

Occurrence of Culturable *Vibrio cholerae* from Lake Victoria, and Rift Valley Lakes Albert and George, Uganda

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Abstract

Vibrio cholerae, a bacterium that causes cholera, poses a human health risk when consumed via untreated or contaminated water. Monthly investigations into the presence of *V. cholerae* from Lakes Albert, George and Victoria were conducted, with the goal being to examine the relationship between the occurrences of *V. cholerae* with various water quality parameters at fish landing sites in major water bodies in Uganda. The pH, temperature and electrical conductivity were measured at three fishing sites in each of the three study lakes; namely Gabba in Lake Victoria, Butiaba in Lake Albert and Kayanzi in Lake George. The pH values varied from 7.76 to 9.36 at Butiaba, 8.68 to 9.85 at Kayanzi and 6.6 to 9.88 at Ggaba. The temperature ranged from 17.9 to 32.3 °C at Butiaba, 22.5 to 29 °C at Kayanzi and 18.2 to 30.5 °C at Ggaba. The electrical conductivity ranged from 129.2 to 984 $\mu\text{S cm}^{-1}$ at Butiaba, 658 to 1090 $\mu\text{S cm}^{-1}$ at Kayanzi and 119 to 218 $\mu\text{S cm}^{-1}$ at Ggaba, for Lakes Albert, George and Victoria, respectively. Enrichment techniques were used to detect culturable *V. cholerae* on TCBS culture media. Seventy-five (75%) of the samples ($n = 90$) were positive for *V. cholerae*. The occurrence of *V. cholerae* was positively associated with water quality parameters over the 10-month period of study. *Vibrio cholerae* was more frequently detected during the dry season (warmer) than during the wet season. These study results suggest the investigated study lakes are natural reservoirs for *V. cholerae*.

Key words

George and Victoria; rift valley, Lakes Albert, Uganda, *Vibrio cholerae*, waterborne diseases.

INTRODUCTION

A safe freshwater supply is a vital for the livelihoods of people, being a requirement for domestic, recreational and industrial purposes, as well as for agricultural supply. Anthropogenic activities, however, including waste being introduced into freshwater sources, make them unsafe for human consumption. Thus, water safety is increasingly threatened by pathogens that can affect humans and other organisms. Introduced pathogens can cause major waterborne diseases, which represent one of the major contributors to human disease burden and mortality (Pruss & Havelaar 2001). Examples of such pathogens include *Vibrio cholerae*, *Escherichia coli* and *Salmonella typhi*. Among these deadly pathogens, *V. cholerae* causes cholera. In fact, cholera is one of the few bacterial

diseases known for pandemicity when infection is acquired through contaminated drinking water or eating food contaminated with the cholera bacterium. Cholera is widespread in sub-Saharan Africa, Southern Asia and South America, which commonly exhibit seasonal outbreaks. This disease can spread rapidly in areas with inadequate treatment of sewage and drinking water, with Faruque *et al.* (1998) reporting the disease was predominantly associated with poverty, poor sanitation and hygiene.

Several studies by Colwell (1996), Lipp *et al.* (2002), Huq *et al.* (2005) and Worden *et al.* (2006) highlighted the pandemicity of *V. cholerae* O1 in different parts of the world. More than 200 sero groups of *V. cholerae* have been described during the course of such investigations (see Huq *et al.* 1983; Broza *et al.* 2005). Only sero groups O1 and O139, however, are associated with epidemic cholera. A wealth of information also has been collected on the ecology and biology of *V. cholerae* in natural aquatic

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Accepted for publication 21 November 2012.

systems. Nevertheless, the organism has been reported to persist and grow in water systems in the total absence of a human host (Faruque & Nair 2002; Faruque *et al.* 2005). From the several sero groups of *V. cholerae* isolated from the water environment in different studies, the medical importance of this organism has been demonstrated, enhancing our understanding of its transmission cycle.

Research on cholera in Africa is incident-based, with little effort typically being made for long-term surveillance in any cholera-prone area. Studies on the existence of distinct strains by (Legros *et al.* 2000; Alajo *et al.* 2006, du Preez *et al.* 2010, Mohamed *et al.* 2010), cholera transmission mechanisms during outbreaks, (Bompangue *et al.*, 2012, Khonje *et al.* 2012) and plasmid profiles and antimicrobial susceptibility patterns (Olukoya *et al.* 1995), have been reported. Only a few studies in Uganda have examined the occurrence of cholera as a disease resulting from outbreaks in parts of the country Legros *et al.* (2000) and Alajo *et al.* (2006). The existing studies reported outbreaks of cholera may have been further compounded by ever-changing climate effects throughout the country. The presence of *V. cholerae* in water bodies in Uganda represents a significant threat to populations

living around water bodies. With increased population and more anthropogenic activities associated with them, it is important to examine the natural habitats of *V. cholerae*, to take measures to control disease outbreaks. To this end, this study examined the occurrence and association of *V. cholerae* with water parameters from three fish landing sites on major water bodies in Uganda.

MATERIALS AND METHODS

Study sites

Three fish landing sites each from Lakes Albert, George and Victoria were sampled monthly from September 2009 to August 2010, to determine the physicochemical and biological parameters of the lakes. These water bodies are used as sources of drinking and household water to neighbouring communities, with a total of nine sampling sites between the three lakes being studied. Each location included a landing site with three sub-sites (Fig. 1). The criteria used for sampling site selection included human population numbers and the availability of health services to communities. For Lake Victoria, the Gabba fish landing site (N 00° 15.374', E 032° 38.264'; N 00° 15.407' E 032°

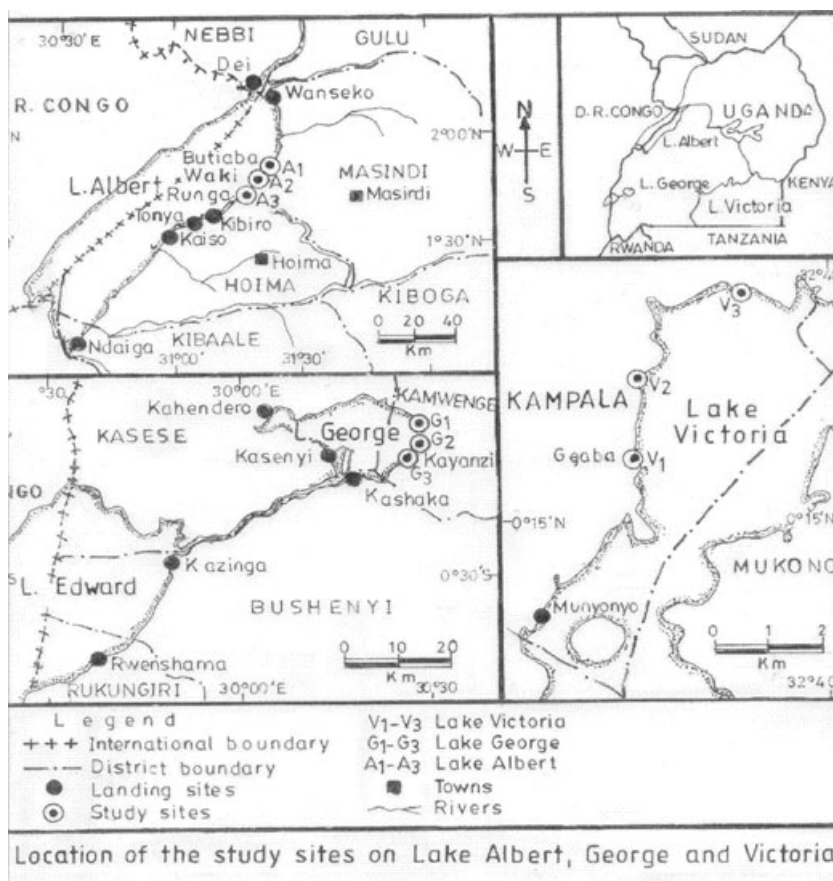


Fig. 1. Sampling sites on Lakes Albert, George and Victoria.

38.270'; N 00° 15.464' E 032° 38.262') in Kampala District was sampled, with three sampling spots (V1, V2, V3; see Fig. 1). Lake Albert and Lake George lie in the East African Rift Valley. For Lake Albert, the Butiaba fish landing site (N 01° 49.097' E 031° 19.224'; N 01° 49.141' E 031° 19.223'; N 01 49.181' E 031° 19.225') consisted of three sites, identified as sampling sites A1, A2 and A3 in Masindi District. For Lake George, the Kayanzi fish landing site (S 00° 05.506' E 029° 45.705'; S 00° 05.469' E 029° 45.713'; S 00° 05.418' E 029 45.722') in Bwera, Kasese District consisted of three sites, identified as G1, G2 and G3.

Sample collection

Water samples were collected at each sampling site with a standard sterile loop and sampling bottles. A boat was used to access the sampling sites for deeper waters. All water samples were transported to the laboratory in an insulated cool box and processed within 6–12 h of collection. Water parameters measured in this study include *in situ* water temperature and electrical conductivity, using Wissenschaftlich-Technische Werkstätten (WTW) hand-held portable multi-probes, and WTW 330 combined pH and temperature electrode. Water samples were taken from a depth of dipped 10 cm below the water surface, after the probes were calibrated according to manufacturer instruction.

Microbiological analysis – *Vibrio cholerae*

Environmental water samples were collected from three samplings sites each on Lakes Albert, George and Victoria (Fig. 1). Triplicate water sample volumes, ranging from 100 to 500 mL, were serially diluted, 1–10 serial dilutions, and filtered on 0.22- μ m membrane filters. The filter papers, containing the trapped microorganisms, were enriched with alkaline peptone water (APW) (Difco, Detroit, MI, USA) consisting of 1% peptone and 1% sodium chloride, with the pH adjusted to 8.5. They were then incubated at 37 °C for 16–24 h. The un-culturable cells were enriched to make them more culturable. Approximately 5 μ L of enriched APW broth was streaked with an inoculating loop onto plates containing Thiosulfate-citrate-bile salts-sucrose (TCBS; (Huq *et al.* 1983; APHA 1989). Yellow, flat 1–3-mm-diameter colonies were picked, streaked onto nutrient agar (VWR, Merck, Basingstoke, Hampshire) without sodium chloride (NaCl) and incubated overnight at 37 °C. Colonies that formed were counted and selected for further testing. Colonies exhibiting growth on both nutrient agar plates with (NaCl+), and without NaCl (NaCl–) were selected and subcultured onto gelatine agar (Merck). The gelatine-positive cultures were subjected to the oxidase test. Oxi-

dase-positive colonies were then subjected to further biochemical characterization, using API 20E (Biomereux).

Absorbance analysis

The collected water samples were filtered, using 0.22 μ m pore-size (sterile) polycarbonate membrane filters (Cyclo-pore, Whatman) for absorption measurement. A spectrophotometer (Lambda 40 UV/VIS; Perkin Elmer, Zaventem, Belgium) was used to measure the absorption at wavelengths of 250, 365, 430 and 436 nm, in a 1-cm quartz cuvette. Distilled water was used as a blank in all cases. The absorbance of water samples was measured against those of double-distilled water. These data were used to determine yellow/brown colour organic acids (humic substances) within the water, noting that it has recently been shown that humic substances most probably inhibit *V. cholerae* growth (Kirschner *et al.* 2008).

Statistical analysis

Analyses were done using the programme R 2.10 (R Development Core Team, Vienna, Austria). Physicochemical data were analysed with descriptive statistics. Analysis of variance (ANOVA) was used to determine any seasonal variations in water quality parameters (e.g. temperature, pH, conductivity). Comparisons were made between data collected for the different seasons, using the *t*-test. Correlation analysis between the presence of *V. cholerae* and water quality parameters was conducted with Spearman rank correlation. The isolation rate from the environmental water samples was calculated as follows: Isolation rate (%) = number of positive samples (NP)/number of samples tested (NT) \times 100. Ninety-five percentage (95%) confidence intervals for the Odds ratio (OR) for each environmental variable were computed. The absorbance ratio at 250 nm, to that at 365 nm, was calculated to determine possible shifts in the molecular size spectrum during the experiments.

RESULTS AND DISCUSSION

The mean values of the physicochemical parameters are summarized in Table 1. These values represent the means of the samples from the three sampling sites for each fish landing site. The highest physicochemical mean values were observed for the Kayanzi fish landing site on Lake George, with the pH and electrical conductivity ranging from 8.6 to 9.85 units and 658 to 1090 μ S cm^{-1} , respectively. The pH and electrical conductivity values for Lake Albert ranged from 8.77 to 9.26 °C and 618.4 to 984 μ S cm^{-1} , respectively, while the values for Lake Victoria pH ranged from 6.6 to 9.8 °C and the electrical conductivity from 119 to 218 μ S cm^{-1} .

Table 1. Physicochemical characteristics at Lakes Victoria, Albert and George Fish Landing Sites (mean \pm sSE)

Variable	No. of samples (n)	Mean			Minimum value			Maximum value		
Lake site	90	A _B	G _K	V _G	A _B	G _K	V _G	A _B	G _K	V _G
pH (units)	90	8.77 \pm 0.07	9.08 \pm 0.037	8.67 \pm 0.15	7.76	8.68	6.6	9.36	9.85	9.88
Cond. (μ S cm ⁻¹)	90	618.3 \pm 36.7	918.6 \pm 23.4	154.1 \pm 3.7	129.2	658	119	984	1090	218
Temp (°C)	90	26.34 \pm 0.87	26.37 \pm 0.39	26.28 \pm 0.55	17.9	22.5	18.2	32.3	29	30.5
Col.nos ($\times 10^4$)	90	75.6 \pm 21.9	87 \pm 25.7	58.9 \pm 27.7	129.2	0	0	565	564	548

Cond., electrical conductivity; Temp, temperature; Col. nos, plate colony numbers; A_B, Lake Albert Butiaba site; G_K, Lake George Kayanzi site; V_G, Lake Victoria Gabba site; values are means and standard errors of sampling sites at fish landing sites on each study lake).

The lowest pH values for Lakes Albert and George were observed during the wet season, while those for Lake Victoria occurred during the dry season (Fig. 2a). These results of the varying pH, temperature and electrical conductivity values are in agreement with those reported by others (e.g. see Talling 1992; Kasozi *et al.* 2006; Muwanga & Barifaijo 2006; Lake Albert Eastern Catch-

ment Management Initiative 2006; Ssanyu & Schagerl 2010).

The water column temperatures for Lake George and Victoria were lowest during the dry seasons, while those measured for Lake Albert were relatively constant. Lake Albert exhibited the lowest temperature (17.9 °C), which may be attributable to the large riparian vegetation at this

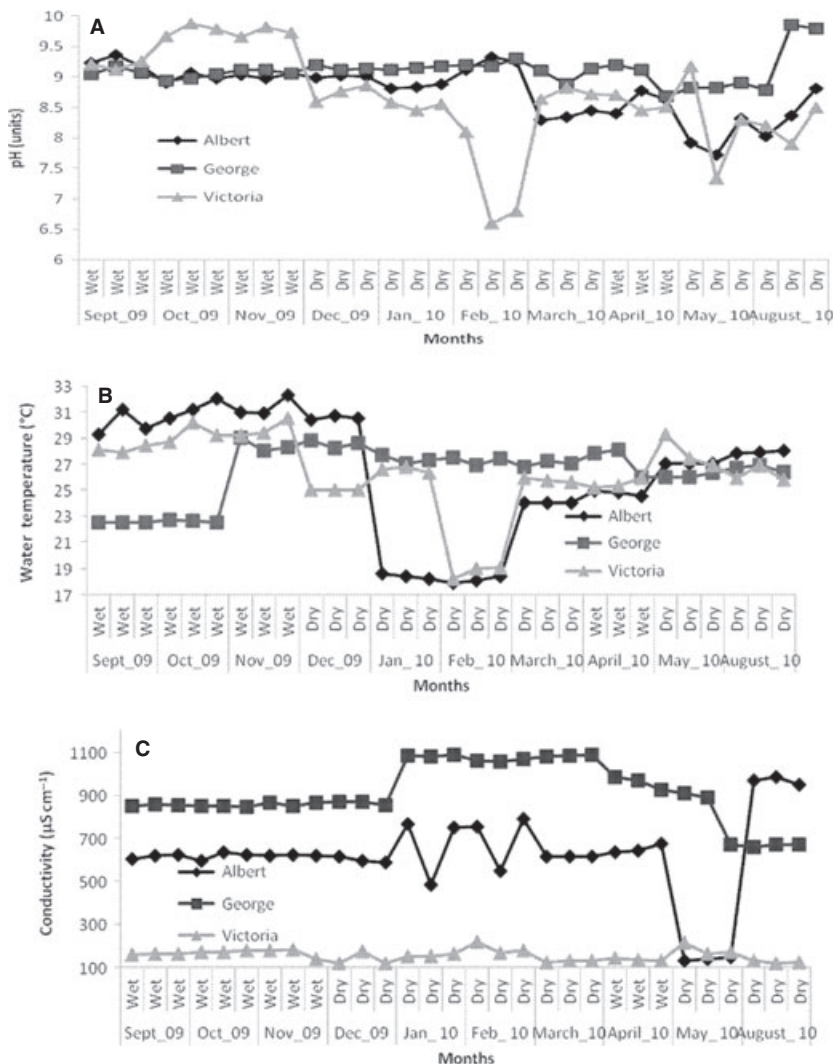


Fig. 2. Mean abundance of *Vibrio cholerae* from cell plate counts ($\times 10^4$) for Lakes Victoria, George and Albert.

site, which shades the water and possibly reduces its temperature. The water temperature ranges did not vary significantly over the sampling period. Those during the wet season were significantly higher ($t = -2.258$; d.f. = 96.469; $P = 0.0262$) than those during the dry season (Fig. 2b).

Figure 2c compares the electrical conductivity across the sampling sites and seasons (ANOVA; $F = 537.2$; d.f. = 39.46; $P = 2.39E-29$). The highest electrical conductivity values were observed at the Kayanzi fish landing site on Lake George, with an average of $910.3 \mu\text{S cm}^{-1}$, followed by the Butiaba fish landing site on Lake George and the Gabba landing site on Lake Victoria, with values of 618.4 and $154.1 \mu\text{S cm}^{-1}$, respectively. The mean electrical conductivity values for Lakes Albert and George were $>600 \mu\text{S cm}^{-1}$, suggesting a high ionic content in these waters. The mean electrical conductivity value for Lake Victoria was $154.1 \mu\text{S cm}^{-1}$, which was much lower than that for Lakes Albert and George. The normal electrical conductivity for Lake Victoria is $80 \mu\text{S cm}^{-1}$, suggesting the observed values in this study indicate a high ionic content. A significant decrease in the electrical conductivity was observed in May 2010 for Lake Albert. All the study sites were threatened because of the significant anthropogenic activity surrounding them. The associated refuse and rubbish would likely be washed into the river during the rainy season, exerting pollution pressures on the sources of the lake water.

Presence of *V. cholerae* determined by selective enrichment technique

Overnight growth (18–24 h) of *V. cholerae* on TCBS produced large (2–4 mm diameter), slightly-flattened, yellow colonies with opaque centres and translucent peripheries. The yellow colour was caused by the fermentation of sucrose in the medium (Appendix 1). Preferential selection on the TCBS with nutrient agar plates allowed isolation of *V. cholerae* O1 from 68 of 90 samples tested, which constituted 75% of all water samples collected from the three study lakes. Table 2 presents the samples that tested positive for culturable *V. cholerae* O1, as observed on TCBS agar. The October samples exhibited the least presentation of colonies. Findings from this study and others suggest *V. cholerae* O1 remains viable in water, and in association with other aquatic organisms, throughout the year, irrespective of epidemic and inter-epidemic periods of cholera outbreaks. This is the case even though they may not be found cultivable in traditionally used culture media, as observed by Huq *et al.* (1983) and Colwell (1996).

Table 2. Samples testing positive for culturable *Vibrio cholerae* O1 on TCBS agar (expressed as per cent)

Sampling month in 2009/10	No. of water samples analysed	Positive plates	No. of water samples (%) positive for <i>V. cholerae</i>
September	9	6	67
October	9	4	44
November	9	6	67
December	9	7	78
January	9	9	100
February	9	9	100
March	9	9	100
April	9	6	67
May	9	6	67
August	9	6	67
Total	90	68	75

Figure 3 presents changes in the numbers of *V. cholerae* during the wet and dry seasons. Positive colonies were observed in the wet and dry seasons from the plate cultures for all three study lakes. The presence of *V. cholerae* was evident from monthly average abundance of the bacterial total plate counts after isolation from each study lake. These study results suggest the presence of *V. cholerae* from the cultures in all cultures. Positive cell cultures were observed only for the months of December 2009–March 2010 for Lake Victoria. All three study lakes exhibited peaks of counts from December 2009 to February 2010. The plate colony numbers were significantly higher during the dry season (118.08) than during the wet season (19.31) ($t = 3.8815$; d.f. = 51.812; $P = 0.0003$). This may be attributed to more favourable and stable conditions for the growth of the organisms. The results

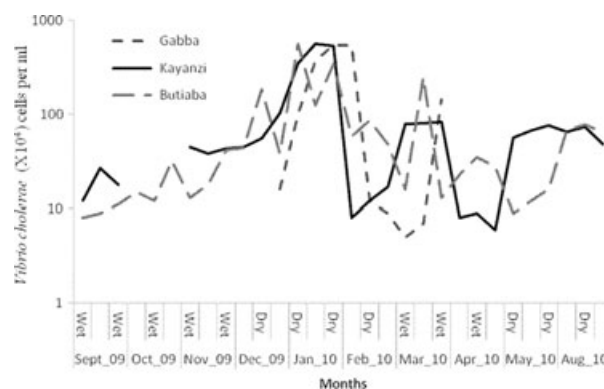


Fig. 3. Changes in *Vibrio cholerae* colony concentration over seasons.

of the present study also agree with previous findings reporting that *V. cholerae* cells do not necessarily die when discharged into the aquatic environment, but rather remain viable and capable of transforming into a cultivable state if the environmental conditions become stable. It is reported that *V. cholerae* O1 responds to environmental stresses (Brayton *et al.* 1987, Colwell *et al.* 1985; Huq *et al.* 1990 and Borroto 1997). Such stresses could be caused by nutrient limitation, unsuitable temperatures, pH or any other environmental parameter that could trigger its conversion to a non-culturable state.

Peak counts of *V. cholerae* were observed during the month of March 2010 during the rainy season. This may be attributed to the inflow of run-off consisting of wastewater with faecal material from surrounding settlements. This is in addition to favourable water conditions, specifically water temperature and pH conducive for organisms to multiply at the onset of the rainy season. When the temperature changes after extensive rains, much of the wastewater rich in fecal material has been washed off and diluted by the lake water, conditions change, resulting in a reduced abundance of *V. cholerae*. *Vibrio cholerae* has been observed to grow in freshwater (Vital *et al.* 2007; Huq *et al.* 1983; Colwell *et al.* 1996), confirming the hypothesis that *V. cholerae* reservoir was in the environment. *Vibrio cholerae* was observed to grow best within an alkaline – pH range above 8.5 (Huq *et al.* 1984). Within Lakes George and Albert, the pH was >8.5 throughout the sampling months, providing a suitable pH for the growth and survival of *V. cholerae*. The water pH at the Gabba fish landing site was within the range suitable for growth only during January–March 2010, while it was below 7 for the other months. A pH value below 7 was shown to not favour the multiplication of *V. cholerae* (Huq *et al.* 1984).

The survival of *V. cholerae* in the environment may be dependent on such factors as the occurrence of particular physical and chemical conditions, a specific association of the bacteria with aquatic plants or animals and/or the existence of specific ecological associations involving several aquatic environment components. It has been postulated that the vibrios are converted to a viable but non-culturable (VBNC) form under stressful conditions that cannot be recovered by standard culture techniques, and that such VBNC forms are able to cause infection and can revert to the culturable form (Colwell & Huq 1994). The bacterium exists in a viable, but non-culturable (VBNC) state, when such environmental parameters as temperature, salinity, osmotic pressure, pH and nutrient concentrations become less favourable for growth. The appearance of colonies on plates during both the dry and

the wet season may be attributed to the influence of human activities through secondary transmission. The seasonality of the presence of *V. cholerae* in the different study lakes also may be attributed to secondary transmission. Several studies have reported the severity of secondary transmission is associated with local environmental variables, predominantly water sources for household consumption (Hughes *et al.* 1982; Birmingham *et al.* 1997). Humans who use contaminated surface water for drinking, cooking and bathing are more likely to contract cholera than those who do not. Thus, populations living around these water bodies are highly dependent upon them for survival.

The abundance of *V. cholerae* varied with the absorbance (Fig. 4). It was observed in all three study lakes that the abundance of *V. cholerae* decreased when the ratio was high. For Lake Albert, the abundance of *V. cholerae* exhibited a close relation with absorbance throughout the study (Fig. 4b), and the association was present for only 3 months for Lake Victoria (Fig. 4c). A close association was observed between the presence of *V. cholerae* and absorbance among the sampling sites (ANOVA; $F = 12.05$; d.f. = 35; $P = 0.0024$ for Lake Albert; $P = 0.0014$ for Lake George; $P = 0.048$ for Lake Victoria). It was shown recently that humic substances most probably inhibit *V. cholerae* growth (Kirschner *et al.* 2008). The abundance of *V. cholerae* was reduced with high humic substances, as revealed by the high ratio. The humic substances might have reduced the absorbance ratio in the different study lakes, thereby affecting the abundance of *V. cholerae*. It was noted that there was a negative association between the presence of *V. cholerae* and rainfall during the study. The number of *V. cholerae* decreased with increasing rainfall, which may be attributed to environmental conditions that would become unfavourable for the organisms.

Table 3 highlights the effects of different water parameters on the occurrence of *V. cholerae*. A unit increase in temperature results into a significant decrease ($P < 0.05$) in the number of colonies by 8.57, while an increased pH causes a corresponding nonsignificant increase in the number of colonies by 25.6 (Table 3). However, a unit increase in electrical conductivity leads to an increase of 0.21. Based on these results, a unit increase in temperature, pH and electrical conductivity of water results into a 72.94 increase in the colony numbers recorded in the vicinity of the Gabba fish landing site in the dry season.

CONCLUSIONS AND RECOMMENDATIONS

Cholera has been reported in many Africa countries, including Uganda. This study demonstrated that

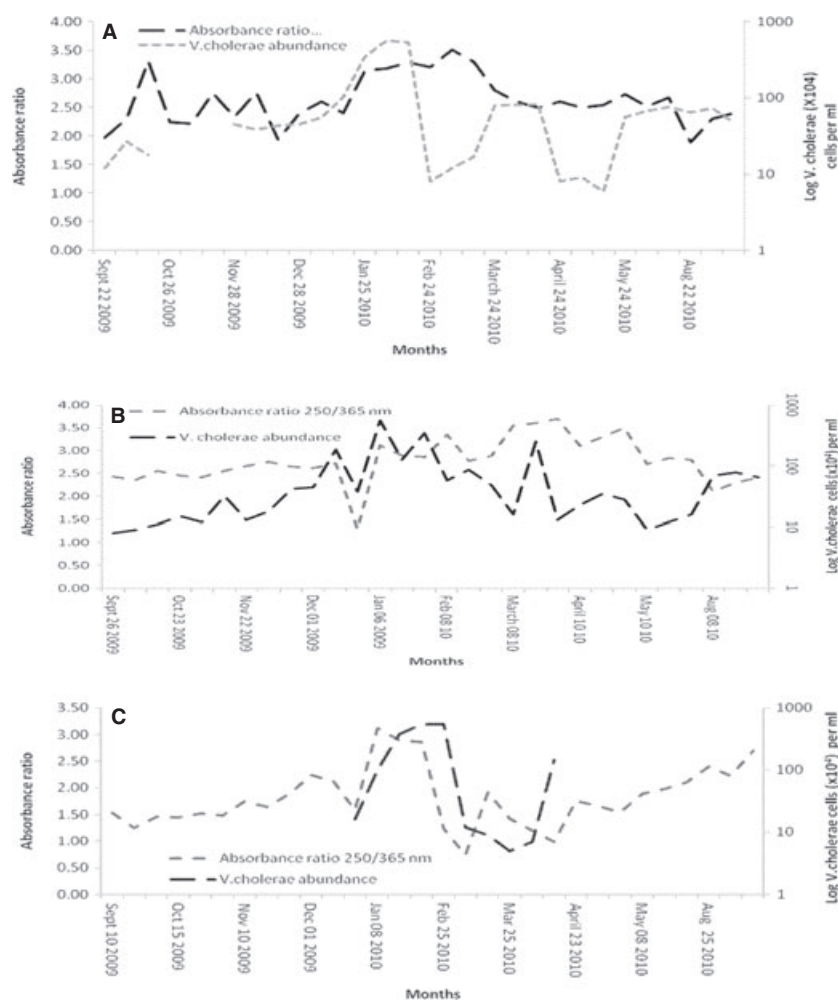


Fig. 4. Variation of *Vibrio cholera* with absorbance.

Table 3. Climatic parameter-related effects of *Vibrio cholerae* in the sample localities (districts) (after logistic regression with coefficients of correlation, standard error, level of significance and confidence intervals)

Site/Parameter	Coef.	SE	$P > t$	CI lower limit	CI upper limit
Wet season	-87.09	28.97	0.00	-144.71	-29.475
Kayanzi fish landing	-151.64	81.23	0.07	-313.20	9.913
Butiaba fish landing	-82.02	55.91	0.15	-193.23	29.186
Temp	-8.57	4.19	0.04	-16.90	-0.238
pH	25.60	28.37	0.37	-30.82	82.021
Conductivity	0.21	0.10	0.04	0.01	0.406
Constant (Gabba fish landing)	72.94	229.28	0.75	—	—

V. cholerae is present in a culturable state from Lakes Victoria, Edward and George. Further, its presence is influenced by environmental factors. The presence of *V. cholerae* is greatly influenced by the pH value. The microorganisms from Lake Victoria, for example, were present when the pH was above 8.5. The numbers of *V. cholera*, however, varied during seasons, being more abundant in the dry season than during the wet season.

This study offers a unique insight into the presence of *V. cholerae* in the study lakes in Uganda. Nevertheless, further research is needed to validate the trends observed in the present study.

ACKNOWLEDGEMENTS

The financial support from the International Foundation of Science is gratefully acknowledged. Part of the

research funds were obtained from IFS grant W/4678-1 Water safety and pollution (Uganda) – from IFS – 2009 for Martha Kaddumukasa. We also acknowledge the support of Pithon Kabiro (ICIPE, Nairobi) for assisting in map setting, Mark Kaddumukasa (Makerere University Medical School, Uganda) and all the logistical support from Alexander Kirschner (Professor, Institute of Medical Hygiene, University of Vienna). The staff of the Department of Biological Sciences, Makerere University who helped in various ways also is acknowledged.

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APPENDIX 1

Plates illustrating positive colonies of *Vibrio cholerae*.

