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WATER FROM DIFFERENT SOURCES USED FOR THE IRRIGATION OF VEGETABLES TO BE MARKETED: RESEARCH ON *Cryptosporidium* spp., *Giardia* spp., AND COLIFORMS IN PARANA, BRAZIL

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SUMMARY

The aim of this work was to compare, from a parasitological (*Cryptosporidium* spp. and *Giardia duodenalis*), bacteriological (total and thermotolerants coliforms) and physicochemical perspective, water sources used for drinking and irrigation of vegetables intended to be sold for human consumption. From January 2010 to May 2011, samples of different water sources from vegetable producing properties were collected; 100 liters for parasitological analysis, 200 mL for bacteriological analysis, and five liters for physicochemical analysis. Water samples were filtered under vacuum with a kit containing a cellulose acetate membrane filter, 1.2 µm (Millipore®, Barueri, SP, Brazil). The material retained on the membrane was mechanically extracted and analyzed by direct immunofluorescence (Merifluor®kit). From 20 rural properties investigated, 10 had artesian wells (40 samples), 10 had common wells (40 samples), and one had a mine (four samples), the latter contaminated by *Cryptosporidium* spp. In samples from artesian wells, 90 to 130 meters depth, 42.5% were positive for total coliforms and 5.0% were identified to have abnormal coloration. From the samples of common wells, 14 to 37 meters depth, 87.5% were contaminated with total coliforms, 82.5% were positive for thermotolerant coliforms, and 12.5% had color abnormalities. We did not detect the presence of *Giardia* spp. or *Cryptosporidium* spp. in artesian and common wells. The use of artesian or common wells is an important step in the control of the spreading of zoonoses, particularly *Cryptosporidium* spp. and *Giardia* spp., as well as artesian wells for coliform control in local production of vegetables to be marketed.

KEYWORDS: Water; Irrigation of vegetables; *Cryptosporidium* spp.; *Giardia* spp.; Thermotolerant bacteria; Total coliforms.

INTRODUCTION

Water is a natural resource that is indispensable not only for human life, but for all living organisms[23]. It is considered safe to drink when it has no microbiological, chemical, physical, or radioactive contamination and is within the quality limits for several parameters, such as pH, turbidity, color, total suspended solids, and total dissolved solids[2]. However, more than one billion people have no access to treated water, including 19 million Brazilians[12].

The intensification of anthropogenic activities is compromising water sources that are available for human consumption, with risk of infections that are transmitted through water, and are caused by pathogenic bacteria and protozoa, the latter being mostly resistant to conventional water treatment[10]. Among the existing water quality monitoring parameters that are used there is bacteria; *Escherichia coli* is an indicator of fecal contamination from the intestinal tract of warm-blooded animals that can eliminate elevated amounts of bacteria in their feces. The presence of these bacteria in the water, along with others from the coliform group, indicates the presence of fecal material or sewage contamination[7], which can infect individuals when drinking this water or ingesting food, fruit, and vegetables that were irrigated with this contaminated water [13].

Giardia duodenalis and *Cryptosporidium* spp. are the most frequent protozoa that can cause gastroenteritis. It is normally self-limited but can be more severe in immune deficient individuals[22]. Identification of the source of contamination is critical for health authorities in several parts of the world[16]. In the last 30 years, both developed and developing countries have experienced epidemic outbreaks of gastroenteritis caused by giardiasis[4].

Water quality requirements have evolved and continue to evolve along with advances in technical and scientific knowledge. The standards of quality have gradually become more stringent. However, the presence of thermotolerant coliforms is still often used to verify water potability[11]. When combined with other indices, such as turbidity, it is an indirect indicator of the presence of protozoa1.

The northwest region of Parana State has several small rural

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properties where the population uses water from alternative sources for consumption and crop irrigation, such as fruit and vegetable plantations, including vegetables that are consumed raw[8]. This production supplies farmers' markets, supermarkets, and CEASA (a central supplier in the region and other localities). Studies performed on vegetables collected in farmers' markets and CEASA in the same region showed the presence of contaminant forms of protozoan cysts, worms, and eggs of helminths[8,9]. Investigations performed in daycare facilities in the city of Maringa, in northwestern Parana State, revealed that *Giardia duodenalis* was the most prevalent protozoa, which was identified in 23.6% of the children[21]. Children who were hospitalized in the *Hospital Universitário de Maringá* presented 10.3% and 6.6% positivity for *Giardia* and *Cryptosporidium*, respectively[17]. A study performed with 5,000 children in a county located in northwestern Parana found that *G. duodenalis* was the second most prevalent species (5.2%)[18]. Research performed in water fountains in a river that supplies Maringa, in southern Brazil, reported the presence of the protozoa *Giardia* spp. and *Cryptosporidium* spp., although turbidity, pH, and thermotolerant coliforms (*Escherichia coli*) were within acceptable limits[19]. In northwestern Parana, several rural properties produce fruit and vegetables where the families use water from supply sources such as wells and mines, for consumption and irrigation. When contaminated, the fruit and vegetables can serve as a carrier for several enteric diseases[14]. The aim of the present study was to compare the parasitological (*Cryptosporidium* spp. and *Giardia duodenalis*), bacteriological (total and thermotolerant coliforms), and physicochemical aspects of different alternative water sources used for consumption and irrigation of fruit and vegetables to be marketed.

MATERIAL AND METHODS

Study location: We investigated water sources in rural properties that produce vegetables intended for raw ingestion. The properties were registered in the Parana State Institute of Technical Assistance and Rural Extension (EMATER), and participants of the producers' fair (*Feira do Produtor de Maringá*) agreed to participate in the study. This region is located in the mesoregion of Parana State (between longitude 52º20' and 52º50' west, and latitude 23º20' south)[15].

From January 2010 to May 2011, twenty rural properties were investigated, with 21 points of water that consisted of 10 artesian wells (90-130 m deep) where 40 samples were collected, 10 common wells (14-37 m deep) where 40 samples were collected, and one mine where four samples were collected. At each sampling point, four water samples were quarterly collected being 100 L of water for parasitological analysis, 200 mL for bacteriological analysis, and 5 L for physicochemical analysis. The samples for parasitological analysis were first conditioned in a container that was previously sanitized with 10% sodium hypochlorite solution and sent to the Environmental Parasitology Laboratory at Maringa State University. The water samples for bacteriological and physicochemical analyses were stored in sterile bottles and transported under refrigeration to the Laboratory for Microbiological Analysis of Water at Maringa State University[19].

Laboratory procedures

Identification of *Giardia* spp. and *Cryptosporidium* spp.

Technique for material filtering: According to NISHI *et al.,* 2009[19], the water samples were vacuum-filtered with a filtration kit that contained a cellulose acetate membrane (Millipore, Barueri, Sao Paulo, Brazil), with 1.2 µm porosity and 47 mm diameter. The material retained in the membrane was mechanically extracted by scraping the surface with a plastic spatula, alternating with washes for 10 min. in a Petri dish with 1% Tween 80 solution for elution[19]. The material was centrifuged at 600 × *g* for 15 min. The supernatant was removed with pipettes until a residual volume of 3 mL was reached, and the sediment was re-suspended in distilled water[19]. This new suspension was centrifuged again at 600 × *g* for 15 min. All of the supernatant was removed, and the sediment was re-suspended to a final volume of 1 mL[19].

Merifluor technique for the detection of Giardia spp. *and Cryptosporidium* spp. *in water:* From the sediment obtained through the filtering process, 5 µL was used for direct immunofluorescence using the Merifluor kit (Meridian Bioscience, Cincinnati, Ohio, USA), following the manufacturer's instructions. Fluorogenic vital DAPI stain was added to allow the detection of the morphological characteristics of the protozoa. The samples were analyzed by the same researcher under microscope, considering size (4-6 µm for oocysts and 5-15 µm for cysts), typical shape and typical fluorescence[24] and the examination was conducted at a 200X magnification. The reading of the reaction was performed with an Olympus BX51 epifluorescence microscope (Merifluor: 450-490 nm excitation wavelength, 520 nm emission wavelength; DAPI: 365-400 nm excitation wavelength, 395 nm emission wavelength) using 200X magnification. The number of oocysts and cysts per liter in the positive samples were estimated with the following equation[19]:

$$\left(x = \frac{\text{n}^{\text{o}}.\text{oo(cysts)} \times 10^{6}}{\text{volume used/slide } (\mu\text{L})} \times \frac{\text{pellet volume (1 mL)}}{\text{sample volume (mL)}} \right)$$

Quantification of total coliforms (TC) and thermotolerants coliforms (ThC): We used 100 mL of water for each kind of bacterium. The samples were vacuum-filtered with a cellulose acetate membrane (47 mm diameter, and 0.45 µm pore size) and seeded in M-ENDO medium at 35 °C ± 2 °C and M-TEC medium at 41 °C ± 2 °C according to the recommendations of the Standard Methods for the Examination of Water and Wastewater[2].

Physicochemical analysis: The water samples were characterized with regard to physicochemical parameters, including turbidity (maximum value allowed 5.0 UNT), color (maximum value allowed 15 Hu; mgPt-Co/L), and pH (6.0-9.5)[3]. Turbidity was determined using a portable HACH model 2100P turbidimeter according to the methods recommended by the Standard Methods for the Examination of Water and Wastewater,[2] and the results were expressed in Nephelometric Units of Turbidity (NUT). Color was measured with an HACH DR 2010 spectrophotometer (method 8025, program 120, 455 nm wavelength) and by visual comparison with a cobalt-platinum standard according to the procedures recommended by the Standard Methods for the Examination of Water and Wastewater,[2] and was expressed in Hanzen units (Hu; mgPt-Co/L). pH was determined with a Digimed DM-2 pH meter according to the methodology described by the manufacturer.

Statistical analysis: We performed a descriptive data analysis. The prevalence of oocysts and cysts, total coliforms, thermotolerant coliforms, color, and turbidity of water samples was compared for artesian and common wells. Samples from the mines were not included in the comparisons because of low data variance. Logistic regression models were developed to verify the risk of contamination between common sources and artesian sources, performed with the R Statistical Programming Language 3.2 software for windows. The adopted level of significance was 5%.

RESULTS

For the parasitological analysis, the samples were within the standard levels according to Health Ministry Resolution 2914/2011[3] (which recommends the absence of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts in water), except for the mine water, which presented two *Cryptosporidium* spp. oocysts per liter, increased levels of total coliforms (1,564 colony-forming units [CFU]/100 mL), and thermotolerant coliforms (691 CFU/100 mL), beyond alterations in the physicochemical parameters of color (38.5 Hu) and turbidity (7.6 UNT) (Table 1). The pH results were normal for every sample.

Among the 21 points of water collection, only three (14.3%) from artesian wells were negative for total and thermotolerant coliforms, thus meeting the requirements of Health Ministry Resolution 2914/2011[3], which recommends the absence of *E. coli* in 100 mL of water. The presence of this bacterium in the artesian wells varied from < 1 to 6.24×10^4 CFU/100 mL. For common wells, the presence of this bacterium varied from < 1 to 6.24×10^4 CFU/100 mL. The water from the mine presented 3 to 691 CFU/100 mL of *E. coli*. However, we observed an increased number of samples from the common wells that were contaminated with total and thermotolerant bacteria, and some of the turbidity and color data were outside the acceptable parameters compared with the artesian wells (Table 1). We also found that season did not influence the results.

Table 1

Frequency of *Giardia* cysts, *Cryptosporidium* oocysts, bacteria (TC and ThC), and physical parameters in water samples from wells and mines of rural properties in Parana State, Brazil, 2010-2011

	Artesian wells (*n* = 40) *n* (%)	Common wells (*n* = 40) *n* (%)	*p*	Mine (*n* = 4) *n* (%)	Total (*n* = 84) *n* (%)
Parasites	< 0.01	< 0.01		2* (50)	2 (2.3)
Total coliforms	17 (42.5)	35 (87.5)	< 0.001	4 (100)	56 (66.7)
Thermotolerant coliforms	17 (42.5)	33 (82.5)	< 0.001	4 (100)	54 (64.2)
Color** (mg Pt-Co/l)	0 (0.0)	5 (12.5)	0.995	4 (100)	9 (10.7)
Turbidity*** (UNT)	0 (0.0)	1 (2.5)	0.997	4 (100)	5 (5.9)

**Cryptosporidium* spp. oocysts; **sample number with altered color; ***sample number with altered turbidity.

DISCUSSION

The results indicated that the water used for the consumption and irrigation of fruit and vegetables to be marketed came mainly from groundwater, such as artesian and common wells that exist on the rural properties, and water from a mine. The artesian wells were the least contaminated by coliforms and also presented better physicochemical parameters, which can be explained by their greater depth. On the other hand, water from the mine was the most contaminated.

The comparison of contamination based on total and thermotolerant coliforms in artesian and common wells demonstrated that contamination is not influenced by factors related to seasonal variations. However, the higher contamination in the common wells compared with the artesian wells may be explained by its lower depth, thus increasing the probability of being exposed to fecal sources as sewages or animal feces. For example, in the same rural property that has both types of wells, we observed the presence of contamination, reflected by total and thermotolerant coliforms in the common well, whereas these coliforms were absent in the artesian well. According to OTENIO *et al.*[20], deeper wellsprings may have lower amounts of contaminants. COSTA *et al.*[6] studied an urban environment in Fortaleza, in northeastern Brazil, and found that 40% of the artesian wells were contaminated with total coliforms, and 12.2% were contaminated with *E. coli*. Importantly, water may also be contaminated during the course of collection and distribution and by a failure to protect and clean water tanks[5].

The mine source of water was contaminated with *Cryptosporidium,* spreading the oocysts to the production. The commercialization of these vegetables can favor the dissemination of this protozoan. NISHI *et al.*[19] also observed the presence of *Cryptosporidium* spp. oocysts in water from a mine in an indigenous territory in Parana State. In the artesian and common wells, these protozoa were not detected, indicating that their use can be considered an important control measure for protozoa in cultivation areas and for maintenance by rural workers. However, the development of laboratory techniques by public agencies that are more sensitive, affordable, and easy to use, is necessary to perform monitoring activities in the production locations. Turbidity was generally an adequate criterion for the indirect indication of the presence of protozoa when comparing the water from the mine with the water from artesian wells, which is consistent with data in the literature[1].

Overall, the physicochemical parameters of the water from the sources that supply rural properties met the requirements of Health Ministry Resolution 2914 of 12/12/2011[3], with rare cases of color alterations in the samples from some artesian and common wells, including alterations in turbidity in the latter. According to OTENIO *et al.*[20], water from artesian wells tends to have fewer alterations because they are deeper and more protected from exposure to wind, rain, and dust.

The use of artesian wells with depths of 90-130 m, or common wells with depths of 14-37 m, constitutes an important action to control the dissemination of waterborne zoonosis, especially *Cryptosporidium* spp. and *Giardia* spp. For artesian wells, such depths also control coliforms in areas where vegetables are irrigated to be marketed.

To achieve a better control of water used for the irrigation of fruits and vegetables which are consumed raw, and maintenance by rural workers, educational measures need to be implemented for the population that produces and consumes these fruits and vegetables with incentives for monitoring the quality of water with regard to the presence of coliforms and protozoa.

RESUMO

Água de diferentes fontes utilizadas na irrigação de hortaliças comercializadas: pesquisa de *Cryptosporidium* spp., *Giardia* spp., e coliformes, Paraná, Brasil

O objetivo do estudo foi investigar fontes de água utilizadas para consumo e irrigação de hortaliças a serem comercializadas sob o aspecto parasitológico (*Cryptosporidium* spp. e *Giardia duodenalis*), bacteriológico (coliformes totais e termotolerantes) e físico-químico. De janeiro de 2010 a maio de 2011 foram coletadas amostras de água de diferentes fontes de abastecimento de propriedades produtoras de hortaliças; 100 litros para análise parasitológica, 200 mL para bacteriológica e cinco litros para análise físico-química. As amostras de água foram filtradas a vácuo com um kit de filtragem contendo uma membrana de acetato de celulose, 1,2 µm, (Millipore®, Barueri, São Paulo, Brasil). O material retido na membrana foi extraído mecanicamente e analisado por imunofluorescência direta (kit Merifluor®). De 20 propriedades rurais, 10 tinham poços artesianos (40 amostras), 10 semi-artesianos (40 amostras) e um possuía uma mina (quatro amostras). Esta última contaminada por *Cryptosporidium* spp. Das amostras de poços artesianos com 90 a 130 metros de profundidade, 42,5% foram positivas para coliformes totais e 5,0% apresentavam coloração alterada. Em amostras de poços semi-artesianos com 14 a 37 m de profundidade, 87,5% apresentaram coliformes totais, 82,5% termotolerantes, e 12,5% destas amostras tinham alteração de cor. Não foi detectada a presença de *Giardia* spp. e *Cryptosporidium* spp. em poços artesianos ou semi-artesianos. A utilização de poços artesianos ou semi artesianos constitui importante medida no controle da disseminação de zoonoses, principalmente *Cryptosporidium* spp. e *Giardia* spp., assim como de poços artesianos para o controle de coliformes, em locais de produção de hortaliças irrigadas, a serem comercializadas.

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