

Seasonal variations in fecal contamination of shallow groundwater in Altamira, Amazon, Brazil

Variações sazonais na contaminação fecal de águas subterrâneas rasas em Altamira, Amazônia, Brasil

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Abstract

Altamira city, in the north of Brazil, is known to be a support region since the construction of the Belo Monte Hydroelectric Complex. The Xingu River permeates the city, being extremely important as a tributary of the Amazon River. Only a portion of the population is provided with basic sanitation, and alternatively use water wells and septic tanks, which can be sources of contamination. The study sought to understand the seasonal variation of fecal contamination in groundwater resources. The households were divided into six zones, from which 30 wells were sampled in the dry and wet seasons. For the *Escherichia coli* detection, a Compartment Bag Test (CBT) (Aquagenx[®]) was used, and for the *Bacteroides thetaiotaomicron* (*B. theta*) detection, the microbial source tracking (MST) approach, and quantitative polymerase chain reaction assays (qPCR) were used. The presence of *E. coli* and *B. theta* was verified in all seasons. In the dry season, 63.3% of sampled wells showed *E. coli* contamination, and in the wet 76.6%. *B. theta* was detected in 43.3% of the wells in the dry season and 36.6% in the wet season. In the dry season, 26.6% of the wells presented *E. coli* and *B. theta* simultaneously, and in the wet season, 33.3%. The quantification of *B. theta* was lower in wet seasons, indicating that the source of contamination for both, *E. coli* and *B. theta*, may be different, also, the mechanism of reach and maintenance for *E. coli* in the aquifer appears to be related to runoff and percolation of contaminants from the soil surface not just associated with human fecal contamination.

Keywords: Belo Monte; *Escherichia coli*; *Bacteroides thetaiotaomicron*; Water quality; Public health.

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Resumo

Altamira é uma cidade do norte do Brasil, conhecida por ser uma região suporte desde a construção do Complexo Hidroelétrico de Belo Monte. O Rio Xingu permeia a cidade sendo extremamente importante como um tributário do Rio Amazonas. Apenas uma parte da população é abastecida com saneamento básico, e alternativamente utiliza água de poços e fossas sépticas, as quais podem ser fontes de contaminação. O estudo buscou entender a variação sazonal da contaminação fecal em recursos hídricos subterrâneos. Os agregados familiares foram divididos em seis zonas, das quais 30 poços foram amostrados na estação seca e na estação úmida. Para a detecção de *Escherichia coli* foi usado o teste *Compartment Bag Test* (CBT) (Aquagenx®) e para a detecção de *Bacteroides thetaiotaomicron* (*B. theta*) foi usado o método de rastreamento de fonte microbiana (MST) com ensaios quantitativos de reação em cadeia da polimerase (qPCR). A presença de *E. coli* e *B. theta* foi verificada nas duas estações. Na estação seca, 63,3% dos poços amostrados apresentaram contaminação por *E. coli*, e na estação chuvosa 76,6%. *B. theta* foi detectada em 43,3% dos poços na estação seca e 36,6% na estação chuvosa. Na estação seca, 26,6% dos poços apresentaram *E. coli* e *B. theta* simultaneamente, e na estação chuvosa, 33,3%. A quantificação de *B. theta* foi menor nas estações chuvosas, indicando que a fonte de contaminação para ambas, *E. coli* e *B. theta*, pode ser diferente, também, o mecanismo de alcance e manutenção de *E. coli* no aquífero parece estar relacionado ao escoamento e percolação de contaminantes da superfície do solo, não apenas associado à contaminação fecal humana.

Palavras-chave: Belo Monte; *Escherichia coli*; *Bacteroides thetaiotaomicron*; Qualidade da água; Saúde pública.

Introduction

The Xingu River is a major tributary of the Amazon River located from the Midwest to Northern Brazil.⁽¹⁾ Of riverbank cities around Xingu, Altamira is highlighted due to the construction of Belo Monte Hydroelectric Complex proximity, serving as a support city.⁽²⁾

In the North region's Brazil, the basic sanitation services are flawed. Around 57.5% of the inhabitants have access to potable water and only 12.3% receiving sewer services.⁽³⁾ So that to supply the failure of water and sewer disposal provision, the residents have used water wells and septic tanks. These septic tanks can be contamination sources in the soil and groundwater since having open bottoms which allow the liquids percolation.^(2,4)

The waterborne is one the main for transmitting pathogens agents and deficient sewage collection and treatment systems can generate several problems that compromise public health and the environment.⁽⁵⁾

The percolation of sewage liquids can lead to groundwater contamination with pathogenic microorganisms and chemical substances like nitrates. Various anthropogenic activities, such as the discharge of wastewater and the use of septic systems, contribute to releasing microorganisms and nutrients into the soil which infiltrate through the soil zones, reaching groundwater.⁽⁶⁻⁷⁾

In cities along with riverbank, seasonal variations can influence changes in the groundwater resources.⁽⁸⁾ In this regard, changes in water level can favor contaminant transport from septic tanks to water wells resulting in water quality unsafe. In the Amazon region, seasonal variations are according to the rainfall pattern – dry and rainy seasons.⁽¹⁾

The drinking-water quality is determined by the presence of fecal contaminants from humans and animals since feces can display pathogens and *Escherichia coli* detection is the main approach to attesting water safety for human consumption.⁽⁹⁾

Escherichia coli are enteric gram-negative rods frequently found in the feces of most warm-

blooded animals⁽¹⁰⁾ being used as water marker contamination due to their sources and the involvement of some *Escherichia coli* pathotypes in diarrheal diseases.⁽⁹⁾ However, the association of *Escherichia coli* contamination with anthropogenic activities is not possible and human-specific markers are needed.

Currently, studies about contamination sources have used the Microbial Source Tracking (MST) approach and genes detection from intestinal bacteria. In this context, the genus *Bacteroides* have been used due to host-specific, but despite the high specificity, *Bacteroides* can be found occasionally in non-human feces.⁽¹¹⁾ Around the last decade, *Bacteroides thetaiotaomicron* (hereafter *B. theta*) has been appointed as a choice of human fecal marker considering prevalence, specificity and sensibility in the fecal samples.^(12,13)

In this study, we investigated the seasonal variation of fecal contamination in the groundwater resources within a Xingu riverbank city in Brazil's

Amazon. We considered account for seasonality, as the vast differences between the dry and rainy seasons of the Amazon could change contaminant transport and water quality results.

Material and methods

Sampling

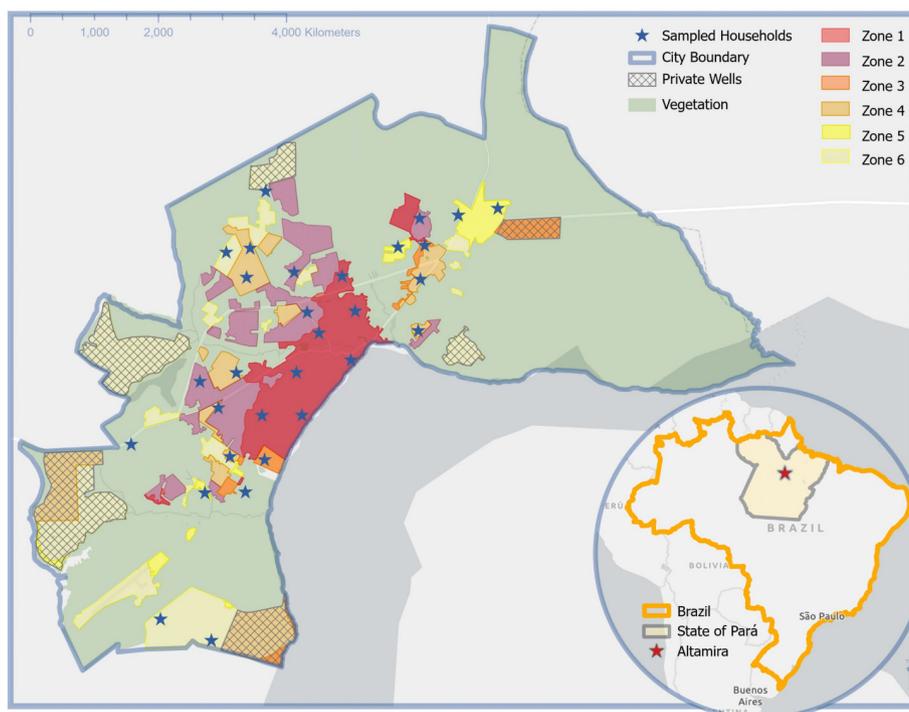
We sampled thirty household wells located in urban Altamira, Pará, Brazil – Xingu riverbank around the downstream course, the homes were organized in six zones according to the terrain elevation and population density as displayed in Table 1. Figure 1 shows the sampled wells allocated to each zone. Water samples were collected from the same household wells during September/October 2018 (dry season) and February/March 2019 (wet season). Temperature and precipitation data were extracted from Instituto Nacional de Meteorologia (<https://tempo.inmet.gov.br/>).

Table 1 - Samples' categories in zones. Numbers of wells, population density, and elevation from each zone.

Zone	Household wells sampled	Population density	Elevation
1	7	high	low
2	5	high	high
3	3	median	low
4	6	median	high
5	4	low	low
6	5	low	high
Σ	30		

Source: the authors.

Figure 1 - Household wells sampled in Altamira, Pará, Brazil, in each zone. The wells shown within vegetation places belong to zones 3 and 6.



Source: the authors.

The elevation of the shallow wells was measured using a 30-meter resolution Advanced Spaceborne Thermal Emission and Reflection Radiometer Global DEM (ASTER GDEM V3) (National Aeronautics and Space Administration Land Processes and Distributed Active Archive Center, 2001) from the NASA Reverb|Echo repository (EOSDIS 2009). Areas up to 105 meters above sea level were categorized as low-elevation areas and areas above were considered high-elevation areas.

We used IBGE's population by neighborhood data⁽¹⁴⁾ to determine the population density changes. Employing urban and neighborhood boundaries provided by Altamira's Municipal Secretary of Planning (SEPLAN), we calculated neighborhood areas in square kilometers through ArcGIS software. Along with IBGE's population data, this yielded houses per square kilometer in each neighborhood. Employing the natural breaks classification method and visual corroboration, we determined sparse urbanization as having low population density (10 to 100 homes/km²), moderate urbanization as a me-

dium density (135 to 800 homes/km²), and intense urbanization as a high (895 homes/km² or more).

Water samples were collected directly into each well, lowering a sterile bottle with a one-liter volume. A bottle of distilled water of the same volume was used as control. All bottles were stored in a cooler at 4 °C for less than 4 hours until tests execute. Residents provided data on home treatment before water consumption.

Escherichia coli detection

We used the Compartment Bag Test (CBT) (Aquagenx[®]) in accordance with manufacturer guidelines. A water quality rating chart, provided in the CBT kit, was used to assess the most probable number (MPN) per 100 mL in each sample showing the presence of *E. coli*. The samples were placed into health risk groups as World Health Organization guidelines,⁽⁹⁾ (safe <1, probably safe 1-10, probably unsafe >10-100, unsafe >100 *E. coli* CFU per 100 mL).

Bacteroides thetaiotaomicron detection

We utilized microbial source tracking (MST) approach using quantitative Polymerase Chain Reaction (qPCR) assays to identify sources of contamination.

We vacuum filtered 150 mL of every water sample through a polycarbonate membrane (47 mm, 0.45 µm pore size) using a sterile funnel for each water sample. Distilled water was filtered as a control for each day of sampling. Two additional replicates were filtered for every sample and filtration blank. The membrane filters were kept refrigerated until DNA extraction (DNEASY® Power-Water Kit QIAGEN).

We performed qPCR assays targeting *B. theta* α-1-6 mannanase gene as previously described.⁽¹²⁻¹³⁾ The DNA from strain *B. theta* ATCC 29148 was used for standard curve and positive control.

The qPCR amplification reactions contained 10 µL of Applied Biosystems® TaqMan® Environmental Master Mix 2.0, 0.4 µL forward and reverse primers, 0.2 µL Probe 62 (Roche), 4.0 µL nuclease-free water, and 5.0 µL of extracted DNA and processed in triplicate.

The thermal cycling was performed using an Applied Biosystems™ QuantStudio™ 3 Real-

Time PCR System with 15 min, 95 °C pre-incubation cycle, followed by 50 amplification cycles, and a 0.5 min 40 °C cooling cycle. We performed the test with DNA samples triplicate and used molecular-grade water for negative control. The detection limit of the assay was ten copies 5 µL⁻¹.

Statistical analysis

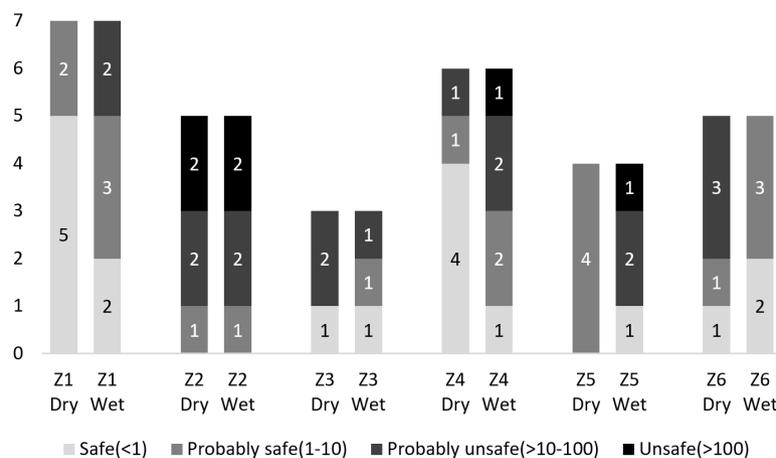
Spatial analysis for all locational data was completed in ArcGIS Desktop. The relationships between dry and wet seasons for *B. theta* and *E. coli* were evaluated by Wilcoxon and McNemar tests (p=0.05).

Results

We assayed water samples from dry and rainy seasons looking for *E. coli* and *B. theta*.

Water quality was assessed by the most probable number (MPN) per 100 mL of *E. coli* and in the dry season, 11 wells were placed into safe group and two into unsafe. In the wet season, seven wells were placed into safe and four in unsafe group (Figure 2). The zone 2 (Z2) showed water contamination in both seasons dry and wet and unsafe samples were found in the zones Z2 (dry and wet), Z4 (wet), and Z5 (wet) (Figure 2).

Figure 2 - Water quality of wells showed in zones (Z). Categories according to most probable number (MPN) categories of *Escherichia coli* per 100 mL sampled in dry and wet seasons.

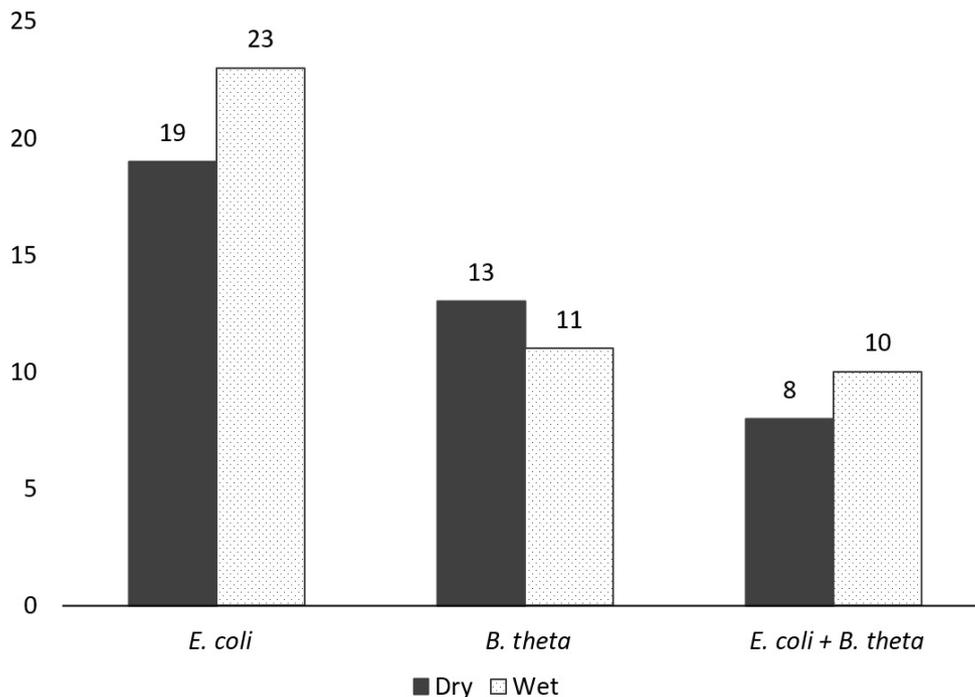


Source: the authors.

About the individual household wells, in the dry season, 63.3% (19/30) of sampled wells showed *E. coli* contamination, and in the wet 76.6% (23/30). *B. theta* was detected in 43.3% (13/30) of the wells in the dry season and 36.6% (11/30) in the wet season. In the dry season, 26.6% of the wells presented *E. coli* and *B. theta* simultaneously, and in the wet season, 33.3%. Figure 3 shows the absolute numbers of positive wells for *E. coli*, *B. theta*, and both. Only one well from zone 3 did not show contami-

nation in any sampling, and two wells from zone 2 and zone 5 showed *E. coli* and *B. theta* contamination in the dry and wet seasons. The other wells displayed *E. coli*, *B. theta*, or both in at least one of the seasonal samples. Although the water from these wells is intended for human consumption, 40% (12/30) of the residents said have not performed any water treatment, 33.3% (10/30) have filtered, 13.3% (4/30) have added chlorine, 10% (3/30) have boiled, and 3.33% (1/30) have used strainer cloth.

Figure 3 - Positive wells for *Escherichia coli*, *Bacteroides thetaiotaomicron*, and both. Sampling in the dry and wet seasons.



Source: the authors.

Overall, *Escherichia coli* presence was continual in seasons and between samples so that the McNemar test showed no difference between wells number *E. coli* positive in the dry and wet season ($p=0.3438$). As for *B. theta* presence in the dry and wet seasons, we also observe that there was no difference between the variables (McNemar test, $p=0.8036$).

We analyzed *E. coli* and *B. theta* association and there was no relationship in both occurrence in the dry season (McNemar test, $p=0.2101$), how-

ever in the wet season there was a significant difference between *E. coli* and *B. theta* proportions, showing less *B. theta* occurrence in the wet season (McNemar test, $p=0.0018$).

Indeed, we found lower quantification indices in qPCR results of *B. theta* in the wet season compared to the dry season (Table 2 and Figure 4). Employing the Wilcoxon test we found significant differences in *B. theta* quantification between the dry and wet seasons from zone 4 ($p=0.0022$) and zone 5 ($p=0.0277$).

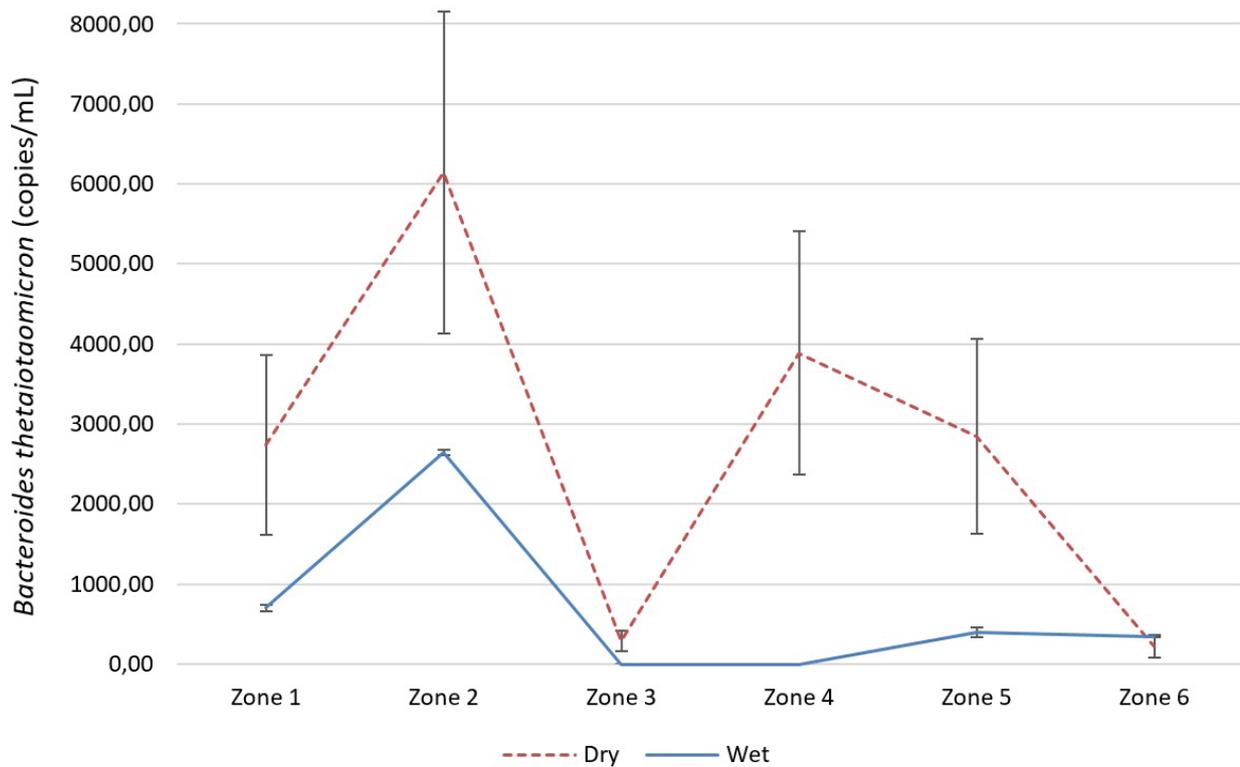
Table 2 - *Bacteroides thetaiotaomicron* qPCR results in the dry and wet seasons. Mean, standard deviation, and range of *Bacteroides thetaiotaomicron* (copies/mL). Mean of temperature and precipitation in September/October 2018 (dry season) and February/March 2019 (wet season).

Zone	Dry			Wet		
	Mean (copies/mL)	Temperature (°C)*	Precipitation (mm)*	Mean (copies/mL)	Temperature (°C)*	Precipitation (mm)*
1	2739.22 ± 7430.73 (0 - 38621.68)			704.28 ± 1957.42 (0 - 7500.872)		
2	6146.07 ± 11021.71 (0 - 41702.42)			2643.58 ± 4260.83 (0 - 13844.958)		
3	288.9 ± 561.58 (0 - 1548.44)			0		
4	3883.82 ± 9381.45 (0 - 43460.58)	30.6 (24 - 37.4)	1.04 ± 6.58 (0 - 45)	0	26.9 (23.1 - 32.9)	3.89 ± 4.94 (0 - 42.9)
5	2850.98 ± 5969.56 (0 - 21794.4)			399.79 ± 1809.90 (0 - 8868.495)		
6	218.61 ± 784.93 (0 - 3348.4)			346.33 ± 1588.09 (0 - 8568.533)		

*Data from Instituto Nacional de Meteorologia (<https://tempo.inmet.gov.br/>).

Source: the authors.

Figure 4 - Average of *Bacteroides thetaiotaomicron* (copies/mL) quantification in each zone. Dry and wet seasons.



Source: the authors.

Discussion

Altamira is the largest city in Brazil in area, but the population is concentrated near the riverbank, the urban Altamira region in which our sampling was conducted.⁽¹⁵⁾ According to Baptista,⁽¹⁶⁾ the dug wells in Altamira have a depth of fewer than 30 m and thus reach shallow aquifers, being more susceptible to contamination.⁽¹⁷⁾

Despite that Belo Monte construction from 2011 has driven the increase of the urban population, agriculture and livestock remain the main economic activity,⁽¹⁵⁾ this factor that impacts on the soil and underground water resources quality.

In the urban region, sanitation services have not kept pace with urban growth so that the population was supplying your demand with open bottom wells and septic tanks, however, septic tanks increase soil microbiological contamination probability and consequently water table contamination.^(2,4)

It is known that in rain and river floods periods, the level of the water table increases, facilitating their contamination due to proximity to the surface. This is more pronounced in shallows aquifers such as the one in Altamira which in addition had their level increased due to constructions of dams.⁽¹⁸⁾ On the other hand, there is also an easier flow of contaminants from the septic tanks and percolation from the most superficial layers of the soil to groundwater.

The World Health Organization⁽⁹⁾ highlights that *E. coli* “must not be detectable in any 100 mL sample” of drinking-water or that it does not have a fecal indicator. We verify that only one of the sampled wells met the drinking-water conditions in both seasons and the others showed contamination by *E. coli* or *B. theta* at some time (dry, wet, or both seasons). This data is worrying because 40% of the residents reported not using water treatment before consuming it.

Our results show that there was no significant difference between *E. coli* detection in the dry season and in the wet season so that the detection was relatively continuous (Figure 3). Comparing *E. coli* and *B. theta* detection (presence and absence), we obtained that in the dry season, the wells with *E. coli* did not necessarily have *B. theta* and vice versa, because there was no relationship between the two occurrences for this study conditions (McNemar test, $p=0.2101$). In the wet season, there was a difference regarding the *B. theta* absence and *E. coli* presence with more *B. theta* negative wells and more positive wells for *E. coli* (McNemar test, $p=0.0018$).

Comparisons between *E. coli* and *B. theta* detection in the two seasons indicate that in the dry season contaminated wells with *E. coli* were also contaminated with *B. theta* and that in the wet season wells contaminated with *E. coli* were not contaminated with *B. theta* necessarily. These *E. coli* results show that the *E. coli* detection parameter to potability was efficient.

Similarly, Shrestha *et al.*⁽¹⁹⁾ and Lima *et al.*⁽²⁰⁾ in their studies in Nepal and Brazil, respectively, discussed the contamination by *E. coli* of the shallow aquifer through runoff and infiltration in wet seasons. However, in our study, there was no pronounced difference in the volume of precipitation between the periods of drought and rain (Table 2), these data support the continuous detection of *E. coli* in both seasons and the hypothesis of sources of contamination on the soil surface.

No difference in *E. coli* detection in seasons shows diverse sources of *E. coli* contamination in the water, as animal feces and garbage waste, probably on the soil surface and that reaches the aquifer through surface runoff and percolation. In contrast, similar studies conducted in Brazil identified a higher rate of *E. coli* contamination during the wet season in Amapá,⁽²¹⁾ Pará,⁽²²⁾ and Mato Grosso.⁽²³⁾ Furthermore, Silva *et al.*⁽²³⁾ discuss the importance of septic tanks in this process and point out that

the greater groundwater contamination during the rainy season may be due to the leaching process being more pronounced than the dilution of contaminants in the aquifer.

On the other hand, the differences in the *B. theta* concentration between the dry and wet seasons suggest a different process in the origin and permanence of contamination in the aquifer. Two zones (4 and 5) showed a higher *B. theta* concentration in the dry season (Wilcoxon test), but Figure 4 illustrates this difference for most of the zones tested. The *B. theta* specificity with the human host indicates wells contaminations by sewage,⁽²⁴⁾ probably from septic tanks located nearby.

We hypothesize that the lower *B. theta* concentration in the wells during the wet season may be due to the contaminant dilution within the aquifer due to the increase in the water level in the wet season, which is possible because of the existence of a confined aquifer fissured in the urban Altamira and due to it refueling linked to the rains.⁽²⁵⁻²⁶⁾

Information about population density and terrain elevation was used for direct sampling. The correlation between these variables and the detection of *E. coli* and *B. theta* in the dry and wet seasons, as well as the differences in methodologies for detecting the two bacteria, can be considered a limitation of this study.

Conclusion

There is an interconnection between the consequences of public policy measures, environmental impacts, and natural variations in the environment. In our study, we observed contaminant agent variations due to seasonal variations in rainfall patterns so that the presence of contaminants on the soil surface seems to be a determining factor for the quality of shallow aquifers.

Given this scenario, educational measures to guide the population to adopt home treatment practices for the consumption of collected water

from wells could be implemented as public health policies. In a broader context, we believe that investments in infrastructure for the collection, removal and treatment of sewage are essential for the municipality.

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