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Microbiological Water Quality Assessment of Chambo Based on the Inen 1108 Standard

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ABSTRACT
This study aimed to assess the quality of drinking water in the Chambo canton, focusing on the detection of pathogenic bacteria that may pose a risk to public health. A total of 50 water samples were collected from strategic locations throughout the area and analyzed using selective culture media and conventional Polymerase Chain Reaction (PCR), a technique that enables precise molecular identification of microorganisms. Specific primers were used for each target bacterium (Listeria monocytogenes, Escherichia coli O157:H7, and Salmonella spp.), along with positive controls to validate the results. The analysis revealed a concerning presence of pathogens: Listeria monocytogenes was detected in 40% of the samples, Escherichia coli O157:H7 in 80%, and Salmonella spp. in 20%. These findings indicate a significant level of microbiological contamination in the drinking water consumed by the population of Chambo. The purpose of this research is to raise awareness about the health risks associated with contaminated water and to emphasize the urgent need for measures that improve water quality in the area, thereby reducing the population's exposure to waterborne diseases.
KEYWORD: *PCR, Water Quality, Pathogens.*

OBJECTIVE

The objective of this research is to determine the quality of water for human consumption in the Chambo canton, using molecular techniques that provide a reliable result and future decision-making in the face of possible threats.

INTRODUCCION

Water is essential for life on Earth and for carrying out all our daily activities. Our planet holds about 1.4 billion cubic kilometers of water, an enormous amount. However, the vast majority around 97.5% is salty and found in the oceans and seas, so it's not directly available for human consumption [(1).]
Freshwater, which is vital for human consumption and the activities we carry out on land, makes up only about 2.5% of all the water on Earth [(2)]. Of this freshwater, the majority exists in the form of ice in polar ice caps, glaciers, and ice sheets, accounting for approximately 68.7%. Groundwater, found in aquifers and underground reservoirs, makes up about 30.1%. The remaining freshwater can be found in lakes, rivers, soil moisture, and in the atmosphere [(3).]

In Chambo canton, located in the Chimborazo province, water is used both for daily human consumption and for irrigation. However, the conditions from the water sources to the reservoirs and distribution system are not ideal. As water flows through channels, streams, and springs, it is at risk of contamination due to the presence of animals such as cows, horses, and even dead animals near the water sources. These animals often come to the channels to drink and unfortunately also to defecate, which contaminates the water and promotes the growth of harmful bacteria and other microorganisms. This contamination poses a serious health risk to those who consume the water, potentially causing severe

illnesses and even threatening lives.

Detecting pathogens in drinking water is of great importance to ensure safety and public health [4]. The presence of pathogenic microorganisms in water intended for human consumption can lead to waterborne disease outbreaks and pose a significant risk to the population [5].

The molecular biology technique known as Polymerase Chain Reaction (PCR) is a widely used and effective tool for detecting pathogens in samples of water for human consumption. PCR allows for the selective amplification and detection of specific DNA fragments from pathogens, even at low concentrations [6].

Hence, the general objective of the current research was to detect pathogens in water for human consumption using the PCR technique. By conducting analyses through PCR, reliable data can be obtained to make accurate assessments of the degree of pathogenic contamination that may be present in drinking water. This information can, in turn, guide appropriate corrective actions in the event of biological contaminants being detected.

MATERIALS AND METHODS

Study Area: The present study is descriptive, experimental, and cross-sectional in nature. It was conducted from January to September 2023, focusing on the probability of the presence of pathogens such as Salmonella spp., Listeria spp., E. coli, and Listeria in 50 samples of drinking water. These samples were collected from 50 strategic points, with 10 samples from each of the North, South, East, West, and Central areas of the Chambo canton, ranging from the sources of human drinking water to households [7].

Georeferencing: This method allowed for the precise or relative location of different sampling points. Coordinates were determined using the GPSMAP 64sx, which provided the necessary data to be processed in the ARcGIS program, resulting in a digital map [8].

Sampling Technique: The collection of samples of drinking water was performed using sterile polyethylene food-grade materials, properly labeled. Strict cold chain procedures were maintained during transportation, and samples were isolated from natural elements [9].

Sample Preparation and Initial Cultivation: For each sample, 100 mL of drinking water were subjected to suction filtration through a filter paper (SKU: 09 790 2E). Once the suction process was complete, the filter paper was removed and brought into contact with specific media for the detection of pathogens: Salmonella spp. (XLD AGAR ISO 9001:2015 M4J2BV01 from TM Media), Listeria spp. (CHROMOGENIC LISTERIA AGAR BASE (Modified) M1GD4HV01 ISO 9001: 2015.ISO 11133:2014 from TM Media), and E. coli (EMB AGAR, LEVINE M3H1FV01 (ISO 21150:2015) from TM Media. The culture plates were incubated at a temperature of 35°C for 24 hours. After this time, colony counts and isolation were performed [10].

Confirmation by Microscopy: Plates that showed microbial growth were selected for Gram staining. By observing the morphology of the pathogen under a microscope, it was determined whether it was Gram-positive or Gram-negative [11].

DNA Extraction: Approximately 5 colonies of the pathogen were suspended in 500 µL of 1X TAE buffer. The suspension was then centrifuged at 16,000 rpm for 10 minutes at room temperature. For DNA extraction of Listeria spp, E. coli, and both Gram-negative and Gram-positive Salmonella spp, the PurelinkTM Genomic DNA Mini Kit, K182001 [12], was used.

DNA Yield: To determine the DNA yield, a UV-Vis microvolume spectrophotometer, such as the Thermo ScientificTM NanoDropTM One/One, was utilized. It measures the concentration and purity of the samples, with measurements conducted on DNA concentrations from 1 to 1.5 µL, and each sample's purity presented optimal percentages [13].

Preparation of E. coli Samples: A solution for E. coli, consisting of R Reverse (5'-GCTATTTCCTGCCGATAAGAGA-3') with a concentration of 39.45 nmol and F Forward (5'-CCAGGCAAAGAGTTTATGTTGA-3') with a concentration of 37.39 nmol, was prepared as a working solution at a concentration of 10 µmoL. Subsequently, for the preparation of the total sample solution, Nuclease-Free Water was added according to the nmol concentration. A 1.5 mL Eppendorf tube was used, and 5 µL of the R primer were added, followed by topping up with 45 µL of Nuclease-Free Water, resulting in a total solution of 50 µL that recognizes the genus Escherichia coli with its serotype O157:H7 in 212 bp [14].

Sample Preparation for Listeria monocytogenes: The solution for Listeria, based on R Reverse (5'-GCCGTCGATGATTTGAACTTCATC-3') with a concentration of 41.88 nmol and F Forward (5'-GAATGTAAACTTCGGCGCAATCAG-3') with a concentration of 42.05 nmol, was prepared as a working solution at a concentration of 10 µmoL. Nuclease-Free Water was added according to the nmol concentration. Using a 1.5 mL Eppendorf tube, 5 µL of the R primer were added, and it was then filled up with 45 µL of Nuclease-Free Water, resulting in a total solution of 50 µL that recognizes the genus Listeria monocytogenes in 388 bp [15].

Sample Preparation for Salmonella spp. in Thermal Cycles: The solution for Salmonella, consisting of reverse invaA3R (5'-TCCATCAAATTAGCGGAGGC-3') with a concentration of 41.05 nmol and forwar inva3F (5'-AACGTGTTTCCGTCGTAAT-3') with a concentration of 52.15 nmol, was prepared as a final concentration of 10 µmoL. Nuclease-Free Water was added. Using a 1.5 mL Eppendorf tube, 5 µL of the R primer were added, and it was then filled up with 45 µL of Nuclease-Free Water, resulting in a total solution of 50 µL that recognizes the genus Salmonella spp. in 244 bp [16].

Table 1 Cebadores utilizados para realizar la PCR.

Bacteria	Sequence	Base pairs bp	References
Listeria monocytogenes	(R,5'-GCCGTCGATGATTTGAACTTCATC-3') (F,5'-GAATGTAAACTTCGGCGCAATCAG-3')	388 bp	(15)
Escherichia coli con su serotipo O157:H7	(R,5'-GCTATTTCCTGCCGATAAGAGA-3') (F,5'-CCAGGCAAAGAGTTTATGTTGA-3')	212 bp	(17)
Salmonella spp.	invaA3R (R,5'-TCCATCAAATTAGCGGAGGC-3') inva3F (F,5'-AACGTGTTTCCGTCGTAAT-3')	244 bp	(16)

Reagents for PCR

In the following tables, the quantities of reagents for use in PCR amplification of DNA obtained from three different microorganisms, namely Listeria monocytogenes, Escherichia coli, and Salmonella spp, are provided. The GoTaq® Green Master Mix, 2X, already contains a 2X solution ready for use. It includes DNA polymerase (GoTaq®), dNTPs, reaction buffer, MgCl, and blue and yellow colorants.

Table 2 PCR Reagents for the Studied Pathogens

Reactives	Volume uni.	Listeria monocytogenes # of Samples	E. coli # of Samples	Salmonella spp. # of Samples
GoTaq® Green Master Mix, 2X	12.5 µL			
Forwar primer,10µM	0.25-2.5µL			
River, 10µM	0.25 2.5µL	20	40	10
DNA Templade	1-5 µL			
Nuclease-Free Wáter to	25 µL			
Total, Volume		830 /20=41,5 µL	1660 /40=41,5 µL	415 /10=41,5 µL

Note: Research Work.

Cycles for the Studied Pathogens in the Thermal Cycler

The cycles were conducted in a thermal cycler (Techne FTC3/05, series TC3000 20X0.5ML, origin Spain) as outlined in Table 3.

Table 3 DNA Amplification Cycles

N°of cycles	T (°C) / T Listeria m.	T (°C) / T Salmonella spp.	T (°C) / T Escherichia coli	Faces
1	95/2 min	95/5 min	95/30 s	Denaturation
	95/1 min	95/1 min	95/20 s	Denaturation
30	53/45 s	63/45 s	53/45 s	Primer Annealing
	72/ 1 min	72/1 min	72/1min	Extension
1	72/ 7 min	72/5 min	72/2min	Extension

Note: [14].

Upon completing these steps in the thermal cycler, a program is set up to carry out the specified cycles for the determined period.

Conventional PCR Electrophoresis:

A 1.5% Agarose gel was prepared in 100 mL of 1X TAE buffer.

The gel was placed into the electrophoresis tank along with a 100 bp leader, and 5 µL of amplified DNA was added to each well.

The electrophoresis run was conducted in the tank at 135 volts for 30 minutes.

After the estimated time had passed, the gel was transferred to a staining tank, submerged in 1X TAE buffer with 1 µL of Diamond MT Nucleic Acid Dye for 15 minutes.

The results were then read and interpreted using the Safe transilluminator photo documentation system, specifically the Invitrogen Serie 12078086 transilluminator, with the aid of a UV transilluminator for visualization [18].

RESULTS:

Georeferencing of Different Sampling Points in Chambo Canton:

Table number 4 presents the georeferencing results achieved using a GPS MAP 64sx through ArcGIS software. This process allowed for the precise location of sampling points within Chambo Canton by using specific coordinates.

Figure 1: Sampling Points in the Urban Area of Chambo Canton.

Table 4 Sampling Points for Human Consumption Water in Chambo Canton.

Sampling Points	Geographic Area
P1, P2, P3, P4, P5	Northwest - Mountainous Area - Sawmill Zone and Storage Tank
P6, P7, P8, P9, P10, P11	Northwest - Mountainous Area - Springs - Storage Tank - 200 meters from Cubillin Estate
P12, P13, P14, P16	Northwest - Storage Tanks - Treatment Tanks - Distribution Tanks
P17, P18, P19, P20, P21, P22, P23, P24	Southwest - First Houses with Potable Water
P25, P26, P27, P28, P29, P30, P32, P33, P34, P50	Southwest - Manuel Zabala and Egidio Fierro Streets
P35, P36, P37, P38, P39	Southeast - Héctor Guevara and 18 de Marzo Streets
P40, P41, P42, P43, P44	Southwest - Héctor Guevara - Edelberto Bonilla -

P45, P46, P47, P49	David Parra Southwest - F Street - Chiriboga - Rocafuerte and García Moreno Streets

Table 4 displays the water sampling points in Chambo Canton, which were collected from areas in the northeastern part of the city, including springs, streams, and rivers. Additionally, in the southwestern and southeastern areas, various points were sampled where treated water is distributed for human consumption.

The microbiological results of the samples from the different sampling points in Chambo Canton

Table 5 Microbiological Results.

Code	monocytogenes Listeria	Salmonella E. Coli	Reference Unit	Analysis	Method
P1	-	+	+		
P2	+	+	+		
P3	+	+	+		
P4	-	+	-		
P5	-	+	-		
P6	+	+	+		
P7	+	+	+		
P8	+	+	+	**CFUs./**	**ISO 9001:2015**
P9	+	+	+	**100mL**	
P10	+	+	+		
P11	+	+	+		
P12	+	+	+		
P13	+	+	-		
P14	-	-	-		
P15	-	-	-		
P16	-	-	-		
P17	-	-	-		
P18	-	-	-		
P19	+	-	-		
P20	+	-	-		
P21	-	-	-		
P22	-	-	-		
P23	+	+	-		
P24	+	+	-		
P25	-	+	-		
P26	-	+	-		
P27	-	+	-		
P28	+	+	-		
P29	-	+	-		
P30	-	+	-		
P31	+	+	-		
P32	-	+	-		
P33	-	+	-		
P34	-	+	-		
P35	+	+	-		

P36	-	+	-
P37	-	+	-
P38	-	+	-
P39	-	+	-
P40	+	+	-
P41	-	+	-
P42	-	+	-
P43	-	+	-
P44	+	+	-
P45	-	+	-
P46	+	+	-
P47	-	+	-
P48	-	+	-
P49	-	+	-
P50	-	+	-

Table 5 presents the results of the sampling of drinking water from different points in Chambo Canton. These results were obtained through microbiological analyses using specific and selective media. It's worth noting that the analyses were conducted in triplicate during the research process to confirm their prevalence.

Table 6 It appears to deal with the microscopic identification and differentiation, likely based on Gram staining, following the microbiological results of the drinking water samples.

Code	**Listeria monocytogenes**	**E. Coli**	**Salmonella**
P1	-	Bacilos (-)	Bacilos (-)
P2	Bacilos (+)	Bacilos (-)	Bacilos (-)
P3	Bacilos (+)	Bacilos (-)	Bacilos (-)
P4	-	Bacilos (-)	-
P5	-	Bacilos (-)	-
P6	Bacilos (+)	Bacilos (-)	Bacilos (-)
P7	Bacilos (+)	Bacilos (-)	Bacilos (-)
P8	Bacilos (+)	Bacilos (-)	Bacilos (-)
P9	Bacilos (+)	Bacilos (-)	Bacilos (-)
P10	Bacilos (+)	Bacilos (-)	Bacilos (-)
P11	Bacilos (+)	Bacilos (-)	Bacilos (-)
P12	Bacilos (+)	Bacilos (-)	Bacilos (-)
P13	Bacilos (+)	Bacilos (-)	-
P14	-	-	-
P15	-	-	-
P16	-	-	-
P17	-	-	-
P18	-	-	-
P19	Bacilos (+)	-	-
P20	Bacilos (+)	-	-
P21	-	-	-
P22	-	-	-

P23	Bacilos (+)	-	-
P24	Bacilos (+)	Bacilos (-)	-
P25	-	Bacilos (-)	-
P26	-	Bacilos (-)	-
P27	-	Bacilos (-)	-
P28	Bacilos (+)	Bacilos (-)	-
P29	-	Bacilos (-)	-
P30	-	Bacilos (-)	-
P31	Bacilos (+)	Bacilos (-)	-
P32	-	Bacilos (-)	-
P33	-	Bacilos (-)	-
P34	-	Bacilos (-)	-
P35	Bacilos (+)	Bacilos (-)	-
P36	-	Bacilos (-)	-
P37	-	Bacilos (-)	-
P38	-	Bacilos (-)	-
P39	-	Bacilos (-)	-
P40	Bacilos (+)	Bacilos (-)	-
P41	-	Bacilos (-)	-
P42	-	Bacilos (-)	-
P43	-	Bacilos (-)	-
P44	Bacilos (+)	Bacilos (-)	-
P45	-	Bacilos (-)	-
P46	Bacilos (+)	Bacilos (-)	-
P47	-	Bacilos (-)	-
P48	-	Bacilos (-)	-
P49	-	Bacilos (-)	-
P50	-	Bacilos (-)	-

Table 7 The concentration and quality of extracted DNA from microorganisms like Listeria monocytogenes, E. coli, and Salmonella spp.

Code	**Listeria monocytogenes** ng/µL	**E. Coli** ng/µL	**Salmonella** ng/µL
P1	-	56,8	69,5
P2	67,5	70,5	51,6
P3	98,2	68,9	87,4
P4	-	93,4	-
P5	-	76,7	-
P6	73,9	87,6	83,7
P7	68,3	101,8	78,9
P8	80,5	99,4	56,8
P9	48,9	102,5	64,8
P10	56,4	89,1	89,7
P11	78,5	97,2	47,9
P12	98,2	83,9	55,6
P13	87,4	68,7	-
P14	-	-	-

P15	-	-	-
P16	-	-	-
P17	-	-	-
P18	-	-	-
P19	102,3	-	-
P20	55,1	-	-
P21	-	-	-
P22	-	-	-
P23	87,3	54,7	-
P24	49,2	77,2	-
P25	-	94,6	-
P26	-	48,9	-
P27	-	62,4	-
P28	78,3	70,6	-
P29	-	58,9	-
P30	-	73,5	-
P31	67,4	54,6	-
P32	-	107,2	-
P33	-	65,4	-
P34	-	48,2	-
P35	63,5	75,8	-
P36	-	76,1	-
P37	-	64,9	-
P38	-	79,1	-
P39	-	89,7	-
P40	54,6	92,4	-
P41	-	100,2	-
P42	-	59,7	-
P43	-	74,8	-
P44	76,8	34,5	-
P45	-	76,0	-
P46	34,5	78,9	-
P47	-	56,7	-
P48	-	49,8	-
P49	-	56,7	-
P50	-	52,7	-

Results of PCR Amplification via Agarose Gel Electrophoresis for Escherichia coli, Listeria monocytogenes, and Salmonella.

Results obtained from photographic documentation on an agarose gel with isolates of Escherichia coli and its serotype O157:H7, considering a positive control (E+), with various points (P) containing amplified DNA, in comparison to a 100 bp molecular weight marker.

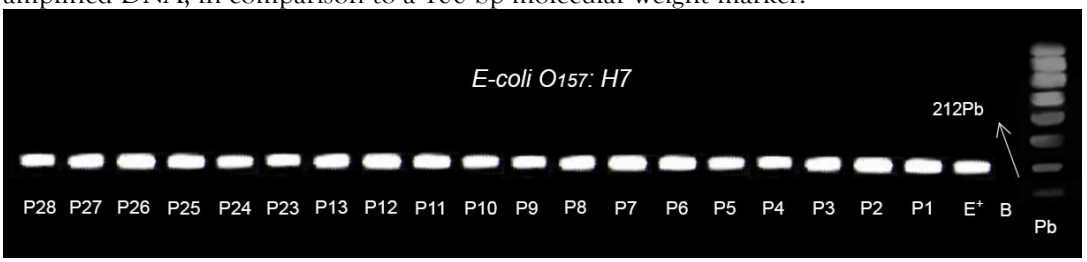

Figure 2: Represents the contaminated samples from P1 to P28.

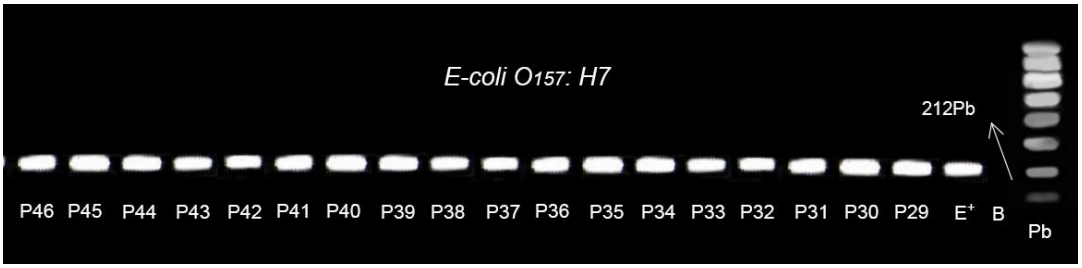

Figure 3: Represents the contaminated samples from P29 to P46.

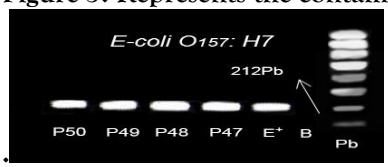

Figure 4: Represents the contaminated samples from P46 to P50

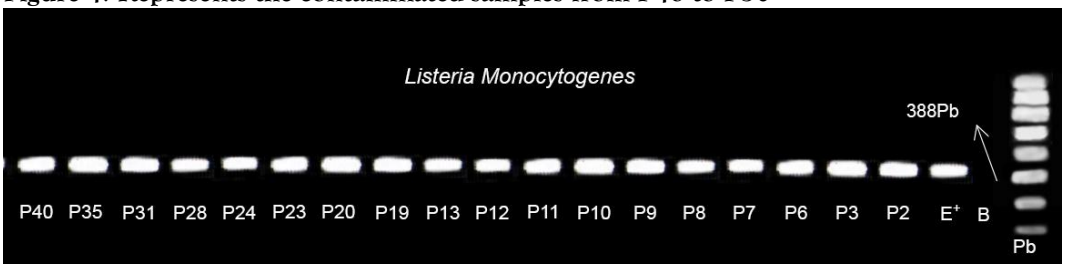

Figure 5: Results obtained from photographic documentation on an agarose gel with isolates of Listeria monocytogenes, considering a positive control (E+), with various points (P) containing amplified DNA, in comparison to a 100 bp molecular weight marker, represents the contaminated samples from P2 to P40.

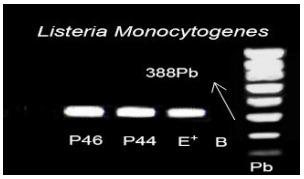

Figure 5: Represents the contaminated samples from P44 to P46.

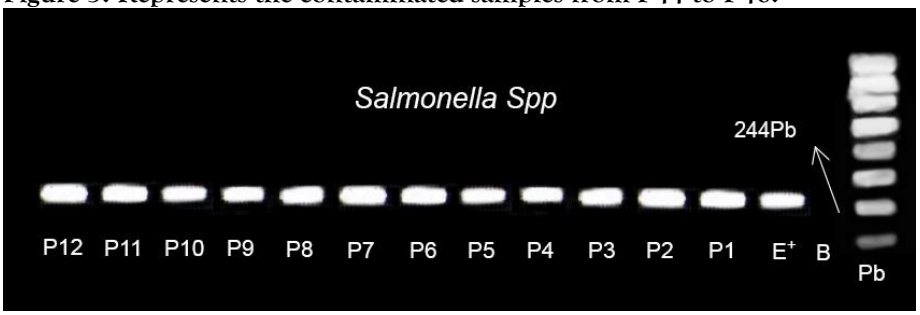

Figure 7: Results obtained from the photographic documentation on an agarose gel with isolates of Salmonella spp., considering a positive control (E+), with various points (P) containing amplified DNA, compared to a 100 bp molecular weight marker, represents the contaminated samples from P1 to P12.

DISCUSSION

Water is a fundamental element for human biological activities, and its quality must be suitable for consumption to prevent diseases that can harm consumers and even lead to fatalities [19]. In this research, the quality of drinking water in Chambo Canton was evaluated, focusing on the microbiology of the water matrix based on the standard [20]. Based on the data obtained, it is concluded that this vital liquid is not suitable for consumption.

Pathogens that can cause foodborne illnesses with gastrointestinal symptoms include Listeria monocytogenes, E. Coli, and Salmonella spp. [21]. Among the 50 points studied, it was observed that the presence of E. Coli was detected in 40 samples, representing 80%, Listeria monocytogenes was found in 20 out of 50 samples, accounting for 40%, and Salmonella spp. was present in 10 samples out of 50, constituting 10%. This suggests that the studied water may potentially transmit diseases such as typhoid

fever, paratyphoid fever, and hepatitis A [22].

CONCLUSIONS

Through georeferencing, the position of water samples was analyzed using GPS coordinates and processed with ArcGIS software. Based on the georeferenced points, it can be concluded that the journey of human consumption water is contaminated both before and after treatment.

There is contamination with Escherichia coli of serotype O157:H7 in 80% of the samples (40 out of 50), followed by Listeria monocytogenes at 40% (20 out of 50), and Salmonella spp. at a rate that isn't specified in the provided text. These findings indicate a significant risk to public health and highlight the need for immediate action to address water contamination in Chambo Canton.

20% (10/50), thus providing a reason to acknowledge that the drinking water in Chambo is contaminated by the studied bacteria. It can be considered that the water, by not complying with the INEN 1108 and INEN 1107 standards, may be influenced by factors such as the deterioration of the drinking water distribution network and the proximity of networks for other uses and wastewater to this network.

Knowing that there is contamination from pathogens that are not heat-resistant, it is recommended to treat drinking water with thermal processing, boiling it at temperatures ranging from 98°C to 100°C. Alternatively, a chemical purification method using chlorine at a minimum concentration of 0.2 mg/L and a maximum of 2 mg/L, as per INEN 409-03: 2021, can be employed.

It is important that local and regional authorities take immediate action to address this contamination and ensure access to safe drinking water for the population of Chambo. Additionally, ongoing water quality monitoring and preventive measures should be implemented to prevent future contamination.

CONFLICT OF INTERESTS

The authors who conducted this research do not have any conflicts of interest.

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