Health Environ, 2024, 5(1): 246-255
DOI: 10.25082/HE.2024.01.002

RESEARCH ARTICLE

Environmental and chemical determinants of contamination and survival of *Vibrio cholerae* in water sources in Bukavu in the Democratic Republic of the Congo

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Received: May 13, 2024;
Accepted: July 18, 2024;
Published: July 22, 2024.

Citation: Mumbumbu AM, Tsongo ZK, Shindano EA, et al. Environmental and chemical determinants of contamination and survival of *Vibrio cholerae* in water sources in Bukavu in the Democratic Republic of the Congo. *Health Environ*, 2024, **5**(1): 246-255. https://doi.org/10.25082/HE.2024.01.002

Abstract: Introduction: Cholera is endemo-epidemic in Bukavu. The aim of the study was to assess the environmental determinants of permanent contamination of spring and well water and to evaluate some of the chemical factors responsible for the persistence of *Vibrio cholerae* in water consumed by cholera patients. Methods: Conducted in the Bukavu health district from September 2020 to September 2021, this was a cross-sectional. The potential of hydrogen (pH) of the water was evaluated before comparing it with the survival of *Vibrio cholerae*. A total of 641 latrines, 92 water sources, and wells were surveyed, with 298 samples analyzed in the laboratory. Results: Out of the 641 latrines surveyed, 367 (57%) were found to be unsanitary; 54 (59%) of the water sources and wells were also deemed unsanitary. In total, 57% of the water samples were found to contain *Vibrio cholerae*, with 90% exhibiting an alkaline pH, of which 54% tested positive for the bacteria. Conversely, 10% of the samples had an acidic pH, with 80% of those containing *Vibrio cholerae*. The pH levels of the water remained alkaline both during the epidemic (95%) and post-epidemic (84%), thereby favoring the survival of *Vibrio cholerae* serotypes Ogawa and Inaba in these water sources. An acidic pH was observed to increase the likelihood of *Vibrio cholerae* survival in these waters by a factor of 3.39. Conclusion: Spring and well water are consistently contaminated with *Vibrio cholerae* due to the unsanitary conditions of nearby latrines. The presence of *Vibrio cholerae* serotypes Inaba and Ogawa in these water sources is further influenced by the alkaline and acidic pH levels.

Keywords: contamination, *Vibrio cholerae*, endemic, Bukavu

1 Introduction

Water is crucial for the survival and well-being of all individuals, constituting a fundamental human necessity and right. However, it also serves as the primary and most critical medium for disease transmission [1]. Consequently, stringent monitoring is essential to ensure that water meets the required standards of quality and quantity. Despite this, it is evident that in numerous instances globally, water is only available in limited amounts, particularly during the wet season, and is often of subpar quality, necessitating several hours of travel from the consumer's residence. This reality leads to hundreds of thousands of individuals worldwide grappling with severe water-related challenges, while others lack adequate access to water that is both of satisfactory quality and quantity [2]. Consequently, millions of people lose their lives annually due to diseases associated with inadequate water supply, poor water quality, and the absence of proper testing and basic sanitation facilities. It is, therefore, imperative to acknowledge that the presence of various contaminants in water necessitates thorough analysis and treatment before use to render it suitable for its intended purposes [3]. It is well known that access to drinking water and the disposal of human excreta are crucial factors in maintaining public health. The are fundamental human needs and remain vital to human dignity [4]. However, access to water is becoming more difficult because of the problems caused by urban development, population growth and the various pressures that people exert on water resources [5]. Dirty water is responsible for 9.1% of illnesses and 6% of deaths worldwide every year [6]. In 2009, two point six billion people (2.6 billion), i.e. half the world's population, had no access to a latrine and 1.1 billion people had no access to a safe water source [4].

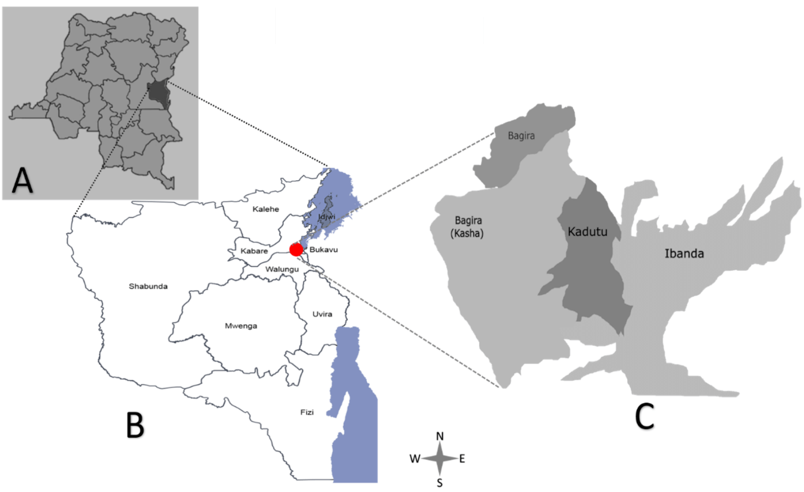
As a direct consequence, 1.6 million people die every year from diarrheal diseases, 90% of them children under 5 years old, most of them living in developing countries [6]. This underscores the significance of noting that 3.1% of global mortality is linked to unsafe water and sanitation [7]. In developed countries, more than 6 billion people had access to improved water sources in 2009, but not necessarily potable. However, in developing countries, only 43% of the population had access to water of sufficient quality and quantity, despite substantial efforts [8]. It is crucial to highlight that Sub-Saharan Africa, in particular, significantly lags behind and still maintains the lowest coverage rate of improved water resources globally [9]. In 59 developing countries, only half the population has access to quality water and improved sanitation, while 16% lack access to sanitation facilities or quality water [10]. In the Democratic Republic of the Congo (DRC), it is noteworthy that many towns and territories are grappling with a shortage of potable water. An estimated 51 million people, or three-quarters of the population, lack access to drinking water, despite the country holding over half of Africa's water reserves. Moreover, only 46% of the population has access to improved water that may not be potable [11]. Additionally, in 2012, only 48% of the population utilized good quality water sources. The humanitarian crisis remains dire in rural areas, where only 20% of the population can access quality water sources, often located in unsuitable areas. Furthermore, 60% of facilities are non-operational due to inadequate resources and maintenance. In the DRC, 36% of children are hospitalized annually, and 20% succumb to dehydration from diarrheal diseases stemming from water source contamination. Moreover, 50% of the population practices open defecation, and 2.3 million lack access to uncontaminated water sources [12]. In South Kivu province, merely 26% of the population can access potable water. Within Bukavu town (Bukavu health district), most neighborhoods or health areas lack a supply of drinking water from the national water distribution company, Régideso, regardless of the season. Consequently, diseases like cholera have been recurring for over 15 years. The study's objective was to evaluate the factors contributing to the persistent contamination of spring and well water, identify sources contaminated with *Vibrio cholerae, and* analyze some of the chemical factors contributing to *Vibrio cholerae*'s survival in this water during and after the cholera outbreak. Ultimately, the study identified the serotype of the cholera strain responsible for the epidemic in Bukavu city for more than 15 years.

2 Materials and methods

2.1 Setting and study period

The study is conducted in the province of South Kivu, within the three health zones of the Bukavu city, in South Kivu Province, in the DRC. It is conducted throughout the endemic cholera epidemic period from September 2020 to September 2021, spanning one year.

The survey sites included water sources, wells, and household latrines in three health zones: Ibanda, Kadutu, and Bagira (Figure 1). These health zones were selected due to their history of enduring endemic cholera epidemics for over 15 years, coupled with similar socio-economic and health profiles. Additionally, a Cholera Treatment Centre (CTC) was established at the Bukavu Provincial General Reference Hospital by the Provincial Health Directorate (DPS).



Legend: A: The Democratic Republic of the Congo; B: The South Kivu Province; C: Bukavu city.

Figure 1 Administrative map of the city of Bukavu

2.2 Type of study

The study is a cross-sectional study.

2.3 Sampling

The sampling technique was exhaustive for cholera patients and systematic for latrine households. Water sources and wells were selected at random by drawing lots without replacement.

2.3.1 Households - latrines

The total population of each health zone was divided by 8 to obtain the average number of households per research unit. From this average, 1% of households were considered for each health zone. A total of 641 latrine households were selected for the survey: 331 households in the Ibanda health zone, 208 households in the Kadutu health zone and 102 households in the Bagira health zone. We selected each household with its latrine to assess the state of sanitation. For each health zone, in each of the health areas, we selected N households in accordance with the Schwart formula: $N = Z^2.p.q/d^2$.

N = Sample size;
p = Proportion of the population with access to drinking water (unexposed);
Z = the deviation corresponding to the 95% confidence level (1.96);
q = proportion of the population without access to drinking water (exposed);
d = desired degree of absolute precision (0.05).

2.3.2 Sources of drinking water (springs and wells)

Sampling was done through simple random selection. For all three health zones in the district, 92 water sources were selected. A total of 298 water samples were collected during and after the epidemic period for bacteriological and chemical analysis. (Table 1)

Table 1 Size of water supply sources by Health Zone

Health zone	Selected sources	Retained wells	Total
Ibanda	31/43 (72%)	19/26 (73%)	50/70 (71%)
Kadutu	13/18 (72%)	18/25 (72%)	31/43 (72%)
Bagira	8/11 (73%)	3/4 (75%)	11/15 (73%)
Total	52/72 (72%)	40/55 (73%)	92/128 (72%)

2.3.3 Eligibility criteria

(1) Any water source or well in any of the district's three health zones;
(2) Any individual aged 18 or above, residing in the chosen households for at least one year, irrespective of gender, occupation, religion, or marital status;
(3) Any household with a latrine that has been in existence for over a year within a 300-meter radius on both sides of a water source or well in any of the health zones chosen for the research.

2.4 Study variables

(1) Dependent variable: positive bacteriology (presence of V. cholerae in water)
(2) Independent variables: chemical factors (potential of hydrogen [pH] of water), sanitation of latrines, water sources, and wells.

2.5 Conduct of the survey

Water samples from springs and wells were collected in sterile 200 ml bottles between 7 a.m. and water samples from springs and wells were collected in sterile 200 ml bottles between 7 a.m. and 8 a.m. They were then transported within an hour to the laboratory at the Bukavu Provincial General Reference Hospital (HPGRB) for analysis. The samples were carried in isothermal boxes maintaining an average temperature of 2° to 8° Celsius.

V. cholerae was isolated from these water and stool samples using the Leminor gallery technique. This consists of:
(1) Take 5 mL of a sterile sample and enrich it in alkaline peptone water for 18 to 24 hours.
(2) Reproduce on the isolation medium (TCBS) and then incubate at 37°C for 18 to 24 hours.
(3) Assess the appearance of colonies that have grown.
(4) If colonies are yellowish, proceed to gram staining and then read;
(5) If gram rods are negative, carry out the leminor identification. After reading the identification, confirm the results with the oxidase and filamentation tests.

(6) If both of these tests are positive, the presence of cholera *vibrios* in the sample is confirmed.

(7) If the oxidase test is positive and the Léminor test is negative, the presence of aeromonases is confirmed.

(8) If the oxidase test is negative, the presence of enterobacteria other than cholera *vibrio* is confirmed.

2.6 Data management and statistical analysis of results

The data collected was directly encoded in Excel v13. Categorical data were presented as frequencies and percentages or graphically using histograms. Data processing and analysis were conducted using Epi - Info version 7.2 software; the chi-square test and the odds ratio were employed for these analyses at the significance threshold of p-value < 0.05.

2.7 Ethical considerations

Authorization for the survey was obtained in advance from the provincial health authorities and the district CTC. No intervention was performed on the patients, and the entire study was conducted in compliance with the code of medical ethics, professional secrecy, and good clinical practice in accordance with the principles of the International Declaration of Helsinki. Moreover, the authors declared no conflicts of interest that could likely influence the results of this study. A three-day information and training session for the investigators was conducted before they entered the field to collect the study data.

3 Results

Of the 641 latrines closest to water sources and wells surveyed in the three health zones, 57% were unsanitary; specifically, 65% in the Kadutu health zone, 55% in the Bagira health zone, and 53% in the Ibanda health zone (Figure 2).

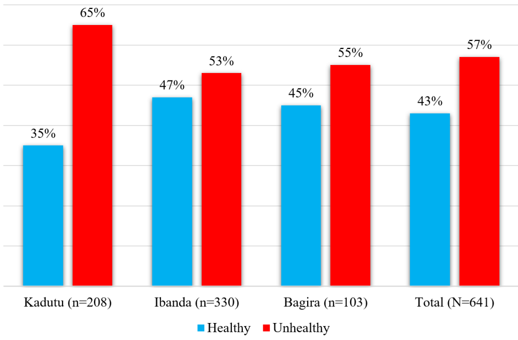


Figure 2 Condition of latrines surveyed in the three health zones in Bukavu city

Overall, in the district's three health zones, 59% of the water sources and wells surveyed were unhygienic (i.e., without a cover for the hole, without a door, with excreta here and there on the ground, on the wall, around the toilet without toiletries, etc.). Specifically, 62% in Bagira health zone, 58% in Ibanda health zone, and 55% in Kadutu health zone (Figure 3).

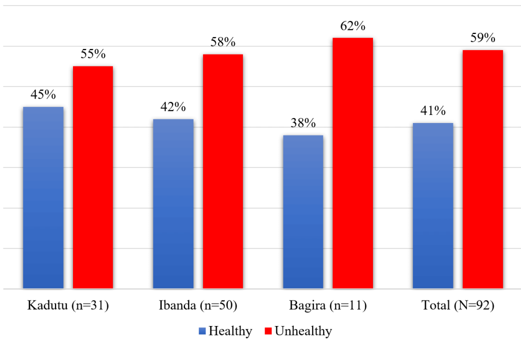


Figure 3 Condition of water sources and wells in the 3 health zones in Bukavu city

In relation to the pH of the water samples analysed, the positive bacteriology of *Vibrio cholerae* was 57%. Out of 298 water samples taken and analysed for the three HZs, 90% of the analyses had either a basic or neutral pH, with 54% *of Vibrio cholerae* detected. However, in the 10% of analyses with an acidic pH, *Vibrio cholerae* was identified in 80% of the samples. The survival of *Vibrio cholerae* in spring and well water was statistically associated with the pH of the water (p = 0.006). An acidic pH increased the chances of survival of *Vibrio cholerae* in spring and well water by 3.39 (OR = 3.39 [95% CI: 1.34 - 8.56]; p-value = 0.006) (Table 2).

Table 2 Distribution of waters according to potential of hydrogen

pH	Bacteriology positive	Bacteriology negative	Total	p-value
Acid	24 (80%)	6 (20%)	30 (10%)	0.006
Basic or neutral	145 (54%)	123 (46%)	268 (90%)	
Total	169 (57%)	129 (43%)	298 (100%)	

Note: (OR = 3.39 [95% CI: 1.34 - 8.56]; p-value = 0.006)

Overall, out of the 298 water samples analyzed across the three health zones, 89.4% exhibited a basic or neutral pH. Specifically, 95.4% had a basic or neutral pH during the epidemic period, while 84.2% did so during the period of low transmission (Figure 4).

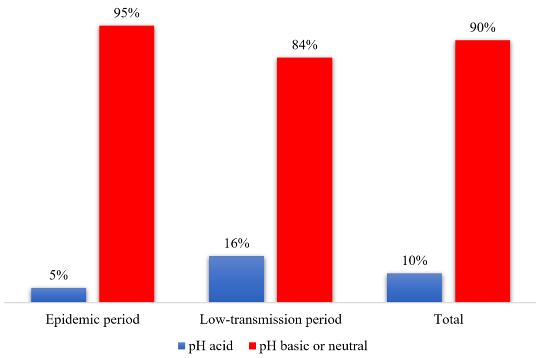


Figure 4 Potential of hydrogen of water during and after the epidemic period in Bukavu city

Among the 63 stool samples analyzed during and after the cholera epidemic, serotype Inaba was identified in 57% of the samples, while serotype Ogawa was found in 11% (Figure 5).

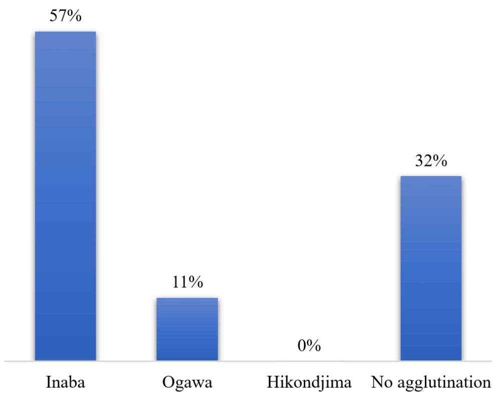


Figure 5 *Vibrio cholerae* serotype (n = 63)

4 Discussion

The aim of the study was to assess the environmental factors of permanent contamination of spring and well waters by *Vibrio cholerae*, as well as the impact of pH on the survival of *Vibrio cholerae* in these waters during and after the cholera epidemic period in Bukavu. The initial hypothesis is that the environment of water sources and wells is unhealthy and likely to permanently contaminate the aforementioned waters with Vibrio cholerae, and that the pH favors the survival of *Vibrio cholerae* in these waters.

4.1 Origin of patients

Of the three health zones in the Bukavu health district, Ibanda recorded the highest number of cholera cases. (47%) than other health zones. This commune is made up of a fairly large peri-urban area (rural - shanty town). In this commune, clean water from Régideso is sometimes inaccessible for many hours, or even several days. As a result, the population drinks water from springs and wells, whose environment is likely to be contaminated with *Vibrio cholerae*. This, among other things, could explain why the majority of cholera cases are recorded in this commune, compared with others. These findings are confirmed by the WHO's statement that: "Cholera transmission is closely linked to inadequate access to safe drinking water and sanitation. Typical areas at risk include peri-urban slums and camps for displaced persons or refugees, where minimum requirements for drinking water and sanitation are not met [13]. It should be remembered that using only clean, potable water from taps and/or well-developed, safe sources for drinking and cleaning is one of the preventive measures against cholera that have already proved effective in several countries, especially industrialized ones [14]. In addition to being a cause of morbidity and mortality, cholera is also an indicator of the underlying inadequacy of water supply, sanitation, food safety and public hygiene systems [15]. During most major epidemics, the role of water in disease transmission was clear [16]. In Africa; in Malawi, the epidemiological study carried out on 784 cases of cholera showed that cholera and its rapid spread in the refugee camp were encouraged by the use of water from shallow wells in the center, contaminated by fecal matter from latrines during the rainy periods two weeks before the epidemic broke out [17]. The results of a study carried out in Zimbabwe in 1991, during an epidemic that threatened to spread to neighboring Tanzania, showed that water contaminated at its source or during storage was cited as one of the probable causative factors [18].

4.2 Assessment of environmental health (households - latrines)

It is crucial to acknowledge that in developing countries, cholera serves as an indicator of the underlying deficiencies in environmental sanitation and hygiene systems [15]. As highlighted by UNICEF, the environmental reservoirs of cholera denote the locations where *V. cholerae* reside, grow, and propagate, potentially leading to its release and consequent human epidemics [8, 19]. Notably, 57% of the latrines in close proximity to the water sources and wells that were surveyed were found to be unsanitary. It is worth mentioning that during periods of heavy rainfall, runoff water carries human excreta that has been openly defecated in various areas. Excreta from inadequately constructed latrines and makeshift drains used as latrines by households without proper facilities are also discharged into rivers, water sources, and even wells that serve as sources of drinking water for the community. This scenario is likely to facilitate the contamination of spring and well water, particularly during the rainy season. Consumption of this contaminated water can result in the cholera cases documented in the district thus far.

However, in sub-Saharan Africa (SSA), cholera epidemics have been reported in both coastal (Kenya, Mozambique, and Tanzania) and non-coastal (Zambia and Zimbabwe) countries, indicating that even non-estuarine aquatic environments could act as reservoirs for *Vibrio cholera* [14, 20]. According to the WHO, in December 2008, a serious cholera epidemic hit Zimbabwe and continues to spread at great speed, as reported by the UN Office for the Coordination of Humanitarian Affairs (OCHA). The factors contributing to the spread of the disease included problems of access to water and toilets, particularly in remote rural areas [8].

This reinforces the findings of our study in this region, where 57% of the latrines examined were found to be unsanitary, highlighting the existing challenge of accessing clean drinking water. The poor hygiene and sanitation conditions observed in this district are likely contributing to the endemic nature of the cholera outbreak in the area. As per the WHO, access to sanitation facilities in Africa has decreased since 1994, and the available facilities are often inadequate. During the same period, the percentage of individuals with proper means of excreta disposal dropped from 36% to 34% [16]. Despite sanitation being a vital component of development, many people worldwide lack access to decent toilets or latrines. The percentage of individuals with hygienic facilities has declined significantly since 1990 due to rapid population growth outpacing construction efforts. The primary consequence can be succinctly described in one word: diarrhoea [17]. According to D'Mello-Guyett *et* al. [15], substantial investments should be directed towards supporting water, sanitation, and hygiene (WASH) interventions to prevent and manage cholera outbreaks. These interventions must consider the social context and rely on the most current evidence and cholera transmission models. It is evident that France remains unaffected by cholera, likely due to the increased level of adequate hygiene practices [18].

4.3 Clean wells and water sources

The results of the study indicate that the majority (59%) of water sources and wells are unsafe. Overall, the cases of cholera recorded in the three health zones are proportional to the degree of insalubrity of the water sources according to the health zones in the district. This situation is likely to encourage contamination of the water, leading to epidemic or endemic cholera in these three health zones, where the population uses spring and well water for drinking. According to A. Tarantola, cholera is endemic in a number of countries where the water sanitation system is inadequate [21].

In 2015, the WHO estimated that 2.4 billion people lacked adequate access to drinking water [22] and satisfactory sanitation [23]. Although studies on the dominant environmental reservoirs of *Vibrio cholerae* in sub-Saharan Africa are still limited, recurrent cholera epidemics indicate the existence of cholera hotspot reservoirs [15, 22]. In the absence of such data, several potential reservoirs could harbor cholera in sub-Saharan Africa (SSA), including on-site sanitation systems, wetlands, landfills, heavily polluted surface water systems, and groundwater systems [19]. In 2001, a study carried out in the same area (Bukavu) on the factors favoring cholera showed, among other things, that 78% of the water sources assessed were unsafe [24]. In 2005, a cholera epidemic broke out in Burkina Faso, with a total of 615 cases, including 9 deaths, reported in the city of Ouagadougou between August 8^{th} and September 4^{th}. The affected neighborhoods were those where water and sanitation conditions were precarious [25].

4.4 pH, serotype, and persistence of *Vibrio cholerae* in water, as well as the contamination of water from springs and wells, are crucial factors to consider in understanding the spread of cholera.

For all three health zones combined, the results of this study indicated that *Vibrio cholerae* was detected in 57% (169) of water samples. Additionally**,** 90% (268) of the samples analyzed in these three health zones had either a basic or neutral pH; 145 (54%) during the epidemic period and 123 (46%) during the period of low transmission, respectively. *Vibrio cholerae* was identified in 54% (145) of these samples. Among the 30 (10%) samples with an acidic pH; 23 (77%) during the epidemic period versus 7 (23%) during the post-epidemic period, *Vibrio cholerae* was detected in 24 (80%) samples. These results confirm the presence of cholera during and after the epidemic period, indicating its endemo-epidemic nature. According to Osei *et al*. [26], in countries with a combination of the main factors favoring a cholera epidemic (socio-economic level, human concentrations, inadequate health structures and environmental factors, etc.), two states can be distinguished: an endemic state (before or after an epidemic) and a paroxysmal outbreak of the epidemic. Additionally, Rebaudet *et al.* [27] specify that in humid zones or during the rainy season, the epidemic is slow to develop, leading to an endemic state which is often latent. The spread of the epidemic is mainly due to polluted water and food, emphasizing the importance of health education and prevention measures.

As for the contamination of drinking water supplies, the results of our study corroborate those of the cholera epidemic that appeared in Europe (Italy in 1973, Portugal in 1974, and France in 1978). The strictly waterborne origin of the infection was clearly demonstrated, with pathogenic *vibrios* isolated from water sources, wells and shells [8]. However, large-scale investment in water supply and sanitation infrastructure has largely contributed to the elimination of cholera in Europe and the Americas [15]. In the Kadutu Health Zone, among the water sources analysed in 2013, during an endemic cholera outbreak, the water from the Saint François, *Chemu Fariala* and *Kadhuru* springs contained high numbers of *V. cholerae* colonies [28].

With respect to the survival of *Vibrio cholerae* in spring and well water, our study results suggest a statistically significant association with the pH of these waters ($p = 0.006 < 0.05$). It is evident that an acidic pH increases the likelihood of *Vibrio cholerae* survival in spring and well water by 3.39, while basic and neutral pH levels promote the survival of *Vibrio cholerae* in river and lake water. Therefore, pH is likely to positively impact the survival of *Vibrio cholerae in* spring and well water during both epidemic and endemic periods.

This may explain the endemo-epidemic nature of cholera in Bukavu city, where drinking water is not regularly available and the population occasionally uses spring and well water for drinking. The results of our study corroborate those of a number of authors who have carried out similar research. According to Merrell *et al*. [29], it is well known that the waters of the lakes in the Great Lakes sub-region, and in particular the lakes to the east of our country, the DRC are salty and have an alkaline pH, factors that favour the survival and development of the *vibrios* responsible for cholera. It should also be remembered that the singular tolerance of *Vibrio cholerae* to alkalis allows it to develop up to a pH of 9.2; on the other hand, it is not resistant to acidic environments [30]. However, the results of our study show that, in water sources and wells, acidic pH also favours the survival of *Vibrio cholerae*. It has also been proven that the waters of Lake Kivu and those of small Rwandan lakes, thanks to their alkalinity with a pH of between 8 and 9, constitute a favourable element for the conservation of enterobacteria [31]. According to Michèle Lavallé, *Vibrio cholerae* grows well in water at a temperature above 15°C, in alkaline (pH above 8) and salty humid environments. It lives for years in deep, brackish water (estuaries).

With respect to the serotypes of the *Vibrio cholerae* strain responsible for the endemic cholera in this setting, our findings reveal that the Inaba and Ogawa serotypes were detected during and post the epidemic, representing 57% and 11% respectively out of the 63 water samples examined. These outcomes align with a study of 40 individuals who contracted cholera in the coastal areas of Texas and Louisiana in 1978 [27]. They also support findings from an outbreak involving 14 cholera cases due to water contamination on an oil platform in the Gulf [5]. Additionally, they coincide with data from the cholera outbreak in the Cyangungu region in 1996, which saw 3,451 reported cases, where the Ogawa serotype was identified in the water samples. Inaba and Ogawa serotypes have been recurrent in recent epidemics, irrespective of the *V. cholerae* or eltor biotype [9].

4.5 Limitations of the study

This study has several limitations that should be acknowledged. Firstly, it is a cross-sectional study, which limits the ability to establish causality between environmental factors and *Vibrio cholerae* contamination. Additionally, the study's reliance on a specific time frame may not capture seasonal variations that could affect water contamination levels. The sample size, while substantial, may still not represent the entire region comprehensively. Furthermore, the study focuses primarily on pH as a chemical factor, potentially overlooking other significant chemical and biological factors that could influence the survival of *Vibrio cholerae* in water sources. Lastly, the study's findings are geographically limited to Bukavu and may not be generalizable to other regions with different environmental and socio-economic conditions.

4.6 Strengths of the study

Despite these limitations, the study has several strengths. It provides a detailed assessment of the environmental and chemical determinants of water contamination with *Vibrio cholerae*, contributing valuable data to the existing body of knowledge. The study's methodology, including the thorough survey of latrines and water sources, and extensive laboratory analysis, ensures robust and reliable data. The identification of specific pH conditions that favor the survival of *Vibrio cholerae* offers practical insights for public health interventions. Additionally, by highlighting the persistent contamination of water sources during and after cholera epidemics, the study underscores the need for continuous and improved water sanitation measures. Finally, the study's findings have significant implications for public health policies and practices in the Bukavu city, potentially guiding future strategies to combat cholera and other waterborne diseases.

5 Conclusion

The unsanitary conditions of latrines near water sources and wells in Bukavu are a significant factor in the contamination of drinking water with *Vibrio cholerae* serotypes Inaba and Ogawa. This contamination persists both during and after cholera epidemics, facilitated by the pH levels of the water, which promote the survival of *Vibrio cholerae*. Notably, an acidic pH increases the likelihood of *Vibrio cholerae* survival by 3.39 times, particularly in the post-epidemic period. These findings highlight the endemic nature of cholera in Bukavu's three health zones. To mitigate this public health issue, it is crucial to enhance the political, administrative, and health authorities' commitment to providing effective health education on the hygienic management of water sources. This initiative would help minimize water contamination and combat diarrheal diseases, including cholera, in the region.

Conflicts of interest

The authors declare that they have no conflict of interest.

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