enteroparasites, associated with several risk factors — low level education. deficient basic of sanitation, handling of bills and coins while selling vegetables, poor conditions of hand hygiene, and deficient control of hygiene food cultivation from to its commercialization — pose risks for consumers.

It is then important that interventions are made in order to reduce the prevalence of enteroparasites stallholders. among consequently reducing the risk of contamination of the food commercialized and infection of the consuming population. Among the forms of guaranteeing the hygiene and sanitary quality of foods are the promotion of continuous education programs for the food handlers and their communities, the conduction of parasitic tests on a regular basis and the treatment of these subjects and their family members, as well as making the sanitary surveillance system more solid for the fiscalization of foods offered to the population, including a proper legislation. In spite of that, the investment in basic sanitation, as well as the improvement of the socioeconomic conditions and of the general infrastructure is of utmost importance for the success of the control programs.

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