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Original Article

Detection of enteroparasites in foliar vegetables commercialized in street- and supermarkets in Aparecida de Goiânia, Goiás, Brazil

Detecção de enteroparasitas em hortaliças comercializadas em feiras e supermercados em Aparecida de Goiânia, Goiás, Brasil

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Abstract

Foliar vegetables contaminated with fecal residues are an important route of transmission of intestinal parasites to humans. The aim of this study was to evaluate the presence of parasitic forms of protozoa and helminths on lettuces (*Lactuca sativa*) and collard greens (*Brassica oleracea*) sold in street- and supermarkets in the city of Aparecida de Goiânia, Goiás, Brazil. A total of 30 samples of each vegetable (15 samples from each supermarkets and street markets) was analyzed. All samples were processed by spontaneous sedimentation method and centrifugal flotation. In 45% of the samples, immature forms of intestinal parasites were identified with 66.7% helminths eggs and 33.3% protozoan cysts or oocysts. Significantly more lettuce samples were contaminated with eggs, cysts or oocyst of at least one parasite than collard green samples (U=216; Z=-3.45; P <0.001). The parasitic forms were identified morphologically up to the family level with eggs of Ancylostomatidae, Strongyloididae, Ascarididae and Taeniidae, or oocysts of Eimeriidae, to the genus with *Cystoisospora* sp. and *Toxocara* sp., and to the species level with *Cystoisospora canis*, *Dipylidium caninum* and *Hymenolepis nana*. The presence of these infective agents in lettuce and collard green from both street- and supermarkets highlights the high risk of spreading parasites by eating raw vegetables sold in Aparecida de Goiânia.

Keywords: contamination, lettuce, collard green, helminths, protozoa.

Resumo

Hortaliças contaminadas com resíduos fecais são importantes vias de transmissão de parasitas intestinais ao homem. O objetivo deste estudo foi avaliar a presença de formas parasitárias de protozoários e helmintos em alface (*Lactuca sativa*) e couve (*Brassica oleracea*) vendidas em feiras e supermercados na cidade de Aparecida de Goiânia, Goiás, Brasil. Um total de 30 amostras de cada vegetal (15 amostras de supermercados e 15 de feiras livres) foi analisado. Todas as amostras foram processadas pelos métodos de sedimentação espontânea e centrífugo-flutuação. Em 45% das amostras foram identificadas formas imaturas de parasitas intestinais sendo 66,7% ovos de helmintos e 33,3% de cistos ou oocistos de protozoários. Significativamente, mais amostras de alface estavam contaminadas com ovos, cistos ou oocistos de pelo menos um parasita do que as amostras de couve (U = 216; Z = -3,45; P <0,001). As formas parasitárias foram identificadas morfologicamente ao nível de família com ovos de Ancylostomatidae, Strongyloididae, Ascarididae e Taeniidae ou oocistos de Eimeriidae, dos gêneros *Cystoisospora* sp. e *Toxocara* sp., e ao nível de espécies com *Cystoisospora canis*, *Dipylidium caninum* e *Hymenolepis nana*. A presença desses agentes infecciosos em alface e couve, provenientes tanto de feiras quanto de supermercados, ressalta o alto risco de veiculação de parasitas pela ingestão de hortaliças cruas comercializadas em Aparecida de Goiânia.

Palavras-chave: contaminação, alface, couve, helmintos, protozoários.

1. Introduction

Intestinal parasites are a serious public health problem that globally affect more than a quarter of the human world population (Jourdan et al., 2018; Li et al., 2020). In countries that still have serious social problems, such as Brazil, a high prevalence of enteroparasitoses is common mainly in regions with lower socioeconomic level (Andrade et al., 2010). This wide occurrence is mainly related to precarious conditions of basic sanitation, inadequate personal hygiene and the lack of care in handling food (Andrade et al., 2010; Fernandes et al., 2015; Rodrigues et al., 2020).

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Foliar vegetables are low-calorie foods with relevant nutritional profiles; they are rich in vitamins, minerals and fibers, essential for the body balance, and, when consumed regularly, they reduce the risk of developing diseases (Wallace et al., 2020). Despite the undeniable nutritional importance, raw or poorly washed vegetables contaminated with fecal residues or irrigated with water contaminated with parasitic forms have been reported as important vehicles for transmission of intestinal parasites to humans (Andrade et al., 2010; Fernandes et al., 2015). In Brazil, contamination of foliar vegetables is one of the main transmission routes of enteroparasites to humans (Soares and Cantos, 2005; Neres et al., 2011), and several studies have confirmed the presence of parasitic forms in vegetables commercialized in all regions of the country (Soares and Cantos, 2005; Esteves and Figueirôa, 2009; Neres et al., 2011; Gomes et al., 2014; Fernandes et al., 2015; Silva et al., 2015; Novacki et al., 2016; Santos et al., 2017; Maldonade et al., 2019; Machado et al., 2020; Rodrigues et al., 2020). However, in the State of Goiás there are still few reports on the occurrence of vegetables contaminated with parasitic forms. Parasitological evaluations of lettuce and collard green were conducted in the city of Anápolis, and in both surveys the contamination with parasitic protozoa and helminths was demonstrated (Neres et al., 2011; Moura et al., 2016). Gomes et al. (2014) also detected enteroparasites in lettuce grown on the edge of the Cascavél river in Goiânia, and Blanco et al. (2019) on lettuce produced and sold in the city of Inhumas. In Aparecida de Goiânia, second city in number of inhabitants of the State with almost 600,000 residents according to IBGE (2019), there is no record of parasitological analysis of foliar vegetables. The evaluation of food contamination has fundamental importance to check the risk of disease transmission, to reflect possible awareness campaigns with the population, to provide health authorities in the municipalities with new information and to encourage the elaboration of public policies of monitoring of vegetable-producing regions.

We report here the occurrence of parasites in *Lactuca sativa* (lettuce) and *Brassica oleracea* (collard) commercialized in street markets and supermarkets in the city of Aparecida de Goiânia.

2. Materials and Methods

2.1. Sample collection

Thirty samples of lettuce and 30 samples of collard acquired between September 2018 and May 2020 were analyzed, with 15 samples of each vegetable coming from street markets and another 15 from supermarkets in the city of Aparecida de Goiânia. One head of lettuce and sheafs of collard were established as the sampling unit, regardless of their weight. The samples were analyzed in the Biology Laboratory of the Federal Institute of Goiás, Aparecida de Goiânia campus or Multiuser Laboratory of IPTSP/UFG.

2.2. Processing

The vegetables were processed as described by Esteves and Figueirôa (2009) with some modifications. The samples were defoliated, and damaged leaves and the stalk were discarded. The remaining lettuce and collard leaves were then placed separately into a plastic vessel containing 250 ml of 0.1% neutral detergent solution and the leaves were washed with a brush. The liquid obtained was filtered through gauze and transferred to a conical glass flask (300 ml). After this procedure, the leaves were transferred, separately, to a clean plastic bag containing another 250 ml of 0.1% neutral detergent solution and manually and carefully shaken for 5 to 10 minutes. The water resulting from the second washing was also filtered through gauze and transferred to another 300 ml conical glass flask. The liquids were kept at rest for spontaneous sedimentation for 24 hours (Hoffman et al., 1934). Then, the supernatants were discarded, and the sediments from the lettuce or collard washing suspensions joined and transferred to a 50 ml Falcon tube and centrifuged at 2,500 rpm for 2 minutes. After centrifugation, the supernatant was carefully discarded, and the pellet was transferred to a 2 ml microcentrifuge tubes. Two microscope slides from each sample were prepared and stained with Lugol´s solution and analyzed using a Leica DM500 optical microscope or Leica DM 750 microscope with integrated ICC50 HD camera at 100x and 400x magnification. The rest of the samples was processed by the Zinc Sulphate Centrifugal Flotation Technique (Faust et al., 1939). Two slides from each sample were prepared and analyzed as described above.

2.3. Statistical analysis

The results were analyzed with non-parametric Mann-Whitney U Test using the program Statistica 7.0 (Stat Soft, Tulsa, USA).

3. Results

Immature forms of parasites were found in 45% of the 60 samples (30 lettuces and 30 collard greens). Of this total of positive samples, 40.7% had bi- or polyparasitism and 59.3% monoparasitism, regardless of the type of vegetable, commercial establishment or parasitic form evaluated. Forty-two infectious forms were identified in all samples, regardless of the type of vegetable, with 66.7% helminth eggs and 30.9% protozoan oocysts and 2.4% cysts. In addition, 20% of the lettuces and 6.7% of the collards were found with unidentified nematode larvae.

Helminths and or protozoa were detected in 70% of all lettuce samples evaluated. A higher percentual of contaminated samples (73.3%) was found in lettuce acquired in supermarkets than in samples from street markets (66.7%), but without statistical difference (U=92.5; Z=0.87; P=0.41). The most prevalent parasitic form detected in lettuce was Ancylostomatidae / Strongyloididae egg from street markets and supermarkets (Table 1). Eggs of *Toxocara* sp. and other Ascarididae, Taeniidae and unidentified nematode larvae were also observed in lettuce from both kind of markets. In 26.7% of the samples from supermarkets *Hymenolepis nana eggs* were found, and in one of these samples a *Dipylidium caninum* egg packet was identified (Figure 1; Table 1). Probable trematode eggs were found in two samples and a *Trypanoxyuris* sp.

Table 1. Frequency of immature forms of intestinal parasites in lettuce (*Lactuca sativa*) acquired in 15 street market and supermarket in Aparecida de Goiânia, Goiás.

Parasites	Street Market		Supermarket	
	N*	%	N*	%
Helminths (eggs)				
Ancylostomatidae/*Strongyloides*	4	26.7	5	33.3
Ascarididae	1	6.7	4	26.7
Hymenolepis nana	-	-	4	26.7
Ovigerous capsule of *Dipylidium caninum*	-	-	1	6.7
Taeniidae	1	6.7	2	13.3
Toxocara sp.	1	6.7	2	13.3
Protozoa (oocyst)				
Cystoisospora canis	1	6.7	-	-
Cystoisospora sp.	1	6.7		
Coccidian oocyst with multiple sporocysts	1	6.7	-	-
Eimeriidae	3	20	1	6.7
Cyst unidentified	1	6.7	-	-

* Number of positive samples.

Table 2. Frequency of immature forms of intestinal parasites in cabbage (*Brassica oleracea*) acquired in 15 street market and supermarket in Aparecida de Goiânia, Goiás.

Parasites	Street Market		Supermarket	
	N*	%	N*	%
Helminths (eggs)				
Ancylostomatidae/*Strongyloides*	1	6.7	-	-
Toxocara sp.	-	-	1	6.7
Protozoa (oocyst)				
Cystoisospora sp.	1	6.7	1	6.7
Eimeriidae	3	20	1	6.7

* Number of positive samples.

egg in one sample from a street market. In only one lettuce sample from a supermarket, a protozoan non-sporulated oocyst of Eimeriidae, was detected. In street markets two samples were found with Eimeriidae oocysts, one with coccidian oocyst containing numerous sporocysts, another non-sporulated *Cytoisospora* sp. oocyst with morphologically similar to *C. belli*, one unidentified cyst and in one sample, oocysts of Eimeriidae and *Cytoisospora canis* were detected (Figure 2; Table 1). A higher number of helminths (26 eggs) were identified in lettuce compared to protozoa (seven oocysts and one cyst) with statistical difference (U=308; Z=-2.1; P=0.01).

Generally, collards had a significantly lower percentage of contaminated samples (20%) regardless where the vegetables were obtained, than lettuces (70%) (U=216; Z=-3.45; $P<0.001$). In 13.3% of the samples from supermarkets and 26.7% from street markets parasites were identified without effect of the type of commercial establishment on the presence of parasites (U=98.5; Z=0.58; P=0.4). Eggs of Ancylostomatidae / Strongyloididae and *Toxocara* were recorded in one sample each and in two other samples nematode larvae were detected (Figure 1; Table 2). Oocysts of *Cystoisospora* sp. resembling to *C. belli* were found in 6.7% of the samples from supermarkets and in 13.4% from street markets. Other Eimeriidae oocysts were found in 6.7% and 20% samples from supermarkets and street markets, respectively (Figure 2; Table 2). There was no statistical difference between the number of helminths and protozoa detected in collards (U=404; Z=0.68; P=0.5).

4. Discussion

An elevated number of intestinal parasites was detected in foliar vegetables commercialized in Aparecida de Goiânia, especially lettuce. The index of 70% positivity of lettuce samples is very high comparing with several others studies carried out in Brazil (Esteves and Figueirôa, 2009; Neres et al., 2011; Fernandes et al., 2015; Silva et al., 2015; Moura et al., 2016; Santos et al., 2017; Machado et al. 2020); the nematode larvae were not included in statistics because they were not identified at the family, genus or species

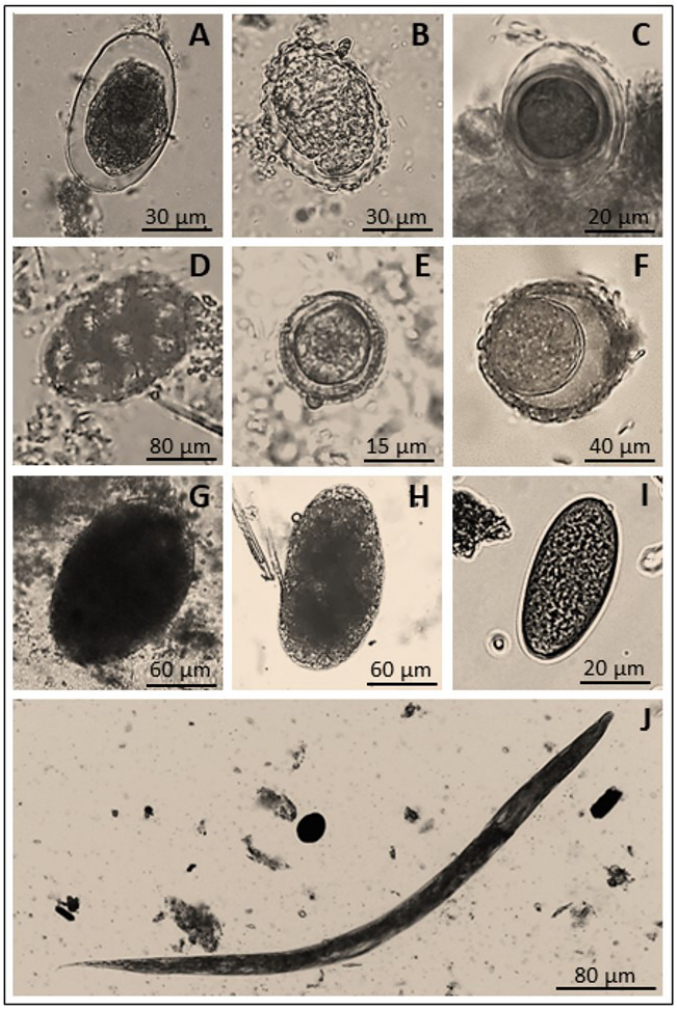

Figure 1. Eggs and larvae of helminths detected in samples of lettuce and collard greens from street market and supermarkets in Aparecida de Goiânia, Goiás. (A) Ancylostomatidae / Strongyloididae; (B) Ascarididae; (C) *Hymenolepis nana*; (D) ovigerous capsule of *Dipylidium caninum*; (E) Taeniidae, (F) *Toxocara* sp.; (G) and (H) probably Trematodae; (I) probably *Trypanoxyuris* sp.; (J) unidentified nematode larvae.

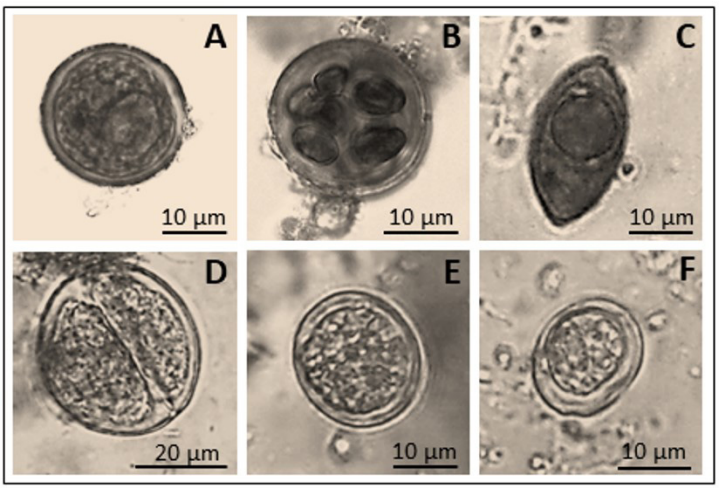

Figure 2. Protozoa detected in samples of lettuce and collard greens from street market and supermarkets in Aparecida de Goiânia, Goiás. (A) Cyst; (B) coccid oocyst with multiple sporocysts; (C) oocyst from *Cystoisospora* sp. resemble the *C. belli*; (D) oocyst of *C. canis*, (E) and (F) oocyst of Eimeriidae.

level, as they could be larvae of free-living non-parasitic nematodes. In addition, probable trematode eggs and *Trypanoxyuris* sp. were also not considered in the analysis. This is the highest percentage of lettuce contamination by parasites ever described in the state of Goiás (Neres et al., 2011; Gomes et al., 2014; Moura et al., 2016; Blanco et al., 2019), and similar or higher percentages have been reported in other cities mainly in the north and northeast of Brazil (Furtado et al., 2015; Novacki et al. 2016; Pinto et el., 2018; Carvalho et al., 2019; Oliveira et al., 2020; Rodrigues et al. 2020). The high prevalence in cities of these regions may be related to the lower percentages of basic sanitation, with emphasis on the sewage network, as described by Ministry of Cities in the National Plan for Basic Sanitation of 2019 (Brasil, 2019) and by Dantas et al. (2012). According data recorded in 2018 and published in the National Sanitation Information System (SNIS, 2018), only 10.5% and 28% of the population in the north and northeast regions, respectively, have access to a collecting sewage network while the national average in Brazil in this year was 53.2%. This information system also indicated that 23.8% of the households in Aparecida de Goiânia were connected to the sewage network (SNIS, 2018).

Regarding collard, the percentual of samples contaminated corroborates other studies carried out in the country and even in the State of Goiás (Esteves and Figueirôa, 2009; Moura et al., 2016; Belinelo et al. 2009). The difference in the level de contamination between the two studied vegetables may be related to the morphology of the plants. While lettuce has numerous large, juxtaposed and flexible leaves with a short stalk close to the ground allowing intense contact with the soil, manure, irrigation water and with fecal residues increasing the probability of contamination with parasitic forms during cultivation. *B. oleracea* is an herbaceous vegetable with a longer stalk than lettuce, and leaves are thick and alternating more distant from soil and possible contaminants (Arbos et al. 2010; Rodrigues et al. 2020). In addition, the health conditions of the environment in which vegetables are grown, the cultivation practices used, storage and distribution influence the level of contamination of vegetables (Belinelo et al. 2009; Rodrigues et al. 2020).

The presence of animal parasites such as *C. canis*, *D. caninum*, *Toxocara* sp. and the probable *Trypanoxyuris* sp. makes us believe that contamination of vegetables often occurs through fecal animal waste. However, the presence of unidentified parasites at the species level that belong to the families Ancylostomatidae, Ascarididae, Eimeriidae, Strongyloididae, Taeniidae, to the genera *Cystoisospora* and the species *H. nana* suggests that the contamination of lettuce and collard should have also occurred by human feces residues. Due to the similarity between the eggs of helminth species of the Ancylostomatidae family and Strongyloididae, the eggs found in a format resembling these two families were identified as Ancylostomatidae / Strongyloididae. Findings strengthen the high risk of consumers to passively acquire parasitoses as a definitive, intermediate or accidental host when eating raw or undercooked vegetables and / or actively via cutaneous invasion by helminth larvae when handling vegetables. The most common parasites found were nematodes belonging to Ancylostomatidae / Strongyloididae and Ascarididae and by cestodes of the family Taeniidae and *H. nana*. The detection of these parasites is not surprising, considering that the species and families of helminths described above are distributed worldwide and affect animals and humans (Dantas-Torres and Otranto, 2014; Gebrie and Engdaw 2015; Muehlenbachs et al., 2015; Savioli et al. 2017; Jourdan et al., 2018). However, there are still few reports on the occurrence of intestinal parasitosis in humans in Aparecida de Goiânia or on the contamination of food or public places by parasitic forms in this municipality. Damasceno and Costa (2017) reported that among the patients treated in the Hospital das Clínicas - UFG, in the city of Goiânia, patients from Aparecida de Goiânia were the second largest group of people attended with enteroparasitosis, without indicating the type of parasites. Silva et al. (2019) recorded the presence of parasites in fruits commercialized in this city, with emphasis on different species of protozoa and helminth eggs of *Ascaris lumbricoides* and *Fasciola hepatica*. In a study on the contamination of squares or parks in this municipality, 36.7% were contaminated with *Toxocara* sp. and hookworm eggs, being the first record of potential causative agents of larva migrans in Aparecida de Goiânia (Monteiro et al., 2018). It is interesting to note that in studies on the occurrence of vegetables contaminated with intestinal parasites sold in other cities in the State of Goiás, eggs of *A. lumbricoides, H. nana* and hookworms were also detected, which shows that these worms are common in the region (Neres et al., 2011; Gomes et al., 2014; Moura et al., 2016; Blanco et al., 2019). In the present study, the identification of most nematodes to the species level was not carried out and, thus, the parasites found could be human parasites or species of animals as the definitive hosts and represent a potential risk to cause anthropozoonoses. In the first case, considering the helminths found, there is a risk of the consumer acquiring ascariasis, hookworm / strongyloidiasis, teniasis, cysticercosis and himenolepiasis. However, as a parasite of animals, it could cause cutaneous larva migrans by larvae of *Ancylostoma* sp. and *Strongyloides* sp., visceral larva migrans by ingesting eggs from *Toxocara* sp. or *Ancylostoma* sp., hydatidosis also by ingestion of eggs, in this case of *Echinococcus granulosus* (Taeniidae). In rare cases, pathology due to accidental ingestion of insects containing *D. caninum* cysticercoid larva.

With the exception of one cyst, all protozoa found were coccids, intracellular parasites commonly found in the intestinal tract of a wide range of vertebrate animals, and one of the main causes of serious diseases and economic losses in livestock. Coccid parasites usually have a high specificity for their hosts, and some species affect humans (Chapman et al. 2013). Most of the coccids identified in the present study belonged to the Eimeriidae family and were found mainly in samples from street markets. In this family, the species *Cystoisopora belli* is a human parasite that has a wide distribution and is found mainly in tropical and subtropical areas (Dubey and Almeria, 2019). Only a few *Cytoisospora* found had characteristics and size similar to *C. belli*, which makes us believe that most of the detected eimerids were parasites of animals. The use of manure, the cultivation of vegetables close to breeding areas, the

free movement of animals at the planting site or the use of water for irrigation that receives animal waste are some of the possibilities of contamination of these vegetables by parasites.

The high number of parasitic structures, especially in lettuce, presupposes a low hygienic care when planting and handling vegetables, putting consumers' health at risk. To our knowledge, this is the first report on the prevalence of enteroparasites in lettuce and collard sold in the city of Aparecida de Goiânia. The presence of parasitic helminths and protozoa highlights the need to strengthen sanitary inspection actions as well as health education campaigns of producers and traders, in order to reduce the contamination of plants by parasites from humans or animals.

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