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Quality of water for human consumption in a rural area community from Brazil

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This work aimed at evaluating the bacteriological, parasitological, physical and chemical quality of water intended for human consumption in a community in a rural area of Recôncavo of Bahia (Brazil) and the factors related to a possible contamination. Samples were collected at two different times: at rainy season (August to September, 2015) and dry season (April 2016). The present work evaluated the presence of total coliforms and *Escherichia coli*; quantified heterotrophic bacteria; performed parasitological techniques of direct examination and modified Faust; analyzed pH, temperature, dissolved oxygen, apparent color, turbidity and salinity; and applied a questionnaire regarding the water source and its storage. Out of the 53 households, 67.9% were in disagreement with bacteriological standards of potability. 5.7% in disagreement with parasitological standards, 92.5% in disagreement with physical and chemical standards and all samples were in disagreement with the parameters allowed and recommended by the Brazilian legislation. The water source, presence of a household reservoir, sewage destination and reservoir cleaning time were significantly related to the bacteriological results. The consumption of this water poses a risk to the population's health and it could provoke outbreaks of food borne diseases. Effective water treatment and surveillance measurements should be taken in order to minimize risks to human health.

Key words: Potable standards, groundwater, indicator microorganisms, contaminated water.

INTRODUCTION

Access to treated water is a basic human right (WHO, 2015). All water intended for human consumption provided from alternative supply sources, regardless of the way of access, is subject to water quality monitoring. Therefore, potable water intended for ingestion, food preparation and personal hygiene, regardless of its source, must meet established drinking standards (Brazil, 2011; Benedict et al., 2017).

Water could suffer contamination at the source, during its distribution, as well as in household reservoirs. About 10% of the world's population does not have access to drinking water and 35% have no access to basic sanitation (WHO, 2015). Brazil possesses the world's largest fresh water reservoirs, however, due to its

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territorial extension, distribution is heterogeneous. Due to the absence of effluent treatment systems, many times water is contaminated from the water table (Brazil, 2005; Rebouças et al., 2015).

Foodborne diseases represent an important public health issue in Brazil and in the world, being associated with multiple etiological agents, such as bacteria, viruses, helminths and protozoa, which invade human body through contaminated water and/or food. Despite the high occurrence rates, most cases of foodborne diseases are not reported (Brazil, 2010; Martins et al., 2015; Neves et al., 2016). Approximately 80% of all diseases in developing countries are caused by non-potable water and poor sanitation (Brazil, 2010, 2016; WHO, 2015).

Considering the shortage of studies correlating microbiological and parasitological, physical and chemical results as well as the shortness of researches regarding possible contamination factors and the relevance of studies to subsidize actions aiming to improve health of the peasant population, this study aimed to evaluate the bacterial, parasitological, physical and chemical quality of water intended to human consumption in a rural area community of Recôncavo of Bahia (Brazil), and also to investigate factors related to a possible contamination.

MATERIALS AND METHODS

Sample collection

This research is characterized as a descriptive and analytical cross-sectional study. Water samples were collected in 53 households of a rural area of Santo Antônio de Jesus-Bahia (Brazil), from August to September 2015 (end of rainy season) and repeated in 34 households in April 2016 (end of dry season), totaling 87 samples. The number of households varied according to availability of residents. Samples were collected after the reservoir. In the absence of faucets, samples were collected directly from the reservoir. Approximately, 1.5 L of water were collected. Samples obtained were stored in first use polyethylene flasks (VidroPET-Goiania, Goias, Brazil), labeled and packed in thermal boxes with recyclable ice packs, kept in the refrigeration temperature (+2 to +8°C), and analyzed within 6 h of collection at the Laboratory of Microbiology and Parasitology of Food and Nutrition Security Center (SANUTRI) from Health Science Center (CCS)/Federal University of Recôncavo of Bahia (UFRB). Along with the water collection, a structured questionnaire based on the Sanitation Manual of the National Health Foundation (FUNASA) with 16 questions regarding the water source and its storage (Brazil, 2014).

Bacteriological analysis

For the analysis of total coliforms and *Escherichia coli*, a 100 mL aliquot of each sample was transferred to a sterile first-use bag Twirl'EM (Labplas™ - Montreal, Quebec, Canada). Then, Readycult Coliforms 100 (Merck KGaA™ - Darmstadt, Germany) was added and homogenized until the lyophile was completely dissolved. Samples were incubated in bacteriological oven at 35±1°C for 24±2 h with subsequent reading. All samples positive for *E. coli* in the ultraviolet light test (366 nm) underwent the indole test with the addition of Kovacs reagent (Laborclin® - Pinhais, Paraná, Brazil).

For quantification of heterotrophic bacteria, a dilution of 10^{-1} with 0.9% NaCl was initially performed. 1 mL of the dilution was inoculated into a Petrifilm Aqua Heterotrophic Count Plate (AQHC, 3M Company™ - Maplewood, Minnesota, USA). After complete gel solidification, the plates were incubated in bacteriological oven at 36±1°C for 44±4 h. Results were expressed in log CFU/mL (APHA, 2012).

Parasitological analysis

For investigation of parasitic forms in water sources sampled, the methods of direct examination and modified Faust were applied in triplicate. Samples were considered positive when at least one parasitic form was found in one of the methods. For the direct examination, 250 mL of the sample were maintained under spontaneous sedimentation for 24 h at room temperature. The sediment was collected, stained with Lugol's iodine and visualized under optical microscopy (Olympus-Tokyo, Japan) at 100 and 400× (Neves et al., 2016; Teixeira et al., 2016).

For modified Faust, a new aliquot of 50 mL was removed from the spontaneous sedimentation of the total sample and centrifuged at 838 relative centrifugal field (RCF) for 1 min (SPLABOR-Presidente Prudente, São Paulo, Brazil). The supernatant was discarded and the sediment resuspended with 10 mL of sterile distilled water and further centrifuged for 1 min. The supernatant was discarded and the sediment resuspended with 10 mL of zinc sulfate solution (density 1.18 g/mL; Synth-Diadema, São Paulo, Brazil) with further centrifugation at 838 RCF for 1 min. The membrane formed on the liquid's surface was removed with a bacteriological loop, stained with Lugol's iodine (Dinâmica-Indaituba, São Paulo, Brazil) and visualized under optical microscopy at 100 and 400× (Neves et al., 2016; Teixeira et al., 2016).

Physical and chemical analyses

For the physical and chemical analyses, pH, temperature, dissolved oxygen and salinity analyses were performed on field, immediately after water collection with a multiparameter meter AK88 (AKSO®-São Leopoldo, Rio Grande do Sul, Brazil), using an approximate aliquot of 100 mL. Turbidity analysis was performed in laboratory, with a AP-2000 (PoliControl®-Diadema, São Paulo, Brazil) microprocessed bench turbidimeter, with 860 nm wavelength. Apparent color analysis was performed with visual colorimeter DLNH-100 (DelLab®-Araraquara, São Paulo, Brazil) (APHA, 2012).

Statistical analysis of data

Data were processed and analyzed using Statistical Package for the Social Sciences (SPSS) version 23 (International Business Machines™-New York, New York, USA). A normality test (Kolmogorov-Smirnov) was carried out with all quantitative variables. Descriptive and analytical statistics, such as median, maximum, minimum, percentage distribution, Spearman's correlation coefficient, Pearson's chi-squared test and variance analysis (ANOVA) were performed. The adopted level of significance was 5% ($p<0.05$).

Ethical considerations

This study was evaluated and approved by the Human Research Ethics Committee from the Federal University of Recôncavo of Bahia (UFRB) (CAAE: 04022312.0.0000.0056 - Authorization 1.167.637), in accordance to National Health Council (CNS)

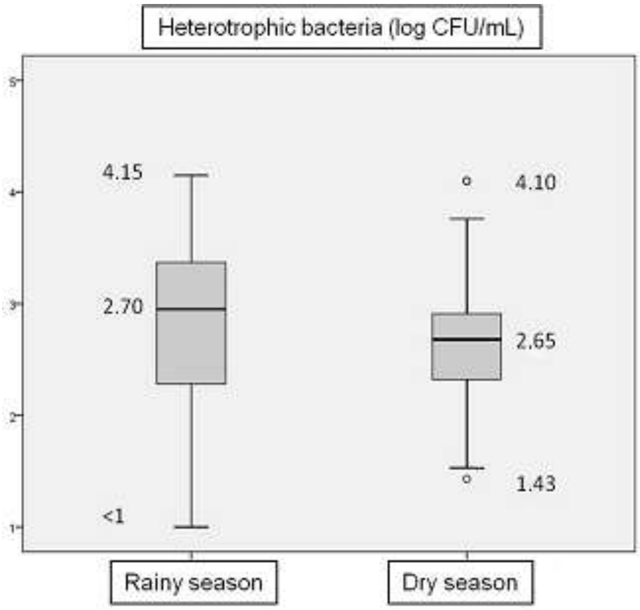

Figure 1. Quantification of heterotrophic bacteria.

Table 1. Total coliform and *Escherichia coli* analysis.

Parameter	Rainy season (N = 53)				Dry season (N=34)			
	Positive		Negative		Positive		Negative	
	n	%	n	%	n	%	n	%
Total coliforms	45	84.9	8	15.1	30	88.2	4	11.8
Escherichia coli	36	67.9	17	32.1	24	70.6	10	29.4

N: Total population; n: sample size; %: percentage.

Resolution 466/2012 (Brazil, 2012). All participants were informed of the study objectives and those who agreed were invited to sign the informed consent form.

RESULTS

Water from every household came from an alternative source of supply, that is, with underground or surface collection, with or without pipeline and without distribution network (Brazil, 2011). Counts of heterotrophic bacteria resulted in maximum values of 4.15 and 4.10 log CFU/mL and minimum values of <1 and 1.43 log CFU/mL by the end of the rainy and dry season, respectively. There were no significant differences between the results of heterotrophic bacteria for both samples (p = 0.071) (Figure 1). Dry season presented a higher proportion of positive samples, with 88.2% (n = 30) positive samples for total coliforms and 70.6% (n = 24) for *E. coli* (Table 1).

No parasitic structures were found in the majority of samples (60.4 and 76.5%). Unidentified protozoa cysts, acari, flagellate protozoa, *Giardia* species and *Endolimax nana* were found in the positive samples, in descending order (Table 2).

Amongst the physical and chemical parameters, there were no significant differences between the means of the two seasons of pH (p = 0.338) and dissolved oxygen (p = 0.859). However, there were significant differences between temperature (p = 0.034), turbidity (p = 0.002) and salinity (p = 0.015) means. It was not possible to perform statistical analysis in the parameter color, because the second water collection was constant, presenting zeroed values. However, there was a significant reduction of this parameter between the rainy and dry season (Figure 2).

Ordinance MS 2914/2011 (Brazil, 2011) subdivides maximum values of potability between allowed and recommended. If a sample is out of the recommended samples, it does not mean it is unsafe for human consumption. However, the source of the problem must be investigated and fixed.

Heterotrophic bacteria, total coliforms and pH have maximum recommended values, however, the presence of *E. coli, Giardia* spp. or *Cryptosporidium* species turbidity and apparent color have maximum allowed

Table 2. Parasitological evaluation.

Parasitological analysis	Rainy season (N = 53)		Dry season (N = 34)	
	Number of findings*			
	n	%	n	%
Negative	32	60.4	26	76.5
Giardia spp.	3	5.7	0	0
Endolimax nana	2	3.8	0	0
Unidentified protozoacyst	15	28.3	3	8.8
Flagellate protozoa	4	7.6	0	0
Acari	0	0	5	14.7

N: Population size; n: sample size; %: percentage; *In a positive sample, one or more protozoa may have been found.

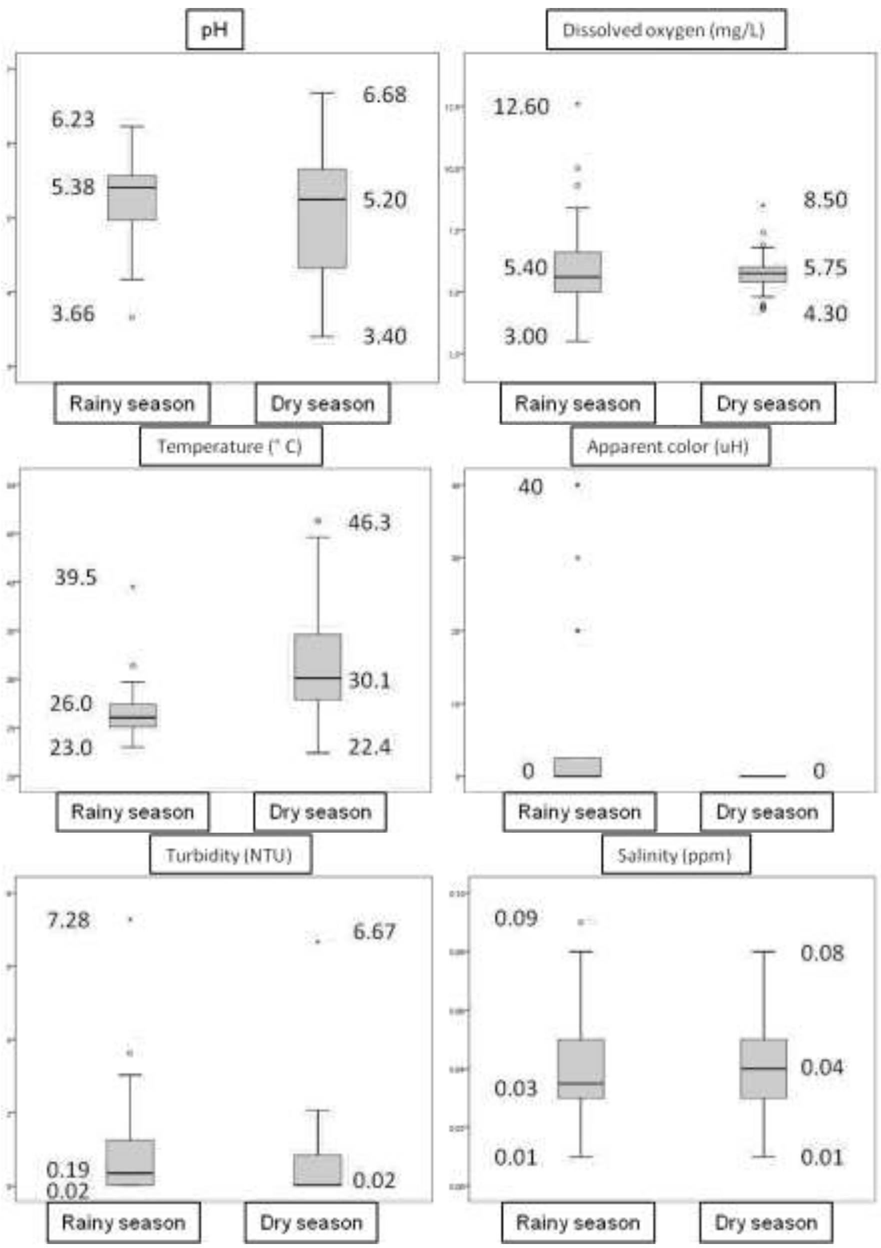

Figure 2. Physical and chemical analysis.

Table 3. Conformity distribution of samples of water intended for human consumption, according to Ordinance MS 2914/2011.

Parameter		In accordance		Not in accordance	
Rainy season (N=53)	**Reference standard**	**n**	**%**	**n**	**%**
Heterotrophic bacteria (log CFU/mL)	≤2.7[x]	27	51	26	49
Total coliforms	Absence in 100 mL[x]	8	15.1	45	84.9
Escherichia coli	Absence in 100 mL[y]	17	32.1	36	67.9
Presence of *Giardia* spp. or *Cryptosporidium* spp.	Absence in 100 mL[y]	50	94.3	3	5.7
pH	Between 6.0 and 9.5[x]	4	7.5	49	92.5
Apparent color (uH)	≤15[y]	47	88.7	6	11.3
Turbidity (NTU)	≤5[y]	51	96.2	2	3.8
All parameters allowed	-	17	32.1	36	67.9
All parameters allowed and recommended	-	0	0	53	100
Dry season (N=34)					
Heterotrophic bacteria (log CFU/mL)	≤2.7[x]	19	55.9	15	44.1
Total coliforms	Absence in 100 mL[x]	4	11.8	30	88.2
Escherichia coli	Absence in 100 mL[y]	10	29.4	24	70.6
Presence of *Giardia* spp. or *Cryptosporidium* spp.	Absence in 100 mL[y]	34	100	0	0
pH	Between 6.0 and 9.5[x]	4	11.8	30	88.2
Apparent color (uH)	≤15[y]	34	100	0	0
Turbidity (NTU)	≤5[y]	33	97.1	1	2.9
All parameters allowed	-	10	29.4	24	70.6
All parameters allowed and recommended	-	1	2.9	33	97.1

x: Recommended values; y: values allowed; N: population size; n: sample size; %: percentage.

Table 4. Spearman's correlation coefficient between bacteriological, physical and chemical analysis.

Parameter	Heterotrophic bacteria	
	Rainy season	Dry season
pH	0.134	-0.372[a]
Dissolved oxygen	0.283[a]	0.018
Temperature	0.210	0.102
Apparent color	0.548[b]	*
Turbidity	0.554[b]	0.255
Salinity	-0.108	-0.307

[a]The correlation is significant at the 0.05 level; [b]The correlation is significant at the 0.01 level; *constant.

values. 32.1% samples (n = 17) collected during rainy season were in accordance with the permitted parameters. However, none of the samples was in accordance with the permitted and recommended standards. 29.4% samples from dry season (n = 10) were in accordance with the standards, but only one sample (2.9%) was classified as allowed and recommended (Table 3).

Spearman's correlation coefficient analysis between bacteriological, physical and chemical analyses showed a weak, directly proportional and significant correlation between dissolved oxygen in the results from rainy season (p = 0.040); a moderate, directly proportional and significant correlation between apparent color and turbidity in the results from rainy season (p = 0.000); and a weak, inversely proportional and significant correlation between pH in the results from dry season (p = 0.030) (Table 4).

Results from the parasitological examination presented statistically significant differences between results of heterotrophic bacteria analysis (p = 0.012), with a higher proportion of negative parasitological results in samples in disagreement with the established parameters for heterotrophic bacteria. The other variables are independent (Table 5).

E. coli and heterotrophic bacteria presented statistically significant differences between the distributions of various sources of water (p = 0.01; p = 0.002) and construction of the tank (p = 0.02; p = 0.01). On the other hand, only results from heterotrophic bacteria presented

Table 5. Percent distribution of the bacterial analysis according to the results from the parasitological examination.

Microbiologic		**Parasitologic**		
		Positive	**Negative**	**p value[1]**
Heterotrophic bacteria	In accordance(%)	18.4	28.7	0.012[a]
	Not in accordance(%)	8.0	44.9	

1: *p* value result from the Pearson's chi-squared test; a: statistically significant differences (p<0.05); %: percentage.

Table 6. Percent distribution of *Escherichia coli* and heterotrophic bacteria analysis, according to the results from the verification list.

Parameter	***Escherichia coli***			**Heterotrophic bacteria**		
	In accordance (%)	**Not in accordance (%)**	**p value[1]**	**In accordance (%)**	**Not in accordance (%)**	**p value[1]**
Water source						
Shallow well	12.5	51.7	0.01[a]	25.3	38.0	0.002[a]
Semi artesian well	29.6	17.2		27.5	9.2	
Construction of the tank						
Dweller	13.8	54.0	0,02[a]	27.6	40.2	0,01[a]
Government	17.2	15.0		25.3	6.9	
Openings or cracks at the water tank						
Present	11.5	31.0	0.487	11.5	31.0	0.001[a]
Absent	19.5	38.0		41.4	16.1	
Sewage destination regarding the water source						
Above	11.5	48.3	0.004[a]	24.1	35.6	0.004[a]
Same level	19.5	0.7		28.7	11.6	
Reservoir cleaning time						
Less than six months	17.2	28.7	0.229	32.1	13.8	0.003[a]
More than six months	13.8	40.3		20.7	33.4	
Water reservoir in the household						
Present	18.4	34.5	0.423	35.7	17.2	.,004[a]
Absent	12.6	34.5		17.2	29.9	

1: *p* value result from the Pearson's chi-squared test; a: statistically significant differences (p<0.05); %: percentage.

statistically significant differences between the distribution of variables openings or cracks in the water source (p = 0.001), sewage destination regarding the water source (p = 0.004), reservoir cleaning time (p = 0.003) and presence of a reservoir in the household (p = 0.004) (Table 6).

DISCUSSION

Every household studied had alternative water supply solutions, either individually or collectively. Therefore, it is necessary for the Municipal Health Department to control and monitor the water quality (Brazil, 2011).

A high percentage of improper samples (67.9%) and not recommended (99%) for human consumption was found. Some samples presented 28 times (4.15 log CFU/mL) the maximum recommended for heterotrophic bacteria based on Brazilian legislation (2.70 log CFU/mL). It has been observed that 51% samples were above this reference standard during the first water collection and 44.1% in the second.

Heterotrophic bacteria constitute an indirect indicator of water safety, not identifying the microorganisms, which may be pathogenic or from the water microbiota (Brazil, 2006; Richards et al., 2018). Its high count may indicate bacterial colonization, water treatment ineffectiveness and even a formation of biofilms in the distribution system, from the presence of organic matter in water (Bargellinia et al., 2011; Chowdhury, 2012; Richards et al., 2018).

Sudden changes or values above the potability standards should be investigated in order to identify irregularities and measures must be taken to restore the integrity of the distribution system, such as the maintenance of a minimum free residual chlorine content of 0.5 mg/L (Brazil, 2011).

Ordinance MS 2914/2011 recommends the absence of total coliforms and determines the absence of *E. coli* in 100 mL of water (Brazil, 2011). The total coliforms group comprises bacteria of both enteric and non-enteric origin (Madigan et al., 2016). Therefore, this parameter is not considered a good sanitary indicator of water intended for human consumption, not of crude water, meaning the presence of the bacteria in crude water does not indicate that water is unsafe for consumption (Cabral, 2010).

In order to differentiate enteric coliforms from total coliforms, the thermotolerance characteristic of enteric coliforms is used. *E. coli* is considered the main thermotolerant coliform, being an ideal sanitary indicator in the bacteriological analysis of the water, since its presence indicates that fecal contamination occurred and, consequently, there is a potential risk of presence of other microorganisms of enteric origin, including pathogenic (Cabral, 2010; Brunkard et al., 2011; Madigan et al., 2016). Several strains of *E. coli* are known to be pathogenic to humans. They may present virulence genes, which are involved in colonization, adhesion, and host invasion; pathogenic tissue processes; and avoidance mechanisms (Mainil, 2013).

Parasitic structures were identified in 33.3% of the total samples. Of these, three samples were contaminated by pathogenic protozoa (*Giardia* spp.) and two by commensal protozoa (*Endolimax nana*).

Giardia duodenalis, the protozoa responsible for causing giardiasis, may lead to diarrhea, but most cases are oligosymptomatic or asymptomatic. In Brazil, even with high prevalence of giardiasis (12.4 to 50%), researches of these protozoa are scarce in alternative sources of supply. Its main form of transmission occurs by ingestion of water contaminated by cysts. Population clusters without basic sanitation and sanitary preparation tend to increase the spread of this pathogen. Even with treatment, it is still possible to find cysts in water, due to their resistance to chlorination, filtration and temperature increase (Fregonesi et al., 2012; Santana et al., 2014).

As with *Giardia* spp., protozoa *E. nana* presents fecal-oral contamination through food or water, but is not pathogenic to humans and can be eliminated by conventional water treatment (Poulsen and Stensvold, 2016). The presence of commensal parasites in water, due to their life cycle, can be used as an indicator of fecal contamination, just like *E. coli* (Xavier et al., 2011; Poma et al., 2012).

Free-living protozoa and heterotrophic bacteria are subjected to disharmonious interspecific relations of predation and interspecific competition (Ricklefs, 2016). This fact may be related to the greater number of samples with higher quantifications of heterotrophic bacteria when the result of the parasitological examination was negative.

Due to the lack or inefficiency of sewage collection systems, water can become a vehicle and source of diseases when transporting pathogens. Generally, high levels of parasitic diseases and cases of foodborne diseases prevail where the socioeconomic conditions of the population are more precarious. Less-favored classes are more affected by food contamination due to difficulty or lack of access to basic sanitation, treated water, education and safe food (Andrade et al., 2010; Neves et al., 2016).

Regarding the physical and chemical parameters, apparent color had the highest standard deviation due to the amplitude of its results. As most of the water samples came from shallow wells and it did not underwent filtration, turbidity interfered with the values, increasing them due to suspended particles, thus not demonstrating the actual color of the samples. However, Ordinance MS 2914/2011 establishes apparent color instead of true color as an organoleptic standard of potability, with a maximum value of 15 uH (Scorsafava et al., 2010; Daneluz and Tessaro, 2015).

Turbidity was the parameter with the highest number of samples in conformity (96.2 and 97.1%). These values reflect the low presence of suspended solids, such as inorganic particles and organic debris. High values may be related to the presence of metals in water, such as iron, from the soil itself or from precarious conditions of pumps and plumbing (Adolfo Lutz Institute, 2008; Satake et al., 2012; Daneluz and Tessaro, 2015).

pH was the parameter with the lowest number of samples (7.5 and 11.8%) within the range of recommended values (6.0 to 9.5), presenting a more acidic pH. Temperature, salinity and dissolved oxygen are not described as potability standards in Ordinance MS 2914/2011; however, they have a direct relationship with bacterial multiplication. The decrease of dissolved oxygen levels is related to decomposition of organic matter (Araujo et al., 2011; Brazil, 2011). Dissolved oxygen reached maximum levels of 15.40 mg/L and minimum levels of 3 mg/L. Temperatures recorded were within the standards for mesophilic microorganisms (minimum of 23.0°C and maximum of 39.5°C). Salinity remained in a range of low values, with minimum of 0.01 ppm and maximum of 0.08 ppm.

Temperature presented significantly higher mean values in the dry season. However, turbidity and salinity presented significantly lower mean values in the dry season. This can be explained by the existence of openings or cracks in most shallow wells, allowing rainwater to conduct soil and organic matter to the wells located in abysses, increasing the amount of organic and inorganic particles in suspension. As a result of the increase in organic matter in the medium, there is an increase of heterotrophic bacteria, since they are chemotrophic, heterotrophic and organotrophic organisms.

Similar bacteriological, parasitological, physical and chemical results were found in other studies with water from shallow and semi-artesian wells in rural regions of Paraná, Rio de Janeiro, Minas Gerais and several cities in the state of São Paulo. These studies have small variations in the quantifications of potability standards, however, the results are unanimous, showing that most samples were unfit for human consumption (Dias et al., 2008; Menezes et al., 2009; Scorsafava et al., 2010; Araujo et al., 2011; Satake et al., 2012; Daneluz and Tessaro, 2015).

Heterotrophic bacteria will consume the oxygen dissolved in water, due to the aerobic respiration process. From this consumption, oxygen levels fall and consequently there will be a greater presence of facultative aerobic and anaerobic bacteria (Santos et al., 2008). Microorganisms are sensitive to pH changes, since the optimum pH ranges from 6.5 to 7.5 for most bacteria, thus lower values can inhibit or delay bacterial multiplication (Machado et al., 2012; Daneluz and Tessaro, 2015).

From the comparison of the bacteriological analyses with the questionnaire, it was possible to identify that results of *E. coli* and heterotrophic bacteria had significant differences between several distributions of the variables.

Water from shallow wells, tanks constructed by dwellers and sewage destination above the level of the water source had higher percentage distributions of samples in disagreement regarding *E. coli* and heterotrophic bacteria.

Results of heterotrophic bacteria also showed significant differences between the variables, in which the presence of openings or cracks in the water source, cleaning time of the reservoir superior to six months and absence of reservoir in the household had larger distributions of samples in non-conformity. Relationships between the other variables were not identified, thus they were considered independent.

FUNASA recommends a minimum distance of 15 m between the wells and the septic tank, and sewage destination should not be at the same level or above the water source (Brazil, 2006). It is also recommended to position breeding farms far from the sources (> 45 m), and both the reservoir and the water source should always remain covered. Finally, reservoirs must be cleaned every six months (Brazil, 2006; Capp et al., 2012; Scalize et al., 2014).

Overall, the study carried out from bacteriological, parasitological, physical and chemical analyses, in association with the verification list, points out to the nonconformity of 99.9% samples from a bacteriological, physical and chemical point of view, considering the MS Ordinance 2914/2011. Therefore, the consumption of this water represents a risk to the population's health of that area, and may lead to the involvement of food borne diseases.

Considering that this rural community is deprived of treated water supply by the state water and sanitation company, alternative and effective measures of water treatment must be taken, such as filtration for the correction of physical, chemical, bacteriological and parasitological parameters, as well as the use of chlorination, or even a solar water disinfection system (SoDiS) in order to eliminate pathogenic microorganisms.

Corrective measures, such as the presence of water reservoirs in the households, protection against animals in the water source, elimination of septic tanks and animal breeding sites near the source, can minimize this risk and guarantee the health of the community.

It is necessary to increase the presence of state with surveillance and quality control of water for human consumption. Sanitary education actions, regular evaluation of potability standards of wells and households, correction of detected failures related to the quality of supply and maintenance of the articulation between the departments of health of the municipalities, states and the union are measures that can be taken to reverse the current situation.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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