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Association among the Presence of Rotavirus Group A and Types of Sources Located in Rural Communities

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Abstract: Among the etiological agents of acute gastroenterocolitis, group A Rotavirus stands out. Proper hygiene and basic sanitation are the prophylactic means. The present research aimed to carry out the tracking of Rotavirus in waters of rural communities. The collections were carried out in 86 residences, distributed in 16 communities, considering the types of sources: tubular wells, excavated wells, surface springs, cistern with rainwater and springs. The elution of membranes with negative charges was used to obtain viral particles. Extraction of the genetic material and cDNA synthesis were performed according to the rules of the kit used, then qPCR was performed with an Applied Biosystems StepOne® thermal cycler. Statistical analysis was performed using Stata® 16.0, BioEstat® 5.3 and Minitab® 1. Rotavirus was detected in 47 samples (54.6%), however, there was no significant association among municipalities and the presence of Rotavirus. There was a strong association with shallow excavated well OR = 3.59 [1.05–12.28, $p = 0.04$]. The virus to infect humans and non-human animals, it can infect poultry, cattle and pigs. The presence of Rotavirus in surface water qualifies it as a biological marker of water quality and would occur through fecal contamination in water surface and underground.

Keywords: contamination; microbiology; virus; epidemiology

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1. Introduction

Rotavirus group A (RVA) is the main etiological agent of acute gastroenterocolitis in children and young people. The infection has a higher incidence in children under five years of age, this greater involvement is due to susceptibility and reinfection, which occurs countless times, and the susceptible individual presents greater severity [1]. Due to acquired immunity to subsequent infections, patients have mild symptoms or asymptomatic infection [2].

Responsible for high rates of morbidity and mortality in childhood, group A Rotavirus has a higher incidence and prevalence in underdeveloped countries, with populations without access to basic sanitation, mainly affecting children residing in these regions [3,4]. Immunization through the attenuated virus vaccine is effective in prevention and has reduced epidemiological indices [5–7].

Rotavirus serogroup A was the causative agent of 440,000 deaths per year before vaccine introduction and according to studies on the global burden in 2016, mortality reached approximately 128,500, the data refers to the public under five years of age. Thus, when considering the causes of deaths due to diarrhea in this age group, Rotavirus was responsible for 28.8%. In underdeveloped countries, mortality is largely related to dehydration [1]. The mortality rate in developed countries is considerably reduced, however, it is the largest

cause of hospitalizations for acute gastroenterocolitis with two million hospitalizations per year and approximately 25 million outpatient consultations worldwide [1].

Diarrhea, caused by RVA, is a worldwide health problem, responsible for 9% of mortality in children, among vulnerable populations indigenous people and populations without basic sanitation stand out. The cases are aggravated by the low economic potential, which reduces life expectancy, resulting in an increase in the infant mortality rate in these regions [3].

The main biological indicator for determining water quality is the bacteria Escherichia coli, however, several other microorganisms may be present, such as viruses, protozoa and fungi [8–10]. Pollution of the environment is almost always a consequence of human actions, either through illegally discharged sewage into surface waters or the use of chemical substances that reach groundwater [11].

The absence or deficiency of sanitation in rural areas is associated with an increased incidence of enteric virus infections, a consequence of contamination of surface water and groundwater wells without adequate infrastructure [12]. Contamination occurs through human and non-human animal waste and may occur directly at the sources or in the surroundings with the mediation of rain. It is estimated that humans with gastroenterocolitis or viral hepatitis can excrete up to 1013 viral particles per gram of feces, therefore, the presence of these viruses are in high concentrations in wastewater of rural communities [13].

Enteric viruses can be transmitted by water, resulting from the fecal-oral cycle, increasing the capacity for infection and subsequent replication in the host's gastrointestinal system. After infection and replication, viral particles are excreted in the feces, among the viruses Rotavirus and Adenovirus stand out as the main causes of gastroenterocolitis in children, the elderly and people with impaired immune systems [14,15].

Surface water contamination occurs through agricultural and domestic effluents, eliminating high concentrations of viral particles in the feces of infected humans and animals [14–17]. Much of the treated and untreated sewage flows into environmental water bodies, standing out as having the potential to negatively impact recreation, agriculture and beverages for human consumption [18]. Despite the confirmation of enteric viruses in surface water, the only bioindicators used to assess water quality are bacterial, with emphasis on total and fecal coliforms (*Escherichia coli*), an assessment used mainly in South America [19].

Industrialized countries and urban areas are privileged with access to the distribution network with treated water, however, in rural regions and countries with little development in basic sanitation, they present heterogeneity in the type of water source. With the absence of water distribution, the resources used are through surface sources such as rivers, lakes, springs, shallow wells, deep wells and shallow wells with rainwater [20,21].

Water obtained from underground sources is preferably used for human consumption, considering the microbiological quality when compared to surface water. Groundwater used in rural areas is preferably from springs and shallow vertical wells, considering the low cost and ease of installation [20].

The promotion of basic sanitation does not reach the entire population, limited to urban regions. The absence or disability of the supply system in rural regions resulted in the exploitation of new solutions such as artesian wells, deep tube wells, wells shallow tubes [22,23], which can be influenced by the precariousness related to sanitation found in these regions [10,20–26].

Although the planet is made up mostly of water, only 2.5% is classified as sweet and a small part of that percentage is in a liquid state, being used for consumption and recreation. Surface water is characterized by accumulating in the surface and can be found in rivers, lakes, streams, swamps, seas and other types of sources [27].

Other types of water are those classified as groundwater, the presence of this type of water can occur naturally or artificially underground. Groundwater is present in the subsoil of the planet, largely found among empty spaces in rocks and in aquifers [28]. Unlike surface water, groundwater extraction is complex, as a result of the depth of the

water table or rocks, which makes access difficult and costly. Due to the lack of fresh water, many regions use groundwater for various activities (agriculture, residential and industrial). Communities far from large centers use groundwater for consumption through artesian wells and shallow wells [29].

The presence of viruses in the rural environment stands out as an extensive field of research, having a relationship with public policies as resources water resources, sanitation, coastal management, epidemiological surveillance and health [9]. Enteric viruses are targets of investigations environments, are represented by several families and genera and are associated with various infectious conditions. Among the most common enteric viruses incidents stand out: Norovirus, human Adenovirus, Enterovirus and the Rotavirus [30,31].

Rotavirus A (RVA) belongs to the genus Rotavirus of the reoviridae family. The viral particle is of the non-enveloped type and has icosahedral symmetry with three concentric capsids of protein characteristics, called: internal, intermediate and external. The genome consists of 11 segments of double-stranded RNA (dsRNA), responsible for encoding six structural proteins (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5, NSP6) [32,33].

The outer capsid is formed by the structural protein VP7, encoded by genomic segments 7, 8 or 9. The coding of segment 4 is responsible for the formation of the VP4 protein. The VP6 protein forms the intermediate capsid, encoded by genomic segment 6. Finally, the structure of the inner capsid is formed by the VP2 protein encoded by segment 2. The VP1 and VP3 proteins are encoded by segments 1 and 3, respectively [32,33].

The great variability in its 11 genomic segments is responsible for the expression of different antigens. Thus, there are different combinations among viral genotypes that vary over time. However, due to the prevalence of the G/P combination (Glycoproteins/Protease-Sensitive Proteins), the variability occurs between two genotypes, which are respectively present in the VP7 and VP4 genes that segregate independently, responsible for the synthesis of external structures: VP7 glycoprotein that determines the rotavirus serotype and VP4, proteins that bind to host cells, known as sensitive to proteases, both present in the external capsid and due to locality, are responsible for the production of neutralizing immunity [34].

Considering Rotavirus, 36 G genotypes and 51 P genotypes are known, human viruses and non-human animals viruses, where the most incidents in the human population are the combinations: G12P [8], G9P [8], G4P [8], G3P [8], G2P [4] and G1P [8]. Despite the diversity of genotypic combinations, the P [8] region is conserved, with the exception of the G2P [4] genotype [4,33].

The presence of RVA in rural areas is favored by the ability of the virus to infect humans and non-human animals viruses, it can infect poultry, cattle and pigs [35]. The feature Rotavirus structure favors its fixation/adhesion and consequently adsorption to organic and non-organic matter present in water [36], conferring resistance to harmful environments [37].

The objective of this research was to identify the Rotavirus group A through molecular techniques, considering water sources used for consumption and recreation in rural communities in 15 counties in the state of Goiás. Evaluate a possible association among the presence of Rotavirus and the independent variables municipalities and font types.

2. Materials and Methods

2.1. Location of Communities Considered for Water Collections

This research qualifies as analytical, considering the descriptive character. The collections were carried out in 14 quilombola communities (groups with their own historical trajectory) and two rural settlements distributed in 16 counties (Table 1), the sample size was 86. Data collection was developed *in loco* from April 2019 to October 2019, cooperatively with the project called *Saneamento e Saúde Ambiental em Comunidades Rurais e Tradicionais de Goiás* (SanRural). Samples from quilombola communities were collected in 16 counties: Nova Roma (Magalhães community), Colinas do Sul (Jose de Coleto), Monte Alegre de Goiás (Pelotas), Mineiros (Cedro), Cavalcante (São Domingos), Flores de Goiás (Canabrava),

Iaciara (Extrema), Padre Bernardo (Sumidouro), Cidade Ocidental (Mesquita), São João da Aliança (Forte), Simolândia (Castelo/Retiro e Três Rios), Mimoso de Goiás (Queixo Dantas), Niquelândia (Rafael Machado) and Campos Belos (Taquarussu). The settlements are located in the municipalities: São Miguel do Araguaia (Lageado) and Silvania (São Sebastião da Garganta) (Figure 1). Collections were performed in 500 mL autoclaved containers, totally sterile. For each water sample, 0.3 g of chloride was added of magnesium ($MgCl_2$) for sample stabilization. The samples were transported to the laboratory at a temperature of 4 °C.

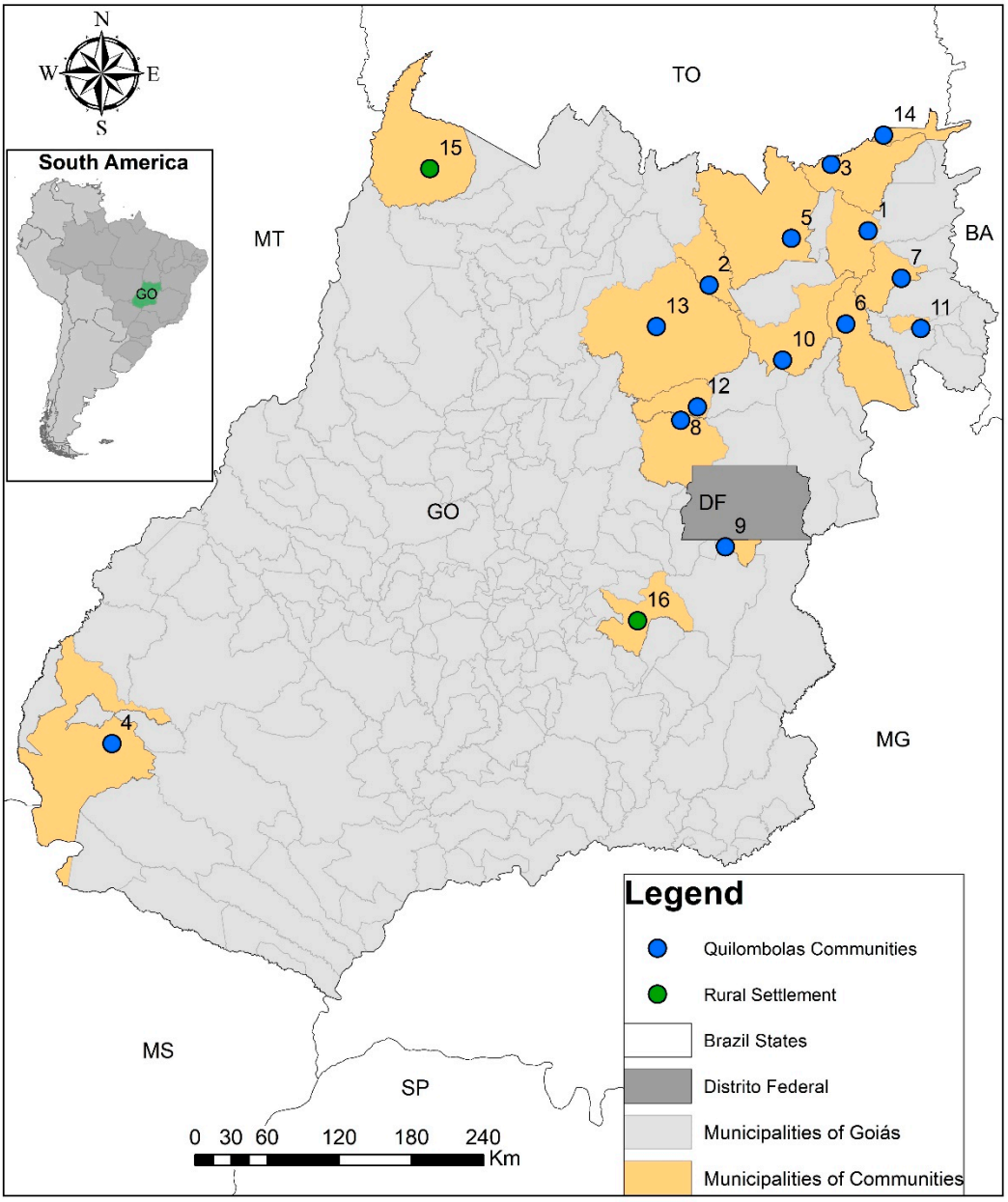

Figure 1. Location of municipalities where collections were carried out in the state territory. Nova Roma (Magalhães community-1), Colinas do Sul (Jose de Coleto-2), Monte Alegre de Goiás (Pelotas-3), Mineiros (Cedro-4), Cavalcante (São Domingos-5), Flores de Goiás (Canabrava-6), Iaciara (Extrema-7), Padre Bernardo (Sumidouro-8), Cidade Ocidental (Mesquita-9), São João da Aliança (Forte-10), Simolândia (Castelo/ Retiro e Três Rios-11), Mimoso de Goiás (Queixo Dantas-12), Niquelândia (Rafael Machado-13) and Campos Belos (Taquarussu-14). The settlements are located in the municipalities: São Miguel do Araguaia (Lageado-15) and Silvania (São Sebastião da Garganta-16).

Table 1. Location in coordinates of the municipalities considered in the research.

Municipalities (Community)	Latitude	Longitude
Simolândia (Castelo, Retiro e Três Rios)	14°28′18″ S	46°29′11″ W
Nova Roma (Magalhães)	13°44′25″ S	46°52′52″ W
Mineiros (Cedro)	17°34′43″ S	52°32′33″ W
Mimoso de Goiás (Queixo Dantas)	15°3′29″ S	48°9′33″ W
Cavalcante (São Domingos)	13°47′51″ S	47°27′20″ W
Flores de Goiás (Canabrava)	14°26′18″ S	47°2′55″ W
Cidade Ocidental (Mesquita)	16°6′19″ S	47°57′0″ W
São João d'Aliança (Forte)	14°42′31″ S	47°31′17″ W
Niquelândia (Rafael Machado)	14°27′28″ S	48°27′59″ W
Colinas do Sul (Jose de Coleto)	14°8′47″ S	48°4′19″ W
Campos Belos (Taquarussu)	13°1′31″ S	46°45′54″ W
Iaciara (Extrema)	14°5′45″ S	46°37′55″ W
Padre Bernardo (Sumidouro)	15°9′36″ S	48°17′2″ W
São Miguel do Araguaia (Lageado settlements)	13°16′30″ S	50°9′46″ W
Silvânia (São Sebastião da Garganta settlements)	16°39′32″ S	48°36′28″ W

Note: West (W), South (S).

Water sources were considered (shallow tubular well—up to 50 m, shallow well dug, spring, surface spring, cistern with rainwater and deep tubular well above 50 m).

2.2. Sample Filtration and Concentration

To obtain the concentration of viral particles, the method of adsorption and elution of membranes with negative charges was used, described by Katayama and collaborators [38]. The samples were identified with the name of the collection sites, pH 5.0 was standardized by adding 10% hydrochloric acid (HCL), considering the pH conditions favorable to possible particles viral.

After homogenizing the samples and filtering the negatively charged membranes, the membranes were washed with sulfuric acid (H_2SO_4, pH 3.0) and then the particles adsorbed on the membranes were eluted using sodium hydroxide (NaOH, pH = 10.5) neutralizing the filtrate and then buffering with Tris-EDTA (TE). Subsequently, they were stored at −80° until viral load quantification was processed.

2.3. Extraction of Genetic Material and cDNA Synthesis

The method of extraction in columns (Quick-DNA/RNA Viral kit), according to the process described by the manufacturer Zymo Research® (Irvine, CA, USA). After extraction, the resulting eluted total RNA sample was stored at −80 °C. All sample handling (pipetting) was performed inside the biological safety cabinet previously sterilized with 70% alcohol and ultraviolet light (UV), considering all personal protective equipment (PPE). The tips and eppendorfs used were characterized as free of DNAse/RNAse, all material used was left for 15 min in biological safety cabinet under UV light.

Complementary DNA (cDNA) synthesis was performed using the enzyme reverse transcriptase (RT). According to the protocol, 4 µL of RNA in a 0.2 mL eppendorf and the cDNA reaction was performed using the Platus Transcriber RNAse H-cDNA First Strand kit (Sinapse Inc. (Richmond East, Australia)) following the manufacturer's instructions. Then the PTC-100 thermal cycler was used. (MJ Research Inc. (St. Bruno, QC, Canada)). The cDNA obtained was quantified and stored at −20 °C.

2.4. Real-Time Polymerase Chain Reaction

The positive control, a nucleotide fragment of 311 base pairs for Rotavirus was synthesized in the laboratory by company Molecular Biotechnology LTDA® (Belo Horizonte, Brazil), chosen and aligned through the Bank of Data from NCBI (GenBank), accession number HM348746 (Human Rotavirus A strain mani-265/07 of the VP6 gene structural protein). Positive control sequence at follow: GAAAATCATCAAGATGTACTTTTCCATT CGAAAGAAC-TACTCTTAAAATCTGTGTTGATAGCTATTGGTCAGTCAAAAGAAATC GAAAC-TACTGCTACTGCCGAAGGAGGAGAAA-TAGTATTTCAGAATGCAGCTTTTA CTATGTGGAAATTGACGTATTTA-GATCATAAATTAATGCCTATTTTGGATCAGAATTT CATTGAATATAAAATTACATT-GAATGAAGATAAACCAATTTCAGATGTATGTGTTAA AGAACTTGTTGCTGAATTAA-GATGGCAGTATAACAGATTTGCTATAATAACACATGG.

The positive control concentration was 5 ng/µL, measured using the nanodrop. Subsequently, serial exponential dilutions were performed. The serial dilution of the positive control presented for the titers: 10^{-1} with 5.6×10^9 genomic copies, for 10^{-2} with 5.6×10^8, for 10^{-3} with 5.6×10^7, for 10^{-4} with 5.6×10^6 and for 10^{-5} with 5.6×10^5 (Table 2).

Table 2. Serial dilution of the positive control for quantification of the samples.

Titration	Potential Genomic Copies (GC)	Genomic Copies (GC) in Number
10^{-1}	5.6×10^9	5,600,000,000
10^{-2}	5.6×10^8	560,000,000
10^{-3}	5.6×10^7	56,000,000
10^{-4}	5.6×10^6	5,600,000
10^{-5}	5.6×10^5	560,000

Note: Genomic copies (CG).

After cDNA synthesis, the qPCR reaction was performed. The reading was by quantification of genetic material according to logarithmic titration positive control. The positive control was synthesized in the laboratory by company *Molecular Biotechnology LTDA®*. For qPCR, the reaction volume was considered of 20 µL per sample, being 0.4 µL of Oligonucleotide sense, 0.4 µL of Antisense oligonucleotide at concentration of 5 5 µg/mL, 4 µL of 5 × HOT FIRE Pol Eva Green qPCR mix Methods 23 plus (ROX), 10.2 µL of water and 5 µL of sample (cDNA). The considered mix presents the following components Oligonucleotides, Taq DNA polymerase and dNTPs).

The following oligonucleotides were used: GARV VP6 ROTAFEEVALE FW5′-GATGT CCTGTACTCCTTGT-3′ Sense; position: 7–25. ROTAFEEVALE-REV5′-GGTAGATTACCAA TTCCTCC-3′ antisense; position: 148–167. The amplicon size was 160 base pairs (bp) [39]. For qPCR the recommended cycling was considered by the manufacturer Solis Bio Dyne, with 95 °C for 12 min for initial activation, 95 °C for 15 s for denaturation, 63 °C for 25 s for annealing and 72 °C for 25 s for amplification.

After preparation, the plate was taken to the thermal cycler, considering the StepOne Biosystems® software version 2.3. All materials used were sterile, free of nucleases and other enzymes. For consideration of detections, a cut-off threshold cycle (CT) equal to 0.8 was considered, taking into account consideration the increase in specificity and decrease in sensitivity of the Sybr Green-type qPCR technique. The amplifications were considered positive until cycle 37 or lower [40,41].

2.5. Sample Size and Statistical Analysis

The collections were carried out in the residences of the quilombola communities and surface sources, each type of source had unique characteristics: deep and shallow tube wells are water sources used by the populations or by a single residence, therefore, they are samples that represent the local populations.

Shallow wells are individual wells located in homes, usually near latrines and places where pigs, birds and cats are kept. These characteristics of shallow wells are frequent, therefore, the sampling was carried out in order to represent the type of source, that is, all residences in the communities participated in the research.

The waters are used for leisure and consumption regardless of the sources, however, water from shallow wells, springs and cisterns in some cases, are treated by filtration, chlorination or heating at 100 °C, for later consumption, but consumption is also consumed untreated by the population. The research by Castell and collaborators [42] identified the presence of Rotavirus approximately twice as high in samples used for recreation compared to groundwater, data considered for the sample calculation. With this, the power was 85%, considering the alpha of 0.05.

For statistical analysis, the multinomial logistic regression test was used, considering the presence of genetic material as the dependent variable, determined by qPCR. The independent variables were types of sources and municipalities. Thus, it was possible to determine an association between the dependent and independent variables, by estimating the Odds Ratio (OR).

To determine normality, the Shapiro Wilk test was considered, when the *p*-value was $\leq$0.05, the data were considered not parametric, that is, abnormal distribution. To determine the difference among the average ranks of the number of genomic copies by type of sources, the non-parametric test of Kruskal-Wallis. Statistical analyzes were performed using the software BioEstat® 5.3, STATA® 16.0, GPower® 3.1 and Minitab® 19, considering the limit of 5% significance.

3. Results

3.1. Percentage of Samples Collected by Counties and by Types of Sources

A total of 86 samples were analyzed. The percentages of samples collected by location were: Padre Bernardo with 18%, Silvania with 18%, São Miguel do Araguaia with 16%, Iacara with 9%, Mineiros with 9%, Flores de Goiás with 7%, Monte Alegre de Goiás with 6% and Cidade Ocidental 5%. The other locations had percentages $\leq$ 2% (Figure 2).

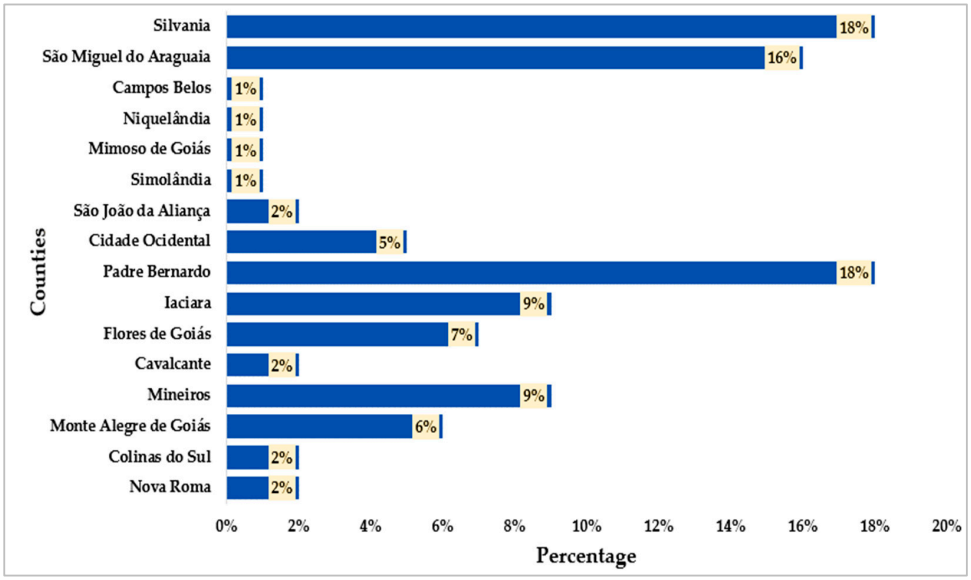

Figure 2. Percentage of samples collected by counties. Nova Roma (Magalhães community), Colinas do Sul (Jose de Coleto), Monte Alegre de Goiás (Pelotas), Mineiros (Cedro), Cavalcante (São Domingos), Flores de Goiás (Canabrava), Iaciara (Extrema), Padre Bernardo (Sumidouro), Cidade Ocidental (Mesquita), São João da Aliança (Forte), Simolândia (Castelo/Retiro and Três Rios), Mimoso de Goiás (Queixo Dantas), Niquelândia (Rafael Machado) and Campos Belos (Taquarussu). The settlements are located in the municipalities: São Miguel do Araguaia (Lageado) and Silvania (São Sebastião da Garganta).

The percentage distribution by type of source was for: shallow well excavated with 36%, spring with 23%, surface source with 21%, cistern with rainwater with 9.2%, shallow tubular well with 8.5% and tubular well deep with 2.3%. The springs had a slightly alkaline average pH (7.4), unlike the other sources with a slightly acidic pH (5.2 to 6.7) (Figure 3).

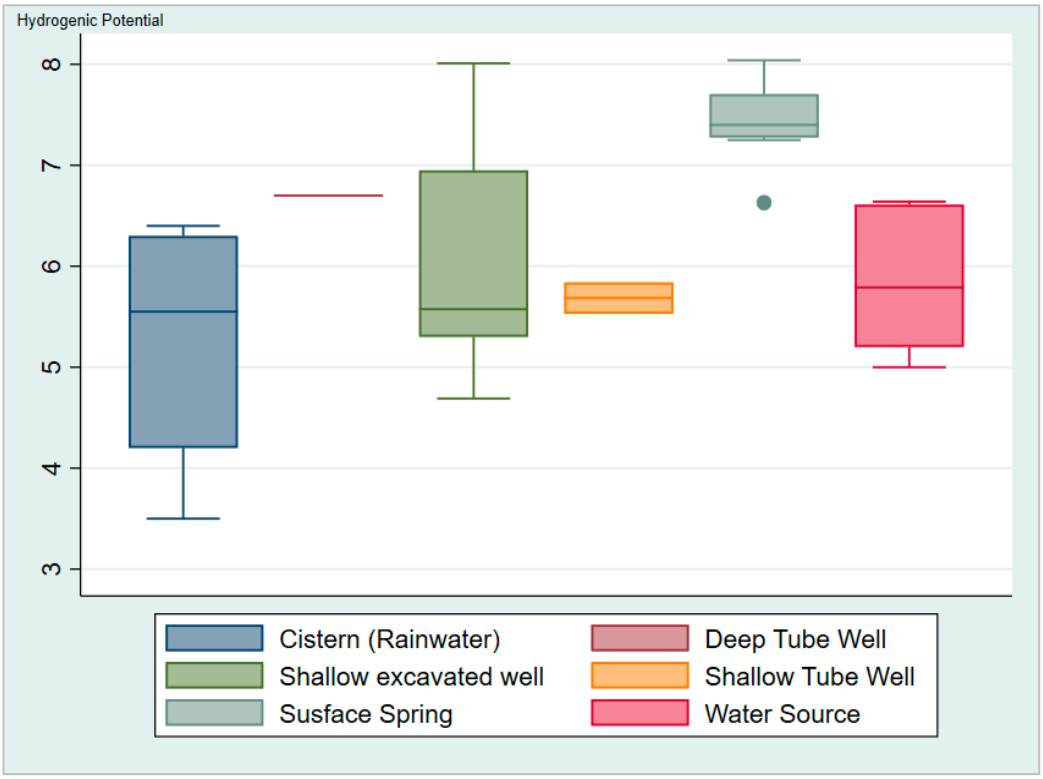

Figure 3. Boxplot with outliers—Mean hydrogen potential by source type. Grey circle (outlier).

3.2. Positive Samples by Counties and Types of Sources

The counties with positive samples were: São Miguel do Araguaia with 86% of 14 samples, Silvania with 53% of 15 samples, Padre Bernardo with 47% of 15 samples, Iaciara with 50% of 8 samples and Monte Alegre de Goiás with 100% of 5 samples (Table 3). The graphic representation of amplification with positive samples can be seen in Figure 4.

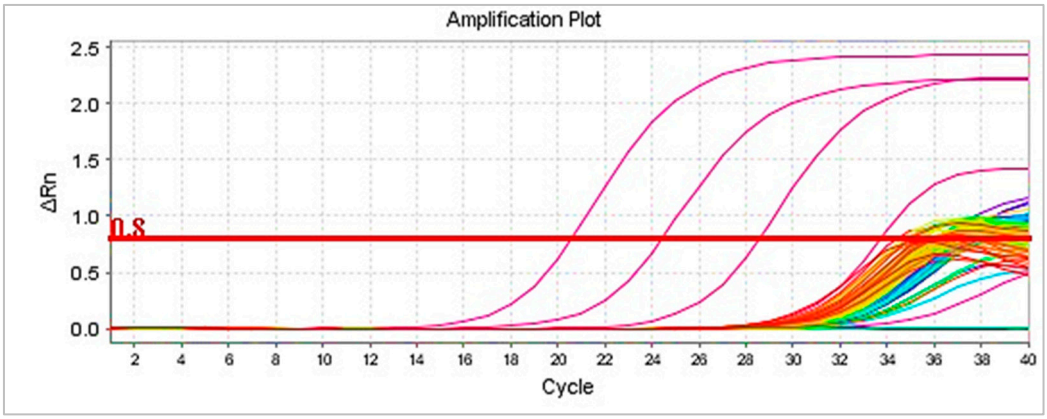

Figure 4. Amplification of titrated positive samples and controls, samples considered positive after reaching the detection limit 0.8, considered amplification until cycle 37 of the reaction. In pink, the four titers of the positive control; green line over delta rn = 0 negative control; the other amplifications are samples (other colored lines).

Table 3. Percentage and description of positive samples by municipalities.

Counties	Total	Events (%)	Description
Silvania	15	8 (53)	n = 4 shallow tubular wells, n = 1 spring, n = 2 shallow wells excavated and n = 1 surface spring.
São Miguel do Araguaia	14	12 (86)	n = 12 of 14 shallow wells excavated.
Campos Belos	2	1 (50)	n = 1 shallow well excavated.
Niquelândia	2	2 (100)	n = 1 spring and n = 1 deep tubular well.
Mimoso de Goiás	2	0 (0)	not detected
Simolândia	1	0 (0)	not detected
São João da Aliança	2	0 (0)	not detected
Cidade Ocidental	4	3 (75)	n = 3 of 4 shallow wells excavated.
Padre Bernardo	15	7 (47)	n = 7 shallow wells excavated from 15 sources analyzed
Iaciara	8	4 (50)	n = 4 of 8 cistern with rainwater.
Flores de Goiás	6	2 (33)	n = 2 of 6 springs.
Cavalcante	1	0 (0)	not detected
Mineiros	7	2 (29)	n = 2 of 5 springs.
Monte Alegre de Goiás	5	5 (100)	n = 5 from surface sources.
Colina do Sul	1	1 (100)	n = 1 spring.
Nova Roma	1	0 (0)	not detected
Total	86	47 (55)	All sources analyzed

Note: n = number or quantity. Nova Roma (Magalhães community), Colinas do Sul (Jose de Coleto), Monte Alegre de Goiás (Pelotas), Mineiros (Cedro), Cavalcante (São Domingos), Flores de Goiás (Canabrava), Iaciara (Extrema), Padre Bernardo (Sumidouro), Cidade Ocidental (Mesquita), São João da Aliança (Forte), Simolândia (Castelo/Retiro and Três Rios), Mimoso de Goiás (Queixo Dantas), Niquelândia (Rafael Machado) and Campos Belos (Taquarussu). The settlements are located in the municipalities: São Miguel do Araguaia (Lageado) and Silvania (São Sebastião da Garganta).

3.3. Association among Sources and Counties with Presence of Rotavirus

Multinomial logistic regression was crucial for evaluating the hypotheses of association among the types of sources and the presence of material genetic. The shallow excavated well source showed a greater association with the presence of Rotavirus, the effect estimate resulted in OR = 3.59 [1.05 to 12.28]. In contrast. Deep tubular well presented OR = 0.62 [0.04 to 8.20; *p*-value = 0.72] (Table 4).

Table 4. Association among type of source and presence of Rotavirus.

Font Type	Total	Events	OR [95% CI]	*p*-Value
Cistern with rainwater	8	4	1.25 [0.23–6.63]	0.79
Shallow tube well	7	4	1.66 [0.28–9.70]	0.57
Deep tube well	3	1	0.62 [0.04–8.20]	0.72
Shallow pit dug	31	23	3.59 [1.05–12.28]	**0.04**
Springs	19	7	0.72 [0.19–2.72]	0.63
Surface source	18	8	1	ns

Note: ns = no significance, 95% CI = confidence interval.

As observed, the results point to a strong association among the presence of genetic material in the type of shallow well source excavated, with a 3.5 times higher chance when evaluated with the other sources. Deep tubular well showed no association significant,

however, there is a contrast when compared to a tubular well shallow and shallow pit excavated, taking into account the absence of material genetic. There was no significant association among the presence of material genetics and counties.

3.4. Comparison of the Number of Genomic Copies per Liter Considering the Types of Sources

A comparison was made among the means of genomic copies per liter, considering as groups the types of sources. There was no difference significant difference among the means of genomic copies per liter, when font types compared.

The absence of correlation difference among the number of copies genomics and types of sources, is a consequence of the great variation (heterogeneity) in the number of genomic copies across all sources analyzed. Several factors contribute to the observed heterogeneity, such as: the locations of the sources, the presence of animals, actions anthropic characteristics and the physical-chemical characteristics of the samples.

4. Discussion

Contact with surface biological materials increases the possibility of surface water contamination, and shallow excavations are more likely to come into contact with excrement. The present research pointed to an association between the excavation of shallow wells and the presence of Rotavirus. The association between Rotavirus and shallow wells is a consequence of the presence of animals close to the sources, with this, they stand out as a potential means of increasing the incidence of gastroenteritis.

Rotavirus is the main viral agent that causes gastroenteritis in animals, being identified in several species of mammals and birds, both wild and domestic. The infection of cattle, pigs and horses has a direct negative impact on the economy, due to the high cost of treatment, aggravated by the weight loss of infected animals. The present research shows a higher prevalence of Rotavirus in shallow wells excavated, with the presence of pigs and birds close to the sources as a striking feature humans and non-human animals [43,44].

All sources analyzed in this research are used for consumption, including shallow wells, increasing the risk of infections by viruses and other microorganisms, however, infection can occur indirectly, through food without proper hygiene or through food sanitized with water from these sources. Considering the target population consisting of children aged less than or equal to five years, the risk of incidence increases, as they tend to have less adherence to their own hygiene.

Urban areas in developed countries have better access to treated water, however, rural areas in countries with low socioeconomic potential have few resources, consequently, communities in these regions seek alternative resources such as water from rivers, lakes, springs, shallow wells, deep wells and rainwater for consumption and recreation [20,21,45].

Although the other sources do not present the presence of Rotavirus statistically significant, demonstrates significance for the quality of isolated sources, mainly in deep tube wells, where the expected is not to present the presence of viruses. Research by Steyer and collaborators [46], presented a proportionally higher presence of Rotavirus in groundwater compared to surface water. Paul and collaborators [47], demonstrated the relatively rapid movement of viruses through the soil, allowing the contamination of groundwater, pumped and used in homes.

The present research showed the presence of genetic material in subterranean water (deep tubular well). Although the water is characterized as high quality and free of contaminants, factors such as processes involved in drilling development or exposure to surface materials during well drilling, can contribute to water contamination. In addition, viral particles can transport through the soil, mediated by binding to organic and non-organic materials. The diameter of viral particles is also a contributing factor in passing through soil pores.

Urban areas in developed countries have better access to clean water, however, rural areas in countries with low potential socioeconomic status, have few resources, consequently,

communities in these regions seek alternative resources such as water from rivers, lakes, springs, shallow wells, deep wells and rainwater for consumption and recreation [20,21,45].

Rainwater is an interesting option in regions where other font types require seasonal availability due to the long period of drought, therefore, it is necessary to build shallow wells to storage [48]. In some places, rainwater can be kept in dug wells (lined to prevent infiltration). In the communities of this research, rainwater is stored in reservoirs above ground level. Drinking water with safe distribution is a fundamental right, however, we often witness exposure to infectious agents through ingestion of contaminated water, causing changes in the well-being of the society.

Through water transmission, bacteria, viruses can be transmitted and other microorganisms. These microorganisms are inserted into the body water, through feces of infected animals and individuals and after ingestion. Microorganisms complete their cycle of infection or parasitism in the gastrointestinal tract and are excreted in the faeces. Water analysis is of paramount importance. Importance to identify these pathogens, allowing the appropriate actions for their disposal, before the water is distributed for consumption human [49].

In a survey carried out by Gonella and collaborators [50], in the stream Ribeirão Preto, whose source is located in the municipality of Cravinhos (São Paulo, Brazil) and which runs through the municipality of Ribeirão Preto, (Sao Paulo, Brazil), 11 water sample collection points were defined, from source to the mouth. Sample analysis showed that 73% were positive for adenovirus, while 36% were positive for Rotavirus (the source had both types of virus), 27% did not no contamination by the studied virus.

The presence of Rotavirus and Adenovirus in the source (located in an ecological park), can be attributed to the presence of animals such as birds and mammals that can transmit these agents. The greater presence of Adenovirus in the samples, according to the study, can be attributed to greater resistance to the disinfection process carried out by different water and sewage treatments, in addition to pH variation [51].

In the research by Ahmad and collaborators [52], samples were collected of surface and underground water on the outskirts of Peshawar, capital of Khyber Pakhtunkhwa (Pakistan). In all, 95 samples were collected for carrying out the analysis using the conventional PCR technique, to verify the presence of Rotavirus, Human Adenovirus, Hepatitis A virus and Enterovirus. Of the samples, 9.47% had Rotavirus, 12.63% were positive for Hepatitis A, 38.94% had an Adenovirus genome human, with the Enterovirus found in 48.42% of the water samples.

The presence of viruses occurs through fecal contamination in water surface and underground, a consequence of population growth substantial part of the region, which overloads the drainage system that transports the sewer water, getting full of dirt in the pipes that don't are consistently maintained. Consequently, the system is not sufficient to support all water, especially during periods of rain, responsible for enabling the contact among contaminated water and surface water, standing out as a recurrent problem in several countries, mainly the underdeveloped or under development, due to the low investment in adequate sewage or treatment facilities, contributing to spread of these viruses in surface waters.

5. Conclusions

The presence of Rotavirus is associated with shallow waters close to the surface such as a shallow excavated pit. The association with water close to the surface in the present research is a consequence of the presence of animals close to the sources. There was no association among the presence of Rotavirus and the counties. There was also no significant difference in the number of genomic copies per liter among the types of sources considered.

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