









## Occurrence of pathogenic microorganisms in small drinking-water systems in Costa Rica

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### ABSTRACT

This study describes the quality of drinking water sampled over 2 years (2018 and 2019) from 20 ASADAS (Spanish acronym for *Administrative Associations for Water and Sewer Systems*) in Costa Rica. The analysis included Rotavirus (RV), somatic coliphages, fecal coliforms, and *Escherichia coli*. The ASADAS were categorized into three regions as temperate rainy (region 1), tropical rainy (region 2), and tropical rainy and dry (region 3) according to biogeographic classification. The concentrations of fecal coliforms and *E. coli* were higher in samples from surface water sources from the ASADAS in region 3 compared to regions 1 and 2. RV-positive samples (24/296) were detected in drinking-water samples from regions 2 and 3 during dry and transition seasons, with higher concentrations more frequently in the dry season. In addition, somatic coliphages were detected in samples from the three regions, with higher concentrations in region 2. Furthermore, a statistically significant relationship was found between somatic coliphages and diarrheal cases, classified as outbreaks or alerts in the region. Thus, the results confirmed that somatic coliphages are a good indicator of the presence of diarrhea cases in a specific region.

**Key words:** Costa Rica, *Escherichia coli*, fecal coliforms, Rotavirus, somatic coliphages

### HIGHLIGHTS

- Rotaviruses (RVs) are frequent waterborne pathogens.
- Fecal coliforms, *Escherichia coli*, somatic coliphages, and RV were analyzed from drinking-water samples in Costa Rica.
- A significant relationship was found between somatic coliphages and diarrheal cases.
- Somatic coliphages are considered a good indicator of diarrhea-causing pathogens in a specific region.

### INTRODUCTION

Drinking water is necessary for life and an essential human right. But, unfortunately, the World Health Organization (WHO) estimates that 700 million people worldwide are not receiving their drinking water from improved water sources, with subsequent devastating effects on their quality of life (UN-Water 2014; WHO 2017).

A variety of pathogen microorganisms are transmitted by contaminated water. Bacteria, protozoa, helminths, and viruses are good examples of pathogens as etiological agents of waterborne diseases. The most significant microbial risks are water contamination with feces from humans or animals (including birds). Thus, feces can be a source of pathogenic microorganisms (WHO 2017).

Fecal coliforms (FCs) and *Escherichia coli* are common bacterial indicators of fecal water contamination. They have traditionally been used to monitor drinking-water quality, and they remain important parameters in monitoring undertaken as part of verification or surveillance. However, water intended for human consumption should contain no fecal indicator organisms (WHO 2017).

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Viruses are the smallest pathogens and may be less sensitive to water disinfection than bacteria. They usually persist for long periods in water, and infective doses are typically low. Rotaviruses (RVs), enteroviruses, and noroviruses have been identified as frequent waterborne pathogens among viruses (WHO 2017). They contaminate water through human activities such as leaking sewage and septic systems, agricultural and urban runoff, estuarine and marine waters, sewage outfall, and vessel wastewater discharge (Fong & Lipp 2005; WHO 2017). RVs are the most important cause of gastrointestinal infection in children, and this infection can have severe consequences, including hospitalization and death, particularly in low-income regions. Occasional outbreaks of waterborne disease have been recorded (WHO 2017).

Before introducing RV vaccines in 2006, approximately 15,000 deaths, 75,000 hospitalizations, and two million medical visits were associated with RV infection in Latin America and the Caribbean annually (Ureña-Castro *et al.* 2019). The effectiveness of RV vaccines against hospitalizations and severe diarrhea in Latin American children has been approximately 70%, with notable reductions in mortality rates in Brazil, Mexico, and Panama. In Costa Rica, since 2019, two live oral RV vaccines have been included in the National Immunization Program. One of these vaccines is derived from an attenuated human strain of RV, and the other combines five bovine-human reassortant strains (Ulloa-Gutierrez & Avila-Aguero 2014; Ureña-Castro *et al.* 2019).

On the other hand, coliphages are a type of bacteriophage, viruses that use only bacteria as hosts for replication. Coliphages use *E. coli* and closely related species as hosts and can be released into humans and other warm-blooded animals (WHO 2017). Moreover, they share many properties with human viruses, such as composition, morphology, structure, and replication mode (Jofre *et al.* 2016). As a result, coliphages are applicable models of enteric viruses in water environments and the sensitivity to treatment and disinfection processes. Therefore, the WHO recommends coliphages for inclusion in verification and surveillance monitoring where source waters are affected by human fecal waste (WHO 2017).

### Drinking-water coverage in Costa Rica

Costa Rica has been considered one of the countries in the Central American region with the best access to improved water sources, reducing the inequity of this resource among rural and urban populations (MINAET 2013). According to the National Institute of Statistics and Census (INEC), drinking-water coverage increased in almost 10 years, from 91.1% in 2010 to 94.1% in 2019 (INEC 2021). Regarding these data, coverage in the urban area increased from 96.7% in 2010 to 97.8% in 2019; however, for rural areas, it increased from 76.1 to 84% in the same period (INEC 2021).

Since 2000, a legal framework for the co-management of rural aqueducts was created at the national level to address the pattern of inequity among rural and urban populations and achieve complete nationwide coverage (Dobbin 2013). This national policy created a new type of water management organization with the legal status of community water organizations and increased governmental oversight, called *Administrative Associations for Water and Sewer Systems* (Asociaciones Administradoras de Sistemas de Acueductos y Alcantarillados Sanitarios or ASADAS) (Dobbin 2013). More than 1,400 ASADAS in the country supply 25.5% of the Costa Rican population. The quality of the water and the supported inputs that an ASADA can obtain, such as technology, human, and financial resources, directly affect the drinking-water quality in a specific population (Soto-Córdoba *et al.* 2016).

The degree of pollution of the source used for water supply is also an important parameter that could affect the drinking-water system's quality. Surface waters in Costa Rica suffer potential contamination, mostly from untreated sewage, runoffs, and agricultural chemicals. This situation limits the value of surface sources for water supply (Bower 2014). Morales *et al.* (2019) described that the contamination of catchment areas used as resources in one drinking-water treatment plant in Costa Rica influences the water quality in the final distribution network, even if the water was treated. For that reason, intensive and sectorized source monitoring is recommended to locate specific contamination points (Morales *et al.* 2019).

Another critical fact to consider is that access to drinking water does not necessarily imply that the resource is of potable quality. Potable means that it complies with the physical-chemical, biological, and microbiological characteristics according to sanitary regulations, and thus guarantees that its consumption does not imply health risks (WHO 2017). However, according to the National Water Laboratory of the Costa Rican Institute of Aqueducts and Sewerage (Instituto Costarricense de Acueductos y Alcantarillados, ICAA), in 2020, only 89% of the population supplied received potable drinking-water quality (LNA-AyA 2021), which means that 11% of Costa Rica's population consume water of non-potable quality, thereby increasing the risk of developing waterborne diseases.

Even though Costa Rica has good water sources and generally good water quality for consumption, there are many diarrhea cases annually (Chacón Jiménez *et al.* 2015). The National Water Laboratory of the ICAA analyzed 115 diarrheal outbreaks

related to waterborne pathogens from 1999 to 2005 (Valiente & Mora 2007). The study showed that in 70% of the outbreaks, bacterial pathogens or FCs were detected (Valiente & Mora 2007).

Thirty per cent of outbreak cases' water samples were tested negative for bacteriological indicators of fecal pollution, such as FCs (Valiente & Mora 2007).

In another study, microbiological indicators of water contamination were analyzed in two rural community aqueducts in the Central Valley of Costa Rica for 1 year (Chacón Jiménez *et al.* 2015). The authors reported an association between viral indicators in the water, such as somatic coliphages, and the increase in diarrhea cases in these communities. In those aqueducts, the water was treated using unsuitable technologies such as chlorination to eliminate viruses and parasites. The bacteriological indicators (FC and *E. coli*) were also analyzed, and they were absent in the samples (Chacón Jiménez *et al.* 2015).

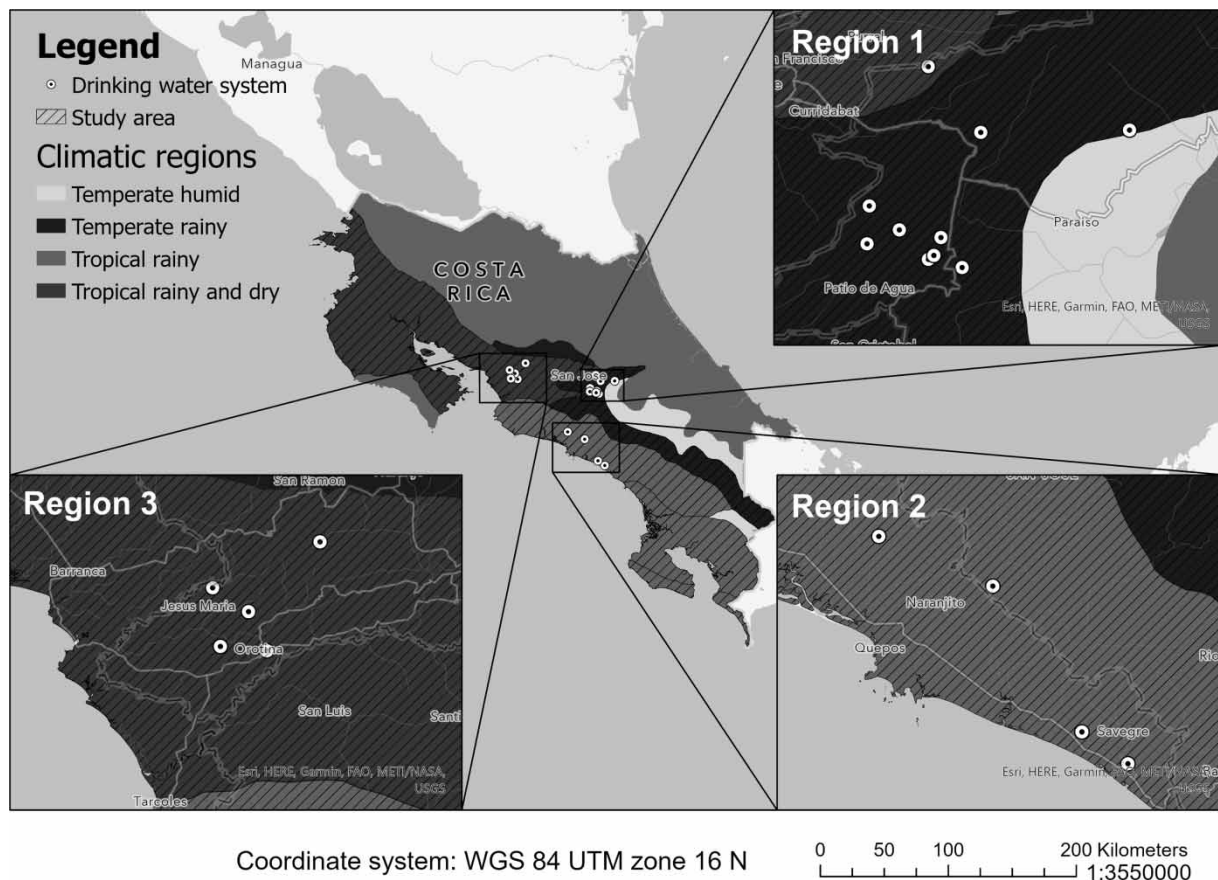
Although several studies described the detection of viral pathogens such as RV or Norovirus in surface and treated water (De Deus *et al.* 2019; Prez *et al.* 2020), information regarding the frequency of viral pathogens in water is scarce in the Latin American region.

The present study describes the quality of drinking water sampled over 2 years (2018 and 2019) from 20 ASADAS in Costa Rica. The analysis included RVs, somatic coliphages, FCs, and *E. coli*. In addition, the ASADAS were categorized into three regions according to climate and humidity parameters. As a result, there were observed differences regarding microbiological parameters in the ASADAS from region 3 compared to regions 1 and 2. Also, endemic channels were elaborated to compare and discuss the diarrhea cases reported in those locations.

## METHODS

### Drinking-water sampling

A total of 296 water samples (treated and non-treated) were collected from 20 ASADAS, located in Cartago, Puntarenas, and Alajuela provinces in Costa Rica (Figure 1).



**Figure 1** | Location of the 20 ASADAS in Costa Rica according to each climatic region. The scale denotes the distance in km.

Figure 1 shows the location of the three regions on the Costa Rica map. Region 1 was categorized as temperate rainy; region 2 was classified as tropical rainy, and finally, region 3 was tropical rainy and dry.

Sample collection was performed during the transition and rainy seasons in 2018 (35 samples in April and May, 65 in October and November, respectively) and dry, transition, and rainy seasons in 2019 (99 samples in March and April, 40 in May and June, and 57 in August and September, respectively).

Each ASADA was visited three times, one per season. During the sampling period five water samples were collected per visit, one from the raw water source and four from the distribution network.

Table 1 indicates the types of water source and treatment used for each ASADA.

### Climatic conditions

The 20 ASADAS were categorized according to the Holdridge life zone system (Holdridge 1971) into three regions as temperate rainy (region 1), tropical rainy (region 2), and tropical rainy and dry (region 3) according to biogeographic classification. The climatic regions were obtained from the Atlas of Costa Rica (Ortiz-Malavassi 2014), which considers the accumulated annual precipitation range, mean temperature, and vegetation type. Thus, region 1 included nine ASADAS; region 2 included four ASADAS; and region 3, seven ASADAS.

### FC and *E. coli* analysis

Microorganisms were enumerated using the most probable number technique (9221E; American Public Health Association 2017). Two hundred milliliters of water were collected using a sterile recipient. The sample was inoculated within less than 30 h of the collection into lauryl tryptose broth (Oxoid) and incubated at 35.0 °C for 48 h. All tubes testing positive after the incubation period were inoculated into EC-MUG broth (Oxoid). After a 24-h incubation period at 44.5 °C, tubes with a positive reaction were checked and reported. *E. coli* ATCC 25922 was used as a positive control and *Pseudomonas aeruginosa* ATCC 14502 as a negative control.

### Somatic coliphages

Somatic coliphages were enumerated using the single layer agar method 9924B and E Parts (American Public Health Association 2017) with modifications (Solano Barquero *et al.* 2012).

Briefly, 250 mL of water was collected using a sterile recipient. The sample was first filtered using an 80- $\mu$ m glass fiber filter (Sartorius Stedim Biotech, Goettingen, Germany) pretreated with beef extract pH 7.2 (Oxoid) to prevent losses associated with viruses sticking to the filter. Then, a second sample filtration using 0.2  $\mu$ m cellulose acetate filters (Sartorius Stedim

**Table 1** | Types of water source, treatment, and disinfection systems used by each ASADA

Raw water source type	Water treatment system	Water disinfection system	Number of ASADAS
Surface	Conventional DWTP <sup>a</sup>	Chlorine	1
	Slow filtration	Chlorine	1
	Settler	Chlorine	2
	Desander	Electrolysis	1
	Multi-stage filtration <sup>b</sup>	Chlorine	1
	None <sup>c</sup>	None	1
Surface/groundwater	Conventional DWTP <sup>a</sup>	Chlorine	1
	Coarse layered filtration	Chlorine	2
	Settler	Chlorine	1
	Desander	Chlorine	1
	None <sup>c</sup>	Chlorine	2
Spring	None <sup>c</sup>	Chlorine	5
	None <sup>c</sup>	Electrolysis	1
Total of ASADAS			20

<sup>a</sup>DWTP represents the *Conventional Drinking Water Treatment Plant*, which uses a water treatment system, including coagulation, flocculation, sedimentation, and filtration.

<sup>b</sup>*Multi-stage filtration* uses a water treatment system, including two pre-treatment units, such as gravel filters, with a final unit corresponding to slow sand filters.

<sup>c</sup>None means no treatment is used to treat water before disinfection.

Biotech, Goettingen, Germany) was pretreated with beef extract pH 7.2. Then it was mixed with 10 mL of a fresh culture of *E. coli* ATCC 13706 (O.D.=0.300 nm), CaCl<sub>2</sub>, to a final concentration of 0.2 M with 2% trypticase soy agar (TSA).

The mix was spread into Petri dishes and incubated at 35 °C overnight. After the incubation period, samples were analyzed to count for plaque-forming units (PFU).

### Rotavirus

Five liters of water were collected using a sterile recipient. Then, according to reference, samples were processed and analyzed to obtain viral quantification (number of copies/100 ml) (Chacón *et al.* 2020).

### Spatial description and data analysis

The locations of the ASADAS were obtained in the field using a GPS Garmin, model Etrex touch 25. The GPX data were transformed into a vector format. The vectorial database was classified and handled using the SQL language.

The climatic regions were obtained from the Atlas of Costa Rica as vector format (Ortiz-Malavassi 2014). The spatial description was performed using ArcGIS 10.7.1. (ESRI) with four data frames (one for each of the three regions and the fourth for the general location).

Descriptive statistics, boxplot graphics, odds ratio, and logistic regression were performed using the R software from CRAN. In addition, censored data analysis and  $\chi^2$  were performed to determine differences between regions and microbiological parameters. The NADA package of the R language for non-parametric censored data was used (Helsel 2012).

Biogeographic regions were established as the analysis group, delimiting regions 1, 2, and 3. The selection of method for estimating descriptive statistics was determined according to the characteristics of the dataset (sample size and proportion of censored data) (Helsel 2012). For FC and *E. coli*, the Kaplan–Meier and Turnbull method was used. For somatic coliphages and RVs, the maximum likelihood estimation method was used. Region 2 was excluded from the analysis for somatic coliphages since more than 80% of samples showed censored data. Differences between groups were obtained using the non-parametric Kendal–Tau correlation method for each microbiological variable.

According to references, the registered cases of acute diarrheal disease for regions 1, 2, and 3 were analyzed to elaborate endemic channels (Bortman 1999; Chacón *et al.* 2021). The geometric mean of historical trends and their confidence intervals were calculated using MS Excel (Office 365). The endemic ranges were obtained using the time-series records for at least 5 years before the sampling for this study (in this case, the diarrheal registered cases per week from 2012 to 2019). Mean values and quartiles were used for a defined time unit (epidemiological week).

Endemic channels were represented as a graphic elaborated with the R language (version 4.1.1) and ggplot2 package (version 3.3.1). The registered cases of diarrheal diseases were indicated by an epidemiological week of the study year as a bar, and they were compared with historical data on this disease for the last quinquennium.

The data on diarrheal cases was obtained from the Ministry of Health Area located in each region. In addition, inhabitants' populations per county during the period under study (2012–2019) were obtained from the official demographic estimates published by the National Institute of Statistics and Census of Costa Rica (INEC; <https://www.inec.cr/anuario-estadistico>).

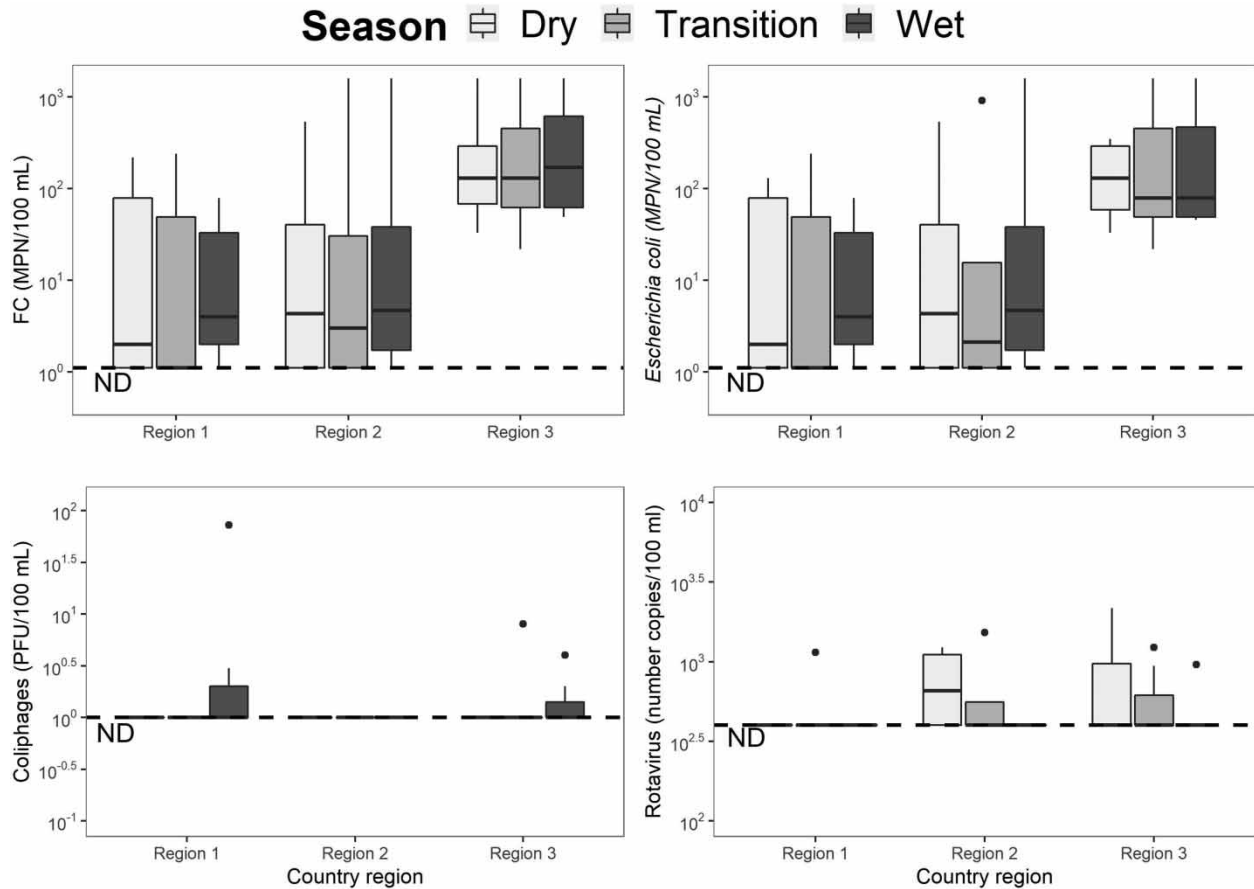
A relationship between somatic coliphages and diarrheal cases was established using multiple logistic regression. This test analyzed the relationship between the presence and absence of each microorganism and the variables such as climatic regions, seasonality, categorized rainfall, and the prevalence of the diarrheal disease. The glm (generalized linear models) function from the R language was used to perform the logistic regression analysis. The exponential coefficient of the logistic regression was calculated to obtain the odds ratios. A significance *p*-value of <0.05 was determined for all analyses.

## RESULTS

### Regions and microbiological analysis

Regarding the microbial indicators and quality of the water source, as shown in Figure 2, the concentrations of FC and *E. coli* were higher in sources from the ASADAS from region 3 compared to regions 1 and 2 ( $p < 0.05$ ). Also, according to Figure 3, they were higher in samples from the distribution networks from region 2.

Furthermore, the higher concentrations from water sources in region 3 were detected during all the sampling periods, in 2018 (transition or rainy seasons) and 2019 (dry, transition, and rainy seasons). In the case of samples from the distribution network, the higher concentrations of FC and *E. coli* were detectable from the ASADAS in region 2.



**Figure 2** | Concentration (mean value) of FCs or FC (MPN/100 ml), *E. coli* (MPN/100 ml), coliphages (PFU/100 ml), and RV (number of viral copies/100 ml) from surface water sources from the ASADAS from regions 1, 2, and 3.

The presence of somatic coliphages was positive in 18% of samples (53/296; 14 samples from sources and 39 from distribution networks) in the three regions, in a range from 1 to 1,464 PFU/100 ml.

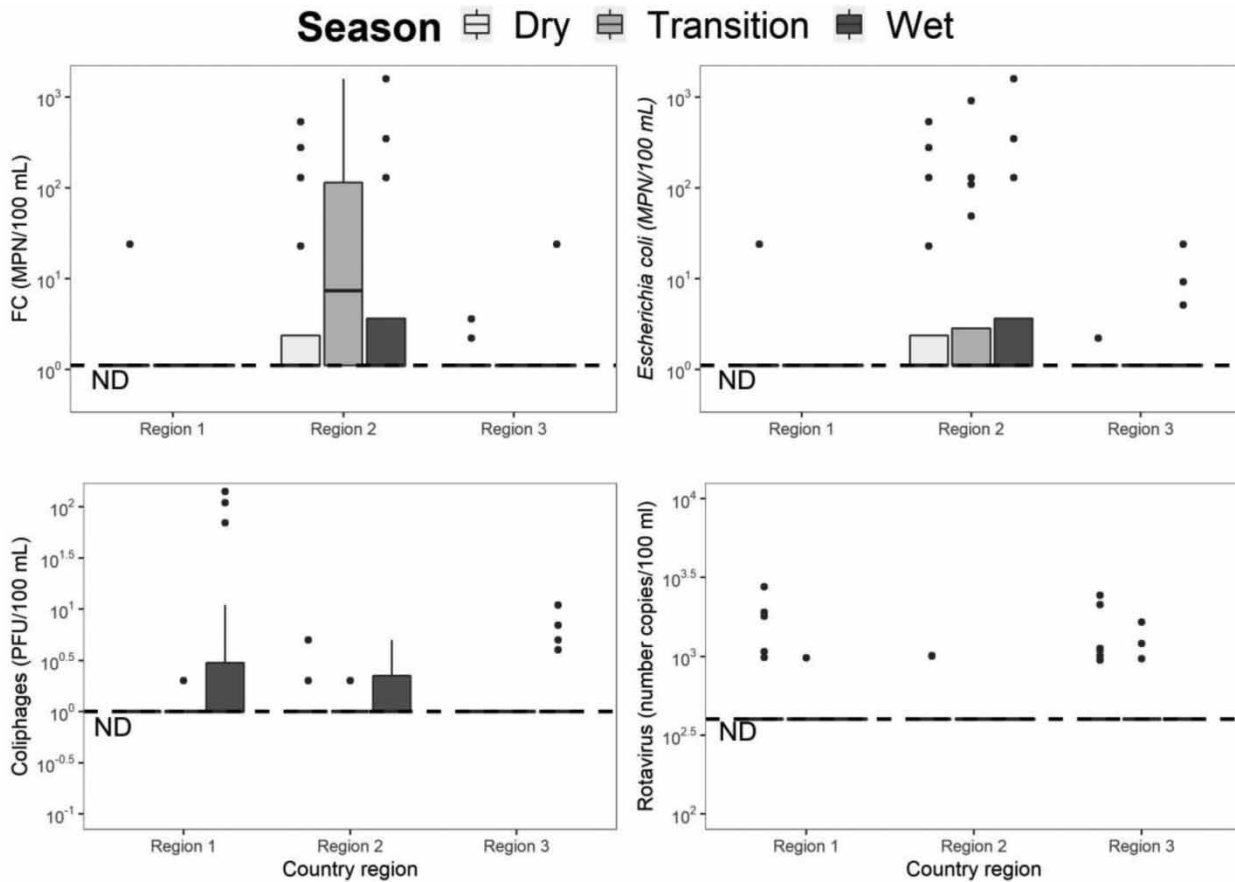
Of coliphages positive samples, 55% belong to region 1 (7/53 from water sources and 22/53 from the distribution network), 26% belong to region 2 (2/53 from water source and 12/53 from distribution networks), and 19% to region 3 (5/53 from water source and 5/53 from distribution networks).

Interestingly, RV was detectable from water sources and distribution networks, as observed in Figures 2 and 3. Its detection was possible in 9% of samples (28/296), with higher concentrations during the dry season (more than 1,000 copies/100 ml) compared to transition (100–1,000 copies/100 ml), mainly in regions 2 and 3. However, it was detected in just one source water sample from region 1.

### Endemic channels of diarrhea cases

Four zones were established for the graphical analysis of the endemic ranges: (1) *Success zone*: diarrhea cases below the 25th percentile of the weekly prevalence (the prevalence of diarrheal cases registered every week); (2) *Safety zone*: diarrhea cases between the 25th and 50th percentiles of the weekly prevalence; (3) *Alert zone*: diarrhea cases between the 50th and 75th percentiles of the weekly prevalence; and (4) *Outbreak zone*: area above the 75th percentile of the weekly prevalence of diarrheal disease (Bortman 1999).

Figure 4 shows the endemic channels of diarrhea diseases from regions 1, 2, and 3 during 2018 and 2019. According to Figure 4(a), in region 1, most of the weeks during 2018 were considered 'success' weeks. Fifty-one weeks in 2018 were successful or safe. One week was considered an 'alarm' week, which means the diarrheal cases were outside the expected normal seasonal range.



**Figure 3** | Concentration (mean value) of FCs or FC (MPN/100 ml), *E. coli* (MPN/100 ml), coliphages (PFU/100 ml), and RV (number of viral copies/100 ml) from water distribution networks from the ASADAS from regions 1, 2, and 3.

Nevertheless, during 2019 (Figure 4(b)), there were more weeks considered ‘alarm’ (16 weeks). In addition, 11 weeks of that year were reported as outbreaks of diarrhea. Thus, the cases of diarrhea registered during 2018 and 2019 were relatively high at the time of sampling and coincided with the RV detection in samples.

Regarding the diarrheal cases in region 2 (Figure 4(c) and 4(d)), it was observed that in 2018, only 2 weeks were considered alarm, no outbreak weeks were reported that year. In 2019, 10 weeks were alarm, and again, no outbreaks were reported.

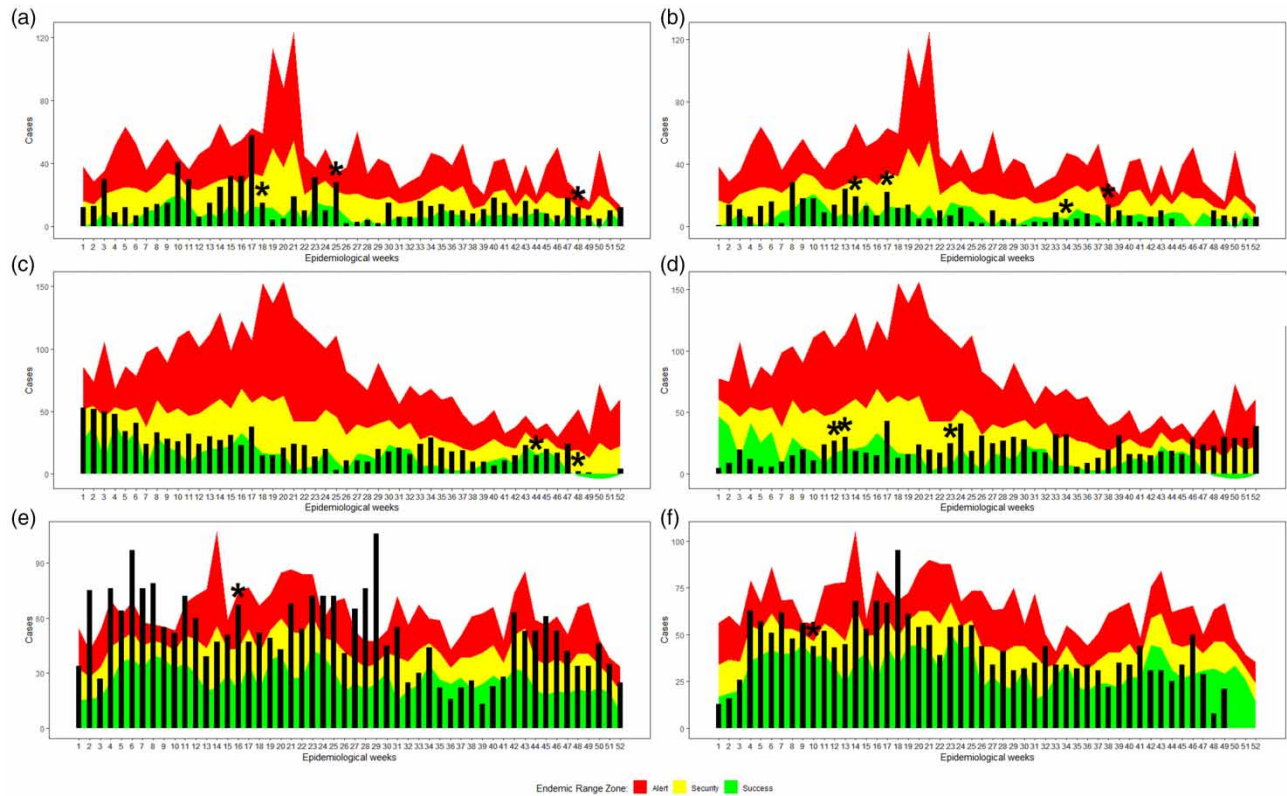
For region 3 (Figure 4(e) and 4(f)), in 2018, 31 weeks of the year (close to 8 months) were considered as ‘alarm’ or ‘outbreak’. In 2019, 14 weeks were also considered in these categories. The observation is congruent with the results obtained from the analysis of microbiological indicators in samples from this region.

A significant relationship between somatic coliphages and the diarrheal cases reported in each region was observed (Table 2). The detection of somatic coliphages in water samples was related to 2.40 (1.21–4.80,  $p < 0.05$ ) times to outbreaks and alert weeks of diarrhea cases in a particular region.

## DISCUSSION

### Regions and microbiological analysis

In general terms, the values of bacterial fecal indicators, such as FC and *E. coli*, sampled from the water source and distribution networks from the three regions were higher according to local and international recommendations for drinking water (WHO 2017). Interestingly, 19 out of the 20 ASADAS in treated water used chlorination (17/20 ASADAS) or electrolysis (2/20 ASADAS). Just one ASADA did not treat the water at all. Therefore, the disinfection process must secure a good quality for water used in human activities (WHO 2017).



**Figure 4** | Endemic channels of diarrhea cases for 2018 (a, c and e) and 2019 (b, d and f), from regions 1, 2, and 3. The number of acute diarrheal cases by epidemiological week is represented with black bars, and sampling weeks are marked with a star symbol. The x-axis denotes the epidemiological weeks, and the y-axis denotes the number of acute diarrheal cases reported.

**Table 2** | Odds ratio values of the epidemiological classification (success, safety, alert, or outbreak) of diarrhea cases and microbiological parameters

	Coefficient $\beta$	Odds ratio	2.50%	97.50%	p-value
Intercept	-0.78	0.46	0.31	0.67	<0.001
FCs	-1.08	0.34	0.05	1.67	0.223
<i>E. coli</i>	0.80	2.22	0.43	16.72	0.371
Somatic coliphages	<b>0.88</b>	<b>2.40</b>	<b>1.21</b>	<b>4.80</b>	<b>0.012</b>
RV	-1.80	0.17	0.01	0.88	0.088

Bold values denotes a p-value less than 5% ( $p$ -value<0.05).

In this study, RVs and coliphages were detectable from treated water (samples from the distribution networks). Virus inactivation in water differs from bacteria. Therefore, if free chlorine is used as a disinfectant for water, a controlled process with stable pH, temperature, and organic matter, among others, is required. Also, it is necessary to include an adequate pretreatment process before chlorination, such as conventional filtration, direct filtration, or sand slow filtration (Shin & Sobsey 2008; Chacón Jiménez *et al.* 2015).

According to these results, the presence of RV in water is a significant risk of waterborne disease. Conforming to the WHO, this pathogen and *E. coli* are responsible for most moderate-to-severe diarrhea cases in low-income countries. In addition, diarrheal disease is the second leading cause of death among children under 5 years old (Anderson & Weber 2004; WHO 2019). Linhares *et al.* (2012) reported a frequency of 53% of RV-positive stool samples in costarrican children with gastroenteritis under 3 years of age. Ureña-Castro *et al.* (2019) reported that RV cases predominated in the country in the first 5 months of the year; meanwhile, the increase of RV hospitalizations coincided with dry and cold weather conditions with a 2-month lag (Ureña-Castro *et al.* 2019).



Most clinicians who care for adults do not recognize the infection caused by typical pediatric pathogens such as RV (Anderson & Weber 2004). This pathogen is considered a directly transmitted disease due to its high infectivity. However, environmental pathways have usually been ignored (Anderson & Weber 2004; CDC 2011). Kraay *et al.* (2018) demonstrated that water could disseminate RV in communities and amplify transmission (Kraay *et al.* 2018). In the model described, RV persists in water sources. Its surface water concentrations and infection incidence vary with temperature, being a critical finding regarding the transmission of this disease (Kraay *et al.* 2018).

Interestingly, RV concentration was increased in regions 2 and 3 during the dry season. Ureña-Castro *et al.* (2019) already reported that RV cases in Costa Rica predominated in the first 5 months of the year, which coincide with the winter months in temperate regions from the northern hemisphere. Climate variations affect the watersheds and the quality and access of the water. Changes in the availability of water through precipitation, for instance, have a significant impact on the development of communities. Water fluctuations that cause scarcity can lead to the use of inappropriate water sources. Therefore, it will increase the risk of contamination for pathogenic microorganisms causing diarrhea and other waterborne diseases (Duran-Encalada *et al.* 2017; Morales *et al.* 2019).

RV infections increase during the winter months in temperate regions, decreasing significantly in summer (Anderson & Weber 2004). However, other authors have indicated that the winter seasonality of RV infections is too simple a generalization. For example, Levy *et al.* (2008) observed a correlation between months, temperature, rainfall, relative humidity, and the incidence of RV infections in tropical countries, concluding that RV responds to climate changes, with the highest number of cases found at the colder and drier times of the year.

Another critical fact to consider is that universal RV vaccination for infants started in Costa Rica in 2019 and long before in the private medical practice (Ulloa-Gutierrez & Avila-Aguero 2014; Ureña-Castro *et al.* 2019). Furthermore, several studies have indicated the shedding of pentavalent RV and RV3-BB (human neonatal) vaccine in fecal samples from vaccinated infants (Yen *et al.* 2011; Cowley *et al.* 2017). Also, Moresco *et al.* (2016) reported the thermal and length of stability of one RV vaccine (RotaTeq) in the aquatic environment (Moresco *et al.* 2016). Therefore, the detection of RV from water in this study cannot be attributable to a wild type or a vaccination RV strain.

On the other hand, somatic coliphages comprise groups of bacteriophages that have been suggested as valuable indicators of fecal pollution in water and the behavior and survival of human viruses (Jofre 2009). These viruses replicate in *E. coli* after infecting it through the cell wall. They may also infect bacterial species closely related to *E. coli*, such as *Klebsiella* spp. and *Shigella* sp. (Jofre *et al.* 2016). Several studies in Costa Rica have visualized the utility of this indicator related to pathogenic virus circulation in water and a higher frequency of diarrheal cases in a particular community (Chacón Jiménez *et al.* 2015; Chacón *et al.* 2020).

### Endemic channels of diarrhea cases

The endemic channel is a graphic representation of the number of diarrhea cases (or any other infectious disease) registered in an area during established periods, in this case, epidemiological weeks in 2018 and 2019. This graphic permits visualization if diarrhea cases in a particular zone increase and start to behave like an outbreak (outbreak is defined as excess cases of one disease in a community, beyond response capabilities). The endemic channel is elaborated using rates of the registered cases of diarrhea for at least the last 5 years. It is used as a criterion to define an infectious disease outbreak from the epidemiological point of view (Bortman 1999).

This study observed that for regions 1 and 2, no outbreak weeks were observed during 2018 and 2019; meanwhile, for region 3, 12 outbreak weeks were detected in 2018 and 1 week in 2019. Interestingly, a significant relationship was found between microbiological parameters and climatic regions, including seasonality, categorized rainfall, and diarrheal disease prevalence. No significant differences were observed between regions 1 and 2 for FC and *E. coli* ( $p > 0.05$ ). However, region 3 showed differences for all fecal indicators ( $p < 0.05$ ). For somatic coliphages, differences were observed between regions 2 and 3 ( $p < 0.05$ ). For RV, no differences were found in any case. Considering the statistical analysis of microbiological parameters and endemic channels of diarrhea cases, region 3 differed compared to other regions.

### Somatic coliphages are more related to diarrheal cases than the other microbiological indicators

Interestingly, somatic coliphages were more related to the diarrheal cases reported in each region (odds ratio=2.4;  $p < 0.05$ ). Meanwhile, no significant results were obtained for bacterial fecal parameters (such as FC and *E. coli*) (Table 1). In addition, Chacón *et al.* (2020) described a statistically significant relationship between somatic coliphage threshold and the detection of

human enteric viruses such as Norovirus, RV, Enterovirus, and Hepatitis A from wastewater (Chacón *et al.* 2020). Also, the same authors have reported the presence of somatic coliphages in treated drinking water and their relationship with diarrheal cases in one community from Costa Rica in the absence of bacteriological indicators in the same samples (Chacón Jiménez *et al.* 2015).

Because coliphages are viruses that closely mimic human viral pathogens in terms of environmental dissemination and survival, they are being investigated as a potentially more reliable indicator of fecal pollution and pathogens' presence in the water. Therefore, the results obtained in the present study confirm that somatic coliphage detection in drinking water is a good indicator of the circulation of diarrhea-causing pathogens and the need to increase the screening of the microbiological quality of the water with other types of indicators of contamination that could correlate better with diarrhea cases.

## CONCLUSIONS

Contaminated water is a source of microbiological pathogens, including bacteria, viruses, protozoa, and helminths, causing waterborne diseases. For example, in Costa Rica, diarrhea outbreaks related to drinking water are reported despite good drinking-water coverage indicators.

This study reported RV and microbiological indicators of contamination in drinking water from 20 ASADAS, categorized into three regions (temperate rainy or region 1, tropical rainy or region 2, and tropical rainy and dry or region 3). The microbiological indicators such as FCs, *E. coli*, and somatic coliphages were higher in the ASADAS from region 3 than regions 1 and 2.

In a few samples in regions 2 and 3, RV was detected with higher concentrations and more frequently in the dry season.

No significant results were observed for bacterial indicators and diarrheal cases. However, somatic coliphages, detected in samples from the three regions, showed a statistically significant relationship with diarrheal cases. Furthermore, when coliphages were detected, it was 2.40 more likely that diarrheal cases were classified as outbreaks or alerts in the region. Thus, this study suggests that somatic coliphages could be a good indicator of diarrhea-causing pathogens in a particular region.

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## DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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