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Bacterial and Parasitic Characterization of the Rivers in Cuenca, Ecuador

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Abstract: *Cryptosporidium* and *Giardia* are infectious parasitic forms widely distributed in aquatic ecosystems and resistant to disinfection of drinking water. Their presence was investigated in the lower areas of the city's four rivers through a four-stage methodology. Between December 2017 and April 2018, three monitoring campaigns were conducted, with results ranging between not detected to 500 oocysts/L for *Cryptosporidium*, and between not detected and 300 for *Giardia*. *Cryptosporidium* was more abundant, especially in the Machángara River. In the same period, the bacteriological quality of the rivers was also reviewed using Total Streptococci and Fecal Enterococci expressed in colony-forming units (CFU)/100 mL as indicators. The results showed a progressive increase in pollution as the course of the rivers progressed. The sensitivity of bacterial indicators to changes in quality is also observed, which is why their use in specific studies is recommended. It is concluded that untreated domestic wastewater discharges may be the main source of contamination by bacteria and parasites and that there is a relationship between their concentration and the seasonal period. In dry weather, the concentration is higher for both microorganisms. This study fills a gap in knowledge in the region, due to the absence of data on parasitic indicators with great impacts on public health.

Keywords: surface water body; *Giardia*; *Cryptosporidium*; cysts; Enterococci; Streptococci

Citation: Pauta, G.; Vázquez, G.; Carrillo, V.; Torres, C. Bacterial and Parasitic Characterization of the Rivers in Cuenca, Ecuador. *Water* **2024**, *16*, 2016. https://doi.org/10.3390/w16142016

Academic Editor: Guilin Han

Received: 2 June 2024
Revised: 3 July 2024
Accepted: 10 July 2024
Published: 16 July 2024

1. Introduction

A river as a constituent part of the environment, among many other functions, serves as a source of natural resources such as water and sediments, among others. These resources have been used to support human development. However, rivers have also been used as a receptor and means of transport for undesirable waste. Therefore, rivers are also analyzed as the vehicle of microorganisms related to waterborne diseases that can be of bacterial, viral, parasitic, and, to a lesser extent, fungal origin [1–3]. The main source of biological contamination in water bodies is fecal matter coming mainly from domestic sewage discharges, which carry bacteria and gastrointestinal parasites from endothermic organisms [4–6]. In this context, most studies on the quality of water bodies have been based on quantifying the coliform group as bacterial indicators of fecal contamination [7–9]. However, for the same purpose, fecal Streptococci and their specific group Enterococci can be used since they also have shown satisfactory performance. They are sensitive to changes in water quality. They appear in low numbers in areas with little contamination and increase their presence progressively as pollution increases. Additionally, their concentration is lower compared to the usual total and fecal coliform indicators. Most of their species do not proliferate in aquatic environments [10–15]. Therefore, they can be seen as more reliable pollution indicators since their presence would reveal the original source of contamination [16,17]. Additionally, they are important in situations where fecal contamination is known but coliforms are not detected, as occurs when discharges are intermittent or older, so that fecal coliforms and E. Coli die, but Streptococci remain. Finally,

due to the frequency of occurrence and wide variety of origins of the contamination the receiving bodies experience, the evaluation of their quality must cover the entire pathogenic spectrum that these bodies of water entail. More details of the advantages and limitations of the use of fecal Streptococci and their specific group Enterococci as pollution indicators are discussed in [14].

However, another equally important (within the pathogenic spectrum) and underresearched group includes *Cryptosporidium* and *Giardia* cysts. These parasites have significant importance in evaluating river water quality, especially when designated for human consumption, because unlike bacteria, they exhibit markedly superior resistance to disinfection. Thus, drinking water can convey them, becoming a risk factor in the transmission of waterborne parasitic diseases. Therefore, some national regulations such as the Ecuadorian regulation INEN 1108 require the absence of these parasites as a quality requirement for drinking water [18–23].

Giardia spp. and *Cryptosporidium* spp. are protozoa that, in an appropriate relationship, contribute to ecological balance within aquatic environments, but as parasites, they can cause diseases. They are found in stagnant waters or in lagoons located in warm climates. Their biological origin is difficult to determine, although the existence of some of them has been demonstrated in livestock and small mammals such as rats and mice, among others. *Giardia* and other protozoa cysts have a thick wall that protects them from adverse environmental conditions and makes them resistant for several weeks or months. They withstand temperatures below 60 °C in water for two months and can remain viable after the action of numerous disinfectants. They cause diarrheal diseases in the species they parasitize, sometimes acting as causal organisms for severe conditions and even death in children, the elderly, and immunocompromised patients. For example, giardiasis is a self-limiting disease typically characterized by diarrhea, abdominal cramps, bloating, weight loss, and malabsorption. It is the third most common cause of diarrheal diseases according to the WHO. *Cryptosporidium* spp. oocysts remain viable in water for around 140 days and are highly resistant to most common disinfectants, making their destruction by normal chlorination of waters difficult and sometimes even impossible. Global outbreaks of cryptosporidiosis have originated from the contamination of surface water, groundwater, and recreational waters with oocysts of the parasite. Ref. [24] detailed the importance of *Cryptosporidium* spp. oocysts as an emerging zoonotic parasitosis with significant economic and public health repercussions, as well as the risk factors that promote it. The symptoms of the cryptosporidiosis are similar to giardiasis, but cryptosporidiosis can evolve into a chronic and in some cases fatal disease, causing pneumonia and pancreatitis, especially in immunosuppressed patients [25–33].

The cysts of both parasites are widely distributed in aquatic bodies, such as wells, lakes, rivers, reservoirs, etc. Therefore, their survival time, which depends on factors such as pH, temperature, water chemical composition, biofilm formation, and the presence of predator organisms, is an aspect that must be considered when estimating their potential as contamination indicators. Bibliographic research reports that *Giardia* tends to be more frequent in water because it withstands more difficult environmental conditions than *Cryptosporidium*. The diseases caused by both parasites are transmitted via the fecal–oral route by ingesting cysts (8–12 μm) of *Giardia* spp. and oocysts (3–8 μm) of *Cryptosporidium* spp. They have often been studied together due to certain common biological characteristics that determine the general epidemiological aspects of both infections [26,27,34–36]. Moreover, environmental contamination with feces from parasitized animals plays an important role in the transmission of both diseases. Some studies reported interactions between human consumption of water and feces from peri-domestic animals that may be infected. It is also reported that in the epidemiology of cryptosporidiosis, more than 80 species of mammals have been compromised. In this regard, new approaches are suggested to address these diseases [29,37–41].

Moreover, it has been reported that conventional wastewater treatments such as primary sedimentation, activated sludge, and biological filters are inefficient in removing

helminths and protozoa. Therefore, effluents from wastewater treatment plants (WWTPs) constitute a significant source of surface water contamination by both parasites, more significant than that produced by agricultural activities and runoff waters. Thus, it becomes necessary to monitor their presence in WWTP effluents to protect the quality of water from rivers that are commonly destined often for animal and human drinking and to define the other potential uses of treated wastewater [26,42–47].

Epidemic outbreaks of giardiasis and cryptosporidiosis linked to drinking water consumption confirm the presence of (oo)cysts once they have passed through purification processes, and although the parasitic forms of trophozoites and cysts or oocysts of both protozoa are mostly retained in the filtration stage of conventional treatment systems, some are resistant to chlorination and the decontamination process [26,31]. Additionally, the number of parasitic forms along with other factors in the distribution network allows the quantitative assessment of microbial risk (ECRM), a very useful tool in epidemiology. This tool allows us to predict the effect of microorganisms on health, and preventive measures can be taken to reduce disease outbreaks. An important aspect of ECRM is the dose–response relationship. That is the relationship between the concentration of the pathogenic agent and the number of infected people among the total number of people exposed in a given population. This relationship can be adjusted by probability mathematical models to determine the safety margin for some uses of treated wastewater. From the mathematical model results and the analysis of the outbreak conditions, it could be deduced that the microbiological quality control in drinking water treatment plants fails when using traditional indicators such as *Escherichia coli* and *Enterococcus* spp., as they are much less resistant to chlorine than, for example, the *Cryptosporidium* spp. oocysts, which can resist concentrations of up to 80 mg/L. This unidentified contamination makes it necessary to consider these new emerging pathogens as representative biological indicators to ensure efficient quality control [13,31,48–52].

Outbreaks reported by water contamination in treatment plants in large cities in the United States and the United Kingdom (developed countries) confirm the hypothesis that the real incidence of these parasites may be much higher than what official epidemiological bulletins declare [53]. Specific data in Ecuador (developing country) report the presence of *Cryptosporidium* spp. and *Giardia* lamblia with concentrations of 5 oocysts/100 mL and 10 cysts/100 mL, respectively, in water destined for human consumption [54]. Thus, *Giardia* and *Cryptosporidium* are not specific to any region in the world, nor are they exclusively located in developing or developed countries. They represent a general threat that requires integrated actions by the health services of all countries and demand a significant budget for the control of epidemic outbreaks, medical care, and public health [38,55–59].

The ability of these organisms to multiply in water supply systems compelled the European Union to introduce a new guideline regarding water intended for human consumption: "free of any microorganism, parasite, or substance, in an amount or concentration that could pose a potential danger to human health". The World Health Organization has adopted a similar standpoint and reports waterborne diseases as those in which the causative organisms complete some part of their life cycle within water; parasites top the list [31,33,55].

The objective of the present study is to perform an evaluation of the sensitivity of fecal Streptococci and their specific group, Enterococci, as alternative indicators of bacterial contamination in the receiving bodies of water, considering a specific case study on the rivers of Cuenca city in Ecuador. The presence of (oo)cysts of *Cryptosporidium* and *Giardia* parasites, considered emerging organisms because they pose a risk to public health, is also investigated. One of the purposes of the present study is to contribute to filling a knowledge gap in the region and to provide important information for the quantitative assessment of microbial risk that allows the establishment of relevant tools for parasitic disease epidemiology.

2. Materials and Methods

2.1. Study Area

The present study is conducted in the four sub-basins of the Paute River, located in southern Ecuador, in the city of Cuenca. The four rivers Yanuncay River, Tomebamba River, Machangara River, and Tarqui River traverse the city from east to west. They hold significant scenic value for the urban area, and some serve as water sources for existing water treatment plants in the city. Information provided by the Public Water and Sewerage Company (ETAPA. EP) indicates that an average of 1710 L/s was processed for the year 2022. The sub-basins of the Yanuncay River (419 km^2), Tomebamba (380 km^2), and Machángara (325 km^2) originate in the Cajas National Park, while the Tarqui River (476 km^2) originates in the paramos of Cumbe and the upper part of Victoria del Portete. This city has an extensive network of marginal interceptors in the four rivers; however, sewerage service coverage is not 100%, with several discharges remaining un-intercepted at the time of monitoring. Current data indicate that sewage coverage for the urban area is 99% and sanitation coverage is only 90% [8,60]. In Figure 1, the study area is presented.

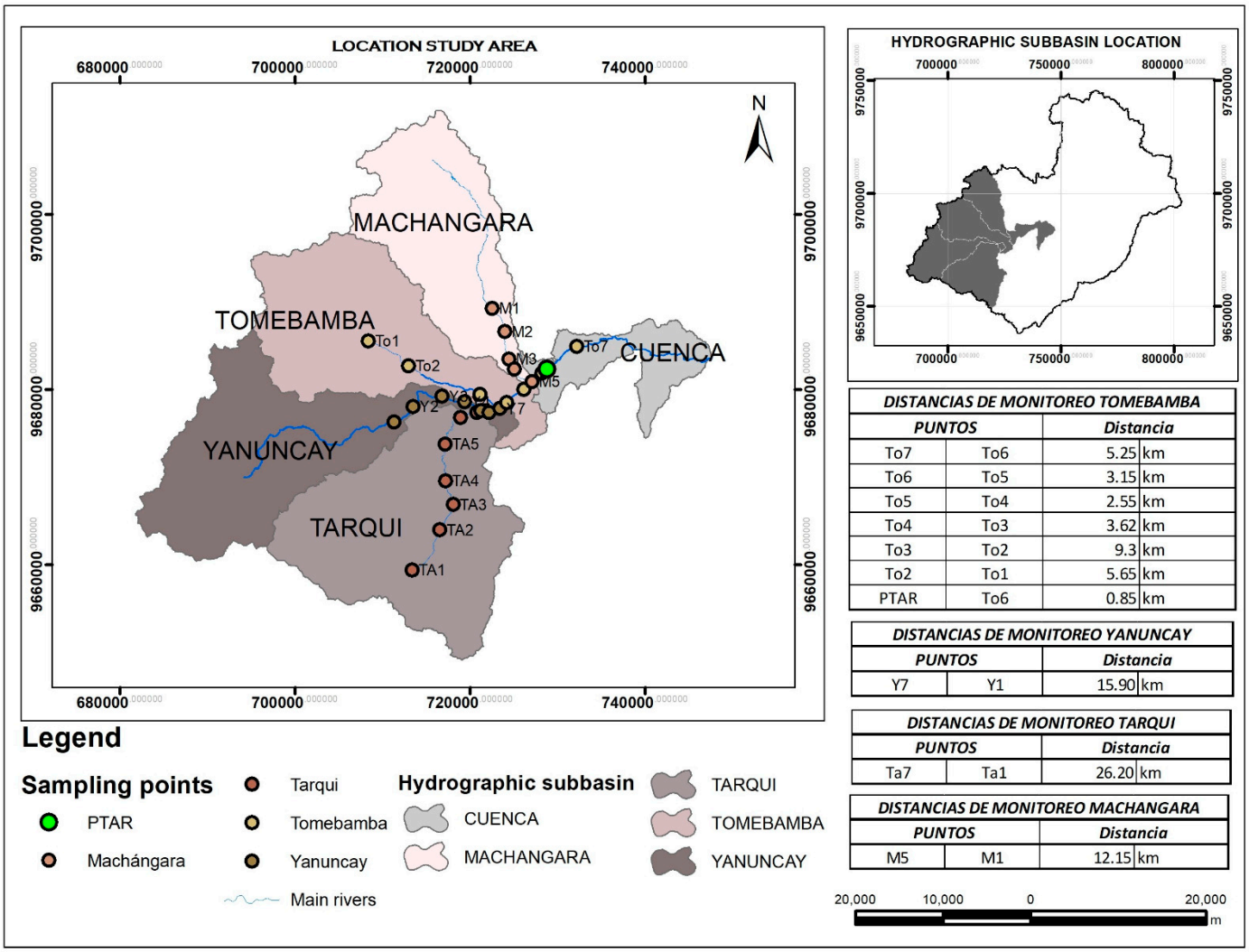

Figure 1. Study area.

2.2. Selection of the Monitoring Stations

For the development of this study, 7 sampling stations were established for the Tarqui (TA), Yanuncay (YA), and Tomebamba (TO) Rivers, and 5 for the Machángara (MA) River. These monitoring stations were already defined in previous studies of water quality and are

representative of the bodies of water to be evaluated [8,14,61]. Three monitoring campaigns were conducted for each river. The time frame for the monitoring was from December 2017 to April 2018 including rainy and dry periods. In Table 1, a description of the data related to the monitoring is presented.

Table 1. Reference of the monitoring sites in the four study rivers.

Sub-Basin	Code	Reference	Monitoring Station (Figures 2 and 3)	Sub-Basin	Code	Reference	Monitoring Station (Figures 2 and 3)
Río Tarqui	TA1	"Portete"	1	Río Tomebamba	TO1	"Llaviuco"	1
	TA2	After the Cumbe River confluence	2		TO2	Sayausí	2
	TA3	"Tarqui"	3		TO3	"Puente del Vado"	3
	TA4	"Zona Franca"	4		TO4	"Empresa Eléctrica"	4
	TA5	After the Zhucay River confluence	5		TO5	Before the Milchichig Stream confluence	5
	TA6	"Parque Inclusivo"	6		TO6	Before the discharge in the Cuenca WWTP **	6
	TA7	Before the Yanuncay River confluence	7		TO7	"Challuabamba"	7
Río Yanuncay	Y1	"Dispensario Barabón"	1	Río Machángara	M1	"Chiquintad"	1
	Y2	"Inmaculada de Barabón"	2		M2	"Ochoa León"	2
	Y3	"San Joaquín"	3		M3	"Feria de Ganado"	3
	Y4	"Avenida Loja"	4		M4	"Parque Industrial"	4
	Y5	"Tres Puentes"	5		M5	Before the Tomebamba River confluence	5
	Y6	"Redondel de la UDA *"	6				
	Y7	"Parque el Paraíso"	7				

Notes: * UDA: University of Azuay. ** WWTP = wastewater treatment plant.

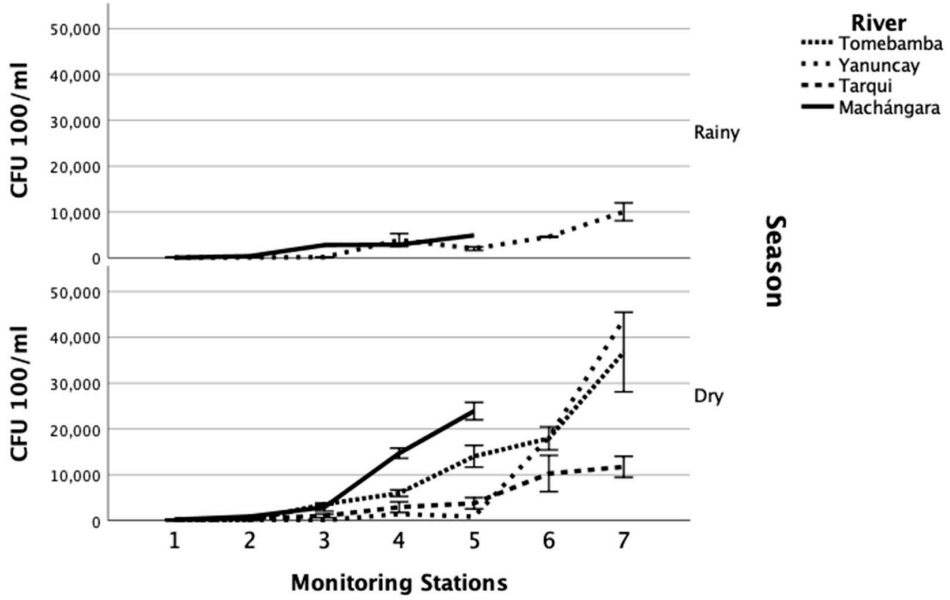

Figure 2. Total Streptococci for the four rivers.

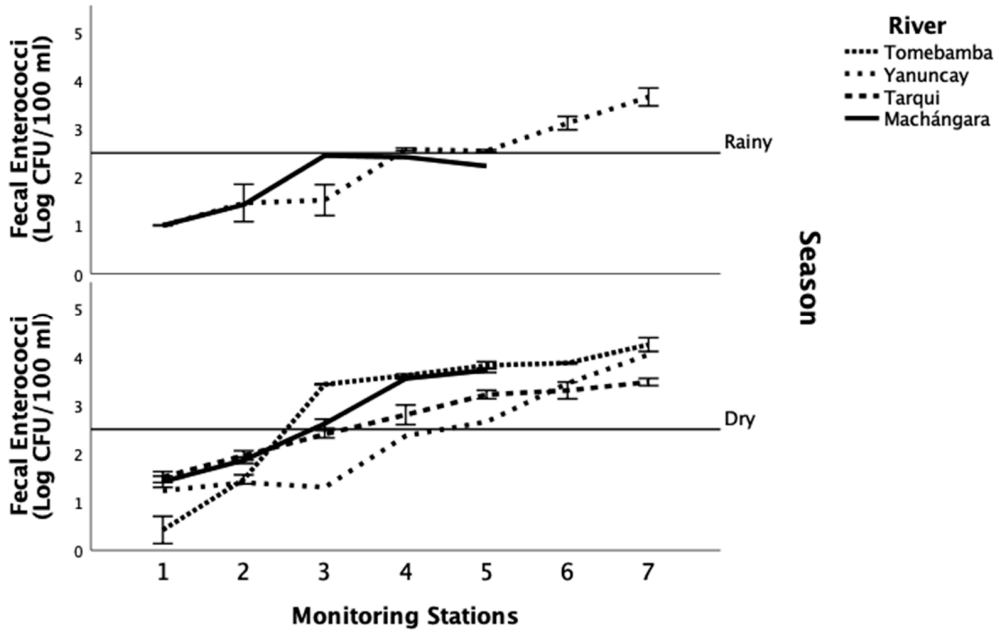

Figure 3. Fecal Enterococci for the four rivers.

For the Tarqui, Yanuncay, and Machángara Rivers, in general terms, the first stations correspond to areas characterized by little urbanization and an absence of industrial activity, but with agriculture and livestock. Progressively, as the rivers pass through urban areas, with greater intensity of agriculture, livestock, and industry, and due to open fecalism, pollution increases. In the Tomebamba River area, on the other hand, the Llaviucu sector is a protected area. A less protected zone is the Sayausí sector. From this, the river crosses densely populated areas in such a way that it progressively loses quality. The WWTP is located between the TO6 and TO7 monitoring points, as shown in Figure 1. These monitoring points represent the points to which all the natural and un-intercepted anthropogenic pollution of the city's river system converges. The approximate distance from TO6 to the WWTP is 0.85 km. This stretch is the last one of the river that has marginal interceptors. Therefore, in the TO7 section of WWTP effluent, about 4.4 km, the river does not present any protection system, acting only as a waste receptor.

Total Streptococci and their specific group of Fecal Enterococci were determined at all stations, with a total of 78 samples obtained. Only in the last two stations of the Tomebamba River, Tarqui River, and Yanuncay River, and in the last one of the Machángara River, were the Crystosporidium and *Giardia* cysts identified and quantified, with a total of 21 samples. The upper and middle zones of the rivers were not monitored because the technique used is not applicable for low-contamination areas. That is to say, the low zones of the rivers were selected for parasitic counts that allow interpretable results.

2.3. Methodology for Determination of Total Streptococci and Fecal Enterococci: Membrane Filtration Technique

For the detection and enumeration of the mentioned groups, the standard method described in ISO 7899:2 was used [62]. Spot samples were collected in sterile 250 mL glass containers and transported in a cooler at 4 °C until the laboratory was reached. A 100 mL amount of sample is filtered through a membrane that retains the bacteria of interest using a vacuum pump. The membrane is placed on a Petri dish with selective Agar KF (Kenner Fecal) medium containing 2,3,5-triphenyltetrazolium chloride (TTC) and incubated for 48 h at 35 °C. The culture develops two groups of colonies: dark red colonies formed by microorganisms capable of reducing TTC to formazan (an insoluble complex in water of intense red color) and pink colonies, which do not have this reducing capacity. The first group of colonies belongs to Enterococci and the second to Streptococci;

the results were expressed in colony-forming units (CFU)/100 mL of sample. Typical colonies of Enterococci from the tested cultures were randomly selected for confirmation, and the following biochemical evaluations were carried out: catalase, esculin hydrolysis (pH 9.6—37 °C), and growth in Brain Heart Infusion (BHI) broth [63].

2.4. Methodology for Determination of Crystosporidium and Giardia Oocysts

The technique used by [64] was used as the reference (MERIFLUOR® Cryptosporidium/*Giardia* IFD kit, Meridian Bioscience, Inc., Cincinnati, OH, USA), which is based on four defined stages: concentration, purification, staining, and cell counting, described below.

In a completely clean bucket, 20 L of water to be investigated was collected. It was homogenized, and a sub-sample of 10 L was extracted, which was concentrated by inorganic flocculation using calcium chloride and sodium bicarbonate. The entire water volume was vigorously stirred, and the pH was adjusted to 10 with 2M NaOH. The sample was allowed to settle for 24 h. The supernatant was removed, and the sediment was recovered in 50 mL centrifuge tubes. The sample containers were washed with sulfamic acid, phosphate-buffered saline (PBS) solution at pH 7.4, and Tween 80 solution to remove any adhered particles from the walls, and the wash product was also placed in the mentioned centrifuge tubes. The sediment was centrifuged at 3000× *g* for 10 min, and the maximum amount possible was recovered in a single tube per sample, with successive washes with PBS until a final pH of 7.4 was obtained. The concentrated samples were stored in the refrigerator at 4 °C until assembly and reading.

Each sample marked with vital stains was taken up with a sterile pipette, and 15 µL was transferred to each of the wells of the slides included in the kit and allowed to dry at room temperature for 30 min. Both the positive and negative controls of the kit were mounted in the same way. The positive control of the kit contained a preparation of formalin-fixed stool with *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts, and the negative control was a preparation of formalin-fixed stool without cysts. In darkness, a drop of detection reagent (preparation of anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies conjugated to Fluorescein Isothiocyanate in a buffered solution containing a protein stabilizer and 0.1% Sodium Azide) and a drop of contrast reagent (Eriochrome Black Solution) were added to each well, with the slide moved circularly to allow the mixing of the reagents.

The plates with the reagents were incubated in the dark in a humid chamber for 30 min at room temperature. A drop of mounting medium (Glycerol Buffered with Formalin and 0.05% Sodium Azide) was added to each well, and they were covered with a coverslip to protect them from light. The samples were stored in a humid chamber at 4 °C until reading. The parasites *Giardia* spp. and *Cryptosporidium* spp. are counted by visualizing cystic forms under a fluorescence microscope using three excitation filters: 450–490 nm for detection of cysts and oocysts stained with fluorescein; 365–420 nm to observe the staining with DAPI; and 520–560 nm for IP.

Counting was performed by observing the entire content of each well on the slide under the microscope and shaking its contents. Viable cystic forms were considered as oval forms with apple green fluorescence, well-defined walls, and a size of 8 to 18 µm in length and 5 to 15 µm in width corresponding to *Giardia* spp., and spherical forms with apple green fluorescence and an approximate diameter of 4 to 6 µm corresponding to *Cryptosporidium* spp.; with the DAPI dye, intense blue fluorescence with 2 to 4 nuclei was seen in the case of *Giardia* spp. and up to 4 nuclei in the case of *Cryptosporidium* spp. With the IP dye, no coloration is observed, as it serves to mark dead or non-viable forms. Finally, with the phase contrast objective, the cysts and oocysts should appear refractive. Any characteristic different from those mentioned indicates that the cystic forms are not viable.

Once the count of only viable forms was conducted, the following formula was applied to obtain the total number of cysts or oocysts per liter:

$$\text{No. Cysts or Oocysts/L} = \text{N}^{\circ}\text{. counted} * \text{Vol. Sediment/Vol. Chamber} * \text{Vol. Sample}$$

where N° counted is the viable cystic forms, Vol. Sediment is the recovered sediment volume, Vol. Chamber is the chamber well capacity (15 µL), and Vol. Sample is the sample volume (10 L).

Interferences for the technique include the presence of organic and inorganic turbidity. Some organisms such as algae, yeasts, and other protozoa can yield false positives as they have a similar size to *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts and exhibit fluorescence similar to that observed in these protozoa when stained with Fluorescein Isothiocyanate [64].

2.5. Statistical Analysis

For all the data, the normality assumption was checked by Shapiro–Wilk tests at a 0.1 level of significance. On the other hand, for the relationship between the variables, Spearman's Rho correlation coefficient was used in each season/river at a 0.10 significance level [65].

3. Results

3.1. Bacterial Indicators

Temporal and Spatial Variation

For both hydrological periods considered (rainy and dry), as rivers progress along their course and pass through urban areas, agricultural areas, livestock farming, industrial activity, and open defecation areas, the value of the two indicators Total Streptococci and Fecal Enterococci increases, as shown in Figures 2 and 3 (average of the three monitoring periods). It is expected that both point and diffuse fecal contamination will progressively increase, making it difficult to observe self-purification processes. However, the increase is significantly higher in the last two stations of the Tomebamba River (Cuenca River, i.e., all four rivers together), due to the contribution of all rivers and because, in these sections, there are no longer marginal interceptors available. Therefore, domestic wastewater is discharged directly into the receiving body, making these last two stations the most contaminated of all rivers. This is a cause for concern because the high bacterial load limits all uses of the resource. Significant differences were observed among the levels of Streptococci and Enterococci at different stations as the rivers progressed along their course, obtaining considerably higher concentrations by the last stations of the rivers. Additionally, in terms of hydrological periods (rainy and dry), considering that rainy data are only available for Machángara and Yanuncay, for both rivers, less favorable conditions are obtained in dry weather. The dilution effect due to rain decreases the concentration of both indicators in all monitoring sites.

To define the use of the resource, emphasizing "recreational through secondary contact", a use that gives merit to the urban scene and that can be used as an environmental indicator, it is necessary to establish a reference value taken from the Guides of International Organizations, since the Ecuadorian TULSMA [66] regulations do not use Enterococci as an indicator organism, but rather fecal coliforms expressed as NMP/100 mL. The value suggested by the Canadian Guide [67] was then selected for Enterococci in sea water ($\leq$70 colony-forming units CFU/100 mL), and to extrapolate it to fresh waters, the value was multiplied by two and five (140 and 350 CFU/100 mL), considering that with equal concentrations of the indicator in fresh and sea water, the pathogenicity is two to five times higher in the sea [68]. Thus, to obtain a standard in all waters and deduce limit values of equal pathogenicity, the limit value of the indicator should be taken five times lower (more stringent) than in fresh water. This deduced value is more appropriate for this study in which three samples were obtained per site. The most permissible value is 350 CFU/100 mL, and 140 CFU/100 mL is the strictest. According to the most permissible reference value and compared with the average of the three samples taken at each station, for the Tomebamba and Tarqui rivers, which could not be monitored in both periods because of logistical reasons, it is observed that they only meet the quality requirement until the second station for the Tomebamba and until the third for the Tarqui. On the other hand,

for the Machángara and Yanuncay, there is a significant difference. In the rainy season, all five stations constitute safe sites, and in dry weather, only three. Moreover, for the Yanuncay River, there are five safe sites (for the rainy period) and four unsafe sites (for the dry period), marking a difference from the results obtained from the average of the three monitoring stations, according to which only two would be suitable for the Machángara and four for the Yanuncay. This trend would probably also be observed in the Tarqui and Tomebamba Rivers.

Relationship between Indicators

As shown in Figure 4, for the dry period, there is a significant correlation between the two indicators (Total Streptococci and Fecal Enterococci) for all rivers with a significant Spearman's Rho correlation coefficient (Table 2). For the rainy period, the correlations slightly decrease for the Yanuncay River compared with the correlation in the dry period. However, for the Machángara, a significant decrease (with respect to the dry period) in the correlation is reported.

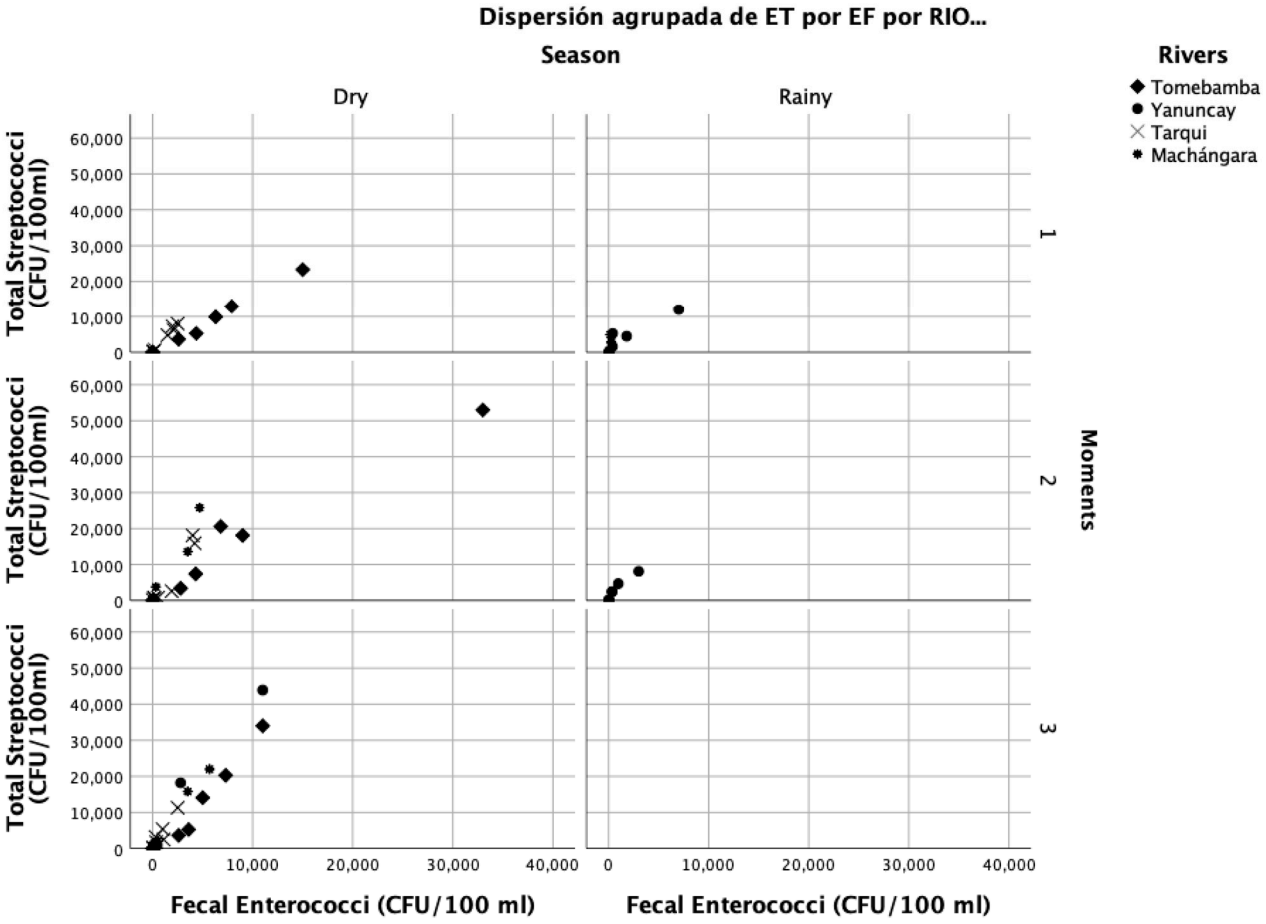

Figure 4. Correlation between Fecal Enterococci and Total Streptococci.

Table 2. Correlation between Fecal Enterococci and Total Streptococci.

River	Correlation	
	Dry	Rainy
Tomebamba	0.967 * (n = 21)	---
Yanuncay	0.967 * (n = 7)	0.955 * (n = 14)
Tarqui	0.954 * (n = 21)	---
Machángara	0.948 * (n = 10)	0.60 (n = 5)

Note: * The correlation is significant at 0.01 (bilateral) according to Spearman's Rho coefficient.

3.2. Parasitic Indicators

Figure 5 displays the average number of parasites obtained in the three monitoring sessions conducted in each river and at the previously established stations, thus representing the parasite level in the final stretch of each river. A predominance of *Cryptosporidium* oocysts is observed in the Machángara River for both periods analyzed. The dilution effect of little significance that rain exerts on the Machángara River is also evident. This can be related to the regulation of flow due to the effect of the reservoir, a situation that does not occur in other rivers. The dilution effect is significant, however, for *Giardia* in the Yanuncay River, which may perhaps be due to the slope of the river that exerts a destructive mechanical action on it, even though it is stated to be more resistant than *Cryptosporidium*. In general, the magnitude of parasites is much smaller than bacteria, and this is because in the feces of parasitized humans and animals, bacteria also predominate.

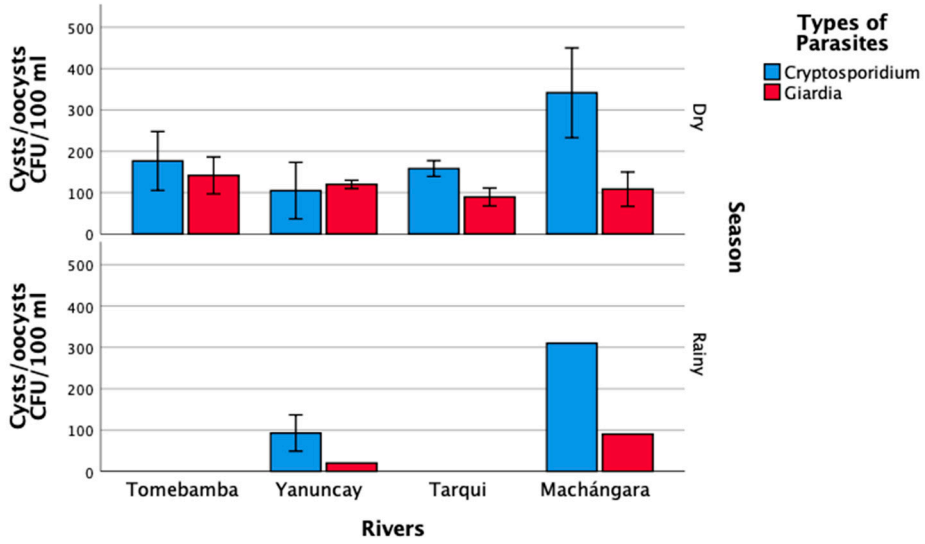

Figure 5. Parasites in all rivers.

4. Discussion

4.1. Regarding Bacterial Indicators

It is observed that Streptococci and Enterococci are sensitive to changes in water quality. The upper reaches of the rivers present low values corresponding to low contamination levels. However, as the water courses progress, the impact of natural contamination, such as erosion, and anthropogenic contamination, such as livestock farming, urbanization, and untreated wastewater discharges, radically modifies the water quality, resulting in high contamination at the end of the courses. The most critical situation is reported for the last two stations of the Tomebamba River. This progressive deterioration in quality is independent of the climatological period. That is, while diffuse contamination, such as erosion, livestock farming, and agriculture, predominantly impacts the streams during the rainy season, some anthropogenic actions, such as untreated domestic wastewater discharges, produce concentrated but continuous contamination. The temporal variation in bacterial quality is evident for the rivers for which information is available. Rain exerts a diluting effect, confirming that the worst conditions for streams from a health point of view occur in dry weather.

River quality studies that report Enterococci as bacterial indicators, expressed in colony-forming units (CFU)/100 mL, are not common, since the universally used indicator is E. Coli. However, international regulations do establish reference values for Enterococci, especially for recreational water uses [67]. These reference values can serve as a guide for the receiving bodies of the present study, due to their sensitivity to change in quality, as reported in the present results.

4.2. Regarding Parasites

In terms of magnitude

In all rivers, a higher concentration of *Cryptosporidium* is observed, meaning that its prevalence is higher than that of *Giardia*, contrary to what some studies suggest [27]. This may indicate that the environmental conditions of the studied rivers are not so inhospitable, allowing *Cryptosporidium* to thrive. Due to time limitations, not all rivers were monitored in the two climatological periods (rainy and dry). This was only possible for the Yanuncay and Machángara Rivers. For the Yanuncay River, it is observed that the figures increase in dry periods. However, for the Machángara, the levels seem to depend less on the seasonal period.

Comparing the concentration values with those obtained in similar studies, the following can be reported. In the Bogotá River [44,69], it can be observed that the average concentrations of *Giardia* were found to be 1.33 cysts/L and 0.88 oocysts/L for *Cryptosporidium*. These values are much lower than those reported in the present study (on average from 86.66 to 141.66 cysts/L for *Giardia* and from 81.38 to 331 oocysts/L for *Cryptosporidium*). However, it is important to note that the Bogota River values correspond to upper basins and probably protected areas of the mentioned river. Additionally, a study conducted in the Po River in Italy [19] reports mean values of *Cryptosporidium* oocysts of 0.2/L and 1.3/L for *Giardia* cysts. In the Llobregat River in Spain [70], average values of 0.77/L for *Cryptosporidium* and 0.73/L for *Giardia* [44] were obtained. In the city of Cayos, Haití, in filtered surface water samples, the number of *Cryptosporidium* spp. oocysts detected ranged from 5 to 100 with an average of 29 oocysts per 100 L (0.29/L). For *Giardia* cysts, the variation was from 5 to 960 with an average of 277 cysts per 100 L (2.77/L), values also lower than those of the present study. This could be seen as a confirmation that filtration is not efficient in retention [71]. In the bays of Mayagüez and Añasco located on the west coast of Puerto Rico, a study by [72] on these indicators reported *Giardia* only at a station corresponding to the effluent discharge zone of a wastewater treatment plant (WWTP), with a value of 74.38 cysts/100 L (0.74 oocysts/L). However, *Cryptosporidium* was not detected at any of the stations studied. All the values reported in previous studies are lower than those obtained in the present study. However, it is important to consider that the present study includes the lower areas of the four rivers (entirely urbanized and populated), therefore representing the most contaminated sites.

Finally, some studies show that the presence of relatively high amounts of nutrients and biodegradable organic carbon, together with warm temperatures and high bacterial loads, favors the proliferation of *Giardia* and *Cryptosporidium*, conditions also present in the final stretches of the studied rivers [8,73,74]. A literature review intended for the creation of an inventory of concentrations reported in surface waters and useful for the quantification of microbial risk indicates reports values up to 8400 oocysts/L and 1000 cysts/L for *Cryptosporidium* and *Giardia*, respectively [27]. In Ecuador, there are few studies on this topic. Research conducted by [75] in eighteen rivers in the province of Pichincha confirms the presence of *Cryptosporidium* in three rivers and of *Giardia* in eight rivers, indicating the high contamination of some rivers and the importance of source protection as a prevention mechanism. Additionally, in a study performed in Quito in paramo areas that serve as a water supply source for the metropolitan district, the absence of these parasites was reported [76]. In contrast, in a study [54] that summarized the most important aspects of water quality in the American continent countries, specifically in the Ecuador chapter, the presence of "*Cryptosporidium* spp. oocysts" was reported in 5 out of 14 untreated water samples from the watersheds in the Quito area. Therefore, with wide variability, both parasites are broadly distributed in the surface bodies around the world, constituting a real danger in the spread of waterborne diseases.

Magnitude in wastewater

Although the determination of these parasites in untreated domestic wastewater was not the main subject of the present study, the figures are also important as they constitute the main mechanism of river pollution. Samples taken at the Ucubamba wastewater

treatment plant (WWTP) had average values for *Giardia* of 1200 cysts/L at the inlet and non-detectable (ND) values at the outlet. For *Cryptosporidium*, on the other hand, there were 300 oocysts/L at the inlet and 69.33 oocysts/L at the outlet. These data show that *Giardia* is more abundant at the plant inlet, but its removal is complete (through the plant treatment process). In contrast, *Cryptosporidium* is more resistant to the lagoon treatment system. Greater efficiency in *Giardia* removal is also observed in research performed on wastewater [44]. Other data from research conducted by [64] and [27] on untreated wastewater show values close to 2200 *Giardia* spp. cysts/L and 62 *Cryptosporidium* spp. oocysts/L in Argentina and from 0 to 680 "*Cryptosporidium* spp. oocysts/L" in Brazil. Finally, a compilation of *Cryptosporidium* and *Giardia* data in wastewater determined concentrations of 60,000 oocysts/L and 100,000 cysts/L, respectively [27]. As observed, figures in untreated wastewater can vary within wide ranges, highlighting the need for prior treatment before discharge into receiving bodies. Most rivers receive untreated domestic and industrial wastewater, with unknown concentrations of present pathogenic microorganisms and effects that natural and artificial barriers can have on them. Therefore, it is important to have systematic analysis tools to evaluate water quality, treatment system efficiency, and potential health risks for the population [64].

Regarding the technique

The present study was based on the use of epifluorescence microscopy. This technique is not a very sensitive method and requires experience on the part of the analyst. However, the results can be used as a confirmation and preliminary quantification of the persistence of parasites in the receiving water courses, indicating the potential threat of a public health problem and identifying the environmental factors involved in their transmission. It is important for future research to develop and optimize new detection methodologies, such as solid-phase cytometry and molecular techniques, to allow more in-depth studies at the parasite species level. The application of molecular biology techniques has allowed the genetic characterization of isolates obtained directly from fecal or environmental samples, validating the existence of different species and/or genotypes of *Giardia* in each host [36,77–81].

Regarding treatment

In developed countries like the United States and some countries in Europe, the disinfection processes used to eliminate these parasites have been shown to be efficient. Methodologies applied include the use of ozone or ultraviolet light to ensure greater inactivation of pathogens such as *Giardia* and *Cryptosporidium*. Additionally, a small dose of chlorine is added to maintain a lasting residual effect when transporting water through the distribution network. However, the application of these types of methodologies is not economically viable in developing countries in most cases. Therefore, the risk of transmitting these parasites through drinking water consumption will continue to be latent [82–85].

5. Conclusions

In the present study, an evaluation of the sensitivity of fecal Streptococci and their specific group, Enterococci, as indicators of bacterial contamination in the receiving water bodies was performed using specific data from the four rivers that cross the city of Cuenca, Ecuador. A satisfactory performance of Streptococci and Enterococci as indicators of bacterial quality is observed, which is useful for understanding the real extent of river water contamination. Enterococci have physical characteristics that confer greater persistence in aquatic environments compared to coliforms in general, which often become autochthonous microorganisms and have been isolated from waters without an identified source of fecal contamination.

The investigated parasites (*Giardia* spp. and *Cryptosporidium* spp.) are present in the lower reaches of all the studied rivers, i.e., in the most contaminated sections. The middle and upper reaches of the rivers were not considered in this study. The presence of these parasites in the water represents a conflict for the potential uses of this resource, especially

for human consumption. Even though the concentration of these parasites detected in the present study would not represent an alarm for the population, they must be a cause for alert for the regulatory entities, given that *Cryptosporidium* and *Giardia* are classified as "emerging contaminants" and water as their vehicle has the potential to simultaneously infect a large proportion of the population.

Despite the fact that water regulations establish the absence of these parasites in water intended for human consumption, due to the difficulty of their determination, most provisioners, especially at the rural level, omit their search. Thus, microbiological quality control is reduced to the presence or absence of fecal coliforms, overlooking dangerous contaminations that are often causes of death.

6. Recommendations

The activities that contribute the most to water parasitological contamination are agricultural such as the movement of animals, the presence of organic fertilizers for crops, and inadequate disposal of domestic wastewater. Therefore, prevention will always be the most opportune and safe mechanism. The implementation of marginal interceptor networks, reinforcement of wastewater treatment systems, and planning of monitoring programs for surface bodies, especially in the headwaters of rivers, are some safety measures applicable against the potential posed by these pathogens.

The present study was performed in the lower portions of the rivers. A complementary study that includes the middle and upper parts of the rivers would be interesting to relate the incidence of this type of parasite and the evolution of river contamination as the rivers cross the city.

Finally, the deterioration of the quality of surface water bodies is a worldwide problem. The presence of new emerging contaminants, a product of anthropogenic interactions, represents a potential health risk and restricts the use of the water from these sources. The data from this study can be seen as evidence that there are more parameters than those usually used to define water quality that require serious consideration.

Author Contributions: Conceptualization, G.P. and G.V.; methodology, G.V.; software, C.T.; validation, G.V.; formal analysis, G.P. and G.V.; investigation, G.P. and G.V.; resources, G.P.; data curation, G.P. and C.T.; writing—original draft preparation, G.P.; writing—review and editing, G.P., V.C. and G.V.; visualization, G.P. and V.C.; supervision, G.P.; project administration, G.P.; funding acquisition, G.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by "Vlir-Integral Water Quality Management".

Data Availability Statement: The data presented in this study are available on request from the corresponding author G.P.

Acknowledgments: The authors express their sincere thanks to the Faculty of Engineering and to the project "Vlir-Integral Water Quality Management", especially to Engineer Felipe Cisneros Espinoza, its director, for the financial support provided, and for the acquisition of materials and reagents used in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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