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ORIGINAL ARTICLE

PARASITOLOGICAL ANALYSIS OF VEGETABLES SOLD IN SUPERMARKETS AND FREE MARKETS IN THE CITY OF TAGUATINGA, FEDERAL DISTRICT, BRAZIL

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ABSTRACT

Parasitosis are the most common diseases in the world, they are responsible for relevant negative consequences in the individuals´ health. The consumption of vegetables in Brazil and in the world is common due to their high nutritional value and they are recommended to be included in the population´s diet. Contaminations in vegetables are most often caused by irrigation water which is contaminated by feces, other forms of contamination are through organic manure containing fecal waste and the contact of vegetables with animals where they are grown. This research evaluated the contamination in vegetables with the purpose of warning the community about hygiene and the correct approach of food handling in order to prevent parasitic infections, contributing to people´s health. Samples were collected from supermarkets and farmers´ markets, where we obtained a total of 30 samples that were analyzed using Hoffman, centrifugation and slide reading´s techniques. In all samples, parasites were found. In six samples non-pathogenic amoebas were found, however this finding means that there was contamination from human or animal feces. And, in the other 24 samples, parasitic structures that cause damage to health were found. This work showed a high level of contamination of the vegetables that are consumed daily by the population and the diseases caused by food which are a concern for the public health. Contaminated vegetables are dangerous considering the transport of parasites that may generate human infections, therefore the importance to create public policies in order to avoid parasitic infections.

KEY WORDS: Parasitic infections; food contamination; parasitology; food parasitology; neglected diseases.

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INTRODUCTION

According to the World Health Organization, parasitic diseases are the most common diseases in the world, they affect about 50% of the world population and they are responsible for significant negative consequences on the individuals´ health. Mainly the poorest population of underdeveloped countries is affected by the parasitic diseases and, even being classified as neglected diseases (WHO, 2017).

The vegetables´ consumption in Brazil and in the world is evident because they have a high nutritional value and they are recommended to be included in the population´s diet (Canella et al., 2018). However, the ways of growing these vegetables are done organically, which makes their cultivation susceptible to the parasitic contamination. Since the water used in the irrigation process may contain fecal material due to the production condition, animals that live in the middle of the plantation, which make their waste near the cultivation areas and the use of organic fertilizer from animal feces and decomposed matter been susceptible to contamination (Robertson & Gjerde, 2001; Takayanagui et al., 2007).

One of the main and the most concerned problems for the public´s health today are foodborne diseases. They affect millions of people annually in both developed and underdeveloped countries (Käferstein et al., 1997). The consumption of food contaminated by parasites may cause severe damage to the population´s health. The clinical symptoms may vary according to the parasite load, and may cause diarrhea, intestinal malabsorption, intestinal obstruction, colitis, anemia and malnutrition, which may damage the individuals´ life quality generating personal, work and educational problems (Colli et al., 2014).

In its fourth report on Neglected Tropical Diseases (2017), the World Health Organization categorizes parasitic infections as neglected diseases, which affect the poorer communities and the underdeveloped countries, because of their collective aspect concerning poverty, lack of basic sanitation, lack of hygiene and education of the population therefore generating a global impact (WHO, 2017; Macedo et al., 2020).

The goal of this research was to evaluate the contamination of vegetables sold in supermarkets and in the farmer´s markets in order to find possible contamination by pathogenic parasites.

METHODS

This is a cross-sectional observational field research (Bordalo, 2006), where the collections came from two supermarkets and two farmers´ markets chosen randomly, in the city of Taguatinga – Distrito Federal. The samples from the establishments were leafy vegetables, the lettuce (*Lactuca sativa*) of the crunchy variety and watercress (*Nasturtium officinale*).

Ten samples from a large supermarket X, 5 of lettuce (*Lactuca sativa*) and 5 of watercress (*Nasturtium officinale*), in the medium-sized supermarket Y 5 samples, 3 of lettuce (*Lactuca sativa*) and 2 of watercress (*Nasturtium officinale*) were collected, with a total of 15 supermarket´s samples, 8 samples of lettuce and 7 of watercress.

One month after, another 10 samples were collected, 5 of lettuce (*Lactuca sativa*) and 5 of watercress (*Nasturtium officinale*) from a large-sized farmers´ market Z, and in the medium-sized farmers´ market W, 5 samples were collected, 2 of lettuce (*Lactuca sativa*) and 3 of watercress (*Nasturtium officinale*), with a total of 15 farmers´ market samples, 7 samples of lettuce and 8 of watercress, 30 samples in total, 15 of lettuce and 15 of watercress.

The people in charge of the supermarkets were asked about the origin of the vegetables, they informed us that they came from large vegetable producers. In the farmers´ markets, they reported that the vegetables were produced in their own rural properties.

The vegetables at the time of the collection were stored separately in clean plastic bags and transported in Styrofoam boxes in order to avoid any kind of contamination from the external environment, then they were sent to the Parasitology Laboratory of the LS University Center, where they remained stored for a period up to 24 hours in a refrigerator at 5º C until the processing of the analyzed material.

The vegetables were defoliated in a clean vessel and washed with 300 mL of distilled water using manual rubbing (using one pair of disposable gloves per sample) and with the support of a soft bristle brush that was sanitized in liquid detergent after each sample rubbing. Then the parasitological method used in the analysis was the spontaneous sedimentation (Hoffman et al., 1934) and simple centrifugation (Rocha et al., 2008). Then the water and the sediment from each sample were transferred into two 200 mL conical-bottomed goblets to perform the sedimentation technique. After 24 hours, most of the supernatant was discarded and the sediment was transferred into tubes containing 15 mL of the samples to be centrifuged at 1,300 rpm for three minutes.

Subsequently, slides were prepared with the sedimentation and centrifugation result homogenized, a 25 μL aliquot was removed and the slides were stained with lugol. Posteriorly, the slides were observed under an optical microscope with 10x and 40x objectives for parasitological analysis. A slide was mounted for each centrifuged sample (n =30), then, the identification of parasite structures was performed with the help of parasitological atlas and the results were expressed in a table in the Excel software.

RESULTS AND DISCUSSION

The results of this study indicate that the samples of the vegetables, lettuce and watercress, sold in supermarkets and in the farmers´ markets in the city of Taguatinga, Federal District, housed various parasitic forms. All 30 samples (100%) presented contamination, 7 samples (23.3%), one of watercress (6.6%) and 5 of lettuce (16.6%) presented non-pathogenic amoebae (*Endolimax nana* cysts and *Entamoeba coli* cysts) and 76.6% of the samples by pathogenic parasites. In the analyzed samples of lettuce (*Lactuca sativa*) a variety of parasites were found, including cysts, eggs, and trophozoites (Board 1).

Board 1. Lettuce (*Lactuca sativa*) analisys

Analysis Parameters	Contamination rate (15 samples = 100%)	Presence of sample contamination
Cyst of *Entamoeba coli*	73,3%	Present in 11 of 15 samples
Cyst of *Endolimax nana*	46,6%	Present in 7 of 15 samples
Cyst of *Entamoeba histolytica*	26,6%	Present in 4 of 15 samples
Trophozoite of *Balantidium* sp.	13,3%	Present in 2 of 15 samples
Egg of *Enterobius vermicularis*	13,3%	Present in 2 of 15 samples
Egg of *Ascaris lumbricóides*	13,3%	Present in 2 of 15 samples
Egg of Hookworm	13,3%	Present in 2 of 15 samples
Egg of *Taenia* sp.	13,3%	Present in 2 of 15 samples
Egg of *Hymenolepis nana*	6,6%	Present in 1 of 15 samples
Egg of *Strongyloides* sp.	6,6%	Present in 1 of 15 samples

The watercress samples were more contaminated with a greater variety of parasitic structures than the lettuce samples. Rhabditiform larvae of *Strongyloides stercoralis* (26.6% of 15 watercress samples), hookworms (13.3% of 15 watercress samples) and a filariform larva of *Strongyloides stercoralis* (6.6% of 15 watercress samples) were identified (Board 2).

Board 2. Watercress (*Nasturtium officinale*) analisys

Analysis Parameters	Contamination rate (15 samples = 100%)	Presence of sample contamination
Cyst of *Entamoeba coli*	20%	Present in 3 of 15 samples
Cyst of *Endolimax nana*	53,3%	Present in 8 of 15 samples
Cyst of *Entamoeba histolytica*	13,3%	Present in 4 of 15 samples
Trophozoite of *Balantidium* sp.	6,6%	Present in 1 of 15 samples
Egg of *Enterobius vermicularis*	20%	Present in 3 of 15 samples
Egg of *Ascaris lumbricóides*	13,3%	Present in 2 of 15 samples
Egg of Hookworm	40%	Present in 6 of 15 samples
Egg of *Taenia* sp.	33,3%	Present in 5 of 15 samples
Egg of *Hymenolepis nana*	6,6%	Present in 1 of 15 samples
Egg of *Strongyloides* sp.	13,3%	Present in 2 of 15 samples
Egg of *Diphillobothrium latum*	13,3%	Present in 2 of 15 samples
Egg of *Hymenolepis nana*	6,6%	Present in 1 of 15 samples
Rhabditiform larvae of *Strongyloides stercoralis*	26,6%	Present in 4 of 15 samples
Rhabditiform larvae of Hookworm	13,3%	Present in 2 of 15 samples
Filariform larvae of *Strongyloides stercoralis*	6,6%	Present in 1 of 15 samples

The non-pathogenic amoebae found in the watercress and lettuce inhabit the large intestine of man and the animals such as pigs, dogs, cats, rodents and others (Nascimento et al., 2010), which means that there is contamination of the soil where the vegetables were planted. The means of cultivation and irregular handling, are facts that imply the contamination of the other 76.6% of the samples by pathogenic parasites, which cause harm to the human body.

It was evident that the watercress samples had a higher diversity of parasites, with its dense foliage as the driving factor for the contamination. The parasitic forms found in 100% of the vegetables show a constant danger when people consume them without the proper hygiene, good preparation and handling practices, even though is known that some of those species are dangerous to humans.

Barreto (2012) analyzed samples from several vegetables, some of them were the same ones analyzed in our research and in cities of the Federal District as well. He found contamination of hookworm and *Ascaris lumbricoides* eggs, hookworm larvae and similar larvae of *Strongiloydes* sp. Silva et al (2013) found 4.6% contamination by nematode larvae in 150 samples of lettuce (*Lactuca sativa*) analyzed in Anápolis – Goiás.

Maciel et al (2014) found contamination in all samples from farmers´s markets analyzed in the Federal District, detecting intestinal parasites such as *E. coli, Strongyloides* sp. larvae, *Ascaris* sp. Also, hookworm eggs and larvae in lettuce and watercress. Tubino et al (2014) purchased vegetables in supermarkets and in farmers´ markets in the Federal District, some of them were lettuce, where 25% of contamination was identified by *Ascaris lumbricoides,* 25% by *Entamoeba hartimann* cysts, 25% by eggs also larvae of hookworms, and 25% by filarioid and rhabditoid larvae of *Strongyloides* sp.

Moura et al (2015) analyzed 28 lettuce samples collected in farmer´s market in Anápolis - Goiás, identifying contamination in 25% of the analyzed samples, with a higher prevalence of *Entamoeba coli* (17.8%), *Ascaris lumbricoides* (3.5%) and *Endolimax nana* (3.5%). Reis et al (2020) brings us an important information in his research, where samples from organic fertilizer were analysed, which were supposedly contaminated and used in the plantations around the Federal District. 51 slides were prepared, and 90% of contamination was found by Hookworm larvae, larvae that showed significant infective viability.

In the samples analyzed in this study pathogenic parasitic structures were found, eggs of *Ascaris lumbricoides*, larvae of *Strongyloides stercoralis* in both evolutionary forms; eggs of *Strongyloides* sp. obtained by sedimentation and centrifugation, eggs and larvae of hookworms, eggs of *Taenia* sp, and cysts of *Entamoeba histolytica*, evidencing parasites that cause damage to the human health and have similar symptoms at early stages of infection for instance nausea, vomiting, malabsorption of nutrients, constipation, diarrhea, weakness within other symptoms according to the level of infection (Soares et al., 2018; Maia et al., 2006; Benincasa et al., 2007; Ferreira et al., 2008; Bosqui et al., 2014; Rey, 2001; García et al., 2003; Castro et al., 2019).

Rhabditoid larvae of *Strongyloides stercoralis*, one of the larvae in the filariform evolutionary form, which is the cutaneous infective form, and two rhabditoid larvae of hookworm were found in the 7 watercress samples (23.3%), all under favorable conditions (Figure 1). The finding of *Strongyloides* larvae is of concern because of the risk of strongyloidiasis, a neglected tropical disease, as well as the Hookworm larvae which cause hookworm disease (Hotez et al., 2005).

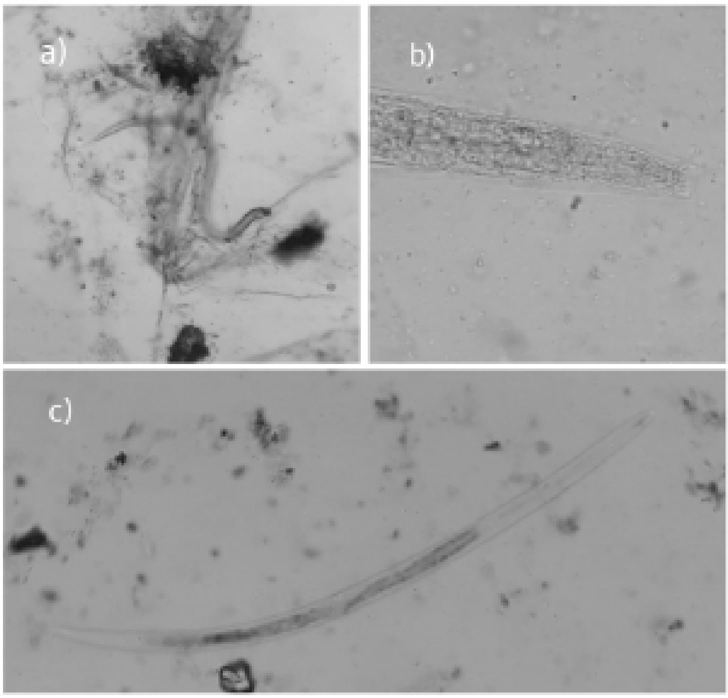

Figure 1. Larval forms: a) Filariform larvae of *Strongyloides stercoralis*; b) *Strongyloides stercoralis* rhabditoid larvae, short buccal vestibule; c) Hookworm rhabditoid larvae, long buccal vestibule.

The present work showed us a high level of contamination in the vegetables (Figure 2) that are consumed daily by the population, and the diseases caused by food are a concern for the public health today. Contaminated vegetables show danger in relation to the carriage of pathogenic parasites that may generate infections in humans.

According to the Global Burden of Disease Study of 2013, intestinal infections caused by protozoan are considered the third cause of death worldwide, resulting in more than 100,000 deaths annually (Castro et al., 2019). Therefore it is important to create public policies for a better prevention of parasitic infections, contributing to public health and fulfilling the two pillars of Prevention and Health Promotion in an attempt to help in the eradication of Neglected Tropical Diseases.

Based on these results, it is evident that more studies on this subject need to be done, and it is necessary to evaluate the viability of the parasitic forms found in vegetables.

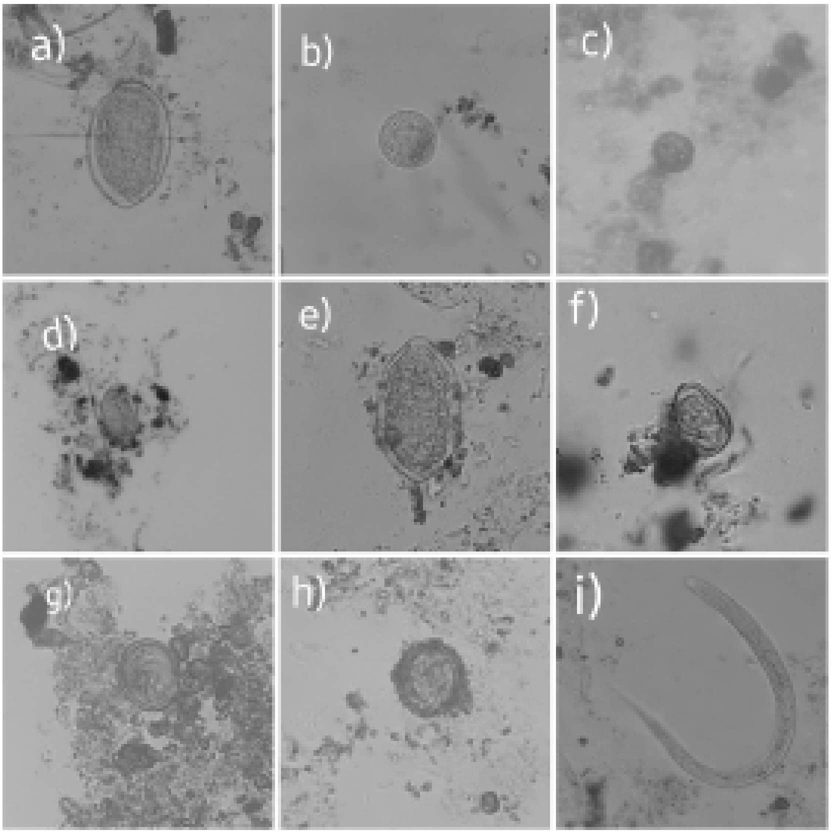

Figure 2. Parasite identification: a) Hookworm egg; b) *Entamoeba coli* cyst; c) *Entamoeba histolytica* cyst; d) *Enterobius vermicularis* egg; e) *Necator americanus* egg; f) *Strongyloides* sp. Egg; g) *Taenia* sp. Egg; h) *Ascaris lumbricoides* egg; i) Hookworm rhabditiform larvae.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

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