Adequate conditions of environmental management: lack of pathogens in community vegetables

Condições adequadas de manejo ambiental: ausência de patógenos em hortas comunitárias

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ABSTRACT
The hygiene and quality of raw food are of concern, as they can transmit bacteria, such as thermotolerant coliforms, protozoa such as Cryptosporidium spp., Giardia spp., Toxoplasma gondii and helminths of public health importance. Thus, in this work, the aim was to investigate environmental management conditions and the occurrence of Escherichia coli in irrigation water, helminths in vegetables, and protozoa in soil, fertilizer, water and vegetables from community gardens in Araçatuba-SP. For this, an epidemiological questionnaire was applied, in the form of an interview to workers and collected from each garden of the six registered with the Agro-industrial Development Secretariat of the City of Araçatuba, 40 liters of treated water or 10 liters of untreated water, a sample of approximately 10 g of soil and 10 g of fertilizer and randomly, a clump of lettuce and one of almond. Microbiological analyzes for the detection of E. coli using the Colilert Kit (IDEXX Laboratories, USA) were performed in irrigation waters. To search for helminths and their eggs in vegetables, it was performed by optical microscopy. The analysis of Cryptosporidium spp., Giardia spp. and T. gondii, was carried out by polymerase chain reaction (PCR) in samples of irrigation water, soil, fertilizer and vegetables. The location in an urban area, use of treated water, absence of pits, limitations of areas by walls, fences or fences, absence of domestic animals in community gardens, were sufficient to prevent E. coli in irrigation water, helminths in vegetables and Cryptosporidium spp., Giardia spp. and T. gondii, in samples of water, soil, fertilizer, and vegetables.

Keywords: Epidemiology, Enteroparasites, Environmental contamination, Fresh foods, Public Health, Vegetables.

RESUMO
A higienização e a qualidade dos alimentos de consumo cru são motivos de preocupação, pois estes podem transmitir bactérias, como as do grupo de Coliformes termotolerantes, protozoários como Cryptosporidium spp., Giardia spp., Toxoplasma gondii e helmintos de importância em Saúde Pública. Dessa forma, neste trabalho o objetivo foi investigar as condições de manejo ambientais e a ocorrência de Escherichia coli na água de irrigação, helmintos nas hortaliças, e protozoários no solo, adubo, água e hortaliças de hortas comunitárias cadastradas na Secretaria de Desenvolvimento Agroindustrial da Prefeitura de Araçatuba- SP. Para isso, foi aplicado um questionário epidemiológico, em forma de entrevista aos trabalhadores e coletadas de cada horta, 40 litros de água tratada ou 10 litros de água não tratada, uma amostra de aproximadamente 10 g de solo e 10 g do adubo e aleatoriamente, uma touceira de alface e uma de almeirão. Análises microbiológicas para detecção de Escherichia coli por meio do Kit Colilert foram

realizadas nas águas de irrigação. Para a pesquisa de helmintos e seus ovos nos vegetais foi efetuada por microscopia óptica. A análise de Cryptosporidium spp., Giardia spp. e T gondii, foi realizada pela reação em cadeia da polimerase (PCR) nas amostras de água de irrigação, solo, adubo e hortaliças. A localização em área urbana, uso de água tratada, ausência de fossas, limitações das áreas por muros, alambrados ou cercas, ausência de animais domésticos em hortas comunitárias, foram suficientes para não ocorrer E. coli nas águas de irrigação, helmintos nos vegetais e Cryptosporidium spp., Giardia spp. e T. gondii, em amostras de água, solo, adubo e vegetais.

Palavras-Chave: Alimentos In Natura, Contaminação Ambiental, Enteroparasitos, Epidemiologia, Hortaliças, Saúde Pública.

1 INTRODUCTION

The consumption of vegetables by the brazilian population has increased due to factors such as changes in nutritional habits and their health benefits, as well as aid in the control of chronic diseases. However, these foods, when not adequately sanitized, can be a route of transmission of pathogens such as bacteria, viruses (Brazil, 2013) helminths and protozoa (ANTUNES et al., 2013).

The protozoa Toxoplasma gondii, Cryptosporidium spp. and Giardia spp. are considered pathogens propagated by water and food, according to the United Nations Food and Agriculture Organization (FAO/WHO, 2012). These pathological agents cause toxoplasmosis, cryptosporidiosis and giardiasis, respectively. Toxoplasmosis, once in the body, forms cysts in the invaded tissue, which remain viable throughout the life of the host (Jeffers et al., 2018). It may be benign in immunocompetent, severe immunosuppressed patients and pregnant women have the capacity to cause abortion, stillbirth and severe disorders in newborn (WEISS; DUBEY, 2009), being considered the most prevalent infection in humans (LASS et al., 2012; AGUIRRE et al., 2019). In general, toxoplasmosis is the second leading cause of death for foodborne diseases (GAO et al., 2016). Cryptosporidiosis and giardiasis are diseases widely known as common causes of outbreaks of diarrheal diseases in humans (EFSTRATIOU; ONGERTH; KARANIS, 2017). They are responsible for causing nutritional abnormalities and mortality, especially in children and immunocompromised people (THOMPSON; ASH, 2016).

Escherichia coli, in turn, is a gram-negative commensal bacterium of the intestine, however, in some situations such as immunosuppression, it can lead to infections and destruction of intestinal cells, increasing permeability and giving rise to an enteroxygenic response and intestinal disorders (AHMED et al., 2016). The U.S. Environmental Protection Agency (USEPA) determined in 1986 that the presence of E. colli in samples is an indicator of fecal contamination, this relationship was evidenced in studies at the time (EDBERG et al., 2000) that showed the relationship of recreational waters impacted by contaminant sources such as sewage. It should be taken into account that water is a common source of contamination by E. colli for vegetables (LUNA-GUEVARA et al., 2019). The survival of this bacterium in soils can last for long periods in the gardens (MOYNE et al., 2020), facilitating the contamination of vegetables and transmission through them, since many of them are consumed raw (JAY et al., 2007; COOLEY et al., 2013; WADAMORI; GOONERATNE; HUSSAIN, 2017).

Community gardens are installed in a place of collective use, cared for by a group of people who live in the region or grouped in an association, production is shared among the participants and its surplus is commercialized generating income to families. It has the technical support and supervision of the City Hall or accredited institutions that support the supply of supplies, technical guidance and use of treated water for irrigation of vegetables (Rafael et al., 2017). Vegetables can be contaminated at various times, from planting to consumption (COELHO et al., 2001; GUPTA; KHAN; SANTRA, 2009; PINTO-FERREIRA et al., 2019, 2020). Cultivation conditions involving irrigation water quality (AMAHMID; ASMAMA; BOUHOUM, 1999; CHAIDEZ et al., 2005), type of fertilizer, presence of animals on the property (FERREIRA et al., 2018) and direct contamination by farm workers (DIXON, 2016) are the main risk points.

Within this context, the objective of this research was to investigate the epidemiological chain of contamination of vegetables in community gardens registered in the Secretariat of Agro-industrial Development of the Municipality of Araçatuba, in the Northwest region of the State of São Paulo., to evaluate the occurrence of E. coli and protozoa in the water used for irrigation, as well as the presence of parasite in water, soil and fertilizer.

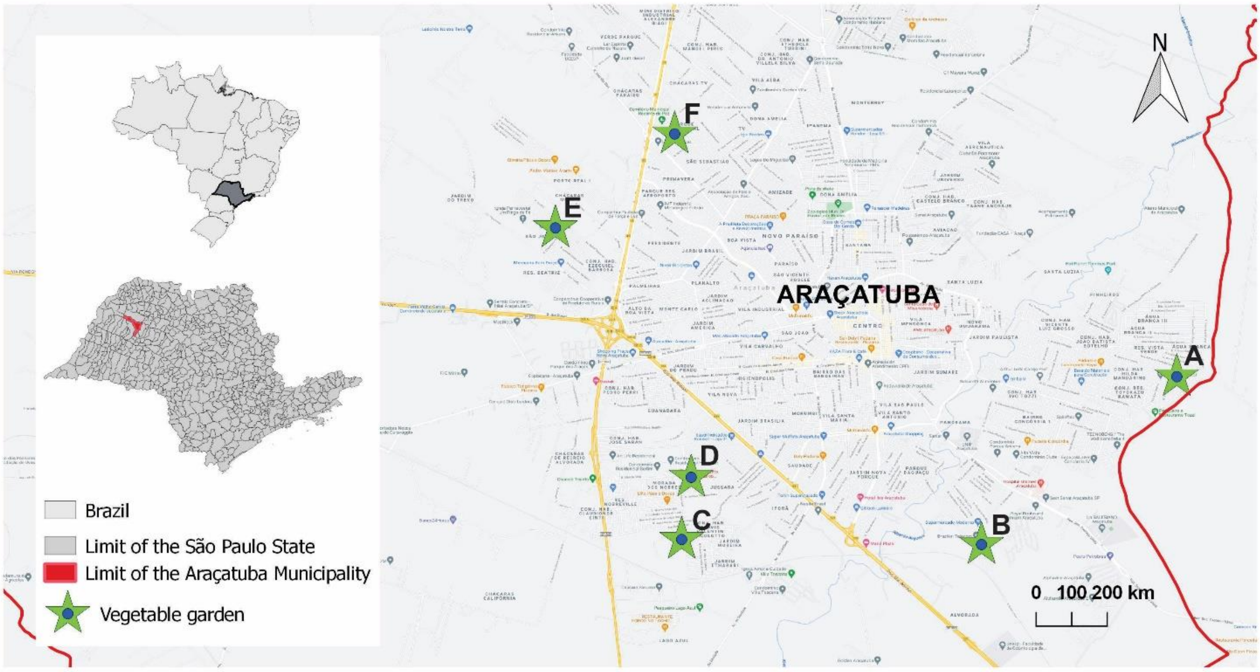
2 MATERIAL AND METHODS

The research was approved by the Ethics Committee involving Human Beings of UNESP - School of Dentistry of Araçatuba/ State University of São Paulo Júlio De Mesquita Filho under opinion no. 3,649,193.

Study area

The study was carried out between October and December 2019, in six community gardens participating in the Community Gardens Program of the Municipal Secretariat for Agro-industrial Development of the Municipality of Araçatuba- SP. Araçatuba is a municipality located in the northwest of the state of São Paulo, is 1,167,126 km² long and the estimated population is 198,129 people. The study gardens were in the peripheral regions of the municipality (Figure 1).

Figure 1- Community Gardens of Araçatuba- SP, 2019.



A, B, C, D, E and F: alphabetical identification of community gardens.

Collection and processing of water samples

For microbiological analysis, using the chromogenic substrate technique (APHA, 2005), six water samples, one per property, were collected directly from the irrigation taps of the vegetable gardens, after previous decontamination with water, soap and iodate alcohol (2%), as recommended by Brazil (2013).

For the detection of T. gondii, Cryptosporidium spp. and Giardia spp. a total of six water samples were collected in plastic drums, the volume variable according to type, when raw water was collected ten liters or 40 liters when the water was treated. In the laboratory, the sample was filtered in a cellulose ester membrane with 47 mm diameter and 1.2 μm milliporosity of Millipore® (Billerica, Massachusetts, USA), in a filter support system using a vacuum pump (4L / min). After filtration, the material was eat in Tween 80 to 0.1% with the aid of flexible plastic handles (Thermo Fisher Scientific, Massachusetts, USA) (BRANCO; LEAL; FRANCO, 2012), The material obtained was concentrated by double centrifugation at 1050 x g for 15 min, at 4° C.

Collection and processing of soil and fertilizer samples

For the detection of T. gondii, Cryptosporidium spp. and G. intestinalis, a sample of 10 g of soil surface and fertilizer were collected at properties A, C, D, E and F and from four different beds of property B, totaling 9 samples. This material was stored in 50 mL conical tubes containing 30 mL of 1M glycine and transported to the laboratory for immediate analysis (Ferreira et al., 2018). In the laboratory, each sample was homogenized with the aid of an agitator for 30 minutes, followed by five minutes of rest for sedimentation. The pellet was centrifuged at 1500xg for 15 minutes. The final concentrate was divided into aliquots in microtubes and stored at -20°C until DNA extraction (FERREIRA et al., 2018).

Collection and processing of vegetable samples

A cline lettuce clump (Lactuca sativa var. crispa) and one of chicory (Cichorium intybus) were randomly collected from the gardens A, C, D, E and F. In vegetable garden B because it presented four types of beds, fertilized in different ways, a clump of lettuce and chicory from each flower bed was collected, totaling 18 samples. The samples were packed in plastic bags of first use and sent to the laboratory for processing in a maximum of four hours.

A total of 50 g of leaves from each sample were homogenized with 300 mL of Tween extraction solution 80 to 1% in a plastic bag, shaking them manually for 10 minutes. The material was filtered in 500 mL glass cups with double gauze. The filtrate was divided into conical tubes, and one of them was transferred to a conical bottom chalice and left at rest for two hours and then 20 µL of the sediment was observed under a 40x objective microscope for helminth detection. The others were submitted to double centrifugation at 2100 x g for 10 minutes, separated the sediment into microtubes and stored at -20 ° C until DNA extraction (FERREIRA et al., 2018).

Molecular analyses

For polymerase chain reaction, the samples were previously submitted to five freezing cycles at -80°C and thawing at 56°C, after this stage, DNA was extracted by means of a commercial kit (NucleoSpin Tissue®, Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. 100 µL of DNA extracted and stored at -20°C was collected until polymerase chain reaction (PCR) was processed.

Polymerase chain reaction (PCR) was performed to amplify a 529 bp fragment of T. gondii (HOMAN et al., 2000), while for Cryptosporidium spp., a nested-PCR reaction was performed with primers described by Xiao et al. (1999) with target in the fragment of the 18S gene of RNAr between 826 and 840 bp. For the detection of G. intestinalis DNA, the samples were submitted to nested-PCR reaction with primers to amplify a fragment of approximately 300 bp of the 18S gene of The RNAr of G. intestinalis (COKLIN et al., 2007).

PCR products were visualized by electrophoresis in 1.5% agarose gel with SYBR Safe DNA (Invitrogen®, California, USA). Known positive samples of the protozoa stored in the laboratory and as negative control ultrapure water were used as control.

Epidemiological questionnaire

An epidemiological questionnaire containing information on the source of water and fertilizer, presence of animals on the property, access to the vegetable garden, destination of sewage, type of cultivation (organic or conventional) among other information, was applied to the workers responsible for the gardens, by signing the Free and Informed Consent.

3 RESULTS

In total, 42 samples were collected from the six gardens registered in the Community Garden Program of the Secretariat of Agro-industrial Development of the Municipality of Araçatuba-SP, being 18 of leafy vegetables, nine of fertilizer and nine of soil for parasitological analysis; and six water samples for microbiological and parasitological analysis.

The samples of vegetables, fertilizer, soil and water did not present DNA of Cryptosporidium spp., Giardia intestinalis and Toxoplasma gondii, as well as, found no other parasitic structure.

Total coliforms were present in irrigation waters in 33.3% (2/6) of the properties, but all were negative for E. coli (Table 1).

Table 1- Epidemiological and parasitological data from community gardens in Araçatuba- SP, 2019.

		Properties					
		A	**B**	**C**	**D**	**E**	**F**
Location		Urban	Urban	Urban	Urban	Urban	Urban
Water		Treated	Treated	Treated	Treated	well	Treated
Area boundary		Wire	Wall	Near	Wire	Near	Wire
Coverage		Shadow	-	-	-	-	-
Visiting animals		Birds	Birds	Birds and Skunks	Birds	Birds, Chickens and Skunks	Birds
Manure		Manure Tiririca extract	Flower Bed 1 - Chemical Flower Bed 2 - Chemical and Ox Manure Flower bed 3 - Ox manure Flower bed 4 - Ox manure	Chicken and ox manure	Chicken and ox manure	Ox manure	Ox manure Saw dust and dry leaves
Total coliforms	Water	-	+	-	+	-	-
E. coli	Water	-	-	-	-	-	-
Helminths	Vegetables	-	-	-	-	-	-
T. gondii, Cryptosporidium spp. and G. intestinalis	Water	-	-	-	-	-	-
	Fertilizer	-	-	-	-	-	-
	Vegetables	-	-	-	-	-	-
	Soil	-	-	-	-	-	-

Regarding the characteristics of the gardens, a single vegetable garden used well water for irrigation, while the others used public water supply. Only one of the vegetable gardens performed the pest control manually or with castor bean extract, the other ones were agricultural pesticides.

The gardens were in the urban space and were surrounded by walls, fences. With the presence of animals in the gardens, those that were limited by about (33.4; 2/6), appeared teyu, skunks and chickens, in addition to 83.4% (5/6) there were doves and other birds.

4 DISCUSSION

The gardens are important environments of study and health education (FERREIRA et al., 2018), during the visits, horticulturists received information about the main critical points of contamination of vegetables, as well as about the main diseases caused by the ingestion of these foods, when contaminated. In these gardens, horticulturists produced for their own consumption, family members, exchanged between them, gave to the needy and marketed the surplus, as verified in an interview with those responsible for the gardens.

Although in the present study all samples of vegetables, soil, fertilizer and water were negative for the parasite scans surveyed, several studies indicate contamination by several agents, besides incriminating water, fertilizer and soil as a source of contamination for vegetables (AMOAH; DRECHSEL; ABAIDOO, 2005; QURESHI et al., 2016; GAD et al., 2020), the negative results may be related to the interference of factors such as the diversity of landscapes, climatic factors, presence of domestic animals and waste at study sites (SPILKI et al., 2013).

Previous studies have shown that there is a higher risk of contamination by T. gondii, Cryptosporidium spp. and Giardia spp., when it is used in irrigation water from mine and river (FERREIRA et al., 2018). Although these parasites are not affected by conventional methods of water treatment (ROSE; HUFFMAN; GENNACCARO, 2002), the World Health Organization considers that treated water is the best way to reduce microorganisms (PAN AMERICAN HEALTH ORGANIZATION, 2001), it is likely that the negativity of the samples in the present study is associated with the quality of water used in the irrigation of vegetable gardens, since most sites had treated water.

E. coli is the main bacterium of the coliform group, whose unique habitat is the intestine, and its presence in water or food is indicative of fecal contamination (ALLENDE; MONAGHAN, 2015), being one of the main causes of diarrhea, causing almost two million deaths, mainly of children, according to the World Health Organization (WHO). Thus, its presence in aquatic environments causes concern to urban public health (TRABULSI; KELLER; GOMES, 2002). Studies associate the presence of parasites such as G. intestinalis and Cryptosporidium spp. with the presence of E. coli (TOLEDO et al., 2017). This corroborates the present study, which did not find positivity for any of the agents surveyed.

Gardens without protection against animals can help the contamination of vegetables, Ferreira et al. (2018), in a study conducted in Paraná, Brazil, reported the association between the presence of wild or domestic animals in the property and contamination of vegetables by parasite. In this work, horticulturists used fences, walls as protection, significantly reducing the access of animals to vegetable gardens, and possibly contamination of food.

Castor bean extract was used in one of the pest control gardens, produced from a macerated of the leaves of the plant with water, and the filtrate was sprayed on the vegetables for protection against nematodes (BARBOSA; SILVA; CARVALHO, 2006). Most vegetable gardens used chemical agricultural pesticides. These products have varied functions according to the chemical group they belong to, fighting bacteria, fungi, molluscs, nematodes, insects and even rodents (PERES et al., 2003). The two forms of control are safe regarding the contamination of vegetables by biological pathogens, favoring the negativity of the samples.

5 CONCLUSION

There was no detection of parasites in any sample analyzed in this study, it is concluded that under adequate conditions of environmental management, there is a clear reduction of contamination. Results that should be used as a stimulus to good practices during production and for the implementation of public policies to control contamination in vegetable gardens.

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