Seasonal and Spatial Variations in the Presence of Giardia and Cryptosporidium in Rural Drinking Water Supply Systems in Different Municipalities of Antioquia, Colombia

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Abstract This study evaluated the seasonal and spatial variations in the presence of Giardia and Cryptosporidium in rural drinking water supply systems of different municipalities of Antioquia. The municipalities evaluated were Envigado, Caldas, Sabaneta, La Estrella, Itagüí, Bello, Barbosa, Copacabana, and Girardota, located in the department of Antioquia, Colombia. The experimentation was carried out over 9 sampling campaigns in the period between July 2019 and November 2020. This period encompassed the two seasons presented in Colombia: dry and wet. Each municipality included in this study has a conventional basic drinking water treatment system (DWTS) which includes sand trap, rapid filtration, and chlorination. The results showed good removal efficiencies of Cryptosporidium and Giardia in the DWTS evaluated. However, evaluation of other characteristics of water quality and of the water quality risk index for human consumption (IRCA) showed concerning water quality conditions in the rural drinking water systems supply. The prevailing risk levels of the drinking water are medium, high, or non-viable sanitary, which means the population is supplied with water that does not meet the minimum quality criteria established by Colombian regulations.

Keywords Environmental engineering · Environmental sciences · Microbiology · Sanitation · Water quality · Water treatment

1 Introduction

Water is a determining factor in public health, as it directly influences human health (Pascual-Benito et al., 2020). Aspects such as the deterioration of water quality and lack of access to water for human consumption have been associated with infectious and parasitic diseases. Children under 5 years of age are those most affected by diarrheal diseases in the world (Presidencia de la República de Colombia, 2004), and in Colombia, a high correlation has been found between infant mortality and water quality. Therefore, the country-wide availability of safe drinking water is fundamental, because this would reduce diarrhea cases by up to 45%, in turn reducing infant mortality.

Cryptosporidiosis and giardiasis are human gastrointestinal diseases caused by the pathogenic protozoa Cryptosporidium parvum and Giardia lamblia (Bing-Mu & Hsuan-Hsien, 2003). The most common symptoms of both diseases are abdominal pain, nausea, and diarrhea (Salamandane et al., 2021). Researchers have found that the main forms of direct transmission of cysts or oocysts are the fecal–oral
route and the consumption of untreated water, while the main indirect route is the consumption of food washed with contaminated water (González-Ramírez et al., 2021; LeChevallier & Norton, 1995; Ramo et al., 2017; Salamandane et al., 2021). The source of oocysts and cysts includes human sewage and feces from many species of mammals and reptiles (Spano et al., 1998).

The presence of oocysts and cysts in drinking water is a critical issue that threatens successful achievement of objective 6 of the Global Goals for Sustainable Development, “clean water for all.” This is mainly because Giardia cysts can survive up to 24 days in the environment, while Cryptosporidium oocysts remain viable for up to 6 months (Alum et al., 2014). This means that both oocysts and cysts can survive in the environment. Another important issue is that they are resistant to conventional disinfection with chlorine (Omarova et al., 2018; Spano et al., 1998). Studies have shown that the time required for inactivation of 90% of Giardia cysts in water is 25–30 min with a free residual chlorine concentration of 1 mg/L, while for Cryptosporidium oocysts, very low or zero effects of chlorination have been documented.

Despite the efforts of government agencies to increase basic sanitation coverage and access to drinking water in Colombia, there remain large gaps in these areas between urban and rural communities. While an adequate supply of drinking water can be found in urban areas, this is not the case in rural areas. Many rural communities have either inadequate or non-existent drinking water treatment systems.

The failings of these drinking water treatment systems are a huge issue that puts the health and well-being of the population at risk, especially in the context of a high deterioration of quality in surface waters. Therefore, there is an imperative need to improve water quality. To achieve this, it is necessary to identify, evaluate, and manage the risk of water for human consumption through intersectoral actions and continuous monitoring of microbiological and physicochemical characteristics, to ensure that water quality is consistent with the potability values established in the regulations.

Due to the susceptibility of water sources to contamination with Giardia and Cryptosporidium as a result of their passage through areas containing cattle or other animals, as well as of the lack of efficiency of the drinking water treatment plants, Giardia and Cryptosporidium may be present for some periods of the year in the drinking water supply systems in Medellín (Betancourt & Rose, 2004). However, studies determining the level of water contamination by these two protozoa in Medellín have not been carried out. Therefore, the objective of this study was to evaluate the occurrence of Giardia and Cryptosporidium in the main rural drinking water supply systems of Medellín, and the relationship of these with land uses and the deterioration of water quality.

2 Materials and Methods

2.1 Study Area and Sampling

The study area was made up of the rural zones of the following 10 municipalities: Medellín, Envigado, Caldas, Sabaneta, La Estrella, Itagüí, Bello, Barbosa, Copacabana, and Girardota, located in the department of Antioquia, Colombia. Twenty sampling points (SPs) were selected (Fig. 1).

The selection of these sampling points was made taking into account that the water was used for human consumption and that it was subject to some type of treatment. Table 1 presents the summary of each sampling point and the description of the drinking water treatment system.

2.2 Sampling Schedule

Assessment of the occurrence of Giardia and Cryptosporidium in the rural drinking water supply systems of Medellín was carried out through 9 sampling campaigns, in the period between July 2019 and November 2020. Colombia, due to its geographical location, has two seasons that can be clearly defined in the year, which are winter (wet season) and summer (dry season). Therefore, sampling campaigns were scheduled with the aim of covering both seasons of the year, dry and wet, to evaluate the influence of this environmental factor on the presence of intestinal parasites. The dry and wet seasons were selected based on the reports given of the study area by the meteorological stations of the Institute of Hydrology, Meteorology, and Environmental Studies (IDEAM). Water samples were taken in the influent and effluent of the
drinking water treatment system, and the sanitary and environmental conditions and land uses of the study area were evaluated.

### 2.3 Chemicals and Analytical Methods

Tests for pH, conductivity, turbidity, and free chlorine were done in the laboratory of the research group known as the Pollution Control and Diagnosis group (GDCON), at the University of Antioquia, following protocols established in the Standard Methods (APHA, 2017). *Total coliforms* and *E. coli* were evaluated according to ISO 9308–1, and enterococci according to SM 9230. *Cryptosporidium* and *Giardia* were evaluated according to the EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. The GDCON laboratory is accredited by the Environmental Research Institute under the Ministry of Environment of Colombia (IDEAM) to carry out these analyses.

### 2.4 Quality Assurance and Quality Control

The sampling of drinking water was carried out based on ISO 5667–5 (2006)- Water Quality – Sampling, part 5: Guidelines for Sampling Drinking Water from Treatment Facilities and Pipeline Distribution System; and ISO 19458 (2006)- Water Quality: Sampling for Microbiological Analysis. Sampling of raw water was carried out based on the guidelines of ISO 5667–6 (2014)- Water Quality—Sampling, part 6: Guidance on Sampling Rivers and
2.5 Water Quality Risk Index for Human Consumption

The water quality risk index for human consumption, also known by its acronym in Spanish, IRCA, is a basic instrument to ensure quality standards for water for human consumption established by Colombian health regulation. Through this basic instrument, it is possible to evaluate the main physical, chemical, and microbiological characteristics of drinking water. Each of these characteristics has a weight on the calculation of the index value according to their respective risks to human health. This study evaluated 5 parameters, with the following weights on the index value: pH (1.5), free chlorine (15), turbidity (15), total coliforms (15), and $E.\ coli$ (25).

The IRCA index for each sample can be calculated through the following expression:

$$\text{IRCA(\%)} = \frac{\sum \text{risk score assigned to unacceptable characteristics}}{\sum \text{risk score assigned to all the characteristics analyzed}} \times 100$$  \hspace{1cm} (1)

The classification of the risk level of water supplied for human consumption through the IRCA index is defined as follows: 80.1–100% (risk: non-viable for sanitary reasons—water not suitable for human consumption); 35.1–80 (risk: high—water not suitable for human consumption); 14.1–35 risk: medium—water not suitable for human consumption); 5.1–14 (risk: low—water not suitable for human consumption); 0–5 (risk free—water suitable for human consumption).

3 Results and Discussion

3.1 Analyses of Environmental and Sanitary Conditions in the Study Area Associated with the DWTS (Each Sampling Point)

Evaluation of the risk factors and the vulnerability conditions of the natural sources of water supply...
was carried out through visual inspection visits. This fieldwork was focused on the following 3 main aspects (Table 2): main type of source, land usage, and organoleptic perception of water. The purpose was to identify the main factors of contamination that could favor the presence of *Cryptosporidium* and *Giardia* in the water.

The results of the visual inspection visits indicated that natural water sources are of the surface type. This means that there may be a greater probability of the presence of the study parasites. This probability is increased due to the fact that water surfaces are directly impacted by the present land use and/or the anthropogenic activities carried out on the land surface. According to Morales-Mora et al. (2022), the surface water could be affected by microbiological pollutants causing diseases. In this way, the urban, agricultural, or livestock uses in soils near water bodies can lead to their contamination with fecal matter.

Eleven kinds of land use were evaluated, taking into account the most common activities carried out in the study area. Table 2 shows how these uses are related to each evaluated sampling point. Households, septic tanks in the study area, and livestock production, which are the land uses related to a greatest number of sampling points, could impact water quality conditions and can be a source of *Cryptosporidium* and *Giardia* contamination in the water. Regarding these parasites, other studies indicated that *Giardia* cysts and *Cryptosporidium* oocysts could be easily found in the surface raw water samples (Bing-Mu & Hsuan-Hsien, 2003). Therefore, it is important to ensure the drinking water treatment system is composed of the suitable treatment stages, including effective source water protection, water treatment, and final distribution. Furthermore, the organoleptic perception of water was evaluated in fieldwork. The perception of oily feel occurred in 5 sampling points, results that could be related to wastewater discharges sources into the natural water.

### 3.2 Physicochemical and Microbiological Analysis of the Drinking Water Quality for Each of the Sampling Points in the Dry and Wet Seasons

Nine parameters of water quality (pH, temperature, free chlorine, turbidity, *fecal coliforms*, *E. coli*, and *Enterococcus*) were evaluated in the drinking water supply in each sampling point during the 9 sampling campaigns. The summary of the results for these characteristics is presented in Table 3. Turbidity is a parameter regulated by Colombian legislation as it can be related to the alteration of microbiological parameters. High turbidity values can affect the chlorination process, because, inside the particulate material, microorganisms can pass unchanged through the chlorination process, reducing the power of the disinfectant agent used. Table 3 shows sampling points with turbidity values above 2 NTU, in which the values for microbiological parameters are highly altered. SPs 2, 9, 10, 13, 16, 17, and 20 present the highest values of microbiological parameters such as *E. coli* and *Enterococcus*, which indicates a high level of contamination by fecal matter in the drinking water supply system.

In the same context, the free residual chlorine values, which must vary between 0.3 and 2.0 mg/L at any point in the distribution network of the drinking water system, are low for the same SPs that present high values for the microbiological parameters. Even for SP 20, there is no available free residual chlorine concentration in the water. These results could be compared with the reported by Rodriguez-Alvarez et al. (2017). They identified that when there is a low concentration of residual chlorine in treatment water, there is a higher probability of finding indicator bacteria. At the same time, in the case of surface water, they highlighted that the potabilization treatment must consider the chlorination process and the level of turbidity as a critical control point, since high turbidity leads to more ineffective disinfection and, consequently, to a high bacterial load.

Seasonality had a significant effect on the quality of drinking water analyzed in each of the sampling points corresponding to a particular drinking water treatment system. From the results found, it is possible to infer that there are difficulties in the adjustment and control of the water treatment processes when there is no precipitation. It could be expected for runoff to be favored during precipitation events and with this the number of pollutants that can reach water sources. Contrary to this expectation, in the dry season, it is possible to find a major concentration of the pollutants that reach the surface water through wastewater discharges, significantly affecting water quality and consequently the drinking water treatment process.
Table 2  Permanent environmental and sanitary conditions found in the study area associated with the sampling points

| Sampling points | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-----------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|
| Type of source  | Surface water | groundwater | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Land usage      | No nearby anthropic use | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Households      | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Discharges      | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Septic tanks in the study area | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Poultry production | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Pig production  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Livestock production | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Deforestation   | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Crops (coffee, onion, among others) | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Recreational    | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Organoleptic perception of water | Unpleasant smell | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
|                 | Oily feel       | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
Table 3: Summary of the evaluation of the physicochemical and microbiological quality of drinking water for each of the sampling points in the dry and wet seasons

| Point | pH | Temperature °C | Free chlorine Mg Cl/L | Turbidity NTU | Conductivity μS/cm | Fecal coliforms CFU/mL | E. coli CFU/mL | Enterococcus CFU/mL |
|-------|----|----------------|------------------------|---------------|-------------------|------------------------|---------------|---------------------|
|       | Dry | Wet           | Dry   | Wet           | Dry   | Wet           | Dry   | Wet           | Dry   | Wet           | Dry   | Wet           |
| SP1   | 7.8±0.3 | 7.4±0.3 | 21±2 | 24±3         | 1.0±0.4 | 1.6±0.7 | 1.6±1.0 | 1.6±0.6 | 155±32 | 121±14 | 6±5 | 8±8 | 1±0.8 | 1±1 | 0±0 | 0±0 |
| SP2   | 7.9±0.2 | 7.7±0.3 | 21±2 | 19±1         | 0.5±0.4 | 2.3±1.5 | 2.3±0.7 | 2.1±0.3 | 227±206 | 166±68 | 30±19 | 8±5 | 6±10 | 1±1 | 0±1 | 1±1 |
| SP3   | 7.7±0.5 | 7.5±0.2 | 22±1 | 24±1         | 1.1±0.4 | 1.4±0.5 | 1.2±0.7 | 1.6±1.0 | 122±29 | 87±21 | 9±6 | 13±12 | 3±3 | 0±0 | 0±0 | 0±0 |
| SP4   | 7.9±1.0 | 8.7±0.8 | 22±1 | 24±2         | 0.6±0.7 | 0.3±0.6 | 1.7±0.2 | 1.2±0.3 | 229±177 | 141±113 | 17±13 | 19±12 | 2±2 | 4±5 | 0±0 | 0±0 |
| SP5   | 7.3±0.5 | 7.4±0.5 | 22±2 | 22±3         | 1.0±0.4 | 1.6±0.8 | 0.9±0.4 | 1.4±0.7 | 118±103 | 104±80 | 48±8 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP6   | 7.5±0.4 | 7.9±0.6 | 23±3 | 21±1         | 1.5±0.9 | 2.3±1.8 | 0.9±0.4 | 1.3±0.9 | 24±12 | 69±22 | 27±22 | 40±38 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP7   | 7.4±0.4 | 7.6±0.5 | 20±2 | 24±3         | 1.3±0.6 | 1.8±1.3 | 0.7±0.3 | 1.2±0.6 | 135±79 | 106±81 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP8   | 7.7±0.5 | 7.6±0.8 | 23±3 | 24±4         | 1.5±0.9 | 1.9±1.2 | 1.0±0.9 | 2.7±1.9 | 36±15 | 138±4 | 0±0 | 0±3 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP9   | 7.7±0.3 | 7.6±0.1 | 22±2 | 24±2         | 0.3±0.4 | 1.7±1.2 | 4.8±2.7 | 5.1±0.6 | 110±16 | 134±20 | 759±66 | 543±488 | 100±109 | 62±76 | 24±17 | 28±26 |
| SP10  | 7.7±0.3 | 7.6±0.4 | 22±2 | 20±1         | 1.9±0.8 | 1.8±0.6 | 13.9±10.1 | 1.6±6.8 | 274±209 | 136±115 | 117±11 | 146±174 | 10±18 | 5±10 | 9±19 | 21±36 |
| SP11  | 7.7±0.5 | 7.6±0.2 | 25±3 | 24±2         | 1.5±0.8 | 2.5±0.8 | 0.7±0.9 | 1.3±0.5 | 39±17 | 38±12 | 9±2 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP12  | 7.7±0.7 | 7.6±0.2 | 22±4 | 24±3         | 1.5±0.4 | 0.8±0.7 | 0.9±0.7 | 1.2±0.5 | 37±6 | 51±11 | 0±0 | 0±0 | 2±0 | 0±0 | 2±3 | 0±0 |
| SP13  | 7.7±0.5 | 7.6±0.2 | 23±3 | 22±2         | 0.8±0.4 | 1.0±0.3 | 2.7±2.4 | 1.2±0.4 | 97±60 | 56±36 | 85±7 | 207±265 | 13±0 | 39±57 | 0±0 | 0±0 |
| SP14  | 7.7±0.8 | 7.6±0.4 | 22±2 | 24±4         | 1.4±0.6 | 1.4±0.3 | 1.2±0.9 | 1.5±0.6 | 128±107 | 160±84 | 4±2 | 5±7 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP15  | 7.7±0.4 | 7.6±0.1 | 23±2 | 25±3         | 0.6±0.6 | 1.1±0.9 | 1.6±0.7 | 1.2±1.0 | 209±109 | 191±48 | 2±2 | 61±102 | 0±0 | 8±14 | 0±4 | 24±41 |
| SP16  | 7.7±0.4 | 7.6±0.1 | 24±3 | 25±3         | 1.2±1.0 | 1.9±1.0 | 2.8±1.9 | 2.4±0.5 | 143±69 | 134±41 | 23±4 | 2±4 | 5±11 | 0±1 | 2±5 | 0±0 |
| SP17  | 7.7±0.4 | 7.6±0.5 | 24±2 | 23±1         | 0.7±0.7 | 2.9±2.4 | 3.9±0.9 | 4.7±0.9 | 157±109 | 153±58 | 158±78 | 927±748 | 8±13 | 12±16 | 3±8 | 14±23 |
| SP18  | 7.7±0.3 | 7.6±0.1 | 24±1 | 25±4         | 1.1±0.9 | 0.4±2.3 | 0.9±0.7 | 1.6±0.6 | 153±92 | 125±36 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP19  | 7.7±0.4 | 7.6±0.6 | 23±3 | 25±3         | 0.7±0.2 | 0.9±0.7 | 1.6±0.6 | 2.3±1.0 | 137±135 | 116±47 | 0±0 | 8±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| SP20  | 7.7±0.4 | 7.6±0.4 | 25±2 | 24±1         | 0.0±0.0 | 0.0±0.0 | 12.7±10.2 | 6.1±3.3 | 77±32 | 56±20 | 1498±617 | 1750±678 | 220±174 | 131±92 | 111±63 | 123±76 |
Other parameters such as pH and conductivity presented adequate values for drinking water despite the alterations analyzed in the other evaluated variables.

### 3.3 Occurrence of *Cryptosporidium* and *Giardia* in Raw and Drinking Water

One of the major challenges of rural drinking water treatment is the removal of *Cryptosporidium* and *Giardia*. The basic nature of the treatment systems used added to the low level of economic investment in water treatment contributes to a great public health risk for vulnerable populations. In this study, the evaluation of *Cryptosporidium* and *Giardia* occurrence in raw and drinking water indicated that DWTS is an important barrier in preventing the spread and inadvertent ingestion of parasites through drinking water.

Seasonality did not have a strong effect on *Cryptosporidium* and *Giardia* occurrence. It was possible to find a slight increase in their presence in raw water during the wet season (Figs. 2 and 3), but the efficiency of the treatment systems was effective in removing them significantly. These results are different with those reported by Morales-Mora et al. (2022). They found that seasonality had a great relevance in his research, identifying in climatic times with high rainfall a behavior of low presence for *Cryptosporidium* sp. and *Giardia* sp., although they emphasize that, due to the low information of historical data, these results cannot be conclusive.

The efficiencies for parasite removal are shown in Figs. 2 and 3. The control and maintenance of treatment units could be a cause for concern with regard to the contamination of the water. To ensure acceptable drinking water quality through treatment systems, the filters must be washed with a certain periodicity. This is because the water may become contaminated when passing through the filter if it is clogged or has had limited maintenance. This condition could alter the microbiological parameters.

### 3.4 Human Health Risk Assessment

The water quality risk index for human consumption (IRCA) was evaluated for each DWTS (Table 4). According to the IRCA classification risk, the results

![Fig. 2](image-url) *Cryptosporidium* and *Giardia* occurrence in the dry season: average count in raw water and efficiency of each drinking water treatment system in the *Cryptosporidium* and *Giardia* removal

![Fig. 3](image-url) *Cryptosporidium* and *Giardia* occurrence in the wet season: average count in raw water and efficiency of each drinking water treatment system in *Cryptosporidium* and *Giardia* removal
indicated a critical state of water conditions in the SP evaluated, in both the dry and wet seasons. The prevailing risk levels were medium, high, or non-viable for sanitary reasons in the drinking water.

The evaluation of this basic instrument showed cause for concern regarding water quality conditions in the rural drinking water systems supply. The population is supplied with water that does not meet the minimum quality criteria established by Colombian regulations.

On the other hand, a relationship was identified between sampling points that have critical sources of pollution such as households, septic tanks, and livestock production, which have the highest index risk values. This scenario shows that there is limited control by environmental authorities of the management of wastewater and its disposal in natural water bodies. The high deterioration in water quality and the low efficiency of the limited treatment systems used put the health of the population at risk.

### 4 Conclusions

The sampling campaigns in 20 locations in the 10 municipalities of the metropolitan area of Antioquia presented different levels of risk as defined by IRCA for water delivered for human consumption. Medium, high, and non-viable for sanitary reasons levels were the most common criteria found. Seasonality did not have a strong effect on Cryptosporidium and Giardia occurrence in the water, having a more visible effect on the parameters turbidity, fecal coliforms, and E. coli, which presented higher values in the dry season than in the wet season. On the other hand, despite the simplicity of the DWTSs, they showed good efficiencies in removing Cryptosporidium and Giardia from the water. This behavior on the part of DWTSs means that it is worthwhile to continue working on the improvement and optimization of their physical and chemical processes in order to improve results for the parameters that do not yet meet regulatory criteria.

Finally, the main results of this study showed that it is important for government agencies to increase their efforts in supporting and strengthening existing aqueduct systems, in order to guarantee the right of the population to drinking water.

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**Table 4** Evaluation of the risk index of water quality for human consumption

| Sampling points | IRCA average | Risk classification | IRCA average | Risk classification |
|-----------------|--------------|---------------------|--------------|---------------------|
| SP1             | 51.2%        | High risk           | 38.5%        | High risk           |
| SP2             | 78.5%        | High risk           | 88.5%        | Non-viable sanitary |
| SP3             | 49.0%        | High risk           | 49.0%        | High risk           |
| SP4             | 44.3%        | High risk           | 67.5%        | High risk           |
| SP5             | 14.0%        | Low Risk            | 10.5%        | Low Risk            |
| SP6             | 18.0%        | Medium Risk         | 49.0%        | High risk           |
| SP7             | 6.0%         | Low Risk            | 10.5%        | Low Risk            |
| SP8             | 12.0%        | Low Risk            | 21.0%        | Medium Risk         |
| SP9             | 92.2%        | Non-viable sanitary | 87.4%        | Non-viable sanitary |
| SP10            | 93.2%        | Non-viable sanitary | 76.9%        | High risk           |
| SP11            | 18.0%        | Medium Risk         | 21.0%        | Medium Risk         |
| SP12            | 60.1%        | High risk           | 100.0%       | Non-viable sanitary |
| SP13            | 66.9%        | High risk           | 5.9%         | High risk           |
| SP14            | 26.3%        | Medium Risk         | 10.5%        | Low Risk            |
| SP15            | 32.3%        | Medium Risk         | 0.0%         | Risk free           |
| SP16            | 29.0%        | Medium Risk         | 10.5%        | Low Risk            |
| SP17            | 33.9%        | High risk           | 59.4%        | High risk           |
| SP18            | 9.0%         | Low Risk            | 10.5%        | Low Risk            |
| SP19            | 22.3%        | Medium Risk         | 11.5%        | Low Risk            |
| SP20            | 95.8%        | Non-viable sanitary | 97.9%        | Non-viable sanitary |
Data Availability  The datasets generated during and analyzed during the current study are available from the authors on reasonable request.

Competing Interests  The authors declare no competing interests.

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