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► **To cite this version:**

Saidou Kabore, Philippe Cecchi, Thomas Mosser, Mylene Toubiana, Oumar Traore, et al.. Occurrence of *Vibrio cholerae* in water reservoirs of Burkina Faso. *Research in Microbiology*, 2018, 169 (1), pp.1-10. 10.1016/j.resmic.2017.08.004 . hal-01826759

**HAL Id: hal-01826759**

**<https://hal.science/hal-01826759>**

Submitted on 2 Jul 2024

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**For publication****Occurrence of *Vibrio cholerae* in water reservoirs of Burkina Faso**

Saidou Kaboré<sup>a</sup>, Philippe Cecchi<sup>b</sup>, Thomas Mosser<sup>c†</sup>, Mylène Toubiana<sup>c</sup>, Oumar Traoré<sup>a,d</sup>,  
Aboubakar S. Ouattara<sup>a</sup>, Alfred S. Traoré<sup>a</sup>, Nicolas Barro<sup>a‡</sup>, Rita R. Colwell<sup>e</sup>, Patrick  
Monfort<sup>c\*‡</sup>

<sup>a</sup>*Laboratoire de Biologie Moléculaire, d'Epidémiologie et de Surveillance des Bactéries et Virus  
Transmissibles par les Aliments (LaBESTA), Centre de Recherches en Sciences Biologiques,  
Alimentaires et Nutritionnelles (CRSBAN), Université de Ouagadougou, 03 BP 7021 Ouagadougou  
03, Burkina Faso*

<sup>b</sup>*MARBEC UMR 248 IRD, CNRS, Ifremer, Université de Montpellier, Centre de Recherches  
Océanologiques, Abidjan, Côte d'Ivoire*

<sup>c</sup>*"HydroSciences Montpellier", UMR 5569 CNRS, IRD, Université de Montpellier, 34093 Montpellier  
Cedex 05, France*

<sup>d</sup>*Laboratoire National de Santé Publique, 09 BP 24 Ouagadougou 09, Burkina Faso*

<sup>e</sup>*Center for Bioinformatics and Computational Biology and Institute for Advanced Computer Studies,  
3103 Biomolecular Sciences Building, 8314 Paint Branch Drive, University of Maryland, College  
Park, MD 20742, USA.*

<sup>†</sup> *In memoriam*

<sup>‡</sup> These authors contributed equally to this work.

\* Corresponding author

E-mail addresses: [saidoukabore@gmail.com](mailto:saidoukabore@gmail.com) (S. Kaboré), [philippe.cecchi@ird.fr](mailto:philippe.cecchi@ird.fr) (P. Cecchi), [mylenetoubiana@yahoo.fr](mailto:mylenetoubiana@yahoo.fr) (M. Toubiana), [marrou4@yahoo.fr](mailto:marrou4@yahoo.fr) (O. Traoré), [as.ouattara@yahoo.fr](mailto:as.ouattara@yahoo.fr) (A. Ouattara), [astraore@gmail.com](mailto:astraore@gmail.com) (A. Traoré), [barronicolas@yahoo.fr](mailto:barronicolas@yahoo.fr) (N. Barro), [rcolwell@umiacs.umd.edu](mailto:rcolwell@umiacs.umd.edu) (R. Colwell), [patrick.monfort@umontpellier.fr](mailto:patrick.monfort@umontpellier.fr) (P. Monfort)

**Abstract**

Africa is currently an important region in which cholera epidemics occur. Little is known about the presence of *Vibrio cholerae* in freshwater bodies in Africa. There are ca. 1,700 lakes and reservoirs in Burkina Faso, most of which have been built within recent decades to secure water resources. The purpose of this study was to investigate the presence of *V. cholera* in the water of reservoirs, using the most-probable-number polymerase chain reaction. Results showed that *V. cholera* could be detected in water samples collected from 14 of 39 sampled reservoirs. The concentrations varied from 0 MPN/l to more than 1,100 MPN/l. Fifty strains of *V. cholera* isolated on CHROMagar™ vibrio were identified as *V. cholerae* non-O1/non-O139, none of which carried the *ctxA* gene.

A significant positive correlation was found between the presence of *V. cholerae* in the reservoirs and both alkaline pH and phytoplankton biomass. *V. cholerae* was present in significantly higher numbers in reservoirs of urban areas than in rural areas. Since *V. cholerae* non-O1/non-O139 has been shown to be a causative agent of endemic diarrheal outbreaks, their presence in Burkina Faso reservoirs suggests they may play a role in gastroenteritis in that country.

**Keywords:** *Vibrio cholerae*; Water; Reservoir; Burkina Faso

## 41 1. Introduction

42

43 Cholera continues to be a disease of significant morbidity and mortality worldwide.  
44 In 2014, 42 countries reported 190,549 cholera cases, including 2,231 deaths [1]. Africa,  
45 notably West Africa, has become a region associated with cholera epidemics. Among recently  
46 reported cholera cases, 55% originated in Africa, 30% in Asia and 15% in Hispanic countries.  
47 More than 100,000 cases, including  $\approx$  2,000 deaths, were reported in 19 African countries in  
48 2014, namely, in Nigeria, the Democratic Republic of the Congo and Ghana. According to  
49 the WHO, the incidence of cholera in sub-Saharan African countries has become critical, as it  
50 is both endemic and epidemic, with high mortality rates [2].

51 *Vibrio cholerae* causes severe diarrhea in humans, but is also a bacterium  
52 autochthonous to riverine and estuarine environments and a natural component of those  
53 ecosystems [3-5]. Today, more than 200 *V. cholera* serogroups have been described, but only  
54 O1 and O139 serogroups have been identified as responsible for epidemics and pandemics.  
55 Cholera toxin (CT) production by these two serogroups rarely occurs in other serogroups,  
56 referred to as *V. cholerae* non-O1/non-O139 [6]. However, *V. cholerae* non-O1/non-O139  
57 have, in fact, also been shown to be associated with endemic diarrheal diseases, as observed  
58 in Nigeria [7].

59 Seven cholera pandemics have been recorded as causing thousands of deaths  
60 worldwide since 1817. *V. cholerae* O1 classical biotype was incriminated in the first six  
61 pandemics, while *V. cholerae* O1 El Tor biotype was described as being responsible for the  
62 7th pandemic that began in Indonesia in 1961 and subsequently was recorded in India, Africa,  
63 southern Europe and finally South America [8,9]. Recently, unusual strains of *V. cholera*  
64 O1 have been isolated from cholera epidemic victims. These strains, referred to as "atypical or  
65 hybrid ElTor", appear to be the result of gene transfer between *V. cholera* O1 classical and

66 EIT or biotypes[10]. *V. cholera* non-O1 and non-O139 serogroups may be associated with  
67 mild to severe diarrhea and extraintestinal infections such as wound infections and  
68 bacteremia [11-15].

69 The seventh pandemic of cholera is believed to have been detected in the Middle East  
70 and West Africa in 1970 [8]. In West Africa, initially reported cases were in Guinea, with the  
71 epidemic also recorded in Sahel countries where case fatality was high. Since then,  
72 outbreaks have occurred regularly in West African regions [16].

73 Burkina Faso is a land-locked developing country located in the Sahel-Sudan area of  
74 West Africa. It is characterized by a single rainy season (July to October) and significant  
75 inter-annual variability in rainfall [17]. That is, rainfalls vary from the south Sudan ( $> 1000$   
76 mm/year) to the northern Sahel ( $< 500$  mm/year), with a regular latitudinal gradient ( $\approx 1$   
77 mm/km). The main hydrological consequence of this substantial seasonality is that almost all  
78 rivers in the country are temporary and remain dry each year for several months. The result is  
79 that water scarcity constitutes a major challenge for Burkina Faso, and water storage is  
80 maintained in reservoirs during the wet season for use during the dry season, thus constituting  
81 an efficient adaptive strategy to cope with this challenging situation [18,19]. As a result, there  
82 are ca. 1,700 lakes and reservoirs in Burkina Faso (ca. 1/3 perennial), most of which have  
83 been built within recent decades to secure water resources for protection against prolonged  
84 dry spells, and historically documented from 1970-1980[20]. These reservoirs mitigate the  
85 most drastic adverse effects of rainfall variability [21], but may have a deleterious effect on  
86 health. Indeed, the increase in the number of small reservoirs in Burkina Faso, coupled with  
87 crowding, poor sanitation, organic water pollution and intense water contact, has increased  
88 transmission of waterborne diseases, a prime example of which is schistosomiasis[22,23].

89 Burkina Faso is not immune to the threat of cholera epidemics [24] and the first cases  
90 of cholera were reported in 1971, with lethality of 28.5%. Five major epidemics have been

91 recorded: 1991,1995, 1998, 2001 and 2005. During the epidemic of 2005,*V. cholera* was  
92 isolated from the stools of 121patients. Two serogroups were identified: *V. cholera*  
93 O1serotype Ogawa in120 patients and*V. Cholera* O139 in one patient [24]. In 2011, 20cases  
94 of cholera were reported, with two deaths [25].

95 Environmental factors associated with cholera epidemics in Burkina Faso, as in other  
96 African countries located far from the ocean coasts, are complex and have not been  
97 extensively studied [16]. Among a number of factors, human population concentration,  
98 season and climate are vital [4]. The presence of *V. cholerae* non-O1/non-O139 has been  
99 detected in water and fish from one reservoir and also in a neighboring channel in  
100 Ouagadougou[26]. However, the ecological dynamics of *V. cholerae* in the reservoirs of  
101 Burkina Faso and the relationship with cholera epidemiology have not been established. In  
102 this study, the occurrence and incidence of *V. cholera* in 39 Burkina Faso reservoirs were  
103 investigated during the dry season (April-June, 2014) to assess the potential health risk.

## 104 2. Materials and methods

105

### 106 2.1. Area of study and sample collection

107

108 Fig. 1 shows the location of sampling sites (N = 39), all of which were in the upper  
109 basins of the Nakambé River (former White Volta) and the Mouhoun River (former Black  
110 Volta) in Burkina Faso. These reservoirs are distributed throughout the entire territory of  
111 Burkina Faso, but some regions were not included in the study for reasons of distance or  
112 security. Because of the diversity of the reservoirs under study, e.g. size and location (Table  
113 1), the reservoirs that were selected for study are classified as rural or urban according to the  
114 administrative district description of the INSD (Institut National de la Statistique et de la  
115 Démographie du Burkina Faso).

116 Five of the reservoirs are used for tap water production (Dem, Loumbila, Ouaga<sup>3</sup>,  
117 Salbisgo, Ziga) and the others were designed for irrigation purposes (Sourou, Boura, Bamsa,  
118 NavriKpè, Bazega, Bagré). Bagré is also a hydroelectric reservoir. All reservoirs except one  
119 (Bala, a natural reserve), are used for domestic activities that include washing, bathing,  
120 livestock watering and brick-making, with fishing being ubiquitous.

121 All reservoirs included in the study are located close to main roads so as to minimize  
122 time for travel from the sampling site (one to three sites per day in the early morning) to the  
123 laboratory (Ouagadougou in the afternoon). All reservoirs were sampled at least once, except  
124 for the Loumbila reservoir, which was easily and quickly accessible ( $\approx 30$  min from the  
125 laboratory) and therefore was sampled six times to evaluate temporal variability of the  
126 measured parameters during the two-month sampling period.

127 For sampling, in situ measurements were first made, after which three subsurface water  
128 samples (5 liters each) were collected in the center of the reservoir from a small boat or close

129 to shore when a boat was not available. Samples for chemical, biological and bacteriological  
130 analyses were stored and transported to the laboratory in a portable refrigerator (4°C), cooler  
131 (25°C) or at ambient temperature, i.e. in an air-conditioned van ca. 35°C, respectively, and  
132 processed within < 1 to 5 h after collection.

133

## 134 2.2. Enumeration and isolation of *V. cholerae*

135

136 Enumeration of *V.cholerae* in water samples was accomplished using enrichment in  
137 alkaline peptone water (APW) and a combined most-probable-number (MPN)-real-time PCR  
138 analysis [27,28]. Water samples (1 liter, 100 ml and 10 ml) were filtered in triplicate through  
139 0.45 µm pore size nitrocellulose membranes (Whatman, GE Healthcare, Versailles, France)  
140 and the filters were incubated in APW at 41.5°C for 18 h. Smaller volumes, i.e. 1 ml of  
141 undiluted sample, were inoculated in triplicate directly into APW broth and incubated at  
142 41.5°C for 18 h. After enrichment, DNA was extracted from 1 ml of the APW by boiling and  
143 used as template (10-fold-dilution) for PCR.

144 Ten µl from each APW broth was inoculated onto CHROMagar™ *Vibrio* medium  
145 (Humeau, La Chapelle-sur-Erdre, France) and plates were incubated at 37°C for 24 h. One  
146 colony per plate showing typical colonies of *V. cholera* was selected and grown overnight in  
147 APW at 37°C. DNA was extracted from 1 ml of culture by heat lysis and diluted 10 times for  
148 further molecular identification.

149 Detection of *V. cholera* in APW broth and confirmation of the species on isolated  
150 strains were performed by real-time PCR using primers for amplification of the 16S-23S  
151 rRNA intergenic spacer region [29,30]. Real-time PCR was performed on a LightCycler 480  
152 (Roche) in 384-well plates. Distribution of reaction volumes was done using the Echo 525  
153 Liquid Handler (Labcyte).



154 Real-time PCR reaction mixture comprised 0.75  $\mu$ l of 2X LightCycler 480 SYBR  
155 Green I Master (Roche Diagnostics, Mannheim, Germany), 0.8  $\mu$ M of each specific primer,  
156 0.5  $\mu$ l of genomic DNA diluted 10 times and nuclease free water adjusted to a final volume  
157 of 1.5  $\mu$ l. Nuclease- and protease-free water was used as negative control, while strains of *V.*  
158 *cholera* O1 and O139 and non-O1/non-O139 from the collection of the Pasteur Institute (CIP  
159 106974, CIP 62.13, CIP 104152, respectively) were used as positive controls. The reaction  
160 was done twice for each sample. The amplification program consisted of one cycle at 95°C  
161 for 10 min and 45 cycles at 95°C for 10 s, 62°C for 10 s and 72°C for 10 s. The program  
162 terminated with a final dissociation curve analysis consisting of one step at 63°C for 30 s and  
163 gradual heating to 95°C. The MPN values were calculated from the statistical tables of de  
164 Man (1983) [31] and expressed as MPN per liter.

165

### 166 2.3. Detection of *rfb* and *ctxA* genes

167

168 Specific *rfb* gene coding for biosynthesis of O1 and O139-antigen of *V. cholera* and  
169 the *ctxA* gene encoding the A subunit of cholera toxin were amplified by end-point PCR on  
170 DNA from isolated strains and from APW broths (only for *ctxA* gene) [32].

171 The reaction mixtures comprised 2.5  $\mu$ l GoTaq reaction buffer, 0.5  $\mu$ l dNTPs (10 mM  
172 each), 0.8  $\mu$ M forward and reverse primers, 0.5 Units GoTaq DNA polymerase (Promega),  
173 0.5  $\mu$ l genomic DNA diluted 10 times and nuclease-free water to a final reaction volume of  
174 12.5  $\mu$ l. Positive and negative controls were the same as those used in the above protocol.  
175 PCR cycles were performed on the Mastercycler thermal cycler (Eppendorf). The  
176 amplification program was 2 min at 94°C for denaturation, followed by 30 cycles of 15 s at  
177 94°C, 15 s annealing at 56°C (O1-*rfb*/O139-*rfb*) or 58°C (*ctxA*), 30 s elongation at 72°C and a  
178 final elongation step of 5 min at 72°C.

179 PCR products were visualized on a 1% agarose gel containing 0.5 mg.ml<sup>-1</sup>ethidium  
180 bromide in 0.5X Tris borate EDTA buffer (TBE) after electrophoretic migration.

181

#### 182 *2.4. Enumeration of thermotolerant coliforms*

183

184 Thermotolerant coliforms (TTCs), used as indicators of fecal contamination, were  
185 quantified following the filtration method of ISO 9308-1 standard protocol, employing 100  
186 ml of water samples filtered in duplicate and collected on cellulose nitrate membranes (0.45  
187 µm pore size) (Sartorius). The filters were incubated on a nutrient pad impregnated with Endo  
188 medium (Sartorius) for 24 h at 44.5 °C. Results are expressed as number of TTC colony  
189 forming units per 100 ml (TTC/100 ml).

190

#### 191 *2.5. Environmental parameters*

192

193 Sampling sites were localized using a GPS receiver (Garmin 45XL). Depth (cm) of  
194 the water column was measured with a hand-held 0.1 m-resolution echo sounder  
195 (PlastimoEchotest II). Measurements of pH, equivalent conductivity at 25°C (µS/cm) and  
196 temperature (°C) were performed in situ using WTW 197 measuring probes. An AlgaeTorch®  
197 (BBE-Moldaenke, GmbH) was used to quantify subsurface phytoplankton biomass (µg Chl*a*/l).  
198 The measurements are based on autofluorescence properties of photosynthetic organisms and  
199 rely on spectrofluorimetry principles [33,34]. Five consecutive measurements were made and  
200 the results averaged.

201 Quantification of suspended particulate matter (SPM) (mg/l) was performed by  
202 filtering known volumes of water sample (pre-filtered using a 200 µm mesh filter to eliminate  
203 large debris) on a pre-weighed (± 0.1 mg) Whatman GF/F glass fiber filter (0.7 µm pore size).

204 The filtered volume (15 ml to 700 ml) depended on the particulate load and was adjusted to  
205 saturate the filter. Weighing ( $\pm 0.1$  mg) the clogged filter after drying at 105°C allowed  
206 determination of total concentration of SPM. This step was done in duplicate and the mean  
207 value calculated.

208

## 209 2.6. Statistical analysis

210

211 Data were analyzed using statistical Sigma Stat 3.5 software (Systat Software, Inc.).  
212 The non-parametric Spearman's rank order correlation was used to evaluate potential  
213 relationships between environmental and microbiological variables. This test was also applied  
214 to compare depth measurements made during sampling and the official declared capacity of  
215 the specific reservoir. Non-parametric Kruskal-Wallis one-way analysis of variance on ranks  
216 was used to assess potential effect of the hydrographic basin (NakambéversusMouhoun) on  
217 environmental parameters and to compare connectivity of sites, according to the presence (or  
218 not) of *V. cholerae*. The Chi2 test was performed to compare results of urban versus rural  
219 sites and, on a national scale, to assess statistical representativeness of the sampling with  
220 respect to site. The Chi2 test was used to evaluate the effect, if any, of the hydrographic basin  
221 (NakambéversusMouhoun) on *V. cholerae* occurrence, to assess frequency of occurrence of  
222 *V. cholerae*, with respect to urban versus rural sites and to test the influence of the sampling  
223 position (near the shore versus the center of the lake) on detection of *V. cholerae*. The Chi2  
224 test was also used to perform similar analyses of thermotolerant coliforms.

## 225 **3. Results**

226

### 227 *3.1. Sampling and sites*

228

229 Forty-four samples were collected at 39 sites in a 24-day field analysis over a seven  
230 week time period (between April 21 and June 4, 2014) corresponding to the height of the dry  
231 season. Thirty-eight sites were sampled once and the Loumbila Lake was sampled six times at  
232 approximately weekly intervals. At that time of year, direct inflow did not reach most of the  
233 lakes included in this study in the interval since the most recent rainy season (5-6 months  
234 earlier), and the water level was at the annual minimum. This period of time also corresponds  
235 to the yearly maximum with respect to water demand and the most frequent visits by humans  
236 and animals to the sites. Also at this time of year, agricultural activities are mainly  
237 concentrated in irrigated plots, around and downstream from the reservoirs, with two  
238 exceptions: Bala, owing to its status as a natural reserve, and Ziga, where agriculture is  
239 strictly prohibited within a 100 m buffer area all around the reservoir to protect its designation  
240 as a source of drinking water. Fishery activities are conducted at all reservoirs. Except for  
241 those reservoirs located in the urban downtown area, all sites are visited intensively every day  
242 by many herds of animals. All sites except Bala are used daily for domestic activities,  
243 especially by children for recreation.

244 All sites are located on temporary rivers of the Nakambé and Mouhoun basins (N = 26  
245 and N=13, respectively; Fig. 1). The depth of 85% of the sampling sites was less than 3 m,  
246 but a large number of sites were very shallow (median of 160 cm). A significant relationship  
247 was observed between depth of sampling site and declared capacity of the reservoir, as  
248 indicated in the national census of reservoirs (Spearman rank order correlation coefficient:

249 0.841;  $p < 0.001$ ;  $N = 31$ ; data not shown). Thus, the different size categories of reservoir were  
250 sampled in this study.

251

### 252 3.2. Environmental parameters

253

254 Water temperature was high (median of ca. 29°C; coefficient of variation <5%).  
255 Sampling was done early in the morning (between 6 and 9 am; median = 7 h 40) and the  
256 water temperature measurements corresponded to daily minimum values (data not shown).  
257 Conductivity varied between 73.8  $\mu\text{S}/\text{cm}$  in Dissin and 480.9  $\mu\text{S}/\text{cm}$  in Pouytenga (median =  
258 114.7  $\mu\text{S}/\text{cm}$ ) (Table 1). The pH was near neutral for all water samples (median = 7.6), except  
259 for the Koubri-ville reservoir (pH = 5.7) and the spillway of the Saaba reservoir (pH = 9.8).  
260 No cause has been identified to explain low pH water, but high pH water can be linked to the  
261 phytoplankton biomass observed at that site. Spectrofluorometric assessments of  
262 phytoplankton biomass were made for 36 sites. Unfortunately, the instrument was not  
263 operational for the other sampling sites because of excessive turbidity. Observed biomass  
264 measurements varied between 1.2  $\mu\text{g Chl}a/\text{l}$  (Petit Bagré) and 184.2  $\mu\text{g Chl } a/\text{l}$  in the Saaba  
265 spillway (median = 17.5  $\mu\text{g Chl}a/\text{l}$ ). Suspended matter (SPM,  $\text{mg}/\text{l}$ ) also showed significant  
266 variation, from < 5  $\text{mg}/\text{l}$  in Bala and > 3000  $\text{mg}/\text{l}$  in Pouytenga (median = 48.8  $\text{mg}/\text{l}$ ).

267

### 268 3.3. *V. cholerae* detection

269

270 *V. cholerae* was detected in water samples collected from 14 of the 39 sites in five of  
271 the administrative regions (Fig. 1; Table 1). Numbers of *V. cholerae* varied between 0 MPN/l  
272 to more than 1,100 MPN/l in the Saaba spillway (region Centre) and in the Moutori-Dano  
273 reservoir (South West region). Among 204 colonies isolated from CHROMagar™ Vibrio

274 medium, 50 were identified as *V. cholera* from samples collected at 12 sites distributed over  
275 four of the administrative regions. However, none was isolated from APW broths incubated  
276 with water samples collected at the Moutori-Dano (> 1,100 MPN/l) and Nabazana sites (0.92  
277 MPN/l) (Table 1). However, some of the APW broths were PCR-positive for the presence of  
278 *V. cholerae*. Conversely, *V. cholera* was isolated from APW broths incubated with 1 liter  
279 water collected at the Tibin, Bamsa and Sian sampling sites, but the broths were PCR-  
280 negative (Table 1). The *V. cholerae* isolates were uniformly *V. cholerae* non-O1 and non-  
281 O139. The *ctxA* gene was not detected in the isolates or the APW broths.

282

### 283 3.4. Statistical analysis

284

285 Of the 39 studied sites, 30 were rural and nine urban, corresponding to partition of the  
286 communes (urban versus rural) at the national level ( $\text{Chi}^2 = 2.304$ ;  $p = 0.129$ ). Among 14  
287 sites where *V. cholera* was detected, half were urban (78% of the sampled urban sites) and the  
288 other half rural (19% of the rural sites). This finding highlights the greater presence of *V.*  
289 *cholerae* in the urban area water supply compared to rural reservoirs, and was statistically  
290 significant ( $\text{Chi}^2 = 14.431$ ,  $p < 0.001$ ).

291 Among the 14 sites positive for *V. cholerae*, 13 were in the Nakambé basin and one in  
292 the Mouhoun basin (42% and 8% of the total number of sites per basin, respectively). These  
293 percentages were statistically different ( $\text{Chi}^2 = 4.951$ ;  $p = 0.026$ ), indicating over-  
294 representation of positive sites in the Nakambé basin.

295 Median distance separating each site from all other sites of the study varied between  $\approx$   
296 90 km for Ziga and  $\approx$  310 km for Bala (median = 136 km overall). Considering only positive  
297 sites, distance was significantly shorter (92 km) than the median distance separating sites  
298 where *V. cholerae* had not been identified (146 km; one-way Kruskal-Wallis analysis of

299 variance on ranks,  $H = 47.549$ ,  $p < 0.001$ ; Fig. 2), suggesting a “clustering effect” for sites  
300 positive for *V. cholerae*.

301         Seven of the 14 sites where *V. cholera* was detected corresponded to samples collected  
302 near the shore (35 %), while the location of the seven other samples had been positioned in  
303 the center of the reservoir (29%). These percentages were not statistically different ( $\chi^2 =$   
304  $0.171$ ;  $p = 0.679$ ), indicating that the precise position of the sampling site may not have  
305 influenced detection of *V. cholerae*.

306         Overall, a series of highly significant statistical relationships were obtained in this  
307 study by exploring potential links between the presence of *V. cholerae* ( $n = 11$  sites) and  
308 environmental parameters (Table 2). Noticeably, the number of *V. cholera* was higher where  
309 the phytoplankton biomass was elevated ( $P = 0.003$ ), particularly in water samples collected  
310 in shallow and turbid sites ( $P = 0.006$  and  $0.018$ , respectively). Conductivity and pH were  
311 positively correlated, but independent of the depth of the sampling location. Conductivity  
312 was not related to phytoplankton biomass that was, in turn, conversely positively associated  
313 with pH. None of these descriptors was dependent on the basin sampled (Nakambé *versus*  
314 Mouhoun) (one way Kruskal-Wallis analysis of variance on ranks,  $P > 0.05$ ).

315         TTCs were present in 17 of 44 samples (Table 1). Concentrations varied from 0 to  
316 30,000 TTC/100 ml. Urban versus rural and Nakambé versus Mouhoun basins were not  
317 significant nor was sampling location (shore versus center). TTC appeared to be independent  
318 of the environmental parameters (Table 2), except for temperature, which was significantly  
319 correlated.

320

321 *3. The Loumbila reservoir*

322

323 A survey of the Loumbila reservoir where six samples were collected led to  
324 assessment of temporal variability over the two sampling months. Five of six samples were  
325 negative for *V. cholera*, but the fifth sample was positive (2.3 MPN/l)(Fig. 3A), matching an  
326 increase in thermotolerant coliforms (from 0-5.5 to 100 TTC/100 ml). Location of the  
327 sampling station was defined by GPS, and the depth (Fig. 3B) showed an association with  
328 evaporation and water withdrawal by the drinking water plant. Significant local rainfall  
329 clearly compensated the water budget during the second part of the survey, and flooding also  
330 occurred, illustrated by a transient but significant (x 4) increase in the concentration of  
331 suspended material (Fig. 3B).



#### 332 4. Discussion

333

334 This study was carried out in Burkina Faso during the dry season of the year,  
335 corresponding to lower water depth of the bodies of water, due mainly to evaporation and  
336 withdrawal of water for various uses. The dry season corresponds to the period of time when  
337 reservoirs are fully utilized and the time of year when the aquatic ecosystem is subject to  
338 greatest pressure from human use.

339 *V. cholera* was detected in fourteen reservoirs of the five regions under study, with  
340 five of the reservoirs located in the central region of Burkina Faso. Temperatures during the  
341 sampling period varied from 25.7to31.5°C. Many studies have shown that temperature is a  
342 major factor associated with the ecological dynamics of *V. cholera* in aquatic ecosystems[35-  
343 37]. Burkina Faso is a country with a tropical climate, and during the dry season, very low  
344 levels of water are present in the reservoirs, the time during which this study was conducted.  
345 The pH of water is another important factor correlated with the incidence of *V. cholerae*[37].  
346 High pH can be linked to abundant plankton, and the incidence of *V. cholerae* has been  
347 associated with plankton blooms. Plankton, notably zooplankton, is also an environmental  
348 parameter affecting the ecological dynamics of *V. cholera* in the aquatic environment [38].  
349 Results of this study showed a significant positive correlation between the presence of *V.*  
350 *cholera* in the reservoirs and both alkaline pH and plankton biomass. The pH of the sample  
351 collected from the spillway located downstream from the Saaba dam, where a large algal  
352 bloom had been observed (chlorophyll *a* level =185µg.l<sup>-1</sup>), was 9.78. It was at this site that  
353 one of the highest number of *V. cholerae* was obtained (> 1100 MPN/l). It would have been  
354 helpful to have directly measured the zooplankton populations, but the necessary equipment  
355 and expertise were not available. Nevertheless, the phytoplankton bloom can be considered a

356 prerequisite for an increase in zooplankton, with which *V. cholerae* has been well documented  
357 as a commensal [4,39].

358         The incidence of *V. cholera* non-O1/non-O139 was significantly higher in the urban  
359 reservoir samples compared to those from the rural reservoirs. Interestingly, the rural  
360 reservoirs where *V. cholera* non-O1/non-O139 were detected were located closer to each  
361 other than other rural reservoirs. Distribution of reservoirs, on a national scale, is more  
362 numerous and close together where population densities are highest[20]. Thus, the high  
363 density of reservoirs in the densely populated areas can be concluded to provide more contact  
364 between humans and bodies of water. Results of this study may indicate a relationship  
365 between the human population and the presence of *V. cholera* non-O1/non-O139 in the  
366 aquatic reservoirs. Thus, there would be a higher probability of both transmission (between  
367 and among humans) and diffusion (within the aquatic environment) of *V. cholera* non-  
368 O1/non-O139 [16,40]. Results of the Loumbila reservoir analysis suggest a possible  
369 association with local rainfall, leading to an increase in the concentration of suspended matter  
370 [16].

371         The observation that the *ctxA* gene was not detected in the isolates of *V. cholera* does  
372 not exclude a risk associated with detection of *V. cholera* non-O1/non-O139 in the aquatic  
373 environment. Indeed, several studies have shown the infectious nature of *V. cholera* non-  
374 O1/non-O139 [7,41]. These strains are responsible for diarrheal disease that can be fatal  
375 [42,43]. Indeed, some studies have shown antigenic conversion of *V. cholera* non-O1/non-  
376 O139 to *V. cholera* O1 when conditions are favorable [43-46].

377         In this study, we have shown that *V. cholera* non-O1/non-O139 lacking genes coding  
378 for production of cholera toxin are present in the water of several reservoirs in Burkina  
379 Faso[26]. The hot climate and proximity of large human populations around the dams and  
380 lakes of Burkina Faso provide favorable conditions for exposure to *V. cholerae* non-O1/non-

- 381 O139 in this environment, raising the question of their role in gastroenteritis in Burkina Faso.
- 382 Epidemiological studies on such diarrheal diseases should be undertaken in that country.

383 **Conflict of interest**

384

385         The authors declare no conflict of interest.

386

387 ***Acknowledgments***

388

389         SK gratefully acknowledges the Action Service of the Embassy of France in Burkina  
390 Faso for allowing him to stay in France at Montpellier University for molecular analyses.  
391 We also thank the International Science Foundation for purchase of reagents, and the IRD for  
392 financial support for the work on *V. cholerae* in Burkina Faso. This work was partially funded  
393 by Johns Hopkins University and the University of Maryland College Park (NIH Grant  
394 2RO1A1039129-11A2). This research was carried out under the umbrella of the W4F project  
395 “Managing water and food systems in the Volta-Niger basins”, with funding from the  
396 European Commission (EC) and technical support from the International Fund for  
397 Agricultural Development (IFAD). The authors are grateful to Philippe Clair for advice and  
398 management of the qPCR platform (qPHD University of Montpellier/Montpellier GenomiX,  
399 France).

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517 **Legends to figures**

518

519 Fig. 1

520 Location of reservoirs sampled in Burkina Faso during the dry season in 2014. The four main  
521 hydrographic basins of Burkina Faso (Comoé, Mouhoun, Nakambé and Niger) are delineated  
522 and the latitudinal gradient of the mean annual rainfall (1960-1990) is indicated. The red  
523 colored sites indicate the presence of *V. cholerae* non-O1/non-O139.

524

525 Fig. 2

526 Box-plots of distances separating sites with and without *V. cholerae* (median values are  
527 indicated).

528

529 Fig. 3

530 (A) Abundance of *V. cholerae* (left scale, dark line with dark dots) and thermotolerant  
531 coliforms (TTC, right scale, dashed-line with white dots) at the Loumbila site. (B) Water level  
532 of the Loumbila Lake (left scale, dark line with dark dots), local rainfall (right scale, gray  
533 bars) and concentration of suspended matter (right scale, dashed-line with white dots).

## 1 Table 1

2 Sites sampled in Burkina Faso between April and June, 2014. Relevant information and measured parameters are provided. Sites are ranked by  
3 sampling chronological order.

Site	Latitude	Longitude	Administrative region	Situation	Sampling place <sup>a</sup>	V <sup>b</sup> (Mm <sup>3</sup> )	Z <sup>c</sup> (cm)	pH	C25 <sup>d</sup> (μS/cm)	SPM <sup>e</sup> (mg/l)	Chl a <sup>f</sup> (μg/l)	<i>V. cholerae</i> (MPN/l)	<i>V. cholerae</i> <sup>g</sup> positive strains	TTC/100 ml <sup>h</sup>
Loumbila-1	12°29'34.0"	01°24'25.8"	Plateau Central	rural	center	42.2	330	7.6	n.d.	11.9	n.d.	0	-	5.5
Bam	13°19'57.4"	01°30'48.8"	Centre Nord	urban	center	31.0	160	7.6	101.1	38.1	10.5	0.36	+	0
Tibin	13°11'11.8"	01°26'36.0"	Centre Nord	rural	shore	0.0	n.d.	7.3	138.6	167.6	15.0	0	+	100
Bamsa	13°15'36.2"	01°06'02.8"	Centre Nord	rural	center	n.d.	220	7.9	115.4	46.7	12.0	0	+	0
Dem	13°11'27.8"	01°09'12.1"	Centre Nord	rural	center	4.0	120	7.8	141.4	20.4	25.8	0	-	0
Sian	13°05'46.1"	01°13'04.7"	Centre Nord	rural	center	2.0	100	7.8	163.3	64.7	14.3	0	+	200
Kaya	13°05'33.4"	01°04'07.5"	Centre Nord	urban	shore	1.1	n.d.	8.5	230.3	391.0	25.4	1.5	+	100
Pouytenga	12°13'41.5"	00°25'54.9"	Centre Est	urban	shore	0.6	60	7.4	480.9	3153.3	n.m.	43	+	30000
Ouaga2	12°23'16.7"	01°31'22.4"	Centre	urban	center	1.2	85	7.7	309.8	144.5	64.5	43	+	0
Ouaga3	12°23'24.9"	01°30'29.3"	Centre	urban	center	1.5	140	8.6	92.5	53.6	65.5	9.3	+	0
Loumbila-2	12°29'38.6"	01°24'22.8"	Plateau Central	rural	center	42.2	260	7.6	155.6	11.4	13.6	0	-	0
Sourou	12°48'07.2"	03°26'55.9"	Boucle Mouhoun	rural	center	370.0	320	8.6	226.0	18.2	33.4	0	-	0
Goundi	12°12'48.4"	02°28'37.1"	Centre Ouest	rural	shore	n.d.	100	8.6	106.4	53.2	17.9	0	-	0
Yako-Kanazoé	13°00'41.9"	02°03'55.1"	Nord	rural	center	75.0	410	7.6	136.1	9.9	11.7	0	-	100
Bala	11°33'37.9"	04°09'24.8"	Hauts Bassins	rural	center	1.5	160	6.6	129.6	5.0	5.3	0	-	0
Salbisgo	12°09'53.6"	02°22'36.1"	Centre Ouest	rural	shore	3.6	100	7.7	362.8	481.3	15.8	0	-	0
Yamtenga	12°20'20.1"	01°26'51.0"	Centre	urban	shore	0.6	60	7.9	350.0	319.0	n.m.	460	+	1000
Saaba (reservoir)	12°20'39.8"	01°24'12.2"	Centre	rural	shore	5.4	60	7.9	369.9	333.0	66.3	24	+	500

Saaba (spillway)	12°20'42.7"	01°24'13.0"	Centre	rural	shore	n.d.	n.d.	9.8	145.7	46.8	184.2	>1100	+	0
Loumbila-3	12°29'37.7"	01°24'21.8"	Plateau Central	rural	center	42.2	270	7.6	83.5	4.7	17.0	0	-	0
Boura	11°02'49.3"	02°29'52.1"	Centre Ouest	rural	center	4.2	330	8.1	75.0	11.9	15.9	0	-	500
Leo	11°05'34.0"	02°06'35.4"	Centre Ouest	urban	shore	0.1	60	6.3	114.0	485.0	n.m.	0	-	500
Sapouy	11°32'38.6"	01°45'45.8"	Centre Ouest	rural	shore	0.7	160	6.3	n.d.	313.2	n.m.	0	-	0
Bapla-Diébouougou	10°52'56.0"	03°15'46.7"	Sud Ouest	urban	center	n.d.	300	8.5	257.0	9.4	23.8	0	-	0
Moutori-Dano	11°09'29.7"	03°04'32.3"	Sud Ouest	urban	shore	n.d.	60	8.3	170.7	63.5	64.6	>1100	-	0
Pa	11°29'23.9"	03°14'33.6"	Hauts Bassins	rural	shore	n.d.	200	7.7	96.4	8.6	17.4	0	-	0
Loumbila-4	12°29'38.3"	01°24'21.6"	Plateau Central	rural	center	42.2	270	8.0	222.4	10.2	10.8	0	-	0
Dissin	10°56'21.7"	02°55'36.4"	Sud Ouest	rural	shore	0.1	60	7.5	73.8	237.9	n.m.	0	-	0
Navri Kpè	10°59'05.3"	02°56'26.7"	Sud Ouest	rural	shore	2.6	n.d.	7.4	117.9	21.2	53.8	0	-	0
Deux Balé	11°43'04.9"	03°00'37.5"	Boucle Mouhoun	rural	shore	6.0	n.d.	7.7	132.7	26.8	42.8	0	-	200
Toukoumtouré	12°15'17.3"	01°19'02.6"	Centre	rural	shore	0.4	60	6.7	75.1	580.0	n.m.	0	-	0
Arzuma	12°13'07.6"	01°17'46.0"	Centre	rural	center	1.4	150	7.8	105.3	20.7	27.1	0	-	0
Tanvi Nakemtenga	12°13'35.7"	01°21'06.6"	Centre	rural	center	0.6	170	7.6	105.3	53.1	15.8	0	-	0
Nabazana	12°12'13.4"	01°21'07.8"	Centre	rural	center	4.0	100	7.7	97.6	51.7	24.7	0.92	-	0
Loumbila-5	12°29'38.2"	01°24'21.6"	Plateau Central	rural	center	42.2	270	7.5	84.3	43.1	14.8	2.3	+	100
Nobili	11°44'27.6"	01°15'24.0"	Centre Sud	rural	shore	0.7	60	7.0	97.0	217.9	n.m.	0	-	0
Bazega	11°44'06.2"	01°21'12.0"	Centre Sud	rural	center	11.2	230	7.4	94.9	25.3	44.1	0	-	5
Ziga (reservoir)	12°29'24.4"	01°05'25.5"	Plateau Central	rural	center	200.0	290	7.4	112.3	23.0	9.0	0	-	0
Ziga (downstream)	12°29'25.2"	01°04'55.1"	Plateau Central	rural	shore	n.d.	n.d.	7.0	n.d.	50.8	38.1	0	-	10
Loumbila-6	12°29'38.8"	01°24'21.6"	Plateau Central	rural	center	42.2	n.d.	7.5	85.6	20.8	17.6	0	-	5
Bagré (reservoir)	11°29'253.7"	00°32'56.9"	Centre Est	rural	center	1700.0	1190	7.5	86.6	44.7	2.6	0	-	5
Bagré	11°28'48.8"	00°32'26.9"	Centre Est	rural	shore	n.d.	n.d.	7.4	86.7	56.0	2.6	0	-	5

(downstream)														
Petit Bagré	11°28'25.4"	00°30'47.0"	Centre Est	rural	shore	3.5	n.d.	7.5	111.1	52.1	1.2	0	-	0
Koubri-ville	12°11'34.0"	01°24'16.2"	Centre	rural	center	9.7	205	5.7	n.d.	1240.0	n.m.	0	-	0

- 4 <sup>a</sup>Sampling place: center of the lake *versus* near shore; <sup>b</sup> V: Total volume of reservoir in millions of cubic meters; <sup>c</sup> Z: depth of sampling site; <sup>d</sup> C25: equivalent conductivity at
- 5 25 °C; <sup>e</sup> SPM: suspended particulate matter; <sup>f</sup> Chl *a*: Chlorophyll *a*; <sup>g</sup> *V. cholerae* positive strains: presence of identified strains; <sup>h</sup> TTC: thermotolerant coliforms.
- 6 *n.d.*: non determined values; *n.m.*: non measurable Chl *a* values owing to excessive turbidity.

1 Table 2

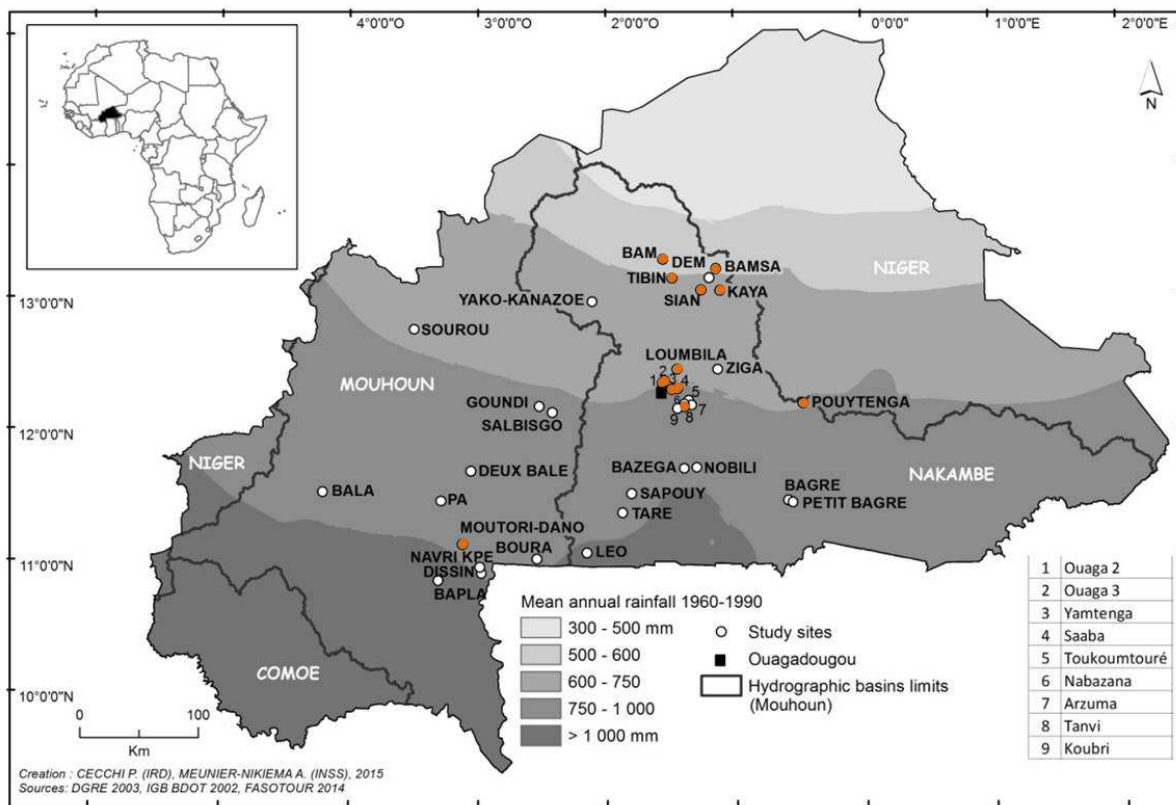
2 Non-parametric Spearman's rank order correlation between environmental and  
 3 microbiological variables.

	T (°C)	pH	C25 <sup>a</sup> (μS/cm)	SPM <sup>b</sup> (mg/l)	Chl <i>a</i> <sup>c</sup> (μg/l)	<i>V. cholerae</i> (MPN/l)	TTC <sup>d</sup> /100ml
Z <sup>e</sup> (cm)	0.515	0.075	-0.226	-0.733	-0.498	-0.454	0.012
	<b>0.003</b> (**)	0.668	0.204	<b>&lt; 0.001</b> (***)	<b>0.008</b> (**)	<b>0.006</b> (**)	0.943
T (°C)		0.327	-0.129	-0.615	-0.318	-0.386	-0.465
		0.052	0.459	<b>&lt; 0.001</b> (***)	0.086	<b>0.019</b> (*)	<b>0.004</b> (**)
pH			0.387	-0.186	0.416	0.388	-0.041
			<b>0.017</b> (*)	0.237	<b>0.016</b> (*)	<b>0.011</b> (*)	0.793
C25 (μS/cm)				0.192	0.273	0.348	0.136
				0.233	0.117	<b>0.028</b> (*)	0.399
SPM (mg/l)					0.201	0.357	0.223
					0.245	<b>0.018</b> (*)	0.145
Chl <i>a</i> (μg/l)						0.488	-0.003
						<b>0.003</b> (**)	0.984
<i>V. cholerae</i> (MPN/l)							0.170
							0.267

4 <sup>a</sup> C25: equivalent conductivity at 25 °C; <sup>b</sup> SPM: suspended particulate matter; <sup>c</sup> Chl *a*: Chlorophyll *a*; <sup>d</sup> TTC:  
 5 thermotolerant coliforms; <sup>e</sup> Z: depth of sampling site.

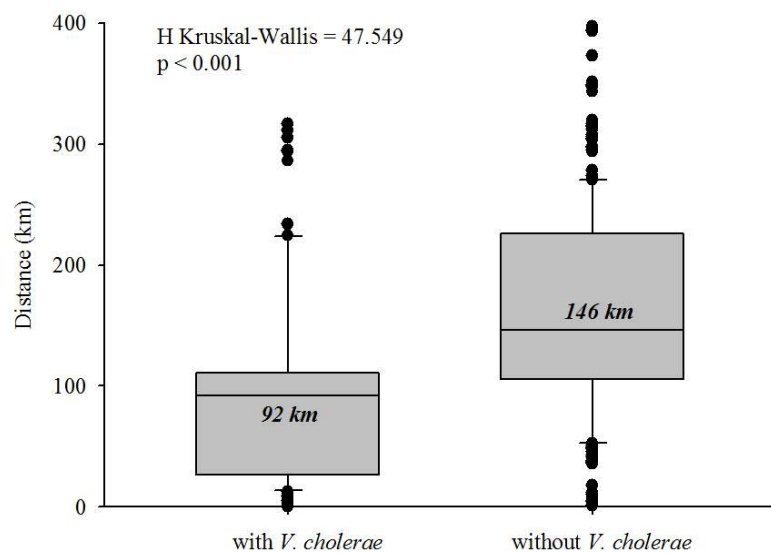
6 (\*) p < 0.05; (\*\*) p < 0.01; (\*\*\*) p < 0.001.

1 Figure 1



2

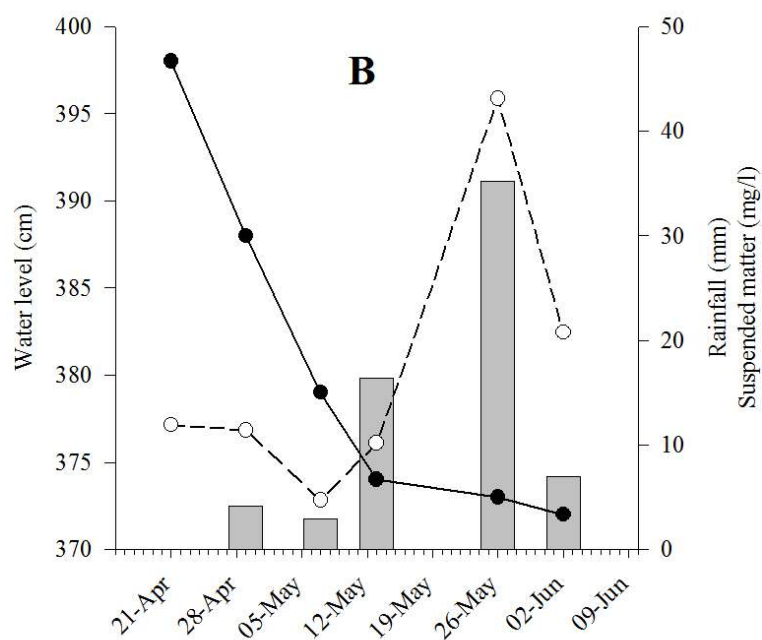
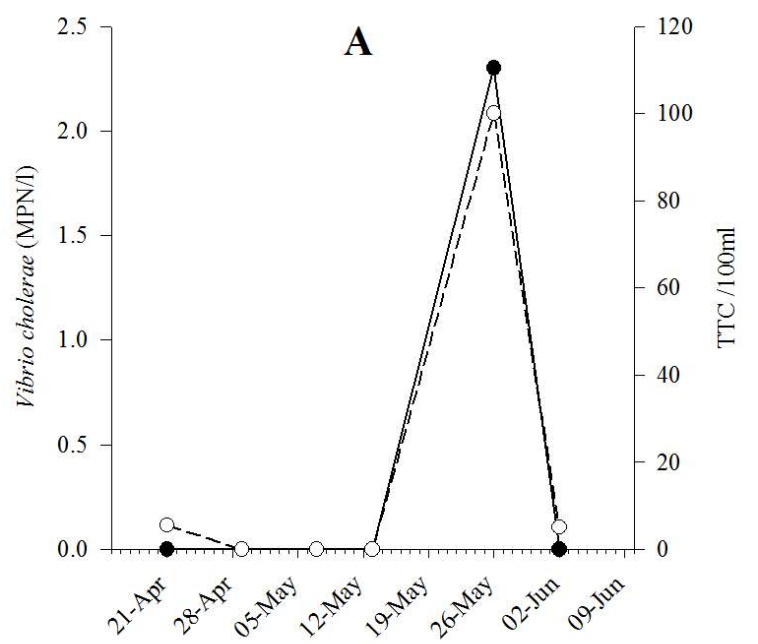
1 Figure\_2



2



1 Figure 3



2