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From cultivation to commercialization: assessing the sanitary quality of various stages of organic vegetable production chain in southern Brazil

Do cultivo à comercialização: avaliação da qualidade sanitária de diferentes estágios da cadeia produtiva de vegetais orgânicos no sul do Brasil

Juliana da Silveira[I]
Jonathan Vieira dos Anjos[I]
Luciana Karbiak[I]
Gabriel Farias Souza[I]
Lucimara Batista Prox[I]
Israel Adrian Ríos Cerezo[I]
Alinne Petris[I]
Gustavo Strieder Scherer[II]
Diego Averaldo Guiguet Leal[I,*]

[I] Laboratório de Parasitologia Ambiental, Departamento de Patologia Básica, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brasil

[II] Laboratório Central do Estado do Paraná (Lacen), Unidade de Fronteira, Foz do Iguaçu, PR, Brasil

* **E-mail:** diego.leal@ufpr.br

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ABSTRACT

Introduction: The concomitant monitoring of parasites and fecal indicator bacteria (FIB) in the organic vegetable production chain is not routinely carried out. **Objective:** To evaluate contamination by intestinal parasites and FIB at different stages of the organic vegetable production chain. **Method:** For this purpose, two groups were defined: group I – five sampling campaigns at three sites of raw water used for irrigation of vegetables, and one site of vegetable washing water (VWW) from Rio Branco do Sul/PR. Group II – curly lettuces (n = 80) from the same municipality sold at organic open-air markets in Curitiba/PR. *Giardia* cysts and *Cryptosporidium* oocysts were searched by membrane filtration and direct immunofluorescence assay and FIB by Colilert® and Enterolert®. Lettuce from group II were eluted with glycine (1M), and the entire resulting sediment was analyzed by optical microscopy to search for parasites. **Results:** Contamination by *Cryptosporidium* and *Giardia* (oo)cysts was detected in 20.00% (n = 4) of the samples from group I. Among these, 40.00% of the VWW samples showed contamination by both protozoa. *Balantioides coli* cysts were detected in 40% of the post-hygienization samples. FIB was detected in all water sites, with a higher concentration of *Enterococcus* sp. In open street markets, 16.25% of the samples were contaminated by parasites, with *B. coli* being the most frequently detected. **Conclusions:** The results denote a wide dispersion of parasites at different stages of the organic vegetable production chain and reinforce the need for continuous monitoring of hygienic-sanitary quality at critical points in the vegetable production chain, aiming to prevent WFD.

KEYWORDS: Irrigation Water; Washing Water; Helminths; Protozoa; Food Safety

RESUMO

Introdução: O monitoramento concomitante de parasitos e bactérias indicadoras de contaminação fecal (BICF) na cadeia produtiva de vegetais orgânicos não é rotineiramente efetuado. **Objetivo:** Avaliar a contaminação por parasitos intestinais e BICF em diferentes etapas da cadeia de produção de vegetais orgânicos. **Método:** Para tanto, dois grupos foram definidos: grupo I – cinco coletas em três pontos de água bruta utilizada para irrigação de vegetais, e um ponto de água de lavagem dos vegetais (ALV), de Rio Branco do Sul/PR. Grupo II – alfaces-crespas (n = 80), do mesmo município, comercializadas em feiras livres de orgânicos de Curitiba/PR. Cistos de *Giardia* e oocistos de *Cryptosporidium* foram pesquisados nas amostras do grupo I, mediante filtração em membrana e imunofluorescência direta, e BICF mediante Colilert® e Enterolert®. As alfaces do grupo II foram eluídas com glicina (1M), e o sedimento resultante foi analisado por microscopia óptica para a pesquisa de parasitos. **Resultados:** A contaminação por (oo)cistos de *Cryptosporidium* e *Giardia* foi detectada em 20,00% (n = 4) das amostras do grupo I. Dentre essas, 40,00% das amostras de ALV apresentaram contaminação por ambos os

protozoários. Cistos de *Balantioides coli* foram detectados em 40,00% das amostras pós-higienização. As BICF foram detectadas em todos os pontos de águas, com maior concentração para *Enterococcus* sp. Nas feiras, 16,25% das amostras apresentaram contaminação por parasitos, sendo *B. coli* o mais frequente. **Conclusões:** Os resultados evidenciam ampla dispersão de parasitos em diferentes estágios da cadeia produtiva de vegetais orgânicos e reforçam a necessidade do monitoramento contínuo da qualidade higiênico-sanitária, em pontos críticos da cadeia produtiva de vegetais visando a prevenção das DTHA.

PALAVRAS-CHAVE: Água de Irrigação; Água de Lavagem: Helmintos; Protozoários; Segurança dos Alimentos

INTRODUCTION

The organic cultivation of vegetables is encouraged as an alternative to conventional cultivation, since the intensive use of pesticides is commonly associated with the emergence of various pathologies, including cancer, hormonal, respiratory, and allergic disorders[1,2,3].

Brazil is one of the world's largest producers of fruit and vegetables and the leading producer of organic food in Latin America[4]. However, the expansion of organic farming in the country has brought challenges related to contamination by enteric pathogens.

This contamination is largely due to the use of organic fertilizers, mainly of animal origin, the presence of domestic and wild animals near the crops and irrigation with untreated water, which favors the contamination of vegetables and the development of parasitic and bacterial infections among consumers[5,6,7,8,9]. In addition, other stages in the production chain, such as harvesting, handling, transportation, and marketing, also contribute to contamination[10,11,12].

The World Health Organization (WHO)[13] establishes guidelines for the quality of water used in irrigation, covering physical-chemical, microbiological, and parasitological parameters, with the aim of reducing the risk of disease transmission and ensuring food safety.

In Brazil, National Council for the Environment (CONAMA) Resolution No. 357 of March 17, 2005, defines physical-chemical and microbiological standards for water for different uses, including irrigation of vegetables consumed raw[14]. Among the parameters that must be analyzed are pH and turbidity, and in the microbiological field, only the analysis of thermotolerant coliforms is required.

For fresh food, Resolutions No. 623, of March 9, 2022, and No. 724, of July 1, 2022, supplemented by Normative Instruction No. 161, of July 1, 2022, both of the Brazilian National Health Surveillance Agency (Anvisa), establish that products intended for human consumption must be free of pathogenic microorganisms, parasites, helminths and protozoa at any stage of development[15,16,17].

In a global call made in partnership by the Food and Agriculture Organization of the United Nations (FAO) and the WHO, with the aim of estimating and classifying the main food-borne parasites, there is great mention and emphasis on the consumption of fresh vegetables, as the main disseminators of various helminthiases and intestinal protozoa that cause gastroenteritis, including zoonotic ones such as *Cryptosporidium* spp, *Giardia duodenalis*, and *Balantioides coli*[18]. In Brazil, according to the Ministry of Health's 2024 report on outbreaks of foodborne diseases (FBD) between 2014 and 2023, only 30% of outbreaks had the causative food identified, of which 2.6% were related to vegetables, which reinforces the need for studies on fresh vegetables[19]. In addition, data on the contamination of organic vegetables by parasites is scarce in the literature, contrasting with the consolidated evidence for conventional crops[20,21].

In view of the above, the aim of this study was to evaluate the hygiene and health quality at different points in the production chain of organic vegetables grown in rural areas of the metropolitan region of Curitiba (Paraná - PR) and sold in the capital of Paraná, as a strategy for understanding the environmental epidemiology of parasites and microorganisms of the fecal-oral route in a relevant fresh produce producing area.

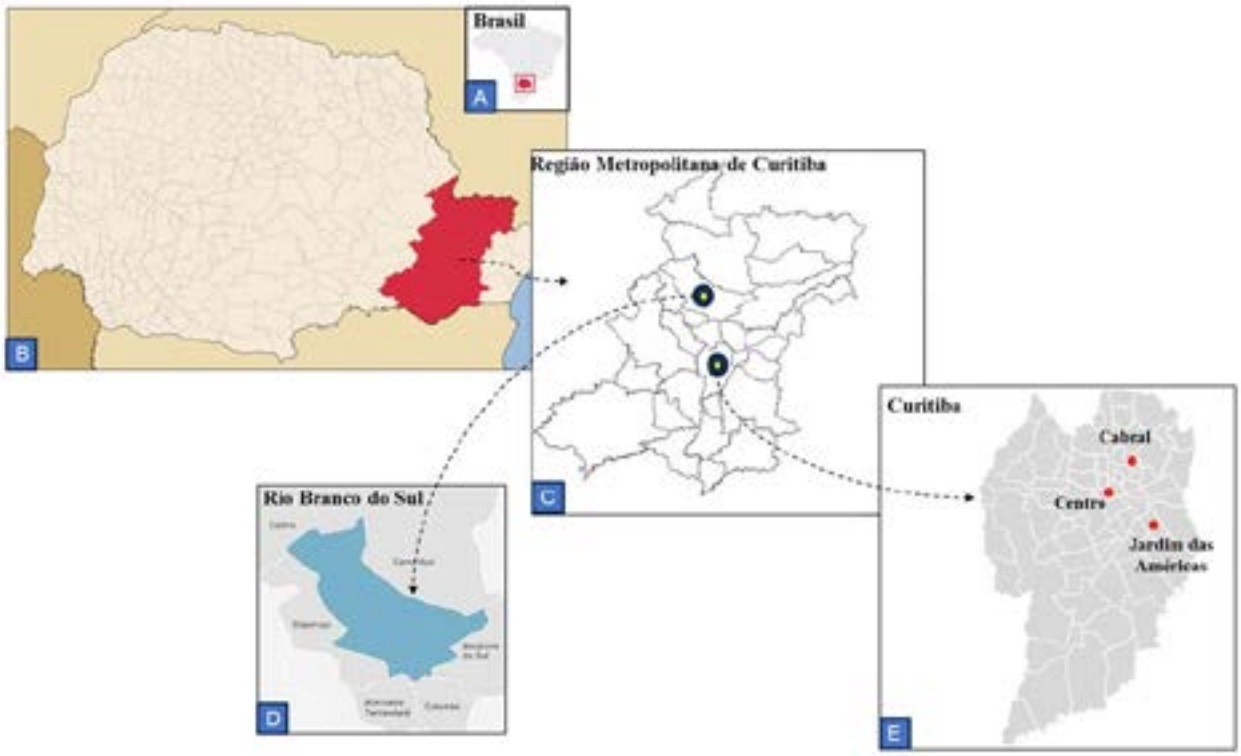
METHODS

Experimental design

The assessment of the hygiene and health quality of the organic food production chain produced in a rural area in the metropolitan region of Curitiba was carried out in two groups: Group I - irrigation water (points 1, 2, and 3) and organic vegetable washing water (point 4) in the town of Rio Branco do Sul (Figure 1), located 30 km from Curitiba. All the points were tested for *Cryptosporidium* spp. oocysts, *Giardia* spp. cysts, *Escherichia coli*, and *Enterococcus* sp., and point 4 was also tested for the presence of other intestinal parasites. Group II - organic vegetables from group I, sold at farmers markets in the capital, for the detection of helminths and protozoa.

Group I - Water collection and sampling points

For the analysis of water samples used to irrigate vegetables from certified properties, as well as vegetable washing water (VWW), five collection campaigns were carried out in the city of Rio Branco do Sul from October 2019 to February 2020. This area is part of the Capivari river basin, one of the state's main watercourses.



Source: Prepared by the authors, 2024.
A and B: Map of Paraná in relation to Brazil; C: Metropolitan Region of Curitiba; D: Area where raw water used for irrigation and vegetable washing water was analyzed in Rio Branco do Sul; E: Map of Curitiba showing the locations of organic fairs.

Figure 1. Location of the water collection points for group I and organic vegetables sold at free markets in Curitiba, group II.

Four analysis points were previously defined (Figure 2): point 1 - raw river water, before being stored in reservoirs (lotic environment); point 2 - water stored in reservoirs after being abstracted from the river (lentic environment); point 3 - irrigation water. The water that irrigates vegetables comes from point 2, being sucked by a pump and sent to polyvinyl chloride (PVC) pipes and then released by sprinkling; and point 4 - water used to wash the vegetables. Moreover, during most of the months of work, at the request of the farmers, the microbiological and physical-chemical quality of the well water ingested by the population and for animal watering was analyzed. This water is also used for the procedure at P4.

For all the points, 250 mL of water was collected in a sterile amber glass container for the physicochemical analysis, and 250 mL for the research and quantification of fecal contamination indicator bacteria (FCIB), in previously autoclaved bottles treated with a chelating reagent, following the guidelines of the Standard Methods for the Examination of Water and Wastewater[22]. For the identification of *Cryptosporidium* spp. and *Giardia* spp., 10-liter volumes of water were collected in first-use gallons pre-treated with 0.1% Tween 80 (Dinâmica Química Contemporânea, Indaiatuba, Brazil), to prevent the adhesion of cysts and oocysts.

In the VWW analysis stage (point 4), the same volume was collected, only after several cultures had been washed. All the samples were identified and then transported to the laboratory under refrigerated conditions for further processing.

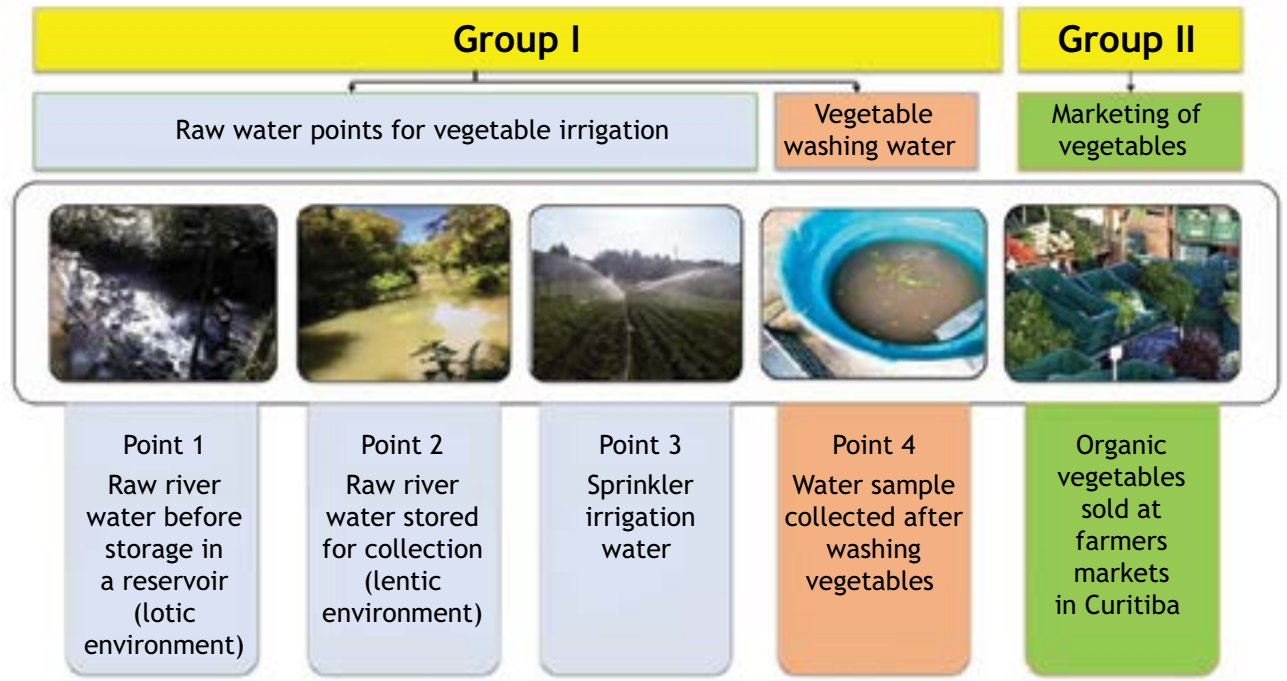
Physico-chemical and microbiological analysis

All the samples were measured for pH using a multisensor device - Mpa-210 (Hanna, Barueri, Brazil) and turbidity (UT) using a turbidimeter - AP 2000 W (Policontrol, Diadema, Brazil).

The detection and quantification of FCIB was carried out by the defined substrate method, using the standard operating procedure of the Colilert® method for total coliforms and *Escherichia coli* and Enterolert® for the detection and quantification of *Enterococcus* (Idexx Laboratories, Inc., Maine, USA), as described in the Standard Methods for the Examination of Water and Wastewater[22]. The results were expressed as Most Probable Number (MPN) per 100 mL.

Detection of Giardia cysts and Cryptosporidium oocysts

The samples collected from points 1, 2, 3, and 4 (n = 20) were processed using the filtration technique on cellulose ester membranes (47 mm in diameter and nominal pore size of 3 µm). The procedure followed the method described by Leal et al.[23]. The filtration system consisted of a vacuum pump (Prismatec, Itu, Brazil) adjusted to a flow rate of between 0.4 and 4 L/min, using the Millipore filter holder apparatus®.



Source: Prepared by the authors, 2024.

Figure 2. Description of the water collection points for irrigation and washing of organic vegetables and the marketing stage, respectively in the Metropolitan Region of Curitiba and the capital.

The elution stage was carried out by scraping and washing each membrane with a surfactant solution of Tween 80 (0.1%) for 20 min. The resulting liquid was transferred to conical tubes and concentrated by centrifugation at 1,500 x *g* for 15 min. All the samples were analyzed by direct immunofluorescence reaction with anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies, directed at the epitopes present on the walls of the oocysts and cysts, respectively, according to the manufacturer's standard operating procedure (Merifluor® Meridian Bioscience, Cincinnati, Ohio, USA).

The morphology of the protozoa was confirmed using the vital dye DAPI in combination with Differential Interference Contrast (DIC) microscopy. The criteria adopted for classifying cysts and oocysts complied with those stipulated by the United States Environmental Protection Agency (USEPA)[24].

Detection of other parasites in vegetable washing water

To detect helminth eggs and (oo)cysts of other intestinal protozoa, 2 L of each sample were used every month, collected exclusively after washing different organic vegetables (carrots, beet, lettuce, tomatoes, and radishes) produced in Rio Branco do Sul (Point 4).

The volume was divided into conical centrifuge tubes of 50 mL centrifuge tubes (Firstlab, China) and subjected to six centrifugations at 1,500 x *g* for 15 min. The supernatant was discarded and the resulting sediment (2 mL per sample) was transferred to microtubes (Olen, China). The entire sediment was analyzed with Lugol's solution (Cromoline Química Fina, Diadema, Brazil) using optical microscopy, resulting in approximately 40 slides per sample.

Group II - Collection and sampling points of vegetables sold at organic farmers markets

To investigate the natural occurrence of helminth eggs and (oo) cysts of other intestinal protozoa in vegetables, lettuce samples were acquired from three organic fairs in the city of Curitiba, which receive products from rural areas, totaling 80 samples. Of these samples, 27 were obtained from market A (central district), 33 from market B (Jardim das Américas), and 20 from market C (Cabral) (Figure 1E).

A head of lettuce was adopted as the sampling unit, taken directly from the available lot, regardless of weight or size, as long as the sample had intact leaves and was in good condition. The samples were then transported and stored in clean plastic bags for parasite testing.

Parasitological analysis

For each sample, 30 g of vegetables were analyzed according to a standardized methodology previously validated inter-laboratory[25,26]. Briefly, each portion of vegetable was washed with 200 mL of 1M glycine solution, pH 5.5, (Synth, Diadema, Brazil), in first-use plastic bags. The eluate was filtered and collected in sedimentation cups, which were kept at rest for 2 hours. The supernatant was then removed, and 10 mL of the sediment was transferred to conical centrifuge tubes (Firstlab, China) and centrifuged at 1,120 x *g* for 5 min.

The entire sediment resulting from the 80 samples was read using lugol (Cromoline Química Fina, Diadema, Brazil) under optical microscopy.

RESULTS

Monitoring the microbiological, physico-chemical, and parasitological quality of the water used for irrigating and washing organic vegetables in the municipality of Rio Branco do Sul (Group I).

The microbiological indicators of fecal contamination, *E. coli* and *Enterococcus* sp., were identified, along with the presence of total coliforms, in all the water samples, as detailed in Table 1.

Regarding *E. coli* contamination, when considering the monthly values and the average for the period, the water used for irrigation and washing vegetables showed the best microbiological quality (Table 1).

However, when measuring the detection and enumeration of *Enterococcus* sp., the average contamination densities were higher, showing an important impact of fecal contamination. The highest contamination averages for this group of bacteria were identified at points 1 and 2.

As for pH and turbidity monitoring (Table 1), almost all the samples analyzed during the period complied with Brazilian guidelines[14], except for P1 in December 2019, as well as P1 and P2 in January 2020, which showed pH levels below the permitted values. In general, the highest pH and turbidity averages were observed in P3 and P4, respectively.

Analyses of the microbiological quality of well water used for cooking, watering, and washing organic vegetables in the rural area showed good sanitary quality in 75.00% of the samples (Table 2).

As for contamination by *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts, contamination by at least one of these genera was confirmed in 20.00% of the samples (n = 4/20) (Table 3).

In P1, *Cryptosporidium* spp. oocysts were detected in 20.00% of the samples and were not identified in the other sampling points related to irrigation water (P2 and P3).

Table 1. Microbiological (MPN/100 mL) and physico-chemical monitoring of raw water and organic vegetable washing water (Group I), in a rural area of the Metropolitan Region of Curitiba, Paraná.

	Total coliforms				Escherichia coli				Enterococcus sp.			
Month / year	**P1**	**P2**	**P3**	**P4**	**P1**	**P2**	**P3**	**P4**	**P1**	**P2**	**P3**	**P4**
10/ 2019	1,413.6	1,299.7	816.4	> 2,419.6	26.3	59.0	61.4	8.4	260,3	416,0	658,6	549,3
11/ 2019	197.1	1,217.2	396.8	1,986.3	30.4	72.2	51.8	52.5	371,9	727	490,7	195,7
12/ 2019	1,986.3	270.3	467.4	866.4	4.1	5.1	3.1	5.2	< 1,0	< 1,0	105,6	122,2
01/2020	1,732.9	307.6	2,419.6	452	51.7	6.1	74.7	< 1.0	< 1,0	< 1,0	< 1,0	< 1,0
02/2020	108.5	> 2,419.6	214.1	231	35.5	161.4	< 1.0	71.7	980	1.046	< 1,0	< 1,0
Average indicators	**1,087.6**	**1,102.9**	**862.8**	**1,191.6**	**29.6**	**60.7**	**38.4**	**27.7**	**322,8**	**438,2**	**251,3**	**173,8**

	pH				Turbidity (uT)			
Month / year	**P1**	**P2**	**P3**	**P4**	**P1**	**P2**	**P3**	**P4**
10/ 2019	6.94	7.87	8.06	7.8	3.47	3.77	5.4	6.8
11/ 2019	6.21	6.98	7.56	7.02	3.98	4.72	5.56	9.94
12/ 2019	5.92	7.86	7.54	6.97	3.02	4.37	3.55	4.32
01/2020	5.12	5.94	6.23	6.52	9.91	6.84	9.98	3.14
02/2020	6.02	6.31	6.53	6.54	4.73	7.12	4.98	12.8
Average	**6.04**	**6.99**	**7.18**	**6.97**	**5.02**	**5.36**	**5.89**	**7.4**

Source: Prepared by the authors, 2024.

Table 2. Sanitary quality of well water used by farmers and for washing vegetables.

Month/year	Total coliforms*	E. coli*	Enterococcus sp.*	pH	Turbidity
October/2019	9.1	< 1.0	9.5	N	N
November/2019	579.4	1.0	437.1	6.52	3.21
December/2019	665.3	< 1.0	< 1.0	5.98	4.36
February/2020	20.4	< 1.0	< 1.0	6.33	1.54

N: Not carried out. *Values expressed in MPN/100mL.
Source: Prepared by the authors, 2024.

Table 3. Monitoring of contamination by *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts in different samples used for irrigating and washing organic vegetables in Rio Branco do Sul.

Month / Year	P1			P2			P3			P4		
	RID	DAPI	DIC	RID	DAPI	DIC	RID	DAPI	DIC	RID	DAPI	DIC
Oct / 2019	(+) Crypto	(a)*	(a)**	ND	NA	NA	NA	NA	NA	(+) Crypto	(a)*	(a)**
Nov / 2019	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dec / 2019	NA	NA	NA	(+) Giardia	(a)*	(a)**	ND	NA	NA	(+) Giardia	(a)*	(a)**
Jan / 2020	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Feb / 2020	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(+): RID positive - compatible size and shape/shiny apple-green fluorescence - no atypical structures on DAPI and DIC filters; (a)*: DAPI negative - light blue staining, no distinct nuclei; (a)** DIC: "empty" (oo)cyst; ND: not detected; NA: not applicable. In the positive samples, a maximum of one (oo)cyst was visualized in each well of the immunofluorescence slides.
Source: Prepared by the authors, 2024.

Similarly, contamination by *Giardia* spp. cysts was detected in P2 (n = 1). Finally, the highest contamination rates were found in P4, after sanitizing the vegetables, in which both protozoa were detected in 40.00% of the samples.

In addition, other parasites were identified in P4. The presence of at least one parasitic form was confirmed in all the samples. Nematode larvae were observed in 80.00% of the samples analyzed. Contamination by *Balantioides coli* cysts was detected in 40.00% of the samples (October and December 2019), with frequencies of six and one cyst, respectively.

Natural occurrence of parasites in organic lettuces sold at farmers markets in Curitiba (Group II)

A total of 80 samples of organic lettuce sold at farmers markets in the capital and produced in Rio Branco do Sul were analyzed. Parasite contamination, including helminth eggs and (oo)cysts of intestinal protozoa, was identified in 16.25% of the samples (Table 4).

Among the positive samples, *B. coli* was the most frequently detected parasite (38.46%) (Figure 3), followed by hookworm eggs (30.76%) and in descending order *Ascaris* sp., *Trichuris* sp., *Hymenolepis nana*, and *Isospora* sp.

Nematode larvae were identified in 3.75% of the samples. The presence of whole arthropods was detected in 5%, and arthropod fragments in 32.5%.

DISCUSSION

The simultaneous assessment of the physical-chemical, microbiological, and parasitological quality of the water used for irrigating and washing vegetables, as well as the parasitological contamination of products at the marketing stage in organic fairs, has never been carried out in the metropolitan region and the capital of Paraná.

Monitoring the sanitary quality of the raw water sites sampled is relevant, as they are part of the Capivari River Basin, one of the main watercourses that runs through four municipalities in the Metropolitan Region of Curitiba: Almirante Tamandaré to the west, Bocaiúva do Sul to the east, Colombo to the south, and Rio Branco do Sul to the north.

It should be noted that in this study the microbiological quality of the water used for irrigation was monitored using parameters that were more restrictive than those recommended by the current resolution. *E. coli* contamination was detected in low concentrations at the raw water points (Table 1). The higher rates of contamination by *Enterococcus* sp. may be related to their greater resistance and long-term survival in the aquatic environment[27]. Thus, *Enterococcus* sp. is a more reliable indicator for assessing fecal contamination in irrigation or drinking water.

Other studies carried out in the west of the state, in the city of Foz do Iguaçu on raw and treated surface water, and in the capital of Paraná on water intended for human consumption, also found that the greatest evidence of fecal contamination was measured by including the enterococci group as a complementary indicator to *E. coli*[27,28].

In addition, it was observed that in the water impoundment in a lentic environment (P2) there was an accumulation of organic matter, which may have favored the detection of a higher bacterial density. These findings differ from the results found in another study where the concentration of indicator bacteria was higher in lotic environments compared to points considered lentic in a large reservoir used to supply water to cities in the interior of São Paulo[29].

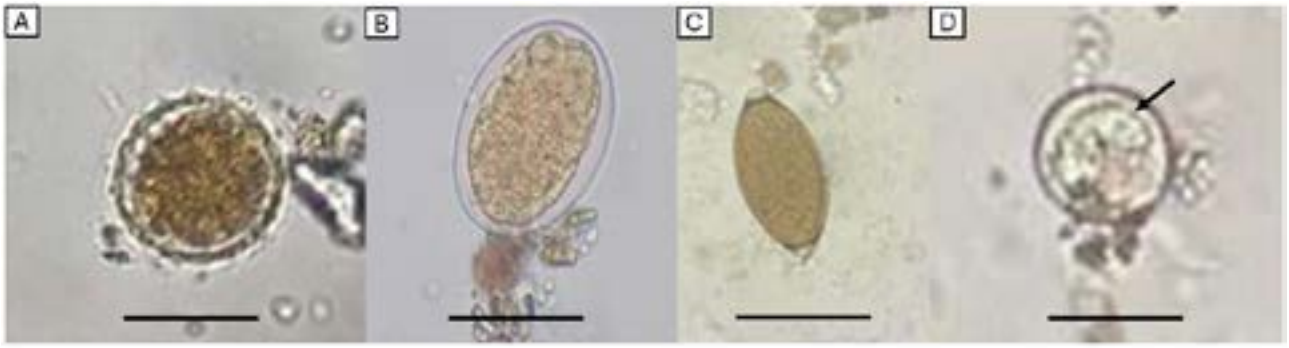
The presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts was confirmed in 20.00% of the water samples from P1 and P2. Although the protozoa were not directly identified in the water used for irrigation (P3), this water originates from P1 and is stored in P2 for later use in crop irrigation.

Both protozoa were identified in a similar number of surface water samples in this study, although *Giardia* cysts are detected more frequently in raw water in Paraná[27,30,31,32]. Giardiasis is

Table 4. Description of parasitic forms and insects detected in positive samples of organic vegetables sold at street markets (Group II).

Sample	Parasitic form	Insects	Location
3	Hookworm	ND	C
6	*Balantioides coli*	ND	C
8	*Balantioides coli*	ND	B
13	ND	Insect fragment	B
14	*Hymenolepis nana*	ND	B
16	*Ascaris* spp.	ND	C
18	*Balantioides coli*	ND	C
22	*Balantioides coli*	Whole insect	B
25	ND	Insect fragment	A
28	ND	Whole insect	C
29	*Trichuris* sp.	ND	C
31	ND	Whole insect	B
34	Nematode larva	ND	C
35	*Isospora* spp.	Insect fragment	C
37	ND	Insect fragment	A
44	ND	Insect fragment	A
47	ND	Insect fragment	A
54	ND	Insect fragment	A
56	Hookworm / nematode larva	ND	A
60	ND	Whole insect	A
61	*Balantioides coli*	Insect fragment	A
63	Hookworm/Ascaris sp.	Insect fragment	B
64	ND	Insect fragment	B
65	ND	Insect fragment	B
66	ND	Insect fragment	B
67	Hookworm / nematode larva	Insect fragment	B
68	ND	Insect fragment	B
69	ND	Insect fragment	B
70	ND	Insect fragment	B
71	ND	Insect fragment	A
72	ND	Insect fragment	A
73	ND	Insect fragment	A
74	ND	Insect fragment	A
75	ND	Insect fragment	B
76	ND	Insect fragment	B
77	ND	Insect fragment	B
78	ND	Insect fragment	B
79	ND	Insect fragment	B
80	ND	Insect fragment	B

ND: not detected.
Source: Prepared by the authors, 2024.



Source: Prepared by the authors, 2024.
A) *Ascaris* sp. egg; B) Hookworm egg; C) *Trichuris* sp. egg; D) *Balantioides coli* cyst with arrow highlighting the macronucleus. 400 x magnification. Scale: 50 µm.

Figure 3. Natural occurrence of parasites in organic lettuces sold at farmers markets in Curitiba.

considered endemic in Brazil, with some states having a prevalence of over 30.00%, including Paraná[33].

It is important to highlight specific cases observed in this study, such as December 2019, when the presence of *Giardia* cysts was detected in P2 and the irrigation water showed excellent microbiological quality for both FCIB.

In light of this finding, the need to monitor intestinal protozoa that show broad environmental resistance in irrigation water becomes evident, given that FCIB are removed or inactivated earlier, in addition to several studies signaling a lack of correlation between FCIB and pathogenic protozoa[6,7,32].

Thus, the results of this study indicate that the water used for irrigation may be responsible for the introduction of both protozoa into organic farming in the city of Rio Branco do Sul.

Contamination by these protozoa may also be related to variations in rainfall, human and/or agricultural activities in the vicinity, the presence of cattle, especially calves, and the movement of wild animals and birds, which can carry cysts and oocysts[34,35,36].

Physico-chemical factors can also be associated with the presence of *Cryptosporidium* spp. and *Giardia* spp. in different aquatic environments, including temperature, pH and especially turbidity[37,38]. In the present study, there was an increase in turbidity throughout the stages from irrigation to vegetable washing (P1 to P4). The highest levels of turbidity were found in the samples from P4. These results are not surprising, since currently several crops are being cleaned in the same container. Therefore, the soil and other impurities present on the surface and/or roots of these foods are transported to the washing water.

Furthermore, the lowest averages for both FCIBs were found at this same point, at the stage of sanitizing and washing leaves, roots, and organic tubers with well water, which is considered to be of good microbiological quality most of the time. This demonstrates the importance of this type of water for irrigating or cleaning vegetables, when compared to surface water (raw) which is subject to greater fecal contamination.

The importance of the analysis conducted at the final stage - post-harvest (P4), which precedes the marketing of the food - is reiterated, as they reflect the contamination rate of the entire cultivation stage, in which 40.00% of the water samples were contaminated with *Cryptosporidium* oocysts or *Giardia* spp. cysts, and *Balantioides coli* cysts.

B. coli is the only intestinal ciliated protozoan with pathogenic potential for humans, with asymptomatic chronic balantidiosis being the most commonly identified form[39,40]. However, in some hosts, the disease can progress to severe dysentery, making it an opportunistic pathogen[41,42].

The parasite has zoonotic potential, infecting the gastrointestinal tract of farm animals such as pigs and cattle, with domestic and wild pigs considered the main reservoirs[39,40].

Several of the organic farms have pig and cattle farms, so it is reasonable to infer that the contamination of vegetables by this protozoan in the washing water could be derived from animals, along with the use of contaminated organic fertilizers. During the initial visit to select certified organic farms in the city, it was observed that more than one type of organic fertilizer is used, including those derived from turkey droppings. However, the possibility of anthropogenic contamination by the indigenous population cannot be ruled out.

B. coli was also the most frequently identified parasite in vegetables sold from rural areas to organic markets in the capital. In addition, the detection of hookworm and *Trichuris* sp. eggs suggests that the contamination and spread of these parasites in the environment can be attributed to the access of domestic animals to the growing areas, as observed during the study, just as the detection of *Hymenolepis nana* eggs may indicate the presence of rodents in the crops[43].

The rural area of the municipality does not have environmental sanitation services and septic tanks are commonly used to dispose of sewage. This fact, together with the presence of pig farming, could explain the detection of *Ascaris* sp. in some vegetable samples, where contamination could also be related to the

transportation of human or pig fecal material by mechanical vectors, or even by the dispersal of this material by the wind[44,45,46].

Therefore, it is clear that multiple factors can influence the results of this study, such as living conditions and sanitation in rural areas, the use of organic fertilizers from sources that do not guarantee the safety of the plants, the contamination of aquatic environments, and the possible runoff of feces from grazing animals.

Moreover, the main factors contributing to food-borne parasitic infections, apart from the inadequate agricultural practices already mentioned, include the consumption of raw vegetables without any cooking process, low infective dose, environmental persistence, and resistance to disinfection procedures of these pathogens[12,21,45].

To summarize, when taken together, the results from the four water points associated with the cultivation or cleaning of Group I vegetables, together with the analysis of vegetables grown in this city and sold at open-air markets in the capital of Paraná, demonstrate a significant impact of parasite contamination, implying a potential risk of acquiring parasitic diseases, including those of a zoonotic nature.

CONCLUSIONS

The quality of irrigation water from an important river basin in Paraná proved to be unsatisfactory when a more restrictive fecal contamination indicator was applied. This indicates that current regulations may be insufficient to predict the quality of water used in organic farming. It is therefore essential to adopt stricter measures, such as monitoring multiple FCIB and, in a future revision of the regulations, to consider including pathogenic protozoa testing in water samples intended for this purpose.

Pre-washing is relevant because it shows contamination by different parasites in different vegetables during the cultivation phase and is considered a critical control point in this stage prior to marketing. This study also highlights the need to trace the sources of contamination and to develop health education strategies and guidelines on good agricultural practices for rural producers, to mitigate contamination and protect public health.

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Author Contributions

Silveira J, Anjos JV, Karbiak L, Souza GF, Prox LB, Cerezo IAR, Petris A – Data analysis, data interpretation, and manuscript writing. Scherer GS – Study planning (study design), data analysis, data interpretation, and manuscript writing. Leal DAG – Conception, study planning (study design), data analysis, data interpretation, and manuscript writing. All authors approved the final version of the manuscript.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.

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