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ORIGINAL ARTICLE

Evaluation of two analytical methods of detection for intestinal parasites in curly lettuce sold in food stalls

Avaliação de dois métodos analíticos para detecção de parasitos intestinais em alfaces crespas vendidas em feiras de rua

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Abstract

This study aimed to investigate the occurrence of parasitic structures in curly lettuce (*Lactuca sativa* L.) sold in food stalls open markets in Cuiabá city, in the Brazilian state of Mato Grosso (MT). For this purpose, 50 samples were evaluated using spontaneous sedimentation and centrifugal flotation methods. These techniques showed positivity in 96% (48/50) and 78% (39/50) of the samples, respectively. The sedimentation method was statistically ($p \leq 0.05$) more effective in detecting parasitic structures of the pathogens as *Entamoeba histolytica*, *Ascaris* spp., *Strongyloides* spp., Larvae and Hookworm eggs, when comparing both methods. However, these methods did not differ significantly for the detection of *Giardia lamblia*. *Ascaris* spp and *Entamoeba coli* occurred in 64% (32/50) and 46% (23/50) of the positive samples, respectively, with the highest percentages observed. The occurrence of *Blastocystis* spp., *Fasciola* spp., *Dipylidium caninum* and *Ascaris lumbricoides* revealed detection exclusively by the spontaneous sedimentation method, while *Iodamoeba butschlii* and *Enterobius vermicularis* were detected by centrifugal–flotation procedure. These results could reinforce the need to use methods based on sedimentation and the fluctuation of parasite structures in the analysis of lettuce. The presence of parasites in lettuce by fecal origin, with recognized pathogenicity, represents a risk to consumer health and demonstrates a deficit in good agricultural practices in the production, distribution and commercialization of lettuce.

Keywords: *Lactuca sativa*; Protozoa; Helminths.

Resumo

Este estudo objetivou averiguar a ocorrência de estruturas parasitárias nas alfaces crespas (*Lactuca sativa*), comercializadas em feiras livres e permanentes da cidade de Cuiabá-MT. Para tanto, 50 amostras foram avaliadas pelos métodos de sedimentação espontânea e centrífugo-flutuação, que revelaram positividade em 96% (48/50) e 78% (39/50) das amostras, respectivamente. O método de sedimentação foi mais eficaz na detecção de estruturas parasitárias dos patógenos *Entamoeba histolytica*, *Ascaris* spp., *Strongyloides* spp. e de larvas e ovos de Ancilostomídeo, quando comparado ao método centrífugo-flutuação, com diferença estatisticamente significativa ($p \leq 0,05$). Contudo, estes métodos não diferiram significativamente na verificação de *Giardia lamblia* nas amostras. *Ascaris* spp. e *Entamoeba coli* ocorreram em 64% (32/50) e 46% (23/50) das amostras positivas, respectivamente, com os maiores percentuais observados. A ocorrência de *Blastocystis* spp., *Fasciola* spp., *Dipylidium caninum* e *Ascaris lumbricoides* foi revelada exclusivamente pelo método de sedimentação espontânea; *Iodamoeba butschlii* e *Enterobius vermicularis*, pela centrífugo-flutuação. Estes resultados reforçam a necessidade de utilizar, na análise da alface, métodos fundamentados na sedimentação e flutuação de estruturas dos parasitas. A presença na alface de parasitas de origem fecal, com reconhecida patogenicidade, representa risco à saúde do consumidor, bem como demonstra deficiência na adoção de boas práticas agrícolas na produção, distribuição e comercialização de alfaces.

Palavras-chave: *Lactuca sativa*; Protozoários; Helmintos.

1 Introduction

Parasites are one of the disease-causing agents in humans, as well as bacteria, viruses, and protozoa, defined by the World Health Organization (WHO) (World Health Organization, 2015). Some parasites can cause diarrheal diseases, as *Cryptosporidium* spp., *Entamoeba histolytica* and *Giardia* spp.. Diseases that affect the abdomen (*Ascaris* spp., *Fasciola* spp. etc.), lung, liver and Central Nervous System (CNS) (*Echinococcus granulosus* and *E. multilocularis, Taenia solium* - cysticercosis). These parasites are among the pathology's causative agents, sometimes disabling the consumer's health (World Health Organization, 2015).

The WHO and the United Nations Food and Agriculture Organization (FAO) endorse the global monitoring of the following genera and species of parasites in fresh vegetables: *Echinococcus granulosus*; *E. multilocularis*; *Cryptosporidium* spp.; *Entamoeba histolytica*; *Ascaris* spp.; *Giardia duodenalis*; *Fasciola* spp.; *Cyclospora cayetanensis*; *Trichuris trichiura*; *Balantidium coli;* and *Toxocara* spp. (Food and Agriculture Organization of the United Nations, 2014). Other parasite species were included recently as *Toxoplasma gondii* and *T. solium* (Food and Agriculture Organization of the United Nations, 2016). Moreover, some genera such as *Giardia* spp., *Cryptosporidium* spp., *Ascaris* spp., and *E. histolytica* are related to parasites that trigger foodborne diseases most frequently (World Health Organization, 2015).

The diversity of ways in which food is consumed worldwide has influenced the occurrence and transmission of different parasites (Robertson et al., 2014). Thus, thousands of consumers have infections due to ingestion of protozoan cysts and oocysts, as well as helminth eggs and larvae. After their complete biological development, they have a direct effect on human health. This infection can also trigger childhood diarrhea and consequences on children's growth rate, especially in developing countries (Doyle, 2003).

The detection of parasitic structures in leaves and vegetables demonstrates the potential carrier parasites to humans and, consequently, a risk to public health (Pinto et al., 2018). Draeger et al. (2019) have suggested that outbreaks triggered by intestinal parasites often do not have the determination of the parasite specie, in view of a low percentage of species identification (between 25% and 30%) was verified for the cases reported from 2016 to 2017. For this, food-borne outbreaks associated with fresh products in Brazil should be monitored to assess control strategies better to prevent diseases (Machado et al., 2018). Outbreaks linked to fruits and vegetables represented an average of 4.3 cases per year, or 0.6% of all food-outbreak reported from 2008 to 2014 (Elias et al., 2018). However, the investigation of outbreaks by parasites requires technician's expertise in evaluating ecological and epidemiological aspects of natural food contamination, one time that food is a complex matrix, and this complexity requires human resources trained in microscopy and analytical methods for accurate diagnosing of parasites in food (Barbosa, 2015).

Lettuce is among the third vegetables, most produced and consumed in Brazil, representing approximately 19 billion dollars to Brazil in 2016 (Brasil, 2017). An increase in good social-environmental measures is desired by consumers, even if it affects its price (Echer et al., 2016).

The occurrence of parasitic structures in lettuce produced in Brazil was analyzed in five regions, in farms of production, commercialization area, and their consumption habits, as well as in farm production (Barbosa et al., 2016), horticulture fair (Tiyo et al., 2016), open markets (Medeiros et al., 2019; Ramos et al., 2019; Pinto et al., 2018; Oliveira et al., 2016), supermarkets (Almeida et al., 2016), bistro (Silva et al., 2017), and two or more types of leaves and vegetable commercialization (Ambrozim et al., 2017; Luz et al., 2017; Trevisan et al., 2017; Brauer et al., 2016; Nogueira et al., 2016; Silva et al., 2016). These mentioned studies were carried out in the Southeast, North, Northeast, South and Midwest regions of Brazil, using diagnostic techniques of spontaneous sedimentation or centrifugal – flotation, or the quantitative FLOTAC technique, based on the flotation of eggs and cysts (Pedroso et al., 2020, Ramos et al., 2019).

The techniques mentioned above are used to analyze leaves, fruits, and vegetables, individually or in combination (Mesquita et al., 2015; Barnabé et al., 2010; Quadros et al., 2008; Paula et al., 2003). These techniques are procedural adaptations used in clinical samples. Fluctuation techniques have the principle that nematode eggs and protozoan oocysts can float in saturated solutions. On the other hand, sedimentation techniques are indicated for the recovery of heavy eggs that do not float in saturated solutions, as is the case of trematode eggs and also some cestodes eggs (Táparo et al., 2006).

In the state of Mato Grosso (MT), studies on the occurrence of parasitic structures in lettuce were carried out at retail supermarkets in Cuiabá (Alves et al., 2013) and at open marketing in Tangará da Serra (Ferro et al., 2012). Therefore, the present study aimed to verify the occurrence of parasitological structure in crisp lettuce (*Lactuca sativa* L.) commercialized in open-markets in Cuiabá (MT) and to compare spontaneous sedimentation and centrifuge-flotation methods.

2 Materials and methods

During one-year (from January 2019 to January 2020), 50 samples of curly lettuce (*L. sativa*) from conventional planting were collected in five open-markets (one permanent and four eventually open-market) in Cuiabá (MT). The sample collection was performed randomly and for convenience. The lettuce plant was packed in a plastic bag provided by the market employee, stored in an isothermal box, and transported to the Parasitology laboratory.

In the laboratory, all lettuces were weighed, removed from the bag, washed, and immersed in 250ml of 0.03% Extram MA02® (Merck, Darmstadt, Germany) aqueous solution (Alves et al., 2013). The leaves were rubbing on both sides with manual movements using our hands to put on a sterile glove. The residual wash was filtered through gauze over a nylon sieve into the Imhoff cup. An aliquot of approximately 15 ml was transferred to a conical tube, the volume remaining in the Imhoff cup was left to stand for six hours. After, two aliquots of the sediment were removed to prepare a microscope slide, stained with Lugol, and covered with coverslips for observation under the optical microscope. The 15 ml of the residual wash transferred to a conical tube was centrifuged at 2500 rpm (1.048 g) (Excelsa Baby® Centrifuge, 206BL) for one minute. Subsequently, the sediment was placed in a tube and a saturated sugar solution was added to the edge of the tube, on which a microscope slide was placed for 5 minutes. Afterwards, the slide in contact with the solution facing upwards, in which a drop of Lugol was included, was covered with a coverslip for further analysis. The examination of all slides was performed using a binocular optical microscope (Micronal, Olympus CBB®), in which the size of the parasitic structures was measured with the aid of an eyepiece and a micrometric slide (Olympus CBB®). The slides were viewed with the aid of objective lenses 10x and 40x. The parasitic structures detected were identified through biometric and image data, compared to those contained in the Atlas of Parasitology (Anderson, 2006; Bowman et al., 2006; Cimerman & Franco, 2009; Foreyt, 2005).

In the comparative assessment of groups, the data set was compared using the Student's t-test. The comparison of data for each group, genus, and species was performed statistically by the Kruskal-Wallis test. Thus, in order to verify if there was a significant difference between the methods, with a significance level of 5%, the statistical analyzes were performed in the PAST. 3 software (Softpedia®) (PAST, 2018).

3 Results and discussion

Lettuces processed by the spontaneous sedimentation method showed positivity for parasitic structures in 96% (48/50) of the samples. While in those samples analyzed using the centrifuge-flotation method, 78% (39/50) were positive (Table 1). The sedimentation method is the most widely used worldwide and is considered the gold standard method for analyzing fruits and vegetables (Medeiros et al., 2019; Ismail, 2016).

Numerous pathogenic parasites to humans are susceptible to transmission by water, fruits, and raw or undercooked vegetables, constituting a significant public health problem (Barbosa, 2015). By using two different methods of parasitological analysis, we found that the lettuces sold at the open-markets in Cuiabá (MT) showed a high frequency of parasitic structures of the different family, genus, and species of the protists (Protozoa) and helminths (Metazoa) (Table 1).

The *Ascaris* spp. egg represented 64% (32/50), and the *Entamoeba coli* cyst 46% (23/50) of the parasitic structures detected in the lettuces, thus constituting the highest percentages of positivity (Table 1). Similar results for the presence of *Ascaris* spp. were found in lettuces evaluated by Corrêa et al. (2012), and Vollkopf et al. (2006) in the state of Mato Grosso do Sul (MS). Otherwise, for *E. coli* cysts, these percentages were similar to the results observed by Beletini et al. (2014) and Nomura et al. (2015) in the state of Paraná (PR). Using a single analytical method (spontaneous sedimentation), *E. coli* cyst was observed in similar percentages in lettuces sold in open-markets in Diamantina, in the state of Minas Gerais (MG) (Silva et al., 2015), and other open-market in Parnaíba, in the state of Piaui (PI) (Melo et al., 2011).

In the fifty samples of lettuce, thirteen genera and eight species of parasites belonging to the groups of protozoa and metazoa were observed, and parasite species were detected using the two applied methods (Table 1).

Table 1. Frequency (%) of positive results for parasitic structures in lettuce (n = 50) from open and permanent markets in the municipality of Cuiabá (MT), diagnosed by spontaneous sedimentation and centrifugal flotation methods.

Parasitic structures	Diagnostic method		Test Kruskal/Wallis
	Spontaneous sedimentation % (*n/N*)	Centrifuge-flotation % (*n/N*)	Value *p* ≤ 0.05
PROTOZOA			
Amoebae			
Entamoeba coli cysts	46% (23/50)	24% (12/50)	0.0217*
E. histolytica/dispar cysts	26% (13/50)	12% (6/50)	0.0465*
Endolimax nana cysts	8% (4/50)	2% (1/50)	0.1808
Iodamoeba butschlii cysts	0%	4% (2/50)	0.1655
Flagellates			
Giardia lamblia cysts	16% (8/50)	14% (7/50)	0.7805
Sporozoa			
Blastocystis spp cysts	4% (2/50)	0%	0.1655
METAZOA			
Trematoda			
Fasciola spp egg	6% (3/50)	0%	0.0801
Cestoda			
Dipylidium caninum egg	2% (1/50)	0%	0.3173
Taenia spp egg	4% (2/50)	4% (2/50)	1
Nematoda			
Strongyloides spp larvae	30% (15/50)	6% (3/50)	0.0018*
Hookworm larvae	18% (9/50)	2% (1/50)	0.0079*
Hookworm egg	20% (10/50)	2% (1/50)	0.0042*
Ascaris spp egg	64% (32/50)	28% (14/50)	0.0003*
Ascaris lumbricoides egg	6% (3/50)	0%	0.0801
Enterobius vermicularis egg	0%	2% (1/50)	0.3271
Non-parasitic structures			
Free-living nematode larvae	36% (18/50)	2% (1/50)	NS
Mite Egg	64% (32/50)	62% (31/50)	NS

NS = not statistically evaluated; * there is statistical difference between evaluated groups.

In the discussion, we highlighted the parasites pathogenic to humans that we observed in the lettuces of the open-markets in Cuiabá (MT), such as the protozoa *E. histolytica/dispar* (26%) and *G. lamblia* (16%). Among the metazoa (helminths), Hookworm's presence (larvae and egg, 18% and 20%), *Ascaris* spp. (64%), *A. lumbricoides* (6%), *Enterobius vermicularis* (2%), *Fasciola* spp. (6%), *Taenia* spp. (4%) and *Strongyloides* spp. (30%) represented a direct risk to consumer's health (Table 1). In addition, commensal parasites such as the cyst of *E. coli* (46%), *Endolimax nana* (8%), *Iodamoeba butschlii* (4%), *Bastocystis* spp. (4%), and or animal pathogens such as *Dipylidium caninum* (2%) were verified.

Previous monitoring of intestinal parasites in lettuce of supermarket, human and animal feces in Cuiabá, showed the occurrence of helminths such as: *Ascaris* spp.; *A. lumbricoides*; Hookworms (*Ancylostoma* spp.); *D. caninum*; *E. vermicularis*; *Hymenolepis nana*; *Strongyloides* spp.; and *Taenia* spp. (Alves et al., 2013; Ramos et al., 2013; Bastos et al., 2008). Protozoa also were detectable as: *Entamoeba* sp.; *E. coli; E. histolytica/dispar*; *E. nana;* and *G. lamblia* (Alves et al., 2013; Ramos et al., 2013; Bastos et al., 2008). In lettuces, the presence of intestinal parasites related to humans and animals reveals that in Cuiabá some factors have influenced the contamination of leaves, for instance: poor economic conditions; inadequate sanitation practices (water and sewage treatment); pollution of gardens soil; and water bodies used in irrigation with fecal material (Amaechi et al., 2016). These factors had an effect upon this scenario for a long time, as after almost ten years later the same parasites were observed in lettuce cultivated in Cuiabá.

High contamination rates of helminths transmitted by the soil such as *A. lumbricoides*, Hookworms and *Strongyloides* spp. in vegetables can be significantly influenced by the high infection rates in humans. In addition to the resistance and persistence of these organisms in the environment, the survivability was estimated in months or years (Rostami et al., 2016). It is noteworthy that the presence of these helminths in the lettuce indicates faecal contamination (Amaechi et al., 2016).

Among the helminths, *Ascaris* spp., *A. lumbricoides*, *E. vermicularis*, *Fasciola* spp., *Taenia* spp. were detected in lettuce and represent intestinal pathogens that have the oral route as the main way of infection of the organism (PAHO, 2003). In addition, Hookworm and *Strongyloides* spp. had the skin penetration as the main route of pathogenicity (Utaaker & Robertson, 2015). However, the ingestion of these parasites can also cause infections in humans (PAHO, 2003).

Vegetables are contaminated by parasitic structures on the farm during production and harvest, via the soil contamination, irrigation or washing water and/or by infected food handlers (Yusof et al., 2017; Ishaku et al., 2013). These routes influence the contamination of lettuces on the farms, as well as those lettuces exposed in commercial places. *Ascaris* spp., *Fasciola* spp., and *Taenia* spp. among metazoa (helminths), and *E. histolytica* and *G. lamblia* among protozoa, were present in the evaluated lettuce, and these parasitic structures are highlighted as important causative agents of infection transmitted by foods of vegetable origin by the WHO and United Nations Food and Agriculture Organization (Food and Agriculture Organization of the United Nations, 2016). Among the important parasites, which have priority control, we still have the helminths *A. duodenale* and *H. nana* (World Health Organization, 2015), which were also found in lettuces. An interesting point is that Cuiabá has different ways to commercialize lettuces, for instance: open markets (permanents or not); greengrocer's shop; warehouse; markets; or super/hypermarkets. However, lettuces are usually displaying on shelves at room temperature in the open market and exposed to dust. In addition, sometimes a market employee can be spray water in leaves to maintain humidity.

Pedroso et al. (2020) reported in their review that *A. lumbricoides*, *Strongyloides* spp., and *S. stercolares* were the helminths frequently detected in lettuces, as well as *Ascaris* spp., and the hookworm group (*Necator* spp., and *Ancylostoma* spp.). These parasites are widely distributed in the lettuce production chain, present in lettuces from the farm, super/hypermarket, greengrocery, fairs, and restaurants, spread throughout the five regions of Brazil (Pedroso et al., 2020).

Lettuces commercialized in different regions of Brazil also showed positivity for parasites, such as: *D. caninum* (Ambrozim et al., 2017; Duque et al., 2014); *E. vermicularis* (Ambrozim et al., 2017; Costantin et al., 2013; Soares & Cantos, 2005); *F. hepatica* (Luz et al., 2017; Duque et al., 2014; Ferro et al., 2012); *H. nana* (Ambrozim et al., 2017; Luz et al., 2017: Duque et al., 2014; Santos & Peixoto, 2007; Soares & Cantos, 2005; Silva et al., 2005); and *Taenia* spp. (Luz et al., 2017; Ribeiro et al., 2015; Duque et al., 2014; Gomes Neto & Pessoa., 2012; Belinelo et al., 2009; Santos & Peixoto, 2007; Freitas et al., 2004). In addition, there are reports of the *D. caninum* and *E. vermicularis* species in lettuce samples from vegetable farms, at the beginning of the production chain (Terto et al., 2014; Costantin et al., 2013). Furthermore, the *D. caninum*, *E. vermicularis*, *H. nana* and *F. hepatica* species were detected in lettuces commercialized in restaurant, which is associated with the final stage of the production chain (Macena et al., 2018; Montanher et al., 2007), thus demonstrating the inefficiency of hygienic practices in the production of this product.

The presence of *E. vermicularis*, *H. nana*, and *Taenia* spp. in lettuce could indicate a potential fecal contamination by human origin (Pedroso et al., 2020; Federer et al., 2016; Georgiev, 2001). The presence of *D. caninum*, probably indicated the contamination with feces of dogs or cats (Pan American Health Organization, 2003), whereas *F. hepatica* can be related to fecal contamination of domestic and wild animals, as well as human feces (World Health Organization, 2015).

The inefficiency of hygienic practices or no application of this tool in the management of lettuce from the vegetable farms was reported by Pedroso et al. (2020), where the occurrence of geohelminths and other intestinal parasites of animals and humans in lettuce were observed. In addition, the presence of *E. vermicularis* was verified in lettuce from the farm (Costantin et al., 2013), in commercialization (Ambrozim et al., 2017), and in a restaurant (Macena et al., 2018). Different contamination source indicated that this vegetable had contact with human feces, directly or indirectly, through vehicles of contamination such as water, dust, cockroaches, flies, or contaminated human hand, during the process of obtaining it until consumption (Amaechi et al., 2016; Oyeyemi et al., 2016; Adenusi et al., 2015). The occurrence in lettuce of this parasite that is exclusive to human reinforces the hypothesis of poor hygiene habits (Georgiev, 2001).

Commensal protozoans from the gastrointestinal tract of animals and humans, such as *E. coli*, *E. nana*, and *Blastocystis* spp., were present in lettuces sold at free markets in Cuiabá (Table 1), as well as the cysts of the pathogenic protozoa *E. histolytica* and *G. lamblia*. In Brazil, in those lettuces collected in vegetable gardens, the occurrence of *G. lamblia* cyst was observed among pathogenic protozoa, and *E. nana* among commensals (Silva et al., 2019). Pathogenic protozoa such as *E. histolytica* and *G. lamblia* were detected in lettuces obtained from restaurants (Silva et al., 2019; Macena et al., 2018; Silva et al., 2017; Queiroz et al., 2014). Both commensal and pathogenic protozoa mentioned above were detected in lettuces at vegetable in super/hypermarket, greengrocer's shop, and fairs (Rodrigues et al., 2020, Ambrozim et al., 2017; Luz et al., 2017; Silva et al., 2016; Gomes Neto & Pessoa, 2012). In the lettuces obtained in fairs and supermarket, the protozoa *Blastocystis hominis* (Rodrigues et al., 2020), and *I. butschlii* were also found (Silva et al., 2016; Gomes Neto & Pessoa, 2012). The *B. hominis* and *I. butschlii* also were observed in lettuce collected in restaurants (Montanher et al., 2007). All the protozoa mentioned above were observed in the lettuces at the fairs in Cuiabá (Table 1).

The presence of pathogenic protozoa, such as *E. histolytica* and *G. lamblia*, and commensal parasites as *Iodamoeba*, *Endolimax*, and *B. hominis* indicated fecal contamination, as the habitat of these pathogens is the human intestine. Other commensal parasites live in the intestinal microbiota of birds, human or nonhuman primates and other mammal animals (Stensvold et al., 2012; Leelayoova et al., 2008; Leelayoova et al., 2004; Orlandi et al., 2002). Among commensal parasites, there are reports of *B. hominis,* causing intestinal inflammation (Hirata et al., 2007), pathogenicity that leads to abdominal pain and diarrhea (Hayashi et al., 2006). Pathogenic protozoa may have contaminated lettuce through the soil in the case of *E. histolytica* that survive in this environment for eight days at temperatures between 28 and 34 °C, and *G. lamblia* by the water where this specie is estimated to survive up to one month at 21 °C (PAHO, 2003).

When comparing the spontaneous sedimentation method "Gold Standard" with the centrifuge-flotation method, there was a difference between the percentages and frequencies obtained for each methodology (Table 1). The Student's t-test revealed that the data set for each method showed a significant difference ($p = 0.0022$). Demonstrating that the spontaneous sedimentation method was more effective in detecting parasitic structure (larvae, eggs, cysts and oocysts) of the different families, groups, genera and species of helminths and protozoa in the lettuces analyzed in Cuiabá (MT) (Table 1). Corroborating to the results obtained in our study, some studies analyzed vegetables such as lettuce in the Dourados (MS), Pelotas (RS), Cascavel (PR), Lages (SC), and São Paulo (SP) (Quadros et al., 2008; Barnabé et al., 2010; Corrêa et al., 2012; Antunes et al., 2013; Beletini et al., 2014), and the spontaneous sedimentation method was more efficient in detecting parasitic structures, with significant statistically differences.

The effectiveness of spontaneous sedimentation and centrifugation methods in detecting parasitic structure in lettuce (larvae, eggs, cysts, and oocysts) of each of the thirteen genera, eight species and a group of intestinal parasites was verified using the statistical test of Kruskal-Wallis with significance level $p \leq 0.05$. Both methods were able to detect protozoa, cysts of the species *E. coli* (p = 0.0217), *E. histolytica*

($p = 0.0465$), *E. nana* ($p = 0.1808$) and *G. lamblia* ($p = 0.7805$). A significant statistical difference was observed in detecting the *E. coli* and *E. histolytica* species' cysts, thus showing the higher effectiveness of the spontaneous sedimentation method (Table 1). These results were similar to those obtained in studies in which this method was effective in detecting the *E. coli* protozoa in lettuces (Beletini et al., 2014; Barnabé et al., 2010; Queiroz et al., 2014; Quadros et al., 2008) and also *E. histolytica*/dispar (Barnabé et al., 2010; Queiroz et al., 2014; Quadros et al., 2008). However, it differed from the results obtained by Queiroz et al. (2014) and Quadros et al. (2008); therefore, we did not obtain a significant difference between the methods used to detect *G. lamblia*, which could contrast with the studies cited in which the spontaneous sedimentation method was more effective in detecting this protozoan. The presence of the species *I. butschlii* ($p = 0.1655$) was also verified only by the centrifugal-flotation method, and also the genus *Blastocystis* spp. ($p = 0.1655$) due to spontaneous sedimentation in lettuces at the open markets in Cuiabá (MT).

Among the helminths, the two methods applied were able to detect parasitic structures such as larvae of *Strongyloides* spp. ($p = 0.0018$), Hookworm (larvae: $p = 0.0079$ and eggs: $p = 0.0042$), *Ascaris* spp. eggs ($p = 0.0003$) and *Taenia* spp. (p = 1). However, the eggs of *A. lumbricoides* ($p = 0.0801$), *Fasciola* spp. ($p = 0.0801$) and *D. caninum* ($p = 0.3173$) were detected only by the spontaneous sedimentation method, whereas the eggs of *E. vermicularis* (0.3271) were detected by centrifuge-flotation (Table 1). A significant difference ($p \leq 0.05$) was observed between the two methods for detecting *Strongyloides* spp., and Hookworm, also for the eggs of Hookworm and *Ascaris* spp. (Table 1). It is noteworthy the effectiveness of the spontaneous sedimentation method in detecting these parasitic structures. Similar results in lettuce and other vegetables were described for the detection of *Ascaris* spp. (Fagbenro et al., 2016; Mesquita et al., 2015; Corrêa et al., 2012; Quadros et al., 2008), Hookworm (larvae and eggs) (Fagbenro et al., 2016; Mesquita et al., 2015; Corrêa et al., 2012), and *Strongyloides* spp. (Fagbenro et al., 2016; Mesquita et al., 2015; Queiroz et al., 2014). The similarity was also observed for the predominant detection of *A. lumbricoides* by spontaneous sedimentation (Fagbenro et al., 2016) and *E. vermicularis* centrifuge-flotation (Corrêa et al., 2012) in lettuce and other vegetables.

The high frequency of detecting parasites in vegetables can be influenced by geographic location, climate, sanitary conditions, water management, and fertilizer or manure contamination. Knowledge of producers and consumers about the possibility of parasite transmission by vegetables and hygiene habits of the population can influence the higher or lesser detection of parasites in lettuce (Fagbenro et al., 2016; Alhabbal, 2015; Eraky et al., 2014). The analytical procedure used to float or sediment the parasitic structures can also influence the detection of these structures (Ezatpour et al., 2013).

To avoid contamination in vegetables and leaves, information regarding the parasites and the consequences of the infection is encouraged for producers and consumers. The understanding of the route of contamination and how to avoid the presence in these products can minimize the risks of food infection and promoting better consumer health.

4 Conclusion

The analytical methods of spontaneous sedimentation and centrifugal-flotation applied to lettuces sold in the open-markets in Cuiabá city (MT/Brazil) showed leaves contaminated with parasitic structures, such as larvae and helminth eggs, protozoan cysts, and oocysts. In addition, the spontaneous sedimentation method was more effective in detecting parasitic structures. Lettuces from Cuiabá city can be considered a potential source of infection in consumers. The present study highlighted the need to implement health education for producers, traders, and consumers. Our study also reinforced the need to increase investments for more preventive actions by environmental and health surveillance agencies in the Brazilian municipalities with respect to the lettuce production chain.

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