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Organic horticulture: a current demand, whose proper management is the only guarantee of safe food

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Resumo

Horticultura orgânica: uma demanda atual, cujo manejo adequado é a única garantia de alimento seguro. O estudo foi realizado entre julho de 2014 e maio de 2016, em 21 propriedades hortícolas do estado do Paraná, Brasil. Foram coletadas duas amostras de vegetais folhosos e uma amostra de água de irrigação por propriedade. As amostras de água foram analisadas pela técnica do substrato cromogênico para avaliar a contaminação por coliformes totais e *Escherichia coli*, e os vegetais foram avaliados pelas técnicas de Willis (1921), Hoffman et al. (1934), Faust et al. (1939) e quanto à contaminação por parasitas. Observou-se presença de *E. coli* em 80,95% (17/21) das amostras de água; com relação aos vegetais, 19 (45,23%) continham pelo menos uma espécie de parasita, tais como: ancilostomatídeos, *Chilomatix* spp., *Dipillidium* spp., *Entamoeba* spp., *Strongyloides* spp., *Trichuris* spp., larva de vida livre, larva de nematódeo, oocisto não-esporulado. Houve associação estatística entre o destino do esgoto (fossa seca) e a positividade aos parasitas. Os dados mostram contaminação fecal em número significativo de amostras e confirmam a necessidade de maiores exigências sanitárias durante o cultivo de hortaliças folhosas, que, na maioria das vezes, são consumidas cruas.

Palavras-chave: *Escherichia coli*; Helminto; Parasita; Protozoário

Abstract

The objective of this study was to evaluate the contamination by *Escherichia coli* in irrigation water and parasites in leafy vegetables cultivated in small organic horticultural properties and to investigate the critical points during cultivation. The study was carried out between July 2014 and May 2016 in 21 horticultural properties in the state of Paraná, Brazil. Two samples of leafy vegetables and one sample of irrigation water were collected per property. Water samples were analyzed by the chromogenic substrate technique to evaluate contamination by total coliforms and *Escherichia coli*, and the vegetables were evaluated by the techniques of Willis (1921), Hoffman et al. (1934) and Faust et al. (1939) for parasite contamination. The presence of *E. coli* was observed in 80.95% (17/21) of the water samples; with respect to vegetables, 45.23% (19/42) contained at least one parasite species, such as: hookworms, *Chilomatix* spp., *Dipyllidium* spp., *Entamoeba* spp., *Strongyloides* spp., *Trichuris* spp., nematode larva, non-sporulated oocyst. There was a statistical association between the fate of the sewage (cesspool) and the positivity to the parasites. The data show fecal contamination in a significant number of samples and confirm the need for greater sanitary requirements during the cultivation of leafy vegetables, which are mostly consumed raw.

Key words: *Escherichia coli*; Helminth; Parasite; Protozoan

Introduction

Organic products have been preferred by consumers because they are associated with a production system that avoids or excludes the use of industrialized chemicals such as synthetic fertilizers, growth regulators and pesticides or agrochemicals (SANTANA et al., 2006; ABREU et al., 2016). On the other hand, there is growing concern about the risk of infection by enteroparasites, since many raw vegetables are served for human consumption, thereby contributing to the oral transmission of these parasites (ESTEVES; FIGUEIRÔA, 2012).

Endoparasites are cosmopolitan and endemic in third-world countries and represent an important problem in public health. They are acquired by ingestion of infective forms contained in contaminated food or water (CACCIÒ et al., 2018). When consumed raw, vegetables can serve as a transmission via (VOLLKOPF et al., 2006; FERREIRA et al., 2018). The cultivation conditions, including quality of irrigation water, the type of fertilizer used, the storage conditions, transport and handling of the crop by the producer are directly related to this contamination (PACHECO et al., 2003).

The objective of this study was to evaluate the contamination by *Escherichia coli* in irrigation water and parasites in leafy vegetables cultivated in small organic horticultural properties and to investigate the critical points during cultivation.

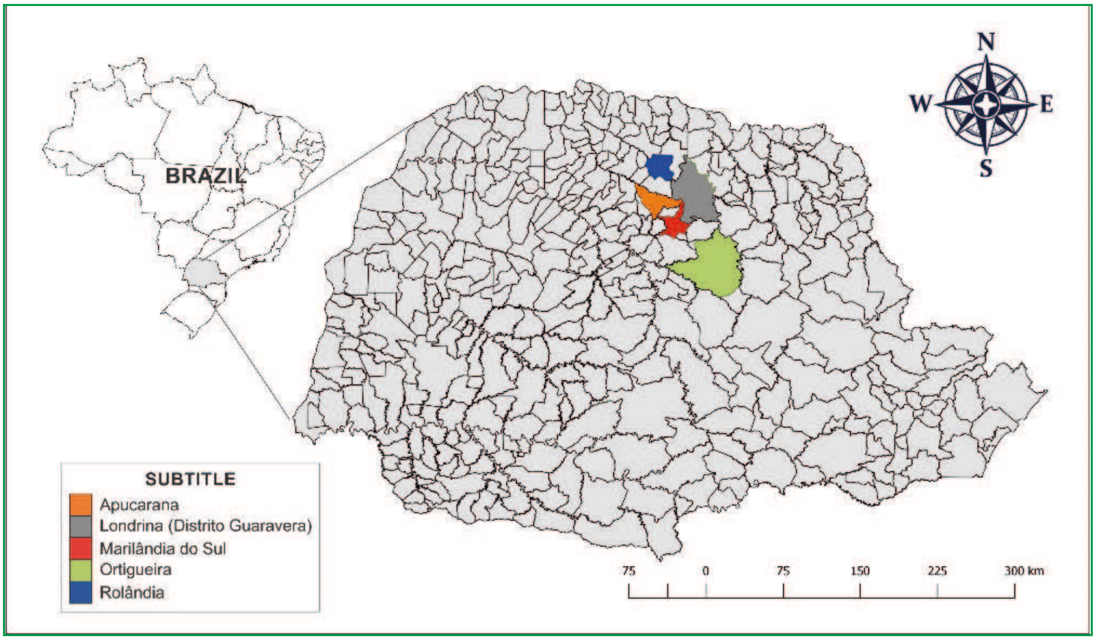
Material and Methods

The study was carried out from July 2014 to May 2016 at horticultural properties in the municipalities of Apucarana, Marilândia do Sul, Ortigueira, Rolândia and Londrina (District Guaravera), in the state of Paraná (Figure 1). Small commercial organic leafy vegetable production properties assisted by the Organic Certification Project of the State University of Londrina were included in analyses.

Forty-two clumps of leafy vegetables were collected (two per property), packed in plastic bags and kept under refrigeration. For detection of helminths, 500 mL of Glycine 1 M and 100 g of the vegetables were homogenized in a plastic bag, manually shaken for 10 minutes, filtered through double gauze, collected in a conical chalice, and allowed to settle for 12 h. Then, 20 µL of the sediment was observed under an objective microscope at 40x (HOFFMAN et al., 1934). The supernatant was removed from the chalice with a pipette, the pellet was centrifuged at 1120 x g for 5 minutes. With the final sediments (approximately 0.5 mL) slides stained with Lugol's iodine (MATOSINHOS, 2012) were mounted to facilitate the detection and identification of eggs and larvae under an optical microscope.

For detection of protozoa, 50 g of the vegetables and 300 mL of Tween 80 (1%) solution were placed in a plastic bag and homogenized with a stirrer for three minutes. Subsequently, the solution resulting from the first step was filtered through double gauze into a

FIGURE 1: Map of the state of Paraná highlighting the municipalities of Apucarana, Londrina, Marilândia do Sul, Ortigueira and Rolândia, sampling sites of vegetables and water subjected to parasitological and microbiological evaluation, from 2014 to 2016, Paraná, Brazil.



500-mL glass beaker. The filtered extract was divided into tubes and centrifuged at 2100 x g for 10 minutes each time (CARELI, 2009). Approximately 5 mL of the extract was processed and observed by the technique of Willis (1921) and Faust et al. (1939).

The microbiological analysis was performed using the chromogenic substrate technique, according to the manufacturer's recommendation (Colilert, Idexx, Westbrook, Maine, EUA), the results are expressed as NMP/100 mL (most probable number of total coliforms or *Escherichia coli* per 100 mL of water), 21 water samples (one per property) were collected directly from the irrigation tap of the gardens vegetables, as recommended by Brazilian protocol (BRASIL, 2013).

A semi-structured questionnaire was applied to all horticulturists participating in the study, with questions regarding the type of planting and fertilization, soil supplementation, use of pesticides, presence of animals on the property, irrigation system and characteristics of the bathroom and sewage. This work was approved by the Research Ethics Committee Involving Human Beings of the State University of Londrina under number 2,481,228.

The program EpiInfo 3.5.4 (DEAN, 1990) was used to tabulate the variables together with the microbiological and molecular results. Statistical analyzes were performed using the EpiInfo 3.5.4 and R 3.4.1 programs (R CORE, 2003) using the Chi-square test or Fisher's exact test, when appropriate. The data were compared using odds ratios (ORs) with confidence intervals (CIs) of 95%, significance level of 5%.

Results

A total of 42 samples of vegetables and 21 water samples were collected from 21 properties in the municipalities of Apucarana (5/21), Marilândia do Sul (8/21), Ortigueira (6/21), Rolândia (1/21) and Londrina (District Guaravera) (1/21). Among the vegetables, 19 samples were of lettuce (*Lactuca sativa*), nine were chicory samples (*Cichorium endivia*), seven were arugula samples (*Eruca sativa*), five were chives samples (*Allium fistulosum*), one was a spinach samples (*Spinacia* *oleracea*) and one was a chard sample (*Beta vulgaris* subsp. *vulgaris*).

Eighteen properties were certified for organic cultivation, and three (14.28%) were in the certification phase. The organic fertilizer source was variable among the properties: eight (38.09%) used cow manure, seven (33.33%) used chicken manure, three (14.28%) used mixed manure (chicken and cow) and three (14.28%) used a commercial compound. Nine properties were regularly supplemented with mineral soil, six with limestone (66.67%), and three with potassium supplement (33.33%). Domestic animals, such as dogs, cats, horses, cattle, were present in all properties studied and, in four (19.04%) of them, the animals had access to the gardens.

Wild animals (deer, hares, skunks, felines and birds) were present in all properties. All the properties performed vegetable washing – 11 (52.38%) in treated tap water and 10 (47.61%) in rinsing tanks. The irrigation was manual in nine (42.85%) and automated in 12 (57.14%) properties; the source of water for irrigation in 12 (57.14%) properties was from mines, three (14.28%) from rivers and six (28.57%) from artesian wells.

Water analysis showed that 18 (85.71%) had total coliforms (CT) ranging from 1 to > 2419,6 CTNMP/100 mL, among these, 10 were mine water, five artesian well and three of river samples. Regarding the presence of *Escherichia coli*, 17 (80.95%) samples were positive, varying from 1 to 218.7 NMP/100 mL, among them, nine were from mine water, five from artesian wells and three from rivers.

Among the properties visited, 71.4% (15/21) had at least one sample contaminated by parasites. In relation to the methods used for parasitological research, 13 (30.95%) vegetables samples were positive in the Hoffman et al. (1934) technique, 5 (11.90%) in the Faust et al. (1939) technique, 5 (11.90%) in the Willis (1921) technique and 19 (45.23%) were positive in at least one of the three parasitological techniques used in the present study. The results are detailed by property, vegetable and parasite found in Table 1. When considering positivity in at least one of the parasitological methods, a statistically significant difference was observed in the fate of the sewage variable ($p = 0.045$), with the cesspool being the most positive sewage destination.

Discussion

In the study, the presence of *E. coli* was observed in 17 (80.95%) of the analyzed water samples, of which three (17.65%) levels higher allowed by CONAMA (National Environment Council) (CONAMA, 2005). Resolution 357/05 recommends that, for the irrigation of raw vegetables and fruit that have yet to fully develop, a CTT count of less than 200/100 mL is appropriate. *Escherichia coli* is a facultative, anaerobic gram-negative bacteria, commensal in the intestinal microbiota; besides being the main indicator of fecal contamination (CONAMA, 2005), it can often become an opportunistic pathogen (NATARO; KAPER, 1998; FRANCO; LANDGRAF, 2003). It is known that the contamination of vegetables by pathogenic microorganisms is directly related to the quality of irrigation water (SCHERER et al., 2016), so it is imperative that this type of water be of high quality, passing through prior treatment, when necessary. The *E. coli* contamination has also been previously described in irrigation water in conventional cultive (SCHERER et al., 2016; SILVA et al., 2016a), which confirms that regardless of the type of cultive, water can be an important pathway for the transmission of pathogens, and chlorination is an efficient technique for the killing of bacteria and viruses.

Among the protozoa found, *Chilomatix* spp. and some species of *Entamoeba* spp. are commensal protozoans, even if not pathogenic, they indicate fecal contamination of human origin (OLIVEIRA; GERMANO, 1992). *Entamoeba histolytica* is important to public health due to its high pathogenicity and cyst resistance to conventional water treatment (MELO et al., 2004; MONTANHER et al., 2007). Arbos et al. (2010) suggested that the presence of parasitic structures in vegetables is due to improper cultivation, with respect to hygiene, either through irrigation water, access of wild or domestic animals to gardens and/or, the use of fertilizers with insufficient composting time.

Samples with non-sporulated oocysts were observed. It was not possible to confirm the species

TABLE 1: Results of the Faust et al. (1939), Hoffman et al. (1934) and Willis (1921) parasitological techniques from samples of vegetables from organic farm properties in Paraná, Brazil, from 2014 to 2016.

Property	Municipality	Faust et al. (1939)	Parasite	Hoffman et al. (1934)	Parasite	Wiilis (1921)	Parasite
1	Mar. Sul	-		-		-	
2	Mar. Sul	-		+ Let + Aru	Chil, Ent, MiE	-	
3	Mar. Sul	-		+ Aru	OoN	+ Let + Aru	OoN, Ent, NeL
4	Mar. Sul	-		-		+ Let	NeL
5	Guaravera	+ Let	Ent	+ Aru	MiE	+ Aru	MiE
6	Ortigueira	-		+ Chi	Ent	-	
7	Ortigueira	-		-		-	
8	Ortigueira	+ Let	Str, Ent	+ Let	OoN	-	
9	Ortigueira	-		-		-	
10	Ortigueira	-		-		-	
11	Ortigueira	-		+ Let	Ent	-	
12	Rolândia	-		+ Chi	Dipil	-	
13	Apucarana	+ Chi	Hoo	-		-	
14	Apucarana	-		-		+ Let	Trich
15	Apucarana	-		+ Let	Str	-	
16	Apucarana	-		-		-	
17	Apucarana	-		-		-	
18	Mar. Sul	+ Chi	Ent	+ Chd	MiE	-	
19	Mar. Sul	+ Chi	Ent	+ Chi	NeL	-	
20	Mar. Sul	-		+ Let	Lar	-	
21	Mar. Sul	-		+ Chv	NeL	-	

* + positive sample; – negative sample. Chd: Chard; Let: Lettuce; Chv: Chive; Chi: Chicory; Aru: Arugula. MiE: Mite egg; Hoo: hookworms; Chil: *Chilomatix* spp.; Dipil: *Dipillidium* spp.; Ent: *Entamoeba* spp.; Str: *Strongyloides* spp.; Lar: free-living larva; NeL: nematode larva; OoN: non-sporulated oocyst; Trich: *Trichuris* spp.

identity through sporulation; however, it is important to consider that several parasites pathogenic to humans present this biological form in their life cycles, such as *Cryptosporidium* spp. (TYZZER, 1907), *T. gondii* (DUBEY et al., 1970) and *Cyclospora* spp. (ORTEGA et al., 1993). One of the main difficulties when working with environmental samples is the fact that many samples go through adverse conditions such as intense sun and low humidity, favoring alterations in the morphologies and metabolism of the microorganisms, hindering the diagnosis by conventional microscopy and sporulation, respectively.

Mites were found in three samples and are the most important aeroallergens in tropical climate regions; they are associated with respiratory allergies, such as allergic asthma and rhinitis. In addition, they can contaminate food, especially if kept in hot and humid environments, facilitating their proliferation. When ingested, they can trigger allergic reactions and anaphylaxis in atopic individuals, so their presence in vegetables presents a potential risk to human health (GELLER et al., 1995; 2009).

A total of 45.23% (19/42) of the vegetables were contaminated by parasites. Cultivation in direct contact with the soil can facilitate this contamination, since these parasites are commonly found in the soil (SILVA et al., 2016b) and animal manure used for fertilization (FERNANDES et al., 2015). *Strongyloides* spp. are opportunistic parasites of animals. The presence of *Strongyloides* spp. deserves special attention because

Strongyloides stercoralis have the capacity to infect humans, mainly immunocompromised individuals (MAIA et al., 2006; SILVA et al., 2016b).

Among the techniques used, a greater percentage of positive results were observed when using the Hoffman et al. (1934) technique. This may have occurred because the test was more sensitive to the parasites found relative to the other methods. A greater sensitivity by the test was also observed by Mesquita et al. (2015) in a study carried out in community gardens in the state of Piauí, Brazil, when they used both the Hoffman et al. (1934) and Willis (1921) methods.

All the properties included in the study had domestic animals; in five of them, the animals had access to the vegetable gardens. Domestic and wild animals are important sources of contamination of soil and water (CASSENOTE et al., 2010; WELLS et al., 2015). The most significant elimination pathway of endoparasites is fecal (NEWELL et al., 2010), which justifies the need to prevent the access of animals to cultivated areas.

A statistical association between sewage destination and positive results was observed in at least one of the parasitological methods used. The form of sewage destination is of extreme importance so that feces do not contaminate the soil and the source of irrigation water (NEWELL et al., 2010; TIYO et al., 2015). The cesspool is the worse sanitary type compared to the septic tank or septic sump biodigester. This is because the cesspool allows slow degradation of pathogens due to anaerobic digestion, and due to the possibility of diffusion of fecal material into the soil, to depths of three meters and a radius of one meter and to groundwater, in which dispersion varies according to the flow level. To avoid risks to the health, safety recommendations must be followed, such as the construction of a cesspool on the lowest ground with a minimum distance of 15 m from any source of water and as far away as possible from cultivation sites (BRASIL, 2007). In conclusion, the presence of parasites in the vegetables, even if apathogenic, may indicate fecal contamination; the main points associated with this contamination were the use of contaminated water for irrigation and the types of sewage destination; the latter may be responsible for soil and water contamination.

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