

Artículos Científicos

## Microbiological quality of drinking water in three rural communities of the Calvas Canton (Loja)

### Calidad microbiológica de agua de consumo humano en tres comunidades rurales del cantón Calvas (Loja)

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**Abstract.-** In Ecuador, rural areas face challenges in accessing safe drinking water due to the contamination of water sources by wastewater or agricultural runoff, which turns water into a vehicle for potentially pathogenic microorganisms. This study shows the results of a microbiological assessment in three communities of the Calvas Canton, analyzing total coliforms, protozoan cysts, and helminth eggs present in drinking water to identify critical contamination sites. A total of 19 samples were processed to evaluate total coliforms and *Escherichia coli* using the multiple-tube fermentation method, and two samples were analyzed to detect intestinal parasites through basic microscopy. None of the samples met permissible limits established by Norma Técnica Ecuatoriana INEN 1108:2014 to be considered safe. Eight sites showed contamination >1600 MPN/100ml, both for total coliforms and *E. coli*. Eighteen resistant structures belonging to four genera of helminths and three cystic structures corresponding to three genera of protozoa were identified. Microbiological data were visualized through concentration mapping. It was concluded that drinking water in these communities is unsafe due to high levels of contamination. Therefore, it is recommended to: (i) implement water treatment technologies, (ii) promote health education, (iii) protect water sources, and (iv) strengthen monitoring and institutional support to ensure access to safe water in vulnerable communities.

**Keywords:** Water quality, coliforms, rurality, *E. coli*, helminths, protozoa.

**Resumen.-** En Ecuador, las zonas rurales enfrentan retos en el acceso a agua segura por la contaminación de fuentes hídricas con aguas residuales o escorrentía de cultivos, lo que convierte al agua en un transporte de microorganismos potencialmente patógenos. En el presente estudio, se realizó un diagnóstico microbiológico en tres comunidades del cantón Calvas, analizando coliformes totales, quistes de protozoarios y huevos de helmintos presentes en el agua de consumo para identificar sitios críticos de contaminación. Se procesaron 19 muestras para evaluar coliformes totales y *Escherichia coli* mediante el método de fermentación multitubo, así como dos muestras para detección de parásitos intestinales por de microscopía básica. Ninguna de las muestras cumplió con los límites permisibles según la Normativa Técnica Ecuatoriana INEN 1108:2014 para ser segura. Ocho localidades mostraron contaminación >1600 NMP/100ml para coliformes totales y *E. coli*. Se identificaron 18 estructuras de resistencia de cuatro géneros de helmintos y tres estructuras quísticas de tres géneros de protozoarios. También se generaron mapas con las concentraciones microbianas. Se concluye que el agua de consumo en estas comunidades no es segura debido a altos niveles de contaminación. Finalmente, se recomienda i) aplicar tecnologías de tratamiento de agua, ii) promover educación sanitaria, iii) proteger las fuentes hídricas y iv) fortalecer el monitoreo y apoyo institucional para garantizar el acceso a agua segura en comunidades vulnerables.

**Palabras clave:** Calidad de agua, coliformes, ruralidad, *E. coli*, helmintos, protozoarios.



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## Introduction

Access to safe drinking water is a basic human need. However, in rural areas around the world, public services that provide this essential resource are inadequate. In 2022, it was reported that 2.2 billion people did not have access to safely managed drinking water for their daily needs (United Nations 2023). In Latin America, despite an abundance of water resources in relation to its surface area and population, has a relatively low coverage of drinking water services (Tribunal Latinoamericano del Agua 2004; BID and CEPAL 2018). In Ecuador, only 48.5% of the rural population had access to safe drinking water in 2019 (INEC 2019). Currently, studies conducted in the country on drinking water focus on reservoirs and treatment plants that generally supply the water resources only to urban areas and nearby peripheries (Baque-Mite et al. 2016; Levy et al. 2021; Molina et al. 2024; Vinueza et al. 2021). In contrast, the few studies conducted in rural areas have revealed that water often does not meet microbiological safety (Sánchez and Guangasig 2023).

For water to be considered safe for consumption, it must meet a series of physical, chemical, and microbiological standards to prevent risk to human health. Safe water requires a microbiological analysis that identifies the presence of microorganisms capable of altering the characteristics of an environment or ecological niche, resulting in possible harmful consequences to humans or the ecosystem (Prieto 2017). To determine whether the water is safe, results of the microbiological analyses must be compared with the permissible limits of a current regulation. Among the microorganisms capable of causing waterborne diseases are bacteria, viruses, protozoa and helminths. However, the Ecuadorian standards for potable water “Norma Técnica Ecuatoriana (NTE) INEN 1108:2014” only considers indicator groups or index microorganisms such as thermotolerant coliforms, *Giardia* spp. and *Cryptosporidium* spp. (Ríos-Tobón et al. 2017).

The coliform group consists of facultative anaerobic organisms that ferment lactose and produce gas, potentially comprising up to 10% of the intestinal microbiota in humans and animals. Therefore, their presence in aquatic environments is often associated with fecal contamination (Michra et al. 2018). *Escherichia coli* is the most clinically relevant species in this bacterial group, with six pathogenic serotypes known (enterohemorrhagic [EHEC], enterotoxigenic [ETEC], enteropathogenic [EPEC], enteroinvasive [EIEC], enteroaggregative [EAEC], and diffusely adherent [DAEC]) that can cause bacteremia, gastrointestinal and urinary tract infections (Hunter 2003; Nowicki et al. 2021). In the case of further complications, these infections may cause death (Rabiu et al. 2022). On the other hand, protozoa are typically found as cysts, while helminths appear as eggs in water samples. These structures remain viable in the water for months and are resistant to both desiccation and chlorination (Grau-Pujol et al. 2021). In humans, they cause diarrheal diseases that primarily affect immunocompromised patients and children (Grau-Pujol et al. 2021; Ríos-Tobón et al. 2017).

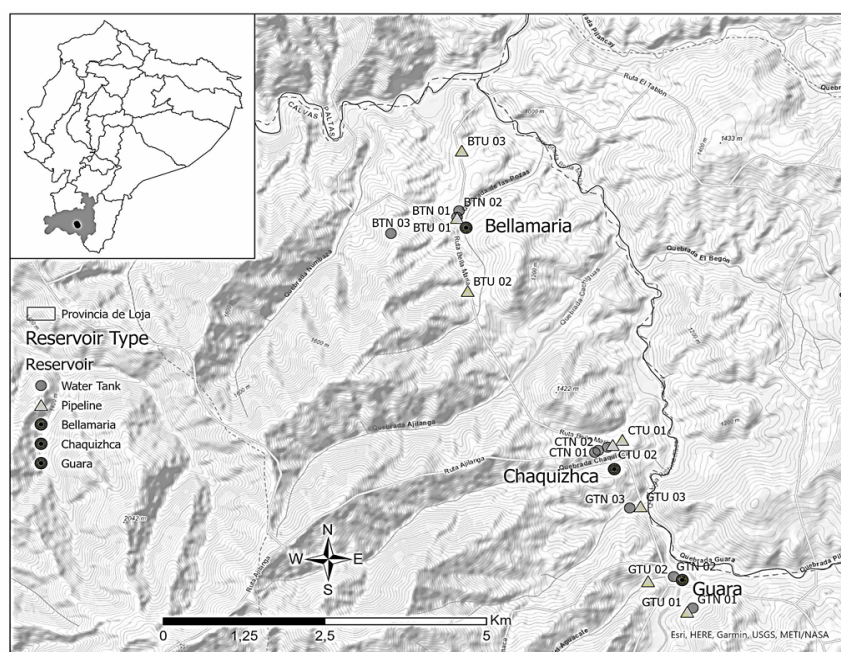
Continuous consumption of water with biological waste (animal and human feces and urine) is known to harm the health of consumers, increasing their risk of gastrointestinal diseases (Hunter 2003; Risebro et al. 2012). Among children, it is a key factor in the prevalence of chronic malnutrition. Early childhood diarrhea, particularly persistent cases, is strongly linked to growth faltering, with effects observed from 18 months up to several years later (Guerrant et al. 2002). Undernourished children experience more frequent and prolonged diarrheal episodes, creating a vicious cycle in which both conditions reinforce each other, further aggravated by enteric diseases that impair nutrient absorption

(Guerrant et al. 2002). In Ecuador, 10,799 cases of poisoning due to consumption of food and water contaminated by microorganisms were documented in the latest report presented by the ministry of Public Health of Ecuador (MSP 2024). Additionally, in 2023 it was reported that 46% of malnourished children in rural areas of the country consume water contaminated with *E. coli*, and in Loja province this percentage is 31.6% (INEC 2023).

Despite progress in expanding water services, access to safe drinking water remains a key determinant of quality of life in rural communities (ONU Ecuador 2023). In the province of Loja, data on non-potable water sources is scarce and largely informal (Alcaldía de Calvas and Aguilera 2018; ARCA 2023). In rural areas of Calvas canton, the absence of public water and sanitation systems forces residents to use rivers and springs, whose quality is unknown. This knowledge gap prevents an accurate understanding of the real situation in the rural sector, which means that new projects aimed at improving the quality of drinking water for these people are not proposed. Consequently, this study sought to evaluate the microbiological quality of drinking water in three rural communities of Calvas canton, located in the province of Loja, during February 2024, in order to determine its safety and identify the areas with the highest contamination levels.

## Material and Methods

The study was carried out in the communities of Guara, Chaquizhca, and Bellamaria located in the Calvas County, province of Loja (Figure 1). Together, these communities comprise a total population of 216 inhabitants, of whom 30 are children under 13 years of age and 27 are elderly adults. The sampling was conducted between the 5<sup>th</sup> and 9<sup>th</sup> of February, 2024 based on NTE INEN 1105:1986. This standard pertains to the sampling methodology and sample transport for analysis of microbiological parameters in drinking water (INEN 2012). The sampling methodology varied depending on whether the collection was conducted in the pipeline, tank, or river. A total of 19 duplicate samples, each with a volume of 75 ml, were collected for analysis of total coliforms and *E. coli*. Additionally, a total of two duplicate samples, each with a volume of 500 ml, were collected for confirmation of the presence/absence of protozoan eggs and helminth cyst (Table 1).



**Figure 1.** Map of the communities of Guara, Chaquizhca, and Bellamaria locations by sample type.

**Table 1.** Location of sampling points in pipeline, tank, and river systems by community.

|  |                                | House code | Sample code | Latitude  | Longitude  |
|--|--------------------------------|------------|-------------|-----------|------------|
| <b>T<br/>A<br/>N<br/>K</b>                         | GUARA                          | GA106      | GTN 01      | -4,252669 | -79,577886 |
|  |                                | *          | GTN 02      | -4,248089 | -79,580523 |
|  |                                | CQ218      | GTN 03      | -4,238073 | -79,586661 |
|  | CHAQUIZHCA                     | CQ 326     | CTN 01      | -4,229987 | -79,591505 |
|  |                                | CQ 307     | CTN 02      | -4,229702 | -79,591036 |
|  |                                | CQ 506     | CTN 03      | -4,229201 | -79,589730 |
|  | BELLAMARIA                     | BMESC      | BTN 01      | -4,195695 | -79,610661 |
|  |                                | BM302      | BTN 02      | -4,194776 | -79,61039  |
|  |                                | BM201      | BTN 03      | -4,198075 | -79,619829 |
| <b>P<br/>I<br/>P<br/>E<br/>L<br/>I<br/>N<br/>E</b> | GUARA                          | GAESC      | GTU 01      | -4,253091 | -79,57867  |
|  |                                | GA221      | GTU 02      | -4,248640 | -79,584110 |
|  |                                | CQ411      | GTU 03      | -4,237737 | -79,58516  |
|  | CHAQUIZHCA                     | CQESC      | CTU 01      | -4,228044 | -79,587687 |
|  |                                | CQ 507     | CTU 02      | -4,228775 | -79,589026 |
|  | BELLAMARIA                     | BMESC      | BTU 01      | -4,195695 | -79,610661 |
|  |                                | BM213      | BTU 02      | -4,20634  | -79,60918  |
|  |                                | BM305      | BTU 03      | -4,185882 | -79,610003 |
| <b>R<br/>I<br/>V<br/>E<br/>R</b>                   | Pishinamaca stream<br>(GUARA)  | -          | GAR 01      | -4,246301 | -79,579589 |
|  | Catamayo river<br>(BELLAMARÍA) | -          | BAR 01      | -4,177247 | -79,614902 |

Note: \* Recently constructed house not yet registered in the CISEAL database.

To sample the eggs of helminths and cysts of protozoa, a phytoplankton net was positioned against the river current for at least 10 minutes. The collection was transferred to a sterile 500 ml container and 2-3 ml of 10% formaldehyde was added to preserve relevant structures (Ferrario et al. 1995; Secretaría de Comercio y Fomento Industrial 1999).

At least one tank and/or piped sample was collected from schools in all three communities. The houses involved in the study were chosen considering water availability and proximity to the community's center. Two rivers near the communities of Bellamaria and Guara were also sampled.

**Report of total coliform and *E. coli*.** - The multi-tube fermentation method was employed to analyze members of the coliform group. Fluorocult medium was used for the analysis and three dilution ratios: 1:1, 1:10 and 1:100. A set of 15 tubes per sample (5 tubes per dilution) was used and incubated for 24 to 48 hours at 35 °C (American Public Health Association 1989). Total coliform results were determined by observing a color change of the medium from yellow to blue, with intermediate shades indicating a positive result. For *E. coli* detection, a positive sample for total coliforms was exposed to UV light, fluorescence indicated a positive result. Confirmation of *E. coli* was achieved by adding a couple of drops of Kovacs reagent to confirm the positive indole reaction (Merck 2024).

The qualitative results were quantified by determining the Most Probable Number (MPN) method. The number of positive results for each dilution was compared to McCrady's table (Standard Methods 9221) (American Public Health Association 1989). The validation of the culture media was performed by inoculating two sets of tubes for each batch used, one with distilled water and the other with a McFarland 0.5 standard with *E. coli*. Sample processing was carried out within a maximum of 4 hours after collection.

**Report of helminth eggs and protozoan cyst.**- The samples were left to settle for 48 hours before analysis to allow for the spontaneous precipitation of parasitic structures and dissolved solids in the water. Subsequently, the supernatant was discarded, leaving a total volume of 100 ml. A volume of 3 ml of Lugol's iodine was added to 100 ml of the sample before microscopic examination using glass petri dishes and a 40X objective lens (Terashima et al. 2009). Sample processing was conducted three weeks after collection, during which the samples were refrigerated at 4 °C.

**Statistical analysis.**- All analyses were performed (mean, median, standard deviation and Shannon diversity index) using RStudio (version 2024.12.1+563) running R (version R-4.5.0). Total coliform and *E. coli* concentrations were represented by bar charts, organized by community and sampling matrix. To evaluate the concentrations, descriptive statistics including mean, median, mode, and standard deviation were calculated for each community. To enhance the interpretation of variability across locations, error bars were used to graphically display the dispersion of values.

The Shannon diversity index assessed the presence of helminth eggs and protozoan cysts. The classification thresholds for the diversity index were established based on researcher-defined criteria, considering acceptable ranges for wastewater quality (the presence of two parasitic structures corresponds to a Shannon index value of 0.693), as outlined by the World Health Organization (WHO 2006).

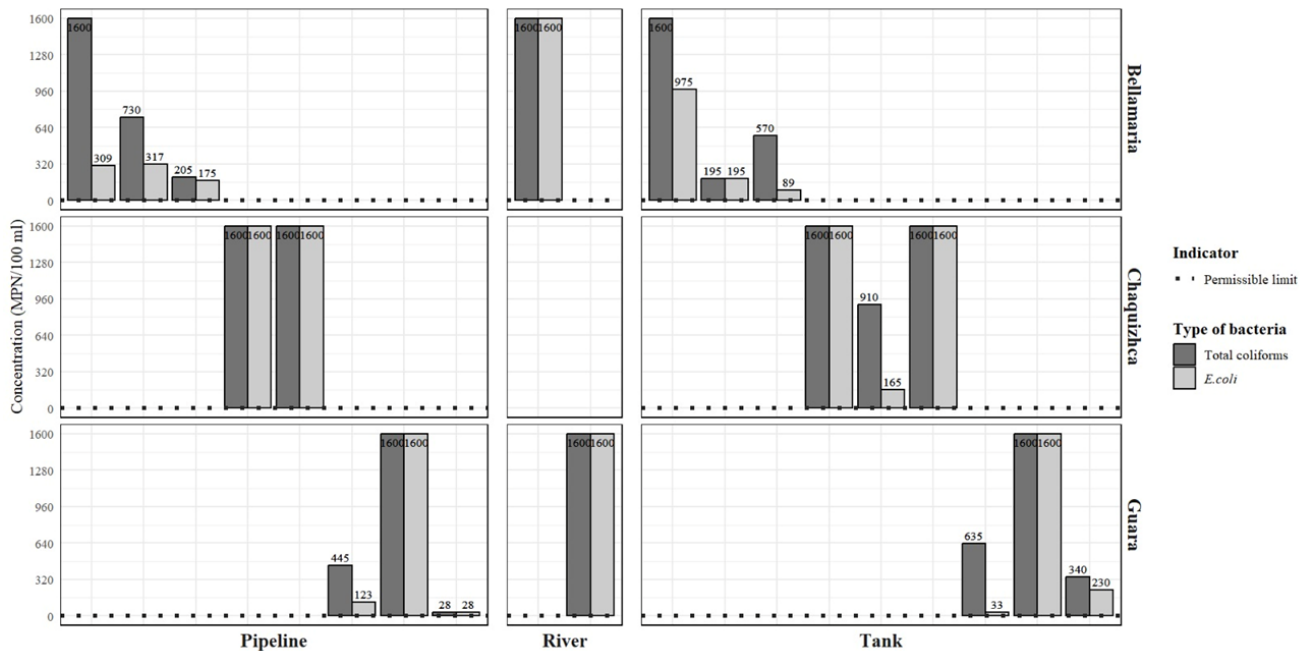
**Map generation.**- ArcGIS Pro software (version 3.3.2) provided map development. This geographic information system facilitated the creation of visually intuitive maps displaying the reported biological contamination concentrations in the study areas.

## Results

The three communities considered in the study are located approximately five km apart, the majority of their population convene in the vicinity the schools. The houses included in the study were either part of these population centers or located just a few meters away. In the community schools, both pipelines and tanks were installed; however, only the tank at the school in Bellamaria remained operational. The tanks in the schools of Guara and Chaquizhca were no longer functional due to damage, and therefore, they could not be sampled. The water pipelines selected for sampling at each school were predominantly the ones used by children when leaving the restroom or returning to class after recess.

Regarding the characteristics of the samples for the analysis of total coliforms and *E. coli* from pipeline and tank sources, these were generally colorless and odorless, with no turbidity. Only the samples labeled CTU01, CTN01 and CTN02 showed slight turbidity. On the other hand, the samples from the rivers were odorless but exhibited slight brown turbidity. As for the samples intended for the analysis of helminth eggs and protozoan cysts, they were odorless, with visible brown turbidity and sediment composed of clay and sand typical of the backwash filtration process in rivers.

**Total coliform and *E. coli*.**- The concentrations of contamination by total coliforms and *E. coli* ranged from 28 MPN/100 ml to >1600 MPN/100 ml. These values indicated that the water from pipelines, tanks, and rivers is not suitable for human consumption. The differentiation of contamination levels, based on the sampling matrix and the communities is illustrated below (Figure 2).



**Figure 2.** Mean concentrations (MPN/100 ml) of total coliforms and *E. coli* by sample type across to sampled communities.

As for piped water, eight samples were collected in duplicate (three from Guara, two from Chaquizhca, and three from Bellamaria). Some pipelines supplying water to the households were located outside the homes; from these points the water was transported to the kitchens using pots, pitchers and containers, or consumed directly from the faucet. The contamination concentrations in this matrix, for both analyzed parameters, ranged from 28 MPN/100 ml to >1600 MPN/100 ml.

In relation to the tanks, plastic or concrete storage containers were observed either near the houses or inside the kitchens. Tanks located inside the homes showed fewer sediments at the bottom. A total of nine samples from this sample type were processed in duplicate (three from Guara, three from Chaquizhca, and three from Bellamaria). Contamination by total coliforms was reported, ranging from 195 MPN/ 100 ml to >1600 MPN/100 ml; for *E. coli*, concentrations ranged from 33 MPN/100 ml to >1600 MPN/100 ml.

At the Guara community school, concentrations of 445 MPN/100 ml for total coliforms and 123 MPN/100 ml for *E. coli* were recorded. In the Chaquizhca community school, concentrations >1600 MNP/100 ml were detected for both total coliform and *E. coli*. In the Bellamaria community school, total coliform concentrations >1600 MPN/100 ml were observed in both matrices, while *E. coli* concentrations reached 309 MPN/100 ml in the pipeline and 975 MPN/100 ml in the tank.

**Helminth eggs and Protozoan cysts.**- This analysis restricted samples to those from the rivers due to the requirement of filtering a minimum of 100 liters of water. In the Pishinamaca stream, a total of 10 helminth eggs were identified, belonging to the genera *Taenia*, *Ascaris* and *Ancylostoma*, along with three protozoan cysts from the genera *Balantidium*, *Isospora*, and *Cryptosporidium*. In the Catamayo river, nine helminth eggs were detected, corresponding to the genera *Taenia* and *Paragonimus* (Table 2).

The identification of the detected genera was based on the determination of differential morphological traits described in the literature. The characteristics considered included size, shape, composition of external layers, and the distribution of intracellular material (Chai 2007; Ladin and Pacheco 2014).

**Table 2.** Taxa of helminth eggs and protozoan cysts identified in river samples.

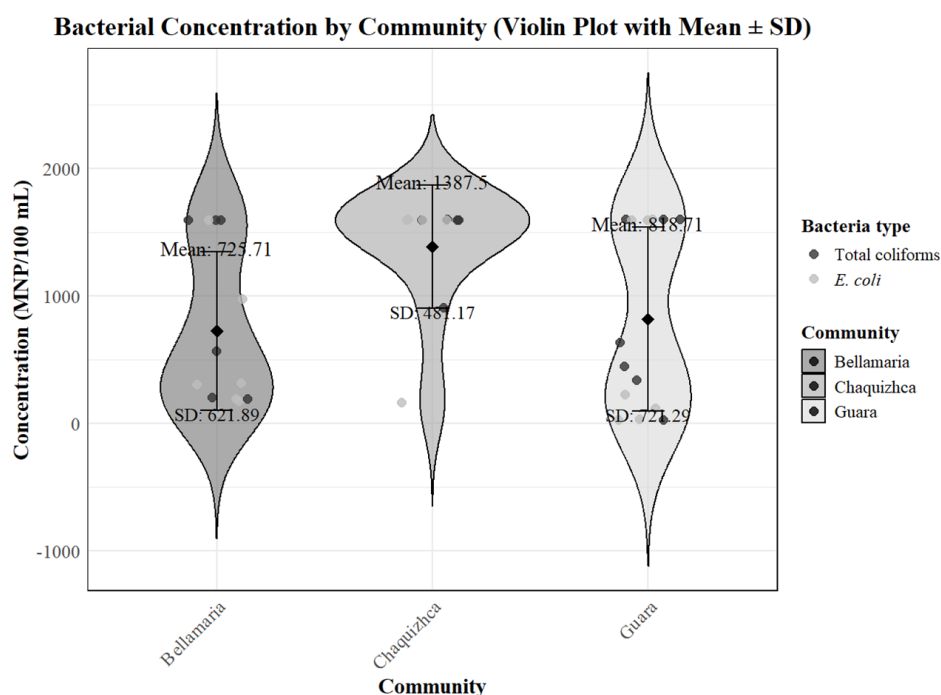
| Location                     | Classification  | Taxa                        | Number of structures |
|------------------------------|-----------------|-----------------------------|----------------------|
| Pishinamaca stream (GUARA)   | Helminth eggs   | <i>Taenia spp.</i>          | 6                    |
|                              |                 | <i>Ascaris spp.</i>         | 2                    |
|                              |                 | <i>Ancylostoma spp.</i>     | 1                    |
|                              | Protozoan cysts | <i>Balantidium spp.</i>     | 1                    |
|                              |                 | <i>Isospora spp.</i>        | 1                    |
|                              |                 | <i>Cryptosporidium spp.</i> | 1                    |
| Catamayo river (BEL-LAMARÍA) | Helminth eggs   | <i>Taenia spp.</i>          | 5                    |
|                              |                 | <i>Paragonimus spp.</i>     | 4                    |

**Statistical analysis.** - The average concentrations of total coliforms and *E. coli* per community exceeded the permissible limit for fecal contamination to be considered suitable for human consumption. In the community of Guara, the mean concentration was 815.71 MPN/100 ml with a median of 540 MPN/100 ml and the highest standard deviation of 761.29 MPN/100 ml. In Chaquizhca, the highest mean value that was observed was 1327.5 MPN/100 ml, with a median of 1600 MPN/100 ml and the lowest standard deviation of 461.17 MPN/100 ml. Conversely, in Bellamaria, the lowest mean concentration was reported at 722.71 MPN/100 ml with a median of 725.71 MPN/100 ml and a standard deviation of 681.89 MPN/100 ml. The mode in all three communities was 1600 MPN/100 ml. These data were visualized using error bar plots to illustrate the variations in concentration (Figure 3).

The Shannon diversity index was applied to quantify the biodiversity of river sampling points on the presence of protozoan cyst and helminth eggs. The complexity of the microbial population is determined by both the number of species detected and their relative abundance.

Regarding microbial diversity, water samples were classified according to Shannon diversity index values; diversity was considered low if the index value was  $\leq 0.5$ , moderate if  $\leq 1.0$ , high if  $\geq 1.1$ , and very high if  $\geq 2.0$ . According to the reported data, the sampling point located in the Pishinamaca stream exhibited high diversity, with a Shannon-Weaver index value of 1.411. In contrast, the Catamayo river, located near the community of Bellamaria, showed moderate diversity with a value of 0.687 (WHO 2006).





**Figure 3.** Mean bacterial concentration per community with standard deviation bars.

**Geospatial analysis.-** The maps allowed the identification of critical areas in terms of contamination. In the case of total coliforms and *E. coli*, the data confirm that the community of Chaquizhca exhibits higher MPN/100 ml concentrations compared to Guara and Bellamaria. Most of the intermediate contamination ranges are in Bellamaria, suggesting that fecal contamination in this area is moderate in comparison to the other communities. This trend is consistent across both microbiological parameters analyzed.

The following map presents the distribution of the genera identified in the samples, represented by pie charts corresponding to each sampling location. In the north, within the Catamayo river, a lower parasitic diversity is observed, characterized by the predominance of *Taenia* spp. This may be attributed to the larger flow volume of the river, which could reduce the representativeness of the sampling area with respect to the river's overall diversity. In contrast, in the southeastern region, the Pishinamaca stream exhibits greater diversity, while still showing a predominance of *Taenia* spp. This pattern is likely associated with a narrower tributary that receives higher contamination loads due to its proximity to the surrounding communities.

## Discussion

None of the processed samples met this standard, which sets the permissible limit for fecal coliforms at < 1.1 MPN/100 ml and requires the absence of *Giardia* spp. and *Cryptosporidium* spp. in 1 L of water. These results are alarming, as they demonstrate that children are exposed to high concentrations of potentially harmful microorganisms at home and at school.

It is essential to raise awareness about the importance of drinking water quality in rural communities and its implications for short- and long-term public health. While access to water services is a central topic in public policy, it is concerning that the water reaching households fails to meet the minimum safety standards established by Ecuadorian regulation (NTE INEN 1108:2014).



A study conducted in 2014 evaluated whether the water supply sources in Cariamanga parish, the cantonal capital of Calvas, met the minimum parameters for safe consumption. Results showed that total coliform concentrations at two of three sampling sites reached 510 and 520 MPN/100 ml during the rainy season, rising to 580 MPN/100 ml in the dry season. Fecal coliform levels ranged from four to nine MPN/100 ml (Cueva et al. 2014). Compared to the present study, which found levels between 28 and >1600 MPN/100 ml for both total coliforms and *E. coli*, the contamination of water sources appears to have significantly worsened.

Based on the results obtained, the community with the highest bacterial counts was Chaquizhca, suggesting that this area experiences the most severe fecal contamination, likely linked to the pollution of water supply sources. In Guara, a high degree of variability was observed, associated with differences in concentrations reported between sampling matrices. Specifically, the counts reported in the river (primary water source) differed from those found in household storage systems (tanks and pipelines), possibly due to preliminary household-level disinfection practices. Finally, Bellamaria, which exhibited the lowest mean contamination level, may have reduced exposure to contamination from its water source. It is important to note that the limited number of collected samples may introduce some bias in estimating the overall contamination level within each community. Nevertheless, the results are considered sufficiently representative to conclude that the drinking water currently consumed by these communities is unsafe.

*Escherichia coli* plays a critical role in the microbiological assessment of water quality due to its ecological niche compatibility with various pathogens. It is therefore considered both an index organism (from the genus *Salmonella*) and an indicator organism (for fecal coliforms) (Food and Agriculture Organization of the United Nations [FAO] 2011; González-Mendoza et al. 2015). Cattle, particularly bovines, are the primary reservoir for several serotypes of *E. coli* (Bettelheim et al. 2005; Hoyle et al. 2021). These strains colonize the gastrointestinal tract of the animals, resulting in feces that serve as a significant source of environmental and foodborne contamination (Bettelheim et al. 2005). Numerous outbreaks have been linked to cross-contamination of food or water with bovine feces (Bettelheim et al. 2005; Costa et al. 2024). Among the most extensively studied and impactful cases are those caused by the O157:H7 strain, which is known to cause severe renal and neurological damage, and can lead to death, particularly in children and the elderly due to serious complications (LeBlanc 2008; Smith et al. 2014). Its presence in this study warrants further investigation, as the high concentrations reported suggest the possible presence of other potentially harmful microorganisms. The reported concentrations are of concern, as infections caused by enteric bacterial pathogens can cause symptoms such as diarrhea, nausea, and vomiting (Wells et al. 2023). These infections can also weaken the immune system, particularly in infants, who frequently present with malnutrition diagnoses due to complications (Petri et al. 2008; Wells et al. 2023).

In contrast, protozoan cysts and helminth eggs represent an aspect that has not yet been addressed in any published study on water quality in the province of Loja. According to the World Health Organization (2006), untreated wastewater typically contains between two and five resistant parasitic structures (protozoan cyst and helminth eggs) per 64.9 L per day. In this study, river water samples showed between 9 and 13 such structures in a 10-minute sampling period. Based on this and in accordance with INEN standard 1108:2014, the water is deemed unfit for human consumption. Although only one *Cryptosporidium* cyst was detected, the findings confirm a high and persistent level of biological contamination in the water source.

According to current secondary environmental legislation helminth eggs must be absent in water intended for irrigation or recreational use (Ministry of Environment, Water and Ecological Transition 2015). The river water analyzed in this study exhibits high contamination levels, which hinder routine agricultural and recreational activities, thereby posing a health risk to local communities.

Helminth eggs and protozoan cysts serve as indicators of fecal contamination in water sources. Their presence is commonly studied in wastewater before and after treatment. Protozoan cysts can remain viable in water for a minimum of two days and up to seven months, whereas helminth eggs can survive from 107 days to as long as 1.5 years (WHO 2006). The presence of these organisms in drinking water poses a significant health risk, particularly for children, as they are more vulnerable to the effects of the effects of intestinal parasitic infections. In children, intestinal parasitic infections can lead to delayed physical and cognitive development due to appetite loss, nutrient malabsorption, and damage to the intestinal mucosa (Celi et al. 2019).

Prevalence and proliferation of microorganisms in water intended for human consumption are primarily caused by the presence of organic matter. Organic waste - especially feces and urine from animals and humans - is a major contaminant of water sources. In addition to introducing microorganisms into the environment, it provides soluble nutrients such as organic molecules and salts that support microbial growth and reproduction (Salazar Coello et al. 2018; Vega Herrera 2019). In the case of total coliform and *E. coli* analyses, it is likely that the water source, distribution systems, or storage containers are continually exposed to such contamination due to limited resources for the maintenance of the distribution system. This would explain why all the samples analyzed showed elevated levels of total coliforms and *E. coli*. Furthermore, protozoan cysts and helminth eggs are directly associated with contamination from fecal matter.

Additionally, soil leachates resulting from the excessive use of chemical fertilizers in agriculture may exacerbate the problem (Salazar Coello et al. 2018; Vega Herrera 2019). Leaching is the process by which water transports nutrients and organic matter from the soil into nearby water sources (Ferrari Noll 2018). This phenomenon is particularly impactful on rivers, as they often run alongside or near agricultural fields in local communities.

## **Conclusion and Recommendation**

This study indicates that the sampled area requires immediate intervention to improve drinking water quality, with the goal of strengthening the public health system. The most highly contaminated sites were the riverine sample and the Chaquizhca community. Therefore, it is recommended that water reaching households undergo preliminary treatment before consumption, to prevent high microbial loads from compromising the health of community members. In the case of river water, direct consumption should be avoided, as traditional household treatment methods are not effective in removing complex structures such as protozoan cysts and helminth eggs.

It is also recommended that future studies include a microbiological assessment of the springs that serve as water sources for the communities, and that household sampling coverage be expanded. Furthermore, it is suggested that future phases incorporate culture-independent methodologies to gain a deeper understanding of microbiome diversity and its potential impact on community health.

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## Author Contributions

EE: experimental design, data collection, data analysis and interpretation, drafting of the initial manuscript; MCT: funding acquisition, manuscript review; AV: data collection, manuscript review; FS: study design, manuscript review, CY: study conception and design, manuscript review.

## References

- Agencia de Control y Regulación del Agua [ARCA]. 2022. Benchmarking de prestadores públicos de los servicios de agua potable y saneamiento en el Ecuador. Boletín Estadístico. 4:41. [accessed 2024 Nov 1]. [http://www.regulacionagua.gob.ec/wp-content/uploads/downloads/2023/12/Boletin-Estadistico-APS\\_dic23-fnl.pdf](http://www.regulacionagua.gob.ec/wp-content/uploads/downloads/2023/12/Boletin-Estadistico-APS_dic23-fnl.pdf)
- Alcandía de Calvas y Aguilera MA. 2018. Mapas. Déficit de agua potable. Alcandía De Calvas. [accessed 2024 Nov 1]. <https://www.gobiernocalvas.gob.ec/index.php/sistema-informacion-local/territorial/mapas>
- American Public Health Association [APHA]. 1989. American Water Works Association & Water Pollution Control Federation. Standard Methods for the examination of Water and Wastewater. 17<sup>o</sup> ed. Washington, D.C.USA. Part 9000.
- Ash, LR, Orihel TC. 2007. Atlas de parasitología humana/Atlas of Human Parasitology. Ed. Médica Panamericana.
- Banco Interamericano de Desarrollo [BID] y Comisión Económica para América Latina y el Caribe [CEPAL]. 2018. Informe regional: América Latina y el Caribe. Comisión Económica para América Latina y el Caribe. [accessed 2025 Apr 28]. [https://www.cepal.org/sites/default/files/news/files/informe\\_regional\\_america\\_latina\\_y\\_caribe.pdf](https://www.cepal.org/sites/default/files/news/files/informe_regional_america_latina_y_caribe.pdf)
- Baque-Mite R, Simba-Ochoa L, González-Ozorío B, Suatunce P, Díaz-Ocampo E, Cadme-Arevalo L. 2016. Calidad del agua destinada al consumo humano en un cantón de Ecuador. Revista Ciencia Unemi, 9(20), 109-117. [accessed 2025 May 20]. <https://www.redalyc.org/articulo.oa?id=582663826015>
- Bettelheim KA, Kuzevski A, Gilbert RA, Krause DO, McSweeney CS. 2005. The diversity of Escherichia coli serotypes and biotypes in cattle faeces. Journal of Applied Microbiology, 98(3), 699-709. <https://doi.org/10.1111/J.1365-2672.2004.02501.X>
- Celi L, Jumbo G, Luzuriaga MDC, Zúñiga I. 2019. Parasitosis intestinal en los niños de 0 a 3 años de los centros infantiles del buen vivir de la zona 7 - Ecuador. Espirales revista multidisciplinaria de investigación científica, 3(28). [accessed 2025 Apr 28]. <https://www.redalyc.org/articulo.oa?id=573263327008>
- Chai JY. 2007. Ash & Orihel's Atlas of Human Parasitology (5th ed.). The Korean Journal of Parasitology, 45(4), 311. <https://doi.org/10.3347/kjp.2007.45.4.31>
- Costa LRM, Buiatte ABG, da Cunha Dias S, García LNH, Cossi MVC, Yamatogi RS, Nero LA, Pereira JG. (2024). Influence of animal production systems on the presence of pathogenic strains of Escherichia coli in the bovine production chain. Food Control, 157, 110155. <https://doi.org/10.1016/j.foodcont.2023.110155>
- Cueva B, Elizalde Briceño CA, Stalin F. 2014. Estudio del estado actual de las principales fuentes abastecedoras de agua de consumo humano de las principales poblaciones del cantón Calvas [Tesis de grado, Universidad Técnica Particular de Loja]. [accessed 2025 Apr 28]. <http://dspace.utpl.edu.ec/handle/123456789/9058>
- Ferrari Noll LA. 2018. Lixiviación de fosfatos y nitratos a partir de fertilizantes inorgánicos y orgánicos bajo lluvia simulada [Tesis de grado, Escuela Agrícola Panamericana Zamorano]. [accessed 2025 Apr 28]. <https://bdigital.zamorano.edu/server/api/core/bitstreams/20d935af-522b-43f9-8523-10469cfc9311/content>
- Ferrario ME, Sar EA, Sala SE. 1995. Metodología básica para el estudio del fitoplancton con especial referencia a las diatomeas [Manual de métodos ficológicos, Universidad de Concepción]. [accessed 2025 Apr 28]. <https://www.researchgate.net/publication/233742221>
- González-Mendoza D, Torrentera-Olivera NG, Ceceña Duran C, Grimaldo-Juarez O. 2015. El agua como fuente de contaminación de Salmonella y Escherichia coli en la producción de hortalizas en México: Una revisión. Revista Bio Ciencias, 3(3), 156-162. <http://dx.doi.org/10.15741/revbio.03.03.02>
- Grau-Pujol B, Cuamba I, Jairoce C, Cossa A, Da Silva J, Sacoar C, Dobaño C, Nhabomba A, Mejia R, Muñoz J. 2021. Molecular Detection of Soil-Transmitted Helminths and Enteric Protozoa Infection in Children and Its Association with Household Water and Sanitation in Manhica District, Southern Mozambique. Pathogens (Basel, Switzerland), 10(7), 838. <https://doi.org/10.3390/pathogens10070838>

- Guerrant RL et al. 2002. Magnitude and impact of diarrheal diseases. *Arch Med Res.* 33(4):351-355. [https://doi.org/10.1016/S0188-4409\(02\)00379-X](https://doi.org/10.1016/S0188-4409(02)00379-X)
- Hoyle DV, Keith M, Williamson H, Macleod K, Mathie H, Handel I, Currie C, Holmes A, Allison L, McLean R, Callaby R, Porphyre T, Tongue SC, Henry MK, Evans J, Gunn GJ, Gally DL, Silva N, Chase-Topping ME. 2021. Prevalence and Epidemiology of Non-O157 *Escherichia coli* Serogroups O26, O103, O111, and O145 and Shiga Toxin Gene Carriage in Scottish Cattle, 2014-2015. *Appl Environ Microbiol* 87:e03142-20. <https://doi.org/10.1128/AEM.03142-20>
- Hunter PR. 2003. Drinking water and diarrhoeal disease due to *Escherichia coli*. *Journal of water and health*, 1(2), 65-72. <https://doi.org/10.2166/wh.2003.0008>
- Instituto Ecuatoriano de Normalización [INEN]. 2012. Aguas. Muestreo para examen microbiológico (1105). [accessed 2025 Apr 28]. <https://www.insistec.ec/images/insistec/02-cliente/07-descargas/NTE%20INEN%201105%20-%20AGUAS.%20MUESTREO%20PARA%20EXAMEN%20MICROBIOLÓGICO.pdf>
- Instituto Ecuatoriano de Normalización [INEN]. 2014. NTE INEN 1108 - Agua potable: requisitos. Instituto Ecuatoriano de Normalización. [accessed 2024 Nov 1]. <https://www.insistec.ec/images/insistec/02-cliente/07-descargas/NTE%20INEN%201108%20-%20AGUA%20POTABLE.%20REQUISITOS.pdf>
- Instituto Nacional de Estadística y Censos [INEC]. 2023. Encuesta Nacional sobre Desnutrición Infantil. Principales resultados. Gobierno del Ecuador. [accessed 2024 Nov 1]. [https://www.ecuadoren cifras.gob.ec/documentos/webinec/ENDI/Presentacion\\_de\\_Resultados\\_ENDI\\_R1.pdf](https://www.ecuadoren cifras.gob.ec/documentos/webinec/ENDI/Presentacion_de_Resultados_ENDI_R1.pdf)
- Ladín, C, Pacheco S. 2014. Helminths: Handbook for Identification and Counting of Parasitic Helminth Eggs in Urban Wastewater. <https://doi.org/10.2166/9781780407159>
- LeBlanc, JJ. 2003. Implication of Virulence Factors in *Escherichia coli* O157:H7 Pathogenesis. *Critical Reviews in Microbiology*, 29(4), 277-296. <https://doi.org/10.1080/713608014>
- Levy K, Nelson KL, Hubbard A, Eisenberg JN. 2012. Rethinking indicators of microbial drinking water quality for health studies in tropical developing countries: case study in northern coastal Ecuador. *The American journal of tropical medicine and hygiene*, 86(3), 499-507. <https://doi.org/10.4269/ajtmh.2012.11-0263>
- Merck. 2024. Fluorocult® LMX Broth Modified (110620). [accessed 2025 Apr 28]. [https://www.humeau.com/media/blfa\\_files/TC\\_Fluorocult\\_14501062054\\_EN\\_080920.pdf](https://www.humeau.com/media/blfa_files/TC_Fluorocult_14501062054_EN_080920.pdf)
- Ministerio del Ambiente, Agua y Transición Ecológica [MAATE]. Acuerdo Ministerial. [083-B; 097-A; 140]. (4 de noviembre de 2015). RO. Año III - N° 387.
- Ministerio de Salud Pública del Ecuador [MSP]. 2024. Enfermedades transmitidas por agua y alimentos, otras intoxicaciones alimentarias. Ecuador, SE52/2021. Gobierno del Ecuador. [accessed 2024 Nov 1]. <https://www.salud.gob.ec/wp-content/uploads/2025/01/ETAS-SE-52.pdf>
- Mishra M, Arukha AP, Patel AK, Behera N, Mohanta TK, Yadav D. 2018. Multi-Drug Resistant Coliform: Water Sanitary Standards and Health Hazards. *Frontiers in pharmacology*, 9, 311. <https://doi.org/10.3389/fphar.2018.00311>
- Molina CA, Quiroz-Moreno C, Jarrín-V P, Díaz M, Yugsi E, Pérez-Galarza J, Baldeón-Rojas L. 2024. Bacterial community assessment of drinking water and downstream distribution systems in highland localities of Ecuador. *Journal of Water and Health*, 22(3), 536-549. <https://doi.org/10.2166/wh.2024.290>
- Naciones Unidas Ecuador [ONU Ecuador]. 2023. Día del agua: garantizar la disponibilidad de agua y el saneamiento en la región andina. Naciones Unidas. [accessed 2024 Nov 1]. <https://ecuador.un.org/es/224762-día-del-agua-garantizar-la-disponibilidad-de-agua-y-el-saneamiento>
- Nowicki S, deLaurent ZR, de Villiers EP, Githinji G, Charles KJ. 2021. The utility of *Escherichia coli* as a contamination indicator for rural drinking water: Evidence from whole genome sequencing. *PLOS ONE*, 16(1), e0245910. <https://doi.org/10.1371/journal.pone.0245910>
- Organización de las Naciones Unidas para la Alimentación y la Agricultura [FAO]. 2011. Prevención de la *E. coli* en los alimentos. Estados Unidos: FAO. [accessed 2025 Apr 28]. [http://www.fao.org/fileadmin/user\\_upload/agns/pdf/Preventing\\_Ecoli\\_es.pdf](http://www.fao.org/fileadmin/user_upload/agns/pdf/Preventing_Ecoli_es.pdf)
- Organización Mundial de la Salud (OMS). 2006. Directrices para el uso seguro de aguas residuales, excrementos y aguas grises - Volumen 4. [Internet]. [accessed 2025 Apr 28]. Available from: <https://www.who.int/publications/i/item/9241546859>
- Petri WA Jr, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. 2008. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest.* 118(4):1277-1290. <https://doi.org/10.1172/JCI34005>
- Prieto Sierra D. 2017. Análisis microbiológico de aguas naturales. [Tesis de pregrado]. Universidade da Coruña. [accessed 2025 Apr 28]. Available from: [https://ruc.udc.es/dspace/bitstream/handle/2183/19635/PrietoSierra\\_Daniel\\_TFG\\_2017.pdf](https://ruc.udc.es/dspace/bitstream/handle/2183/19635/PrietoSierra_Daniel_TFG_2017.pdf)
- Rabiu AG, Falodun OI, Fagade OE, Dada RA, Okeke IN. 2022. Potentially pathogenic *Escherichia coli* from household water in peri-urban Ibadan, Nigeria. *J Water Health.* 20(7):1137-1149. <https://doi.org/10.2166/wh.2022.117>
- Ríos-Tobón S, Agudelo-Cadavid RM, Gutiérrez-Builes LA. 2017. Patógenos e indicadores microbiológicos de calidad del agua para consumo humano. *Rev Fac Nac Salud Pública.* 35(2):236-247. <https://doi.org/10.17533/udea.rfnsp.v35n2a08>

- Risebro HL, Breton L, Aird H, Hooper A, Hunter PR. 2012. Contaminated small drinking water supplies and risk of infectious intestinal disease: a prospective cohort study. *PLoS One*. 7(8):e42762. <https://doi.org/10.1371/journal.pone.0042762>
- Salazar Coello VG, Viteri Poveda CE, Suarez Camacho LA. 2013. Características físicas, químicas y microbiológicas del agua de consumo en comunidades de la Parroquia Manglaralto. *RECIAMUC*. 2(1):690-713. [accessed 2025 Apr 28]. Available from: <https://reciamuc.com/index.php/RECIAMUC/article/view/50/48>
- Sánchez Aroca SA, Guangasig Toapanta VH. 2023. Calidad microbiológica del agua de consumo humano: la realidad en el Ecuador. *REDILAT*. 4(2):2789-3855. <https://doi.org/10.56712/latam.v4i2.690>
- Secretaria de Comercio y Fomento Industrial. 1999. Análisis de agua - determinación de huevos de helminto - método de prueba (113). [accessed 2025 Apr 28]. Available from: <https://biblioteca.semarnat.gob.mx/janium/Documentos/Ciga/agenda/PPD02/DO103.pdf>
- Smith JL, Fratafico PM, Gunther NW IV. 2014. Shiga toxin-producing *Escherichia coli*. *Adv Appl Microbiol*. 86:145-197. <https://doi.org/10.1016/B978-0-12-800262-9.00003-2>
- Terashima A, Marcos L, Maco V, Canales M, Samalvides F, Tello R. 2009. Técnica de sedimentación en tubo de alta sensibilidad para el diagnóstico de parásitos intestinales. *Rev Gastroenterol Perú*. 29(4):305-310. [accessed 2025 Apr 28]. Available from: [http://www.scielo.org.pe/scielo.php?script=sci\\_arttext&pid=S1022-51292009000400002](http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1022-51292009000400002)
- Tribunal Latinoamericano del Agua. 2024. Situación hídrica en América Latina. [Internet]. [accessed 2024 Nov 1]. Available from: <https://tragua.org/situacion-hidrica-en-america-latina/>
- United Nations. 2023. Water and sanitation. United Nations Sustainable Development. [Internet]. [accessed 2024 Nov 1]. Available from: <https://www.un.org/sustainabledevelopment/water-and-sanitation/>
- Vega Herrera JO. 2019. Calidad microbiológica del agua potable de acuerdo a la normativa ecuatoriana NTE INEN 11.08:2014. [Tesis de grado]. Universidad Técnica de Machala. [accessed 2025 Apr 28]. Available from: <http://repositorio.utmachala.edu.ec/handle/48000/14746>
- Vinueza D, Ochoa-Herrera V, Maurice L, Tamayo E, Mejía L, Tejera E, Machado A. 2021. Determining microbial and chemical contamination in Ecuador's main rivers. *Sci Rep*. 11:17640. <https://doi.org/10.1038/s41598-021-96926-z>
- Wells J, Abugo DG, Angong J, Lamwaka NG, Gallandat K, Hassan JL, Deng L, Save D, Braun L, Gose M, Amanya J, Ayoub K, King S, Stobaugh H, Cumming O, D'Mello-Guyett L. 2023. Risk factors for food contamination among children discharged from community management... *Maternal Child Nutr*. 20:e13612. <https://doi.org/10.1111/mcn.13612>