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Investigação Parasitológica de Hortaliças e Microbiológica de Água de Irrigação Oriundas de Hortas Comunitárias em Londrina, Paraná

Parasitological Research on Vegetables and Microbiological Evaluation of Irrigation Water from Community Gardens in Londrina, Paraná

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Resumo

As hortaliças são potenciais vias de transmissão de patógenos de importância em saúde pública, sendo que a contaminação está frequentemente relacionada às condições higiênico-sanitárias das hortas, assim como dos reservatórios de água. Dessa forma, o objetivo do estudo foi analisar a presença de parasitos em vegetais e, *Escherichia coli* em amostras de água de irrigação oriundas de hortas comunitárias, localizadas próximas a fundos de vale e praças públicas, no Município de Londrina, Paraná. O experimento ocorreu entre os anos de 2019 e 2020. Para análise parasitológica, as amostras de hortaliças folhosas foram processadas para concentração e posteriormente processadas e analisadas por exames parasitológicos, sendo o teste de Hoffman, Faust e Willis. Para análise microbiológica, foram coletadas nove amostras de água de irrigação, em recipientes estéreis e utilizou-se o Kit Colilert para detecção de *Escherichia coli*. Ao todo foram coletadas 18 amostras de hortaliças e nove de água. Quanto às amostras de água, foram constatadas as presenças de *Escherichia coli* e coliformes totais em 66.7% (6/9) das amostras coletadas. Nas amostras de hortaliças, foram identificadas parasitos dos gêneros *Ascaris*, *Ancylostoma* e *Entamoeba,* demonstrando que há contaminação de hortaliças provenientes de hortas comunitárias e da água de mina, utilizada na irrigação.

Palavras-chave: Contaminação. Água. Saúde Pública. Parasitos. Vegetais Folhosos.

Abstract

Vegetables are potential transmission routes for pathogens that are important to public health. The incidence of pathogenic contamination is often related to hygiene, sanitary conditions, as well as the quality of the reservoir water used in vegetable gardens. Therefore, this study aimed to analyze the presence of parasites in vegetables samples from community gardens, and the presence of Escherichia coli in the irrigation water samples. These gardens are located near valley bottoms and public squares in the city of Londrina, Paraná, Brazil. During the period of 2019 and 2020, 18 leafy vegetables samples were processed for concentration and later for parasitological analysis, being the test of Hoffman, Faust and Willis. For microbiological analysis, nine samples of irrigation water were collected in sterile containers, and the Colilert Kit was used to detect E. coli which was present in 66,7% of samples. The vegetable samples had parasites from the genera Ascaris, Ancylostoma, and Entamoeba,. demonstrating the contamination of both the vegetables from community gardens and the mine water used for irrigation.

Keywords*: Contamination. Water. Public Health. Parasites. Leafy Vegetables.*

1 Introduction

Community gardens undergo vegetable production, which can be managed by a single person or a group. Their produce can be destined for personal consumption or commercially sold, thus it promotes better health, space revitalization, and even leisure. This program occurs in several cities in Brazil, and involves the direct and indirect collaboration of many persons like seed donation (BIAZOTI; SORRENTINO 2022).

An increase in vegetable consumption has been widely observed owing to their numerous benefits. The World Health Organization - WHO relates the intake of these foods to the control and prevention of chronic non-communicable diseases such as obesity, diabetes mellitus, and cardiovascular diseases (WHO, 2018). Despite their notable benefits, vegetables are potential transmission routes for bacterial, viral, and parasitic pathogens to infect humans. Contamination can occur during production, collection, transportation, and processing. However, the most frequently occurring causes of contamination are related to hygiene and sanitary conditions, which includes the origin of organic fertilizers like the compost, the compost tanning time, and quality of the water used in irrigation or rinsing (FERREIRA et al., 2018; MOHAMED et al., 2016).

A major modern problem is the contamination of water sources by pathogens that are of public health importance. People become infected by drinking contaminated water as well as by consuming raw vegetables that have been washed and/or irrigated with it, contributing to the transmission of diarrheagenic diseases. Only water treatment systems, combined with the proper maintenance of reservoirs, are capable of producing water that is safe for consumption (HAJIPOUR et al., 2021).

The literature highlights parasites of the genera *Giardia,* *Entamoeba, Ascaris, Taenia, Strongyloides, Ancylostoma*, and *Schistosoma* as the main contaminants (FERREIRA et al., 2019). They can contaminate vegetables with eggs, cysts, and larvae during the cultivation stages that extend from planting to consumption, as well as by poor hygiene and sanitary (LUZ et al., 2017).

Considering the benefits of vegetables and the risk of contamination during cultivation, especially by the irrigation water, the present study aimed to analyze the presence of parasites and of E. coli in vegetables and in the irrigation water samples, respectively, from community gardens in the periphereal areas of Londrina, Paraná, Brazil.

2 Material and Methods

The experiment was approved by the Ethics Committee for Research Involving Human Subjects of the State University of Londrina (CEP-UEL) on February 1st, 2018 under opinion number 2,481,228. The research was conducted during 2019 and 2020 in the city of Londrina, located in the Central North Mesoregion of Paraná, at an altitude of 550 m, latitude of 23° 17' 34'' S, and longitude of 51° 10' 24'' W. This study included community gardens from the Community Gardens Program of the Municipal Department of Agriculture and Supply (SMAA) of Londrina City Hall (PML), which was created in 2010. Such gardens are located in public places such as valley bottoms and public squares.

In each community garden, for parasitological analysis, two samples of distinct leafy vegetables, apart from each other, were randomly chosen and collected. The samples were pulled from the soil, the roots were removed, and the leafs stored in a first-time use sterile plastic bag and kept in refrigeration. The samples were processed according to the recovery technique standardized by Ferreira et al. (2018): for helminth detection, 500 mL of 1 M Glycerine and 50g of the vegetables were homogenized in a plastic bag, manually agitated for 10 minutes, filtered through a double gauze, collected in a conical chalice, and left to settle for 12 hours. Then, 20 µL of the sediment was observed under a 40x objective microscope (HOFFMAN et al., 1934). The supernatant was removed from the chalice using a pipette, and the pellet was centrifuged at 1120 x g for 5 minutes. With the final sediments (approximately 0.5 mL), slides stained with Lugol iodine (MATOSINHOS 2012) were prepared to facilitate the detection and identification of eggs and larvae under an optical microscope. The samples also were evaluated for protozoa: 50 g of vegetables were mixed with 300 mL of a 1% Tween 80 solution in a plastic bag and agitated for ten minutes. Subsequently, the resulting solution from the first step was filtered through double gauze in a 500 mL glass beaker. The filtered extract was divided into conical tubes and subjected twice to centrifugation at 2100 x g for 10 minutes each time. Approximately 5 mL of the extract was processed and observed using the Willis (1921) and Faust et al. (1939) techniques.

The microbiological analysis was performed using the chromogenic substrate technique, following the manufacturer's recommendations (Colilert, Idexx, Westbrook, Maine, USA). The results were expressed as NMP/100 mL (most probable number of total coliforms or *Escherichia coli* per 100 mL of water). The water samples were collected directly from the irrigation tap in the vegetable gardens, following the Brazilian protocol guidelines (BRASIL 2013).

During the visit, an epidemiological questionnaire was applied to the leaders responsible for each community garden. These questionnaires enquired about the water source and the use of fertilizer, the presence of animals in the property and their access to the garden, the sewage destination, and other information that helped in the identification of variables associated with parasite contamination. In addition, all gardens were identified and mapped using the Global Positioning System (GPS).

3 Results and Discussion

Eighteen vegetable samples were collected from eight community gardens located in the peripheral areas of Londrina, Paraná. Among the samples, 11 were lettuce (*Lactuca sativa*), four were cabbage *(Brassica oleracea*), one was chicory (*Cichorium intybus*), and one endive (*Cichorium intybus* var. *intybus*) (Table 1). During cultivation, the fertilization type comprised 25.0% (2/8) mixed fertilization and 75.0% (6/8) organic. Furthermore, the fertilization source was variable among the properties: 62.5% (5/8) used chicken manure, 37.5% (3/8) used leaf fertilizer, 50.0% (4/8) used food fertilizer, 25.0% (2/8) used equine manure, and 25.0% (2/8) used ruminant manure. Regarding the irrigation water, only 37.5% (3/8) of the properties used technical irrigation. Water from public supply was used by 37.5% (3/8) of the community gardens, 25.0% (2/8) used water from protected mines and 37.5% (3/8) was from unprotected mines. The vegetables were washed before being packed in 62.5% of the properties, but in only two properties this water was chlorinated.

Table 1- Results of vegetable samples from community gardens assessed using parasitological techniques

Number	Samples	Faust	Hoffman	Willis	TC	*E.coli*
CH01	Lettuce	Negative	Negative	Negative	<1	<1
CH01	Cabbage	Negative	Negative	Ascarid Egg	<1	<1
CH02	Lettuce Mine 1	Negative	Negative	Negative	1,553.1	261.3
CH02	Lettuce Mine 2	Negative	Negative	Negative	1,553.1	261.3
CH02	Endive Mine 2	Mite Egg	Negative	Negative	365.4	5.2
CH02	Chicory Mine 1	Negative	Negative	Negative	365.4	5.2

Number	Samples	Faust	Hoffman	Willis	TC	*E.coli*
CH03	Lettuce	Negative	Negative	*Ancylostoma* spp.	2,419.6	571.7
CH03	Lettuce	Negative	Negative	Negative	2,419.6	571.7
CH04	Lettuce	Negative	Negative	Negative	<1	<1
CH04	Cabbage	Negative	Negative	Negative	<1	<1
CH05	Lettuce	Negative	Negative	Negative	<1	<1
CH05	Lettuce	Negative	Negative	Negative	<1	<1
CH06	Lettuce	Negative	Mite Egg	Negative	2,419.6	1732.9
CH06	Lettuce	Negative	Negative	Negative	2,419.6	1732.9
CH07	Lettuce	Negative	Negative	Negative	2,419.6	3.1
CH07	Cabbage	Negative	Negative	Negative	2,419.6	3.1
CH08	Lettuce	Negative	Negative	Negative	2,419.6	58.8
CH08	Cabbage	Negative	*Entamoeba* spp.	Negative	2,419.6	58.8

CH= Community gardens TC= Total Coliforms

Source: resource data.

Of the 18 samples subjected to the Faust, Hoffman, and Willis techniques, 27.8% (5/18) were positive; of these, 40.0% (2/5) occurred in cabbage, 40.0% (2/5) in lettuce, and 20.0% (1/5) in endive. (Table 1)

Nine water samples were collected for microbiological analysis: each sample came from a community garden and in one of the properties two were collected because they used water from two different mines. Escherichia coli and total coliforms were present in 66.7% (6/9) of the collected samples (Table 1).

Community gardens were selected for this study to evaluate the profile of vegetable contamination, because the occurrence of parasites in this food type is common. It may be associated with poor sanitation practices of the handler, poor sanitary and economic conditions, fertilizer type, cultivation type, and the quality of irrigation and rinsing water (FERREIRA et al., 2018; NORBERG et al., 2008).

Coproparasitological tests are considered cost-effective and easy to perform, besides demonstrating high sensitivity in identifying parasitic structures (COGNIALLI et al., 2017). However, the low occurrence of these parasites in the evaluated vegetables may be a justification for the majority of negative samples, as reported by Pinto-Ferreira et al. (2020), who used flotation, sedimentation, and molecular techniques to investigate enteroparasites in supermarket lettuces, identifying only one positive sample (3.33%, 1/30) through molecular analysis.

Vegetables are important transmission routes because they may be contaminated by parasites present in the soil and fertilizer. Thus, the presence of animals in gardens increases the risk of disease transmission, and this was a very frequent report in the vegetable gardens visited, as well as in other previous studies (FERREIRA et al., 2018; DA SILVA et al., 2019; HAJIPOUR et al., 2021).

Organic products suffer higher contamination pressure due to the intense use of animal manure, and the humid environment promotes the proliferation of microorganisms in poorly tanned fertilize. Therefore, proper fermentation and/or composting of the manure is necessary in preventing pathogen transmission (ALMEIDA et al., 2013). The data in this study showed that 75.0% (6/8) of the community gardens used organic fertilizer for their own production, often not respecting the tanning time, which may have favored 80.0% (4/5) of the positive samples in these places.

Oliveira and Germano (1992) investigated enteroparasites present in curly and smooth lettuce, escarola, and watercress sold in the metropolitan region of São Paulo, SP, and grown by small producers. Of the 200 samples analyzed, 17.0% (34/200) were positive for *Ascaris* sp.; whereas, in this study, a frequency of 5.5% (1/18) was observed. According to Da Costa Dantas (2023), *Ascaris lumbricoides* is among the most prevalent pathogenic helminths found in fresh vegetables, up to 55.1%, as reported in studies from 2016 to 2022.

The presence of *Ancylostoma* sp. was also observed by Oliveira and Germano (1992); where the frequency of positive samples was 22.0% (44/200); however, in this study, it was 5.5% (1/18). The number of positive samples observed by Oliveira and Germano (1992) was clearly higher than that found in this study, but this is because of the sanitary conditions of the environment that each vegetable garden presented. Unlike those used in this study, the samples were also selected in a dry period, where the soil was compacted with the increase in the frequency of irrigation, resulting in vegetable contamination via the water used. Another consequence of this was the survival of helminth eggs for a longer time, due to the increase in humidity (OLIVEIRA; GERMANO, 1992).

Guilherme et al. (1999) investigated parasites in vegetables from the city of Maringá in Paraná, from all stands of the Producer's Fairs, which came from farms in the peripheral Maringa cities, Sarandi, Paiçandu, and Mandaguari. Three lettuce types including scarole, watercress, arugula, endive, parsley, and chives were evaluated. Of the 144 vegetable samples, 16.6% (24/144) were positive for *Entamoeba* sp. in that study, whereas in this study it was 5.0%. Although most species are apathogenic, *Entamoeba histolytica* causes invasive diseases (ALMEIDA; LEITE 2020), which justifies the contamination control in vegetable gardens.

Most community gardens are in valley bottoms, and the water quality of rivers and streams present in these locations is questionable because they are often exposed to solid urban waste. Irrigation water is an important parasite transmission route (FERREIRA et al., 2018), and the highest occurrence of parasites (80.0%) was in vegetable gardens that were irrigated with untreated water from unprotected mines (MOURA et al., 2015).

The presence of *Escherichia coli* indicates fecal contamination of water or food (DRUMON et al., 2018). According to resolution 357/2005 of the National Council for the Environment (CONAMA), it is recommended that the water used for the irrigation of raw fruits and vegetables not exceed the contamination level of 1,000/100 mL for thermotolerant coliforms (TC) and 200/100 mL for *E. coli*. Vegetables consumed after cooking have higher values of 5,000/100 mL for TC and 1,000/100 mL for *E. coli* (CONAMA, 2005; MAROUELLI et al., 2008). Based on these data, the results of this study show a lack of conformity in at least three gardens, which, in the case of consumption without practicing good hygiene prior to consumption, can cause negative health effects.

Weather conditions can interfere with water quality (AKHTAR 2021; NOGUEIRA et al., 2000;). In rainy periods, because of the higher occurrence of floods, there may be greater contamination by coliforms in mines and wells, from contaminated soil and sewage (NOGUEIRA et al., 2000). The collection of samples from the present study began on October 17, 2019 and was finalized on March 12, 2020. The seasons in which these dates fall are characterized by a large volume of rain, which may have been responsible for the greater levels of contamination observed in these properties.

Among all samples collected, only three (33.33%) did not present contamination (<1) because they used water from the public supply, which goes through treatment and chlorination processes before being piped to the properties. Water chlorination is extremely important, as it contributes to the control of water-borne diseases. It is a process that is easily available and has shown proven efficiency in reducing contaminants of bacterial origins (PICKERING et al., 2019).

It was also observed that six of the eight properties (75.0%) washed the vegetables before they were packed. While this was mainly done to cool the leaves and increase shelf time, it also served to remove dirt. Studies conducted by Amoah et al. (2007) indicate that regardless of the way in which washing occurs, and the concentration of disinfectants used, contamination will be reduced. However, they did note that the most efficient method is sanitization using treated running water. In addition, it is known that sanitizing food prior to consumption is important to avoid the dissemination of enteroparasites, thereby ensuring the hygienic and sanitary quality of vegetables consumed in natura.

4 Conclusion

This study demonstrated that the small gardens included in our investigation presented vegetables that were contaminated by free-living and pathogenic parasites. This contamination may be related to the water quality, fertilizer type, or cultivation type. In addition, the use of water from mines (protected or unprotected) resulted in a higher contamination level. Consequently, to reduce the spread of pathogens via vegetables, it is essential to improve the quality of the water used for irrigation because it is one of the main routes by which this type of food contamination occurs.

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