1   **Effectiveness of a single dose of oral cholera vaccine: findings from epidemiological and**

2   **genomic surveillance of *Vibrio Cholerae* in the Democratic Republic of the Congo (PICHA7**

3   **Program)**

4

5   **Authors:**

6   Christine Marie George[1], Alves Namunesha*[2], Kelly Endres[1], Willy Felicien[2], Presence

7   Sanvura[2], Jean-Claude Bisimwa[2], Jamie Perin[1], Justin Bengehya[3], Ghislain Maheshe[4], Cirhuza

8   Cikomola[2,4], Lucien Bisimwa[2], Alain Mwishingo[2], David A. Sack[1], Daryl Domman[5]

9

10   **Affiliations:**

11   1. Department of International Health, Johns Hopkins Bloomberg School of Public Health,

12   Baltimore, MD, USA

13   2. Center for Tropical Diseases & Global Health, Université Catholique de Bukavu, Bukavu

14   B.P 265, Democratic Republic of the Congo

15   3. Bureau de l'Information Sanitaire, Surveillance Epidémiologique et Recherche Scientifique,

16   Division Provinciale de la Santé Sud Kivu, Ministère de la Santé, Bukavu B.P 265,

17   Democratic Republic of the Congo

18   4. Faculty of Medicine, Catholic University of Bukavu, Bukavu B.P 265, Democratic Republic

19   of the Congo

20   5. Department of Internal Medicine, University of New Mexico Health Sciences Center,

21   Albuquerque, New Mexico, USA

22   *Co-first Author

23

24

Abstract (150 Word Limit)

This study investigated whole-cell oral cholera vaccine (kOCV) single-dose effectiveness and transmission dynamics of *Vibrio cholerae* through 4 years of epidemiological and genomic surveillance in Democratic Republic of the Congo (DRC). Whole genome sequencing was performed on clinical and water *V. cholerae* strains from 200 patient households and found annual bimodal peaks of *V. cholerae* clade AFR10e. 1154 diarrhea patients were enrolled with 342 culture confirmed cholera patients. A large clonal cholera outbreak occurred 18 months after a kOCV campaign of >1 million doses of Euvichol-Plus, likely because of low vaccine coverage in informal settlements (9%). Clinical and water *V. cholerae* strains in the same household were more closely related than different households suggesting both person-to-person and water-to-person transmission. Single-dose kOCV vaccine effectiveness in the first 24 month after vaccination was 56.9% (95% CI: 18.6%-77.2%), suggesting a single-dose provided modest protection against medically attended cholera during the 24 months post-vaccination.

38

39

40

41

INTRODUCTION

An estimated 2.9 million cholera cases and 95,000 deaths occur annually in cholera-endemic countries.[1] The Democratic Republic of the Congo (DRC) has one of the highest rates of cholera in Africa.[2] In 2017, the Global Task Force on Cholera Control (GTFCC) released the "*Ending*

47   *Cholera-A Global Roadmap to 2030"* to reduce cholera deaths by 90% and cholera elimination

48   in 20 additional countries by 2030.[3] To address cholera in transmission hotspots, the GTFCC

49   recommends oral cholera vaccine (OCV) campaigns and case-area-targeted-interventions

50   (CATIs) of water, sanitation, and hygiene (WASH) around cholera cases. However, there is

51   currently an OCV stockpile shortage globally resulting in the need for evidence on the duration

52   of protection conferred by OCV to determine the optimal frequency to repeat OCV campaigns.[4]

53   Most studies on OCV effectiveness are from India and Bangladesh, and evaluate the

54   effectiveness of two-dose OCV protection.[5] Only four studies have evaluated single-dose OCV

55   vaccine effectiveness (with only two for a duration of 24 months or longer), and all except one

56   used the no longer produced Shanchol vaccine.[5]

57

58   Few studies combine evaluations of OCV effectiveness with genomic surveillance of clinical and

59   environmental *Vibrio cholerae* strains using whole genome sequencing (WGS) which can

60   provide valuable information on how OCV campaigns impact *V. cholerae* transmission dynamics

61   and the genetic characteristics of *V. cholerae* strains circulating. WGS of *V. cholerae* strains also

62   presents a valuable tool to link epidemics globally and investigate cholera transmission dynamics

63   through distinguishing strains based on single nucleotide polymorphisms (SNPs). Genomic data

64   from 45 African countries revealed that seventh pandemic El Tor (7PET) *V. cholerae* strain was

65   introduced into Africa at least 15 times since 1970.[8] Previous genomic studies (2018-2024) in

66   DRC found *V. cholerae* 7PET strains to be in the AFR10d, AFR10e clade, and ARFR10w clade

67   sublineages.[9,10]

68

69 Only one study has performed WGS on water source and clinical *V. cholerae* strains from patient

70 households to investigate cholera transmission dynamics.[11] This study conducted by our research

71 group in Bangladesh found that 80% of cholera patient households had source water strains

72 closely related to clinical strains, and 20% of households had clinical strains from infected

73 individuals that were more closely related to clinical strains from another household than to

74 source water strains from their own household. These results were consistent with person-to-

75 person and water-to-person *V. cholerae* transmission. There have been no genomic studies to

76 elucidate transmission dynamics of *V. cholerae* infection for cholera patient households in sub-

77 Saharan Africa, with previous studies exclusively in South Asia.

78

79 In this study we conducted 4 years of epidemiological and genomic surveillance of *V. cholerae*

80 in eastern DRC to achieve the following study objectives: (1) to evaluate the effectiveness of a

81 single-dose of killed whole-cell oral (kOCV) Euvichol-Plus during the 24 month period after a

82 preventative kOCV campaign; (2) to investigate *V. cholerae* transmission dynamics among

83 cholera patients, household members, and water sources using WGS; and (3) to investigate the

84 spatiotemporal spread of *V. cholerae* in this hyper-endemic region.

85

86 **METHODS**

87 ***Ethical approval***

88 This study was conducted in urban Bukavu in South Kivu province, DRC. We received ethical

89 approval for this study from the Johns Hopkins Bloomberg School of Public Health and Catholic

90 University of Bukavu. All participants or their guardians gave written informed consent.

91

92 ***Study Design and Protocol***

4

93   For cholera surveillance activities (passive surveillance), diarrhea patients admitted to 115

94   healthcare facilities in Bukavu from March 2020-March 2024 had whole stool tested for *V.*

95   *cholerae* by bacterial culture. Cholera patients were defined as diarrhea patients with a stool

96   bacterial culture result positive for *V. cholerae*. A prospective cohort study of household contacts

97   of cholera patients was conducted from December 2021-December 2023 to investigate cholera

98   transmission dynamics in cholera patient households. Household contacts of cholera patients

99   were defined as those sharing a cooking pot and residing in the same home with the cholera

100   patient for the past three days. Household contacts were enrolled within 24 hours of cholera

101   patients. The sample size for the prospective cohort study was determined by the number of

102   cholera patients that could be screened and were willing to participate in the cohort study from

103   December 2021-December 2023. Cholera patient households in the prospective cohort study

104   were visited on Days 1, 3, 5, 7 and 9 (Visits 1-5) after the household's index cholera patient was

105   admitted at a health facility to conduct clinical surveillance. For clinical surveillance, a stool

106   sample was collected from household contacts at each household visit to test for *V. cholerae* by

107   bacterial culture. An unannounced spot check was conducted at each timepoint to collect a

108   sample of the household's source and stored drinking water to test for *V. cholerae* by bacterial

109   culture.

110

111   During December 28th, 2021-January 2nd, 2022 and March 31st-April 4th, 2022, a preventative

112   OCV campaign delivered 1.04 million doses of Euvichol-Plus kOCV in Bukavu. This campaign

113   was delivered through a combination of door-to-door visits and designated healthcare facilities.

114   The kOCV vaccination status for diarrhea patients and their household members was assessed

115   through self-report or caregiver-report during the time of patient treatment in the healthcare

116   facility or during a home visit conducted the same or the following day. A structured

117   questionnaire was administered obtaining information on kOCV administration and the date and

118   number of doses. A kOCV vaccine card was shown along with a photo of a person consuming

119   kOCV Euvichol-Plus. Informal settlements were defined as areas where there were no household

120   connections to piped water.

121

122   *Laboratory Analyses*

123   All whole stool samples were brought to the PICHA7 Enteric Disease Microbiology Laboratory

124   within three hours of the sample being produced, and water samples were brought to the

125   laboratory within 3 hours of collection for *V. cholerae* analysis by bacterial culture according to

126   our previous methods.[12] Strains were then preserved as stabs in nutrient agar or on Whatman

127   filter paper to preserve their DNA.

128

129   *Whole genome sequencing*

130   Genomic DNA for strains on filter paper was extracted using published Chelex methods and the

131   ZymoBIOMICS DNA Miniprep Kit was used for strains preserved as stabs.[13,14] WGS was

132   performed at the SeqCenter in Pittsburgh, Pennsylvania and the University of New Mexico

133   Health Sciences Center. Short reads were processed using the Bactopia pipeline[15] and SPAdes

134   v.3.10.0[16,17] and annotated using Prokka v.1.520.[18] Genome completeness estimates and checks

135   for contamination were performed using CheckM v.1.0.722 and Kraken v.0.10.6.[19,20]

136

137   *Genomic and phylogenetic analyses*

138   Paired-end reads for 255 7PET strains were mapped and variants called against the *V. cholerae*

139   O1 El Tor reference N16961 (NCBI accession numbers LT907989 and LT907990) using snippy

140   v.4.6.0 (https://github.com/tseemann/snippy) via the Bactopia pipeline[15] to generate a reference-

141   based alignment containing 329 variable SNP sites. All 255 strains mapped over 90% of the

142   reference genome and were considered for further analysis. A pairwise SNP matrix was

143   generated for the 255 7PET strains in this study using `pairsnp`

144   (https://github.com/gtonkinhill/pairsnp). We used ARIBA v.2.14.7 to determine mutations in

145   *wbeT* using a reference wild type Ogawa *wbeT* gene (ENA accession AEN80191.1).

146

147   All 7PET strains from this study were combined with 1,418 globally representative 7PET strains

148   to assess phylogeographic relatedness.[21] The 12,561 variable site (SNP) alignment was used to

149   build a maximum likelihood phylogeny using IQ-Tree v1.6.12 under the GTR model with the

150   gamma distribution to model site heterogeneity (GTR+GAMMA) with 10,000 ultrafast

151   bootstraps and 10,000 bootstraps for the SH-like approximate likelihood ratio tests (SH-aLRT).[22]

152   The phylogeny was visualized with ggtree v.1.6.11[23] and rooted using the pre-seventh pandemic

153   strain M66.

154

155   *Local transmission analyses*

156   Insights on local transmission dynamics were gleaned from a 329 SNP reference-based

157   alignment of the 255 7PET strains. A maximum-likelihood tree was built using IQ-Tree under

158   the GTR+GAMMA model with 10,000 ultrafast bootstraps and 10,000 SH-aLRT. Phylogenies

159   were visualized in Microreact.[24] Minimum spanning trees were created using *GrapeTree*

160   v.1.5.0.[25] Geospatial data for cases was visualized using python with the *geopandas*[26] and

161   *contextily*[27] packages.

162

Data availability

164   All next-generation sequencing data generated in this study have been deposited into the NCBI

165   Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra).

166

Statistical Analysis

168   To assess single dose Euvichol-Plus kOCV vaccine effectiveness during the 24-month period

169   after vaccination using a test-negative design among diarrhea patients, logistic regression models

170   were performed with cholera infection as the outcome (defined as a positive bacterial culture

171   result for *V. cholerae*) and the predictor was kOCV vaccination status (whether diarrhea patients

172   reported receiving one dose of kOCV during the dates of the kOCV campaign. In this model the

173   odds ratio (OR) was the odds of single-dose kOCV vaccination in the cases compared to

174   controls. Vaccine effectiveness was calculated as (1-OR x 100%), and kOCV vaccinated

175   individuals reporting receiving two doses of kOCV were excluded from the analysis. This

176   analysis was performed in SAS (version 9.4). Permutation tests using R and python were

177   performed to analyze pairwise comparisons of genomic data. For pairwise comparisons, SNP

178   counts for each strain were compared to the reference strain reference N16961.

179

Role of funding source

181   The funder has no input in study design, data collection, analysis, or manuscript submission.

182

RESULTS

184 *Epidemiology*

185 During the 4 years of epidemiological and genomic surveillance from March 2020 to March

186 2024, 342 out of 1154 diarrhea patients (30%) were identified to be *V. cholerae* by bacterial

187 culture (Figure 1). Figure 2 shows a map of the 115 surveillance healthcare facilities and

188 diarrhea patient households. An annual bimodal peak of cholera was observed corresponding

189 with the dry season (June to August) and the rainy season (September to January) in 2020 and

190 2023. In December 2021, an kOCV campaign distributed 1.04 million doses of Euvichol-Plus

191 within Bukavu (study surveillance site). After this kOCV campaign there were sporadic cholera

192 patients through January 2023. Then, 18 months after the kOCV campaign from June to

193 November of 2023, there was a large cholera outbreak in this same area. Nine percent

194 (309/3395) of individuals in the informal settlements in our study area reported receiving at least

195 one kOCV dose during our vaccine coverage surveys between December 2021 and April 2022.

196 During the four-year surveillance period, stored and source water samples were collected from

197 177 cholera patient households with 9% (16/172) of households with stored water samples

198 positive by bacterial culture for *V. cholerae* and 5% (8/151) of households with positive source

199 water samples. In total 29 water samples were *V. cholerae* positive by bacterial culture over the

200 study period (Supplemental Figure 1). From October 2021 to November 2022 (including the 12-

201 month period after the kOCV campaign) there was no *V.cholerae* detected in drinking water

202 samples despite there being culture confirmed cholera patients.

203

204 *OCV Vaccine Effectiveness*

205 Seven-hundred-and-fifty diarrhea patients were admitted to surveillance healthcare facilities

206 during the 24-month period after the kOCV campaign (December 2021 to December 2023).

9

207 Demographic characteristics for kOCV vs. non-kOCV vaccinated individuals are included in

208 Table 1. Ninety-four percent (708/750) of diarrhea patients reported residing in their current

209 residence for the three nights before hospitalization, and 12% had running water inside the home

210 (93/748). Twelve percent (93/750) (15 cholera patients and 78 non-cholera diarrhea patients) of

211 diarrhea patients reported receiving at least one dose of kOCV from December 2021 to April

212 2022, with only 2% (14/750) of patients reporting receiving two kOCV doses. Thirteen percent

213 of kOCV vaccinated patients (12/93) showed a vaccination card. During this period there were

214 208 cholera patients (193 non-kOCV vaccinated and 15 kOCV vaccinated). Five-hundred-and-

215 thirty-one diarrhea patients during this 24-month period were >1 years of age. The unadjusted

216 single-dose kOCV vaccine effectiveness in the first 24 month after vaccination was 59.8% (95%

217 CI: 19.7% to 79.9%) for individuals >1 year and after adjustment for age (continuous) the

218 vaccine effectiveness was 58.7% (95% CI: 17.3% to 79.4%). Ten diarrhea patients were

219 excluded from this analysis because they received 2 doses of kOCV. Supplementary Table 1

220 includes the single-dose kOCV vaccine effectiveness by time interval for individuals >1 year.

221

222 *Genomic analyses*

223 We sequenced 255 *V. cholerae* 7PET genomes collected between December 2020 to December

224 2023 from the study area described above. Of these strains, 247 were clinical (158 Ogawa and 89

225 Inaba) strains representing 243 individuals from 200 households (198 patients and 45 household

226 contacts), and 8 were water samples. The WGS analysis included 75% of cholera patient

227 households (255/342) during the surveillance period, all strains with isolates available were

228 sequenced. To investigate the genetic relatedness of the *V. cholerae* strains, we analyzed the

229 pairwise SNP differences across the 255 strains. Of the 200 households in the study, 31

230   households are represented by more than one sample, 26 households had more than one

231   participant with samples, and five households had both a clinical and water sample. Figures 3A

232   and 3B show the maximum-likelihood phylogeny of the 255 7PET *V. cholerae* genomes by year,

233   sample type, and serotype. Our phylogenic analyses indicate *V. cholerae* strains isolated from

234   both clinical and water samples were closely related, indicating limited genetic divergence over

235   the study period. Figure 4A shows a violin-plot of pairwise differences between all *V. cholerae*

236   strains, those from the same households, and those from pre- and post-vaccine campaign.

237   Between all strains, the minimum number of SNP differences was 0 and the maximum number

238   was 65, with a median of 13. Of strains from the same household, the range was from 0-14

239   SNPs, with a median of 0 SNPs (Figure 4B). We found a significant difference in the median

240   number of SNPs between strains from different households and those from the same household

241   (p=<0.0001; Mann-Whitney U test). For strains from the same individual, the range was 0-1

242   SNP, with a median of 0 SNPs. Five households had both a water sample and a clinical sample,

243   where in three households these strains had zero SNP differences between them. The other two

244   households had 3 and 1 SNP differences between the clinical and water samples. This provides

245   strong evidence that the same strain causing infections in the household is also detected in the

246   household water. However, we cannot determine whether the water was the source of infection,

247   or the water was contaminated after an infection was present in the household. The median

248   difference in the number of SNPs was significantly lower for strains from cholera patients pre-

249   kOCV vaccine campaign (median: 6 range: 0-58) compared to post-kOCV vaccine campaign

250   (median: 12 range: 0-31) (Mann-Whitney U test; P-value: p<0.0001)).

251

252   To understand how the 255 *V. cholerae* strains from this study fit within the larger diversity of

253   7PET strains, we contextualized these strains within a collection of 1,422 additional 7PET

254   genomes (Figure 5).[21] Our phylogenetic analysis placed these strains within the T10 lineage

255   within the AFR10e clade. Our strains, sampled from 2020-2023, were similar to other recent

256   samples (2015-2020) from the DRC in the same region of South Kivu.[10] Our genomic analysis of

257   the *wbeT* gene indicated that samples from 2020-2022 have a wildtype, intact gene which would

258   indicate an Ogawa serotype. There is a single exception for a strain sampled in 2020 that had a

259   frameshift mutation (N165fs), which would likely result in an Inaba serotype. All samples in

260   2023 after the OCV campaign, however, appear to have a fragmented *wbeT* gene marked by

261   insertion of mobile elements into this gene – likely conferring an Inaba serotype. In line with

262   other AFR10e strains, our strains harbor mutations in *gyrA* (S83I) and *parC* (S85L) which have

263   been previously described as resulting in reduced susceptibility to fluoroquinolones.[10]

264

265   **DISCUSSION**

266   In our urban cholera endemic setting there were annual bimodal peaks of the *V. cholerae* clade

267   AFR10e corresponding with the dry and rainy seasons. A third of diarrhea patients presenting at

268   our 115 surveillance healthcare facilities were confirmed to have cholera by bacterial culture.

269   Nine percent of stored water and five percent of source water samples from cholera patient

270   households had *V. cholerae* present. This finding suggests that both source water and

271   contamination of stored water may be potential transmission routes for *V. cholerae* infections in

272   cholera patient households. A large clonal cholera outbreak was observed 18 months after a

273   single-dose kOCV campaign where >1 million doses of Euvichol-Plus were distributed. This

274   large outbreak occurred in spite of the kOCV vaccine campaign, perhaps because of the low

275   kOCV coverage (9%) in the informal settlements in Bukavu, which are often hotspot areas for

276   cholera because of limited access to improved drinking water sources and sanitation options and

277   poor hygiene conditions.[28] There were no water samples positive for *V. cholerae* during the 12-

278   month period after the kOCV campaign, despite the presence of cholera patients. Future studies

279   should explore this finding further by investigating the impact of kOCV campaigns on the

280   persistence of *V. cholerae* in the environment.

281

282   Using a test-negative design, we found that a single-dose of Euvichol-Plus provided modest

283   protection against medically attended cholera during the 24 months after vaccination, consistent

284   with previous studies.[5] This test-negative design to evaluate kOCV effectiveness could be

285   incorporated into existing cholera surveillance activities implemented globally and integrated as

286   part kOCV vaccine campaign rollouts. Our study findings suggests that with increased

287   vaccination coverage in informal settlements, single-dose kOCV campaigns present a promising

288   approach to deliver along with water, sanitation, and hygiene programs to reduce cholera in this

289   setting in DRC.

290

291   Our genomic findings highlight several important points. Firstly, we did not detect any samples

292   that grouped into clade AFR10d, a finding that seems to further corroborate that clade AFR10e

293   replaced AFR10d around 2018 in this region.[29] Second, during the subsequent cholera outbreaks

294   after the large kOCV campaign we observed a change in strain serotype to Inaba which was

295   likely related to a fragmented *wbeT* gene marked by insertion of mobile elements. This is

296   consistent with another study that observed a serotype switch following a vaccination campaign.[6]

297   Additionally, we observed an increase in the number of SNPs between *V. cholerae* strains from

13

298   pre to post kOCV campaign. However, both our current findings and the one from the previous

299   study are observational and causality cannot be inferred. Third, this study provided evidence that

300   household water contained the same strains that caused cholera infections among household

301   members. This result combined with the finding that clinical strains in the same household were

302   more closely related than strains from different households, even when no *V. cholerae* was found

303   in household drinking water, suggests a combination of person-to-person and water-to-person

304   cholera transmission. This is the first study, to our knowledge, to conduct both clinical and

305   environmental surveillance of *Vibrio cholerae* in cholera patient households in a sub-Saharan

306   Africa setting. These findings suggest that it is critical that WASH programs put emphasis on

307   both water treatment of household water and handwashing with soap to prevent cholera

308   transmission.

309

310   This study has several strengths. The first is the 4-year duration of epidemiological and genomic

311   surveillance of cholera patients and their water sources during a time before and after a kOCV

312   campaign. Previous longitudinal studies including both clinical and environmental surveillance

313   have been almost exclusively in South Asia. The second strength is evaluating the effectiveness

314   of a single-dose of Euvichol-Plus over a 24-month period building on previous studies which

315   focus on two-doses of kOCV and evaluated the Shanchol kOCV which is no longer produced.

316   The only study we are aware of that evaluated the single-dose kOCV effectiveness of Euvichol-

317   plus for a period of 24 months or longer was from DRC which found a single-dose kOCV

318   vaccine effectiveness of 54% from 12 to 17 months after vaccination, similar to our study.[30]

319   Additionally, the test-negative design for kOCV effectiveness builds on observational studies

320   utilizing community control by reducing the likelihood of differences between cases and control

321   in care-seeking behavior which can introduce substantial bias in observational studies of vaccine

322   effectiveness. The final study strength is the inclusion of the genomics data which allowed us to

323   investigate how water and clinical *V. cholerae* strains were evolving over time during the period

324   before and after a kOCV campaign and to investigate *V. cholerae* transmission dynamics in

325   cholera patient households.

326

327   This study also has some limitations. First, our surveillance focused only on an urban setting so

328   we cannot generalize our findings to rural settings. Additional evidence is needed on single-dose

329   kOCV vaccine effectiveness in rural settings globally. Second, as with all observational study

330   design there is the possibility of misclassification of the patient's vaccination status and cholera

331   infection outcome. Also, it should be noted that the vaccine campaign was a preventive

332   campaign in an area identified as a hotspot; however, currently, because of the global kOCV

333   vaccine shortage, all campaigns are reactive and respond to outbreaks. Future studies are needed

334   to evaluate single-dose kOCV effectiveness during reactive kOCV campaigns globally.

335

336   In conclusion, there were annual bimodal peaks of the clade AFR10e of *V. cholerae* in our urban

337   cholera endemic setting in eastern DRC. A third of diarrhea patients admitted to surveillance

338   healthcare facilities were confirmed to have cholera by bacterial culture. A large clonal cholera

339   outbreak occurred 18 months after a single dose kOCV campaign, and the severity of the

340   outbreak may have been related to the low vaccine coverage in informal settlements. Genomics

341   findings suggest both person-to-person and water-to-person transmission**.** A single-dose of

342   Euvichol-Plus provided modest protection against medically attended cholera during the 24

343   months after vaccination. These results contribute to the evidence base supporting the use of

15

344   single dose kOCV campaigns in combination with WASH programs to reduce cholera in

345   endemic settings globally.

346

347   **Acknowledgements**

362

363   Dr. George is an infectious disease epidemiologist and environmental engineer. Her research

364   focuses on developing and evaluating community and healthcare facility–based water, sanitation,

365   and hygiene interventions to reduce infections in low- and middle-income countries and low-

366   resource settings globally.

367

1.	Ali M, Nelson AR, Lopez AL, Sack DA. Updated Global Burden of Cholera in Endemic Countries. *PLoS neglected tropical diseases* 2015; **9**(6): e0003832.

2.	Lessler J, Moore SM, Luquero FJ, et al. Mapping the burden of cholera in sub-Saharan Africa and implications for control: an analysis of data across geographical scales. *Lancet (London, England)* 2018; **391**(10133): 1908-15.

3.	Organization WH. Ending cholera a global roadmap to 2030. Ending cholera a global roadmap to 2030; 2017: 32-.

4.	WHO. Shortage of cholera vaccines leads to temporary suspension of two-dose strategy, as cases rise worldwide. 2024.

5.	Xu H, Tiffany A, Luquero F, et al. Protection from Killed Whole-Cell Cholera Vaccines: A Systematic Review and Meta-Analysis. 2024: 2024.08.13.24311930.

6.	Karlsson SL, Thomson N, Mutreja A, et al. Retrospective Analysis of Serotype Switching of Vibrio cholerae O1 in a Cholera Endemic Region Shows It Is a Non-random Process. *PLoS neglected tropical diseases* 2016; **10**(10): e0005044.

7.	Chaguza C, Chibwe I, Chaima D, et al. Genomic insights into the 2022-2023Vibrio cholerae outbreak in Malawi. *Nature communications* 2024; **15**(1): 6291.

8.	Weill FX, Domman D, Njamkepo E, et al. Genomic history of the seventh pandemic of cholera in Africa. *Science (New York, NY)* 2017; **358**(6364): 785-9.

9.	Irenge LM, Ambroise J, Bearzatto B, Durant J-F, Wimba LK, Gala J-LJm. Genomic evolution and rearrangement of CTX–Φ prophage elements in Vibrio cholerae during the 2018–2022 cholera outbreaks in The Democratic Republic of Congo. 2024: 2024.03. 30.24305082.

10.	Hounmanou YMG, Njamkepo E, Rauzier J, et al. Genomic Microevolution of Vibrio cholerae O1, Lake Tanganyika Basin, Africa. *Emerging infectious diseases* 2023; **29**(1): 149-53.

11.	George CM, Rashid M, Almeida M, et al. Genetic relatedness of Vibrio cholerae isolates within and between households during outbreaks in Dhaka, Bangladesh. *BMC genomics* 2017; **18**(1): 903.

12.	Organization WH. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world: Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Neisseria gonorrhoea, Salmonella serotype Typhi, Shigella, and Vibrio cholerae: World Health Organization, 2003.

13.	Debes AK, Ateudjieu J, Guenou E, et al. Clinical and Environmental Surveillance for Vibrio cholerae in Resource Constrained Areas: Application During a 1-Year Surveillance in the Far North Region of Cameroon. *The American journal of tropical medicine and hygiene* 2016; **94**(3): 537-43.

14.	ZymoBIOMICS DNA Miniprep Kit. https://www.zymoresearch.com/products/zymobiomics-dna-miniprep-kit.

15.	Petit Robert A, Read Timothy D. Bactopia: a Flexible Pipeline for Complete Analysis of Bacterial Genomes. *mSystems* 2020; **5**(4): 10.1128/msystems.00190-20.

16.	Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of computational biology : a journal of computational molecular cell biology* 2012; **19**(5): 455-77.

17.	Petit RA, 3rd, Read TD. Bactopia: a Flexible Pipeline for Complete Analysis of Bacterial Genomes. *mSystems* 2020; **5**(4).

18.	Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics (Oxford, England)* 2014; **30**(14): 2068-9.

19.	Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GWJGr. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. 2015; **25**(7): 1043-55.

20.	Wood DE, Salzberg SLJGb. Kraken: ultrafast metagenomic sequence classification using exact alignments. 2014; **15**: 1-12.

17

416 21.   Smith A, Sekwadi P, Erasmus L, et al. Imported Cholera Cases, South Africa, 2023. *Emerging*
417 *Infectious Disease journal* 2023; **29**(8): 1687.
418 22.   Minh BQ, Schmidt HA, Chernomor O, et al. IQ-TREE 2: New Models and Efficient Methods for
419 Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* 2020; **37**(5): 1530-4.
420 23.   Yu G, Smith DK, Zhu H, Guan Y, Lam TTYJMiE, Evolution. ggtree: an R package for
421 visualization and annotation of phylogenetic trees with their covariates and other associated data. 2017;
422 **8**(1): 28-36.
423 24.   Argimón S, Abudahab K, Goater RJE, et al. Microreact: visualizing and sharing data for genomic
424 epidemiology and phylogeography. *Microbial genomics* 2016; **2**(11): e000093.
425 25.   Zhou Z, Alikhan NF, Sergeant MJ, et al. GrapeTree: visualization of core genomic relationships
426 among 100,000 bacterial pathogens. *Genome Res* 2018; **28**(9): 1395-404.
427 26.   Kelsey Jordahl JVdB, Martin Fleischmann, Jacob Wasserman, James McBride, Jeffrey Gerard,
428 Jeff Tratner, Matthew Perry, Adrian Garcia Badaracco, Carson Farmer, Geir Arne Hjelle, Alan D. Snow,
429 Micah Cochran, Sean Gillies, Lucas Culbertson, Matt Bartos, Nick Eubank, maxalbert, Aleksey Bilogur,
430 François Leblanc. . geopandas. Zenodo; 2020.
431 27.   Contributors DA-BC. contextily. v1.6.2 ed. github; 2020.
432 28.   Satterthwaite D, Sverdlik A, Brown D. Revealing and Responding to Multiple Health Risks in
433 Informal Settlements in Sub-Saharan African Cities. *Journal of urban health : bulletin of the New York*
434 *Academy of Medicine* 2019; **96**(1): 112-22.
435 29.   Alam MT, Mavian C, Paisie TK, et al. Emergence and Evolutionary Response of Vibrio cholerae
436 to Novel Bacteriophage, Democratic Republic of the Congo(1). *Emerging infectious diseases* 2022;
437 **28**(12): 2482-90.
438 30.   Malembaka EB, Bugeme PM, Hutchins C, et al. Effectiveness of one dose of killed oral cholera
439 vaccine in an endemic community in the Democratic Republic of the Congo: a matched case-control
440 study. *The Lancet Infectious diseases* 2024; **24**(5): 514-22.

441

442

Table 1. Diarrhea patient demographic characteristics by OCV status and cholera status

| | All diarrhea patients | | | OCV status | | | | | | |
| | | | | Received OCV | | | No OCV | | | |
	%	n	N	%	n	N	%	n	N	p-value
Diarrhea Patients			750		93	750		657	750	
Age										
Median ± SD (Min - Max) (Years)		5 ± 19 (0-82)			2 ± 15 (0-63)			7 ± 19 (0-82)		
0-1 Years	29%	217	750	32%	30	93	28%	187	657	0.527
1-4 Years	20%	148	750	30%	28	93	18%	120	657	0.011
5-14 Years	13%	96	750	12%	11	93	13%	85	657	0.893
14 Years or Greater	39%	289	750	26%	24	93	40%	265	657	0.009
Gender										
Female	49%	368	750	48%	45	93	49%	323	657	0.977
Running water inside home	12%	93	748	13%	12	93	12%	81	655	1.000
Working mobile phone in home	85%	565	663	92%	78	85	83%	487	587	0.097
Individuals residing in home										
Median ± SD (Min - Max) (Years)		7 ± 3 (1-23)			7 ± 2 (3-16)			7 ± 3 (1-23)		0.098
Years of formal education*										
Median ± SD (Min - Max) (Years)		9 ± 5 (0-23)			7 ± 5 (0-15)			9 ± 5 (0-23)		0.028

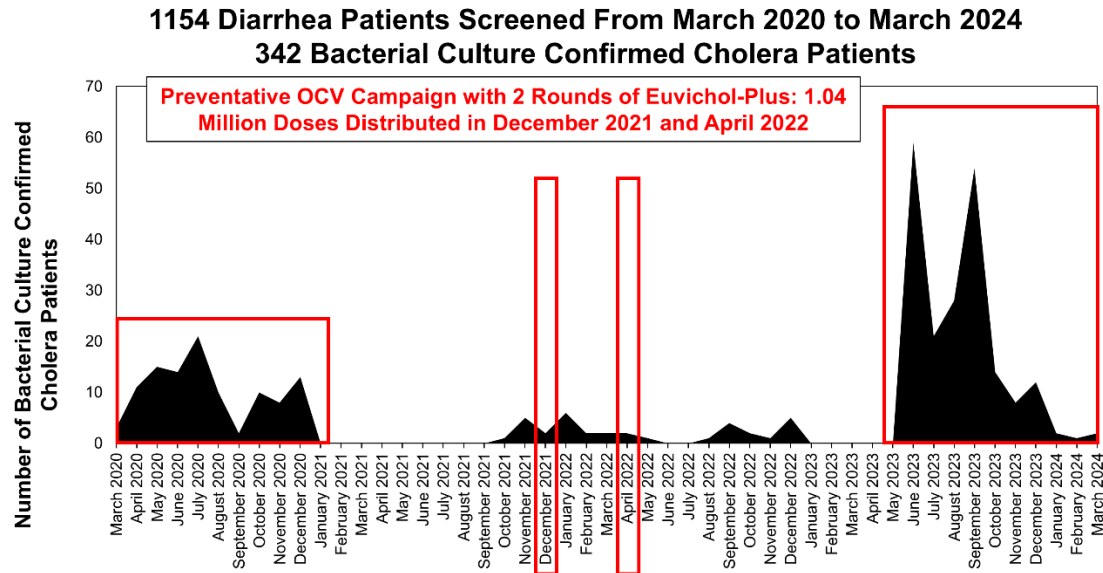SD=Standard Deviation

* for those age 18 and over

443

444

Figure 1. Four-Year Epidemological Surveillance of Bacterial Culture Confirmed Cholera Patients from 115 Health Facilities in Bukavu City of South Kivu Province of the Democratic Republic of the Congo from March 2020 to March 2024. A total of 1154 diarrhea patients were screened at 115 health facilities and 342 cholera patients were confirmed by bacterial culture. A preventative oral killed whole-cell cholera vaccine (kOCV) campaign was conducted in December 2021 and April 2022.
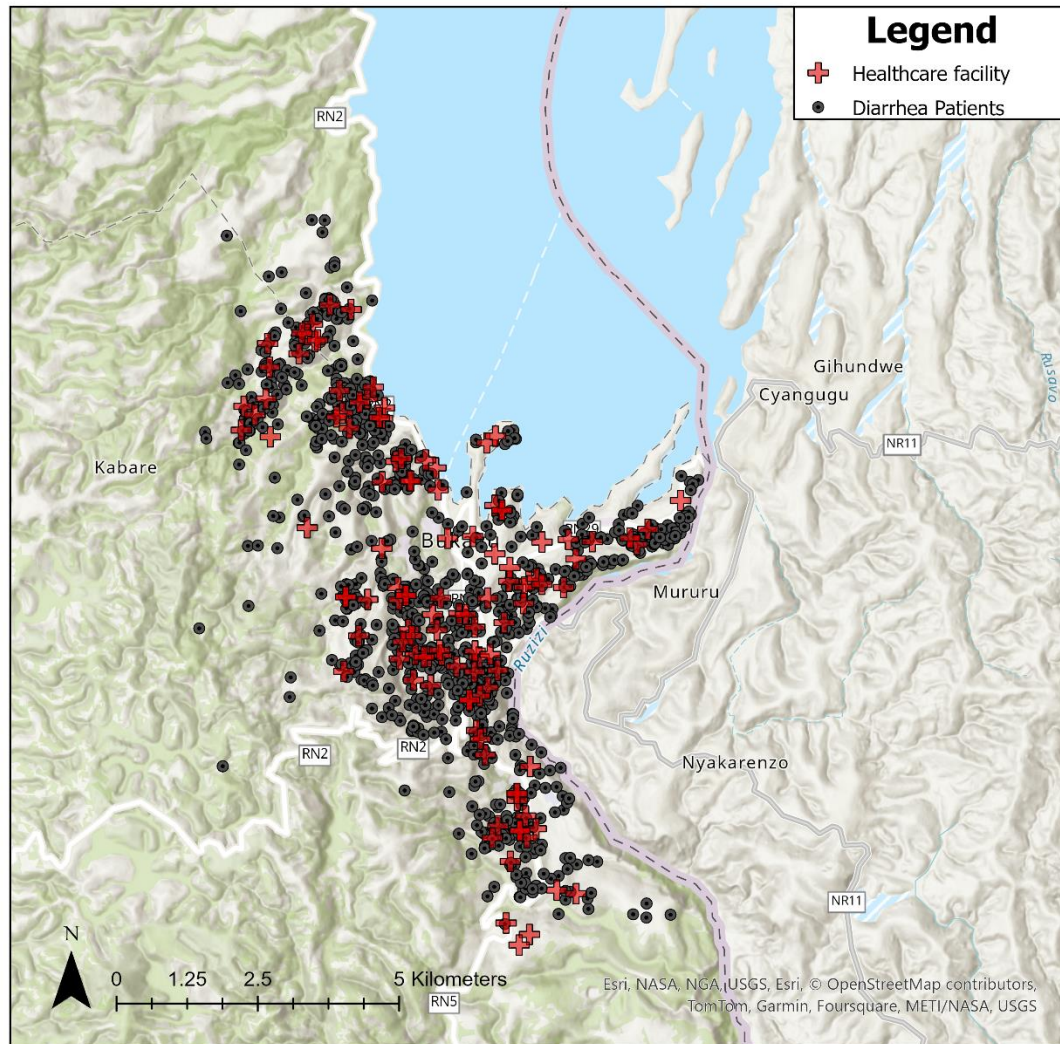
451

Figure 2. Map of diarrhea patients positive for V. cholerae by bacterial culture and health facilities where cholera patient surveillance was conducted between 2020-2024 in Bukavu, Democratic Republic of the Congo. Red cross indicates healthcare facilities (N=115) and black dots indicate diarrhea patient households (N=1098) (56 patient households were missing GPS coordinates).
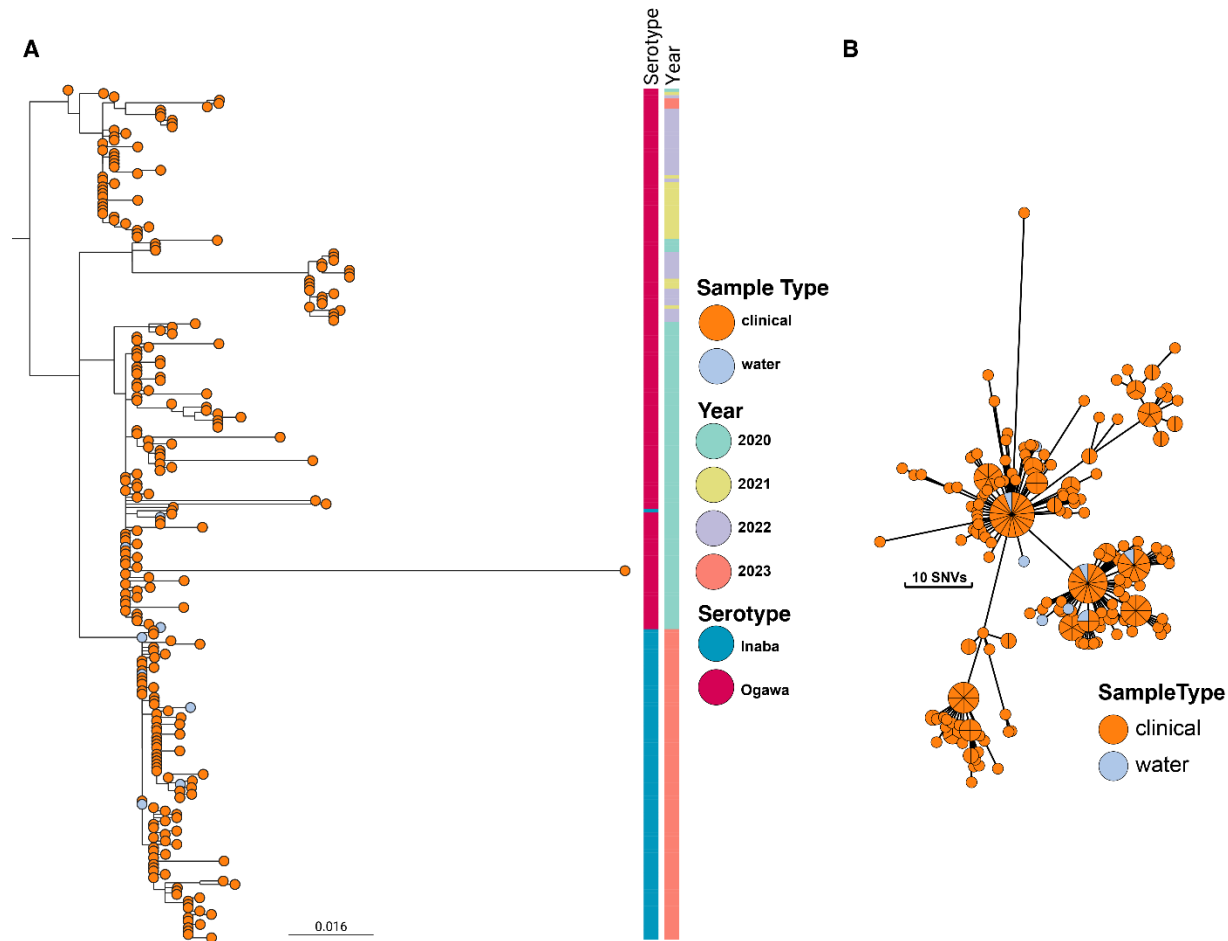
Figure 3. Phylogenetic analyses describing the relationships of the 255 7PET strains. A. Maximum likelihood phylogeny of the 255 7PET *V. cholerae* genomes sampled in Bukavu from 2020 to 2023. The nodes are colored according to sample type. The associated colored metadata corresponds to year of sampling and inferred serotyping results based on genome analysis. Not all strains have serotype data as indicated. **B.** Minimum spanning tree of 255 genomes. Strains that have zero SNP differences between them are collapsed into a single node. Node sizes scale with number of samples. The nodes are colored according to sample type.
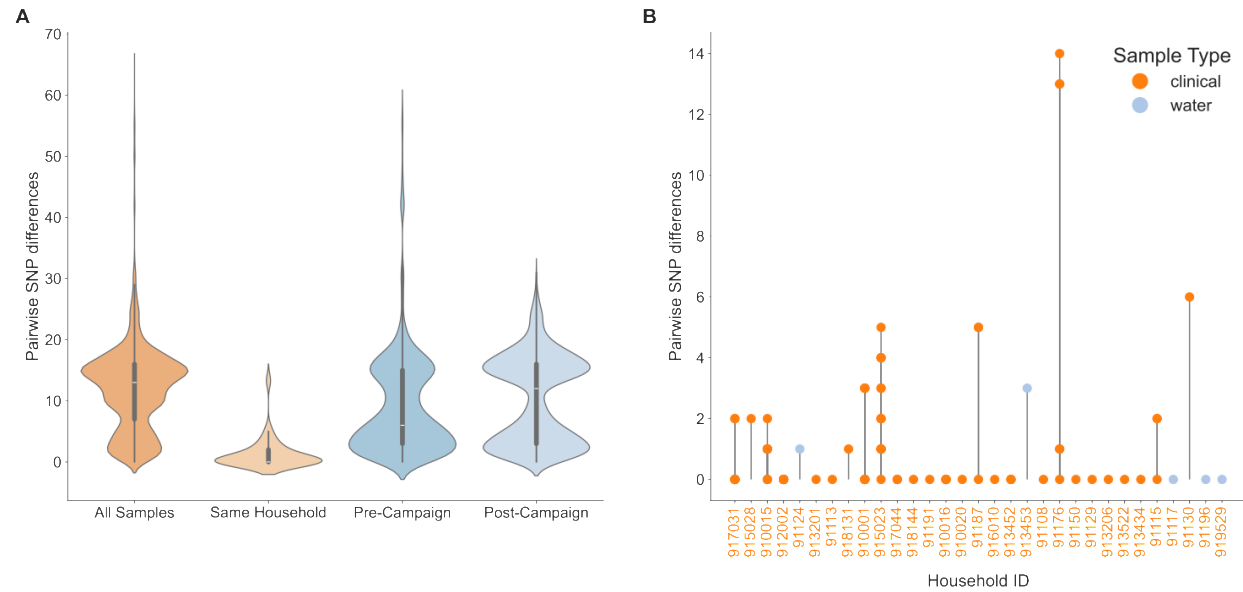
22

Figure 4. Pairwise comparisons of SNPs. (A) Boxplots of pairwise SNP differences. Boxplots depict the distribution of pairwise SNP differences for all strains, those from the same household, and those from the same individual. Each plot shows the median, and interquartile range. The number of pairwise comparisons for each category is as follows: All samples (n=32,385), same household (n=99), Pre kOCV Campaign (n=6,205), and Post-kOCV Campaign (n=10,296). **(B) Stem plots of the SNP differences per household**. Pairwise SNP differences are relative to the first sample in the household. Nodes are colored by sample type and stems connect all samples to their given household.
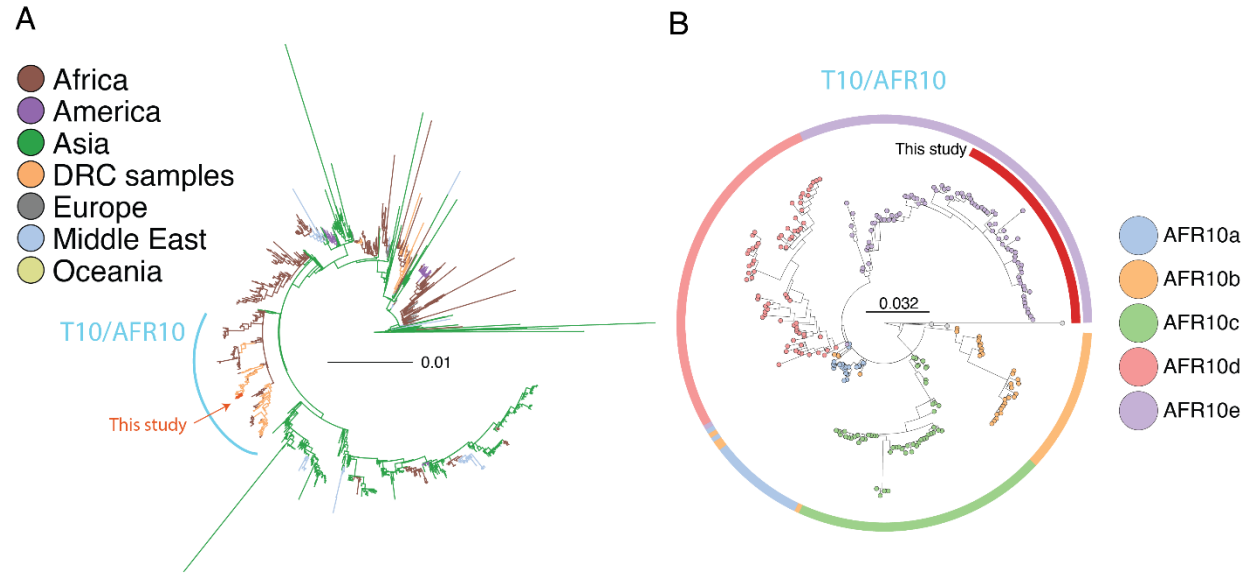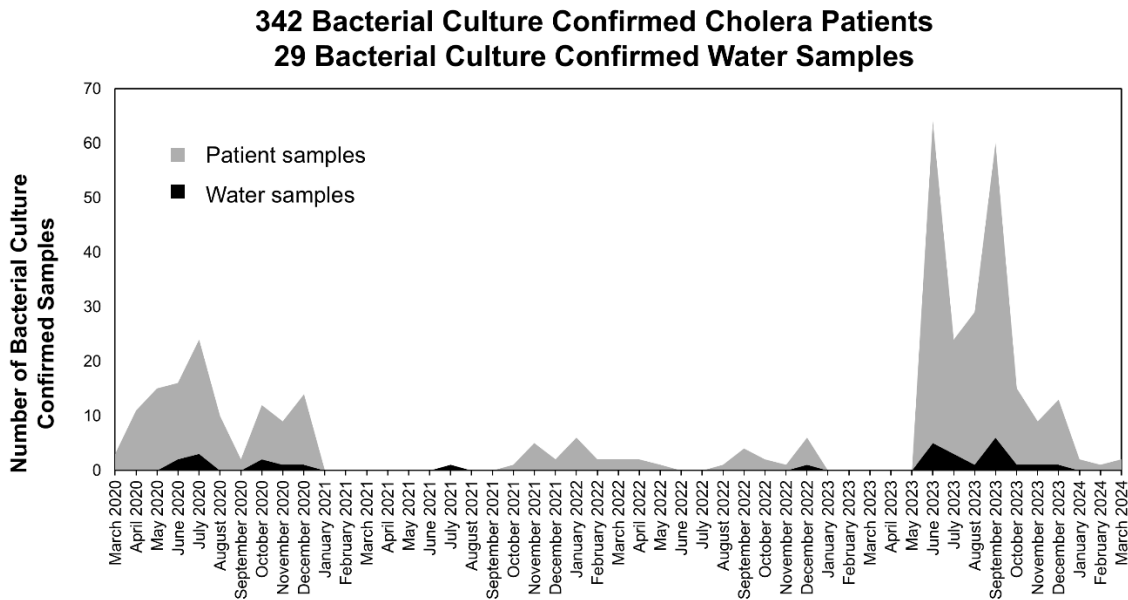
Figure 5. Maximum likelihood phylogenetic trees of seventh pandemic strains. (**A**) **Globally representative phylogeny of 1,428 7PET strains.** Five representative strains from this DRC study were placed within the larger context of 1,423 7PET strains. The tree was rooted on A6 strain. The branches are colored according to geographic origin of the strain. (**B**) **Phylogeny of the T10/AFR10 lineage.** Representative strains from this DRC study (n=46) were placed within the context of 221 T10/AFR10 lineage strains. The tree was rooted on the reference strain N16961.

342 Bacterial Culture Confirmed Cholera Patients
29 Bacterial Culture Confirmed Water Samples

495

Supplementay Figure 1. Four-Year Epidemological Surveillance of *Vibrio Cholerae* Bacterial Culture Confirmed Cholera Patients and Water Samples Bukavu City of South Kivu Province of the Democratic Republic of the Congo from March 2020 to March 2024. A total of 1154 diarrhea patients were screened at 115 health facilities and 342 cholera patients were confirmed by bacterial culture. 29 water samples were positive for cholera (9 source and 20 stored).

501

502

503

504

505

506
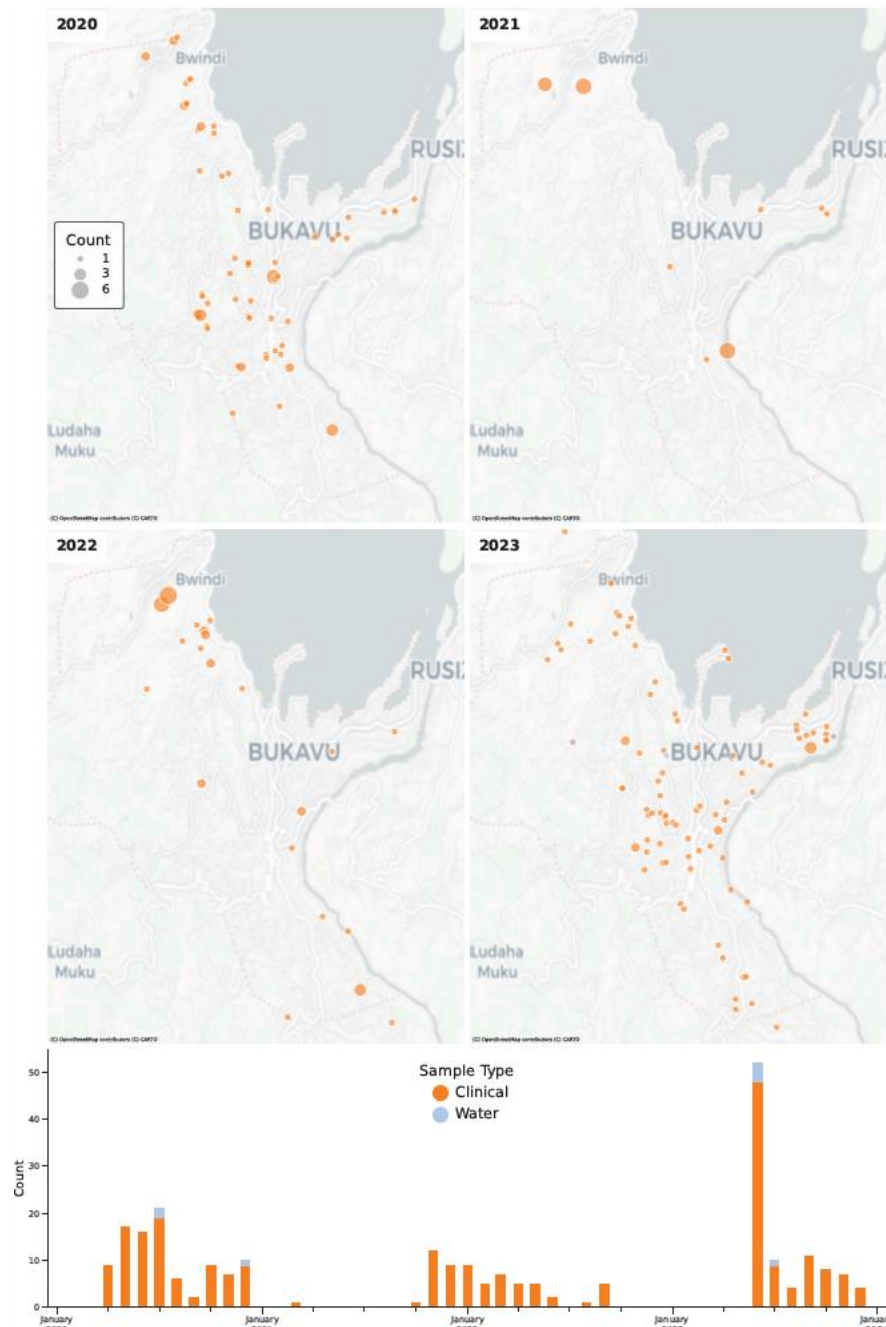
Supplementary Figure 2. Case counts and locations of sampling for genomic analysis in Bukavu, Democratic Republic of the Congo between 2020 - 2023. Top, map indicates spatial distribution of strains and the sample type per year. Bottom, the number and sample type of strains per month.