

1 **Typhoid fever outbreak in the Democratic Republic of Congo:**

2 **Case control and ecological study**

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21 **Short title:** Typhoid fever in DRC: Case control study

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23 water

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27 Abstract

28 During 2011 a large outbreak of typhoid fever affected an estimated 1430 people in Kikwit,
29 Democratic Republic of Congo. The outbreak started in military camps in the city but then
30 spread to the general population. This paper reports the results of an ecological analysis and
31 a case-control study undertaken to examine water and other possible transmission
32 pathways. Attack rates were determined for health areas and risk ratios were estimated
33 with respect to spatial exposures. Approximately 15 months after the outbreak,
34 demographic, environmental and exposure data were collected for 320 cases and 640
35 controls residing in the worst affected areas, using a structured interview questionnaire.
36 Unadjusted and adjusted odds ratios were estimated. Complete data were available for 956
37 respondents. Residents of areas with water supplied via gravity on the mains network were
38 at much greater risk of disease acquisition (risk ratio = 6.20, 95%CI 3.39-11.35) than
39 residents of areas not supplied by this mains network. In the case control study, typhoid was
40 found to be associated with ever using tap water from the municipal supply (OR = 4.29, 95%
41 CI 2.20-8.38). Visible urine or faeces in the latrine was also associated with increased risk of
42 typhoid and having chosen a water source because it is protected was negatively associated.
43 Knowledge that washing hands can prevent typhoid fever, and stated habit of handwashing
44 habits before cooking or after toileting was associated with *increased* risk of disease.
45 However, observed associations between handwashing or plate-sharing with disease risk
46 could very likely be due to recall bias. This outbreak of typhoid fever was strongly associated
47 with drinking water from the municipal drinking water supply, based on the descriptive and
48 analytic epidemiology and the finding of high levels of faecal contamination of drinking
49 water. Future outbreaks of potentially waterborne disease need an integrated response that
50 includes epidemiology and environmental microbiology during early stages of the outbreak.
51

52 Author summary

53

54 There was a large outbreak of typhoid fever in Kikwit, DRC, in late 2011. The outbreak
55 started in military camps in the city but then spread to the general population. Multiple
56 investigations were undertaken to understand how the disease spread. The worst affected
57 areas of the city were mapped and compared to the water network. In early 2013,
58 demographic and exposure data were collected for 320 cases and 640 controls residing in
59 the worst affected areas, using a structured interview questionnaire to try to better
60 understand individual risk factors. Residents of areas with water supplied via a gravity fed
61 network were about six times more likely to have been ill with typhoid fever than residents
62 of areas not supplied by the mains network. The most important individual risk factor was
63 ever using tap water. Visible urine or faeces increased risk of getting typhoid but having
64 chosen a water source because it is protected was linked to lower risk. Not handwashing
65 and regularly sharing plates of food were also linked to less illness, but these findings may be
66 especially subject to recall bias. The water network was also found to be heavily
67 contaminated, including with faecal bacteria of human origin in multiple microbiological
68 studies. Spatial, microbiological and case-control studies all implicate the water supplies in
69 Kikwit to be unsafe and linked to spread of typhoid fever in 2011. Improvements to the
70 mains water network in Kikwit are urgently needed to prevent future typhoid fever
71 outbreaks.

72

73

74 Introduction

75 Typhoid fever (TF) is an infection caused by the bacterium *Salmonella enterica*
76 serovar typhi (*S. Typhi*). The primary symptoms are fever and related malaise, but serious
77 complications, such as intestinal haemorrhage or perforation (1-4% of all cases [1]),
78 encephalitis, respiratory infections and metastatic abscesses can occur. In the absence of
79 treatment, there is a case fatality rate of 10-30%, which drops to ~1% with timely treatment
80 [2]. Data from 2010-2013 suggested that the TF disease burden in Africa was 4.3 million
81 cases per year (95%CI 3.7 – 5.1 million) [3]. Data to estimate the total case fatality rate in
82 sub-Saharan Africa are unreliable [1,3]. The mean case-fatality rate after *S. Typhi* caused by
83 intestinal perforation has been reported at 19.5% (95%CI 16-22%) in African countries [4].

84 Typhoid is a strictly human infection and spreads from one person to another
85 especially through faecal oral or urine oral pathways including consumption of
86 contaminated food or water. Spread is exacerbated by poor sanitation and hygiene.
87 Outbreaks have regularly been reported, but those occurring in low income countries are
88 not well researched. Instead, most studies have described outbreaks in industrialized
89 settings [2]. Consequently, opportunities to reduce transmission in these low-resource
90 settings may not be identified or properly understood.

91 Repeated and sometimes severe outbreaks of typhoid have occurred in the
92 Democratic Republic of Congo [5]. After a large outbreak of typhoid in the city of Kikwit,
93 Bandundu Province, in the year 2006, typhoid became endemic, with low but persistent
94 numbers of cases reported annually until another large outbreak in November 2011 to early
95 January 2012. The 2011-2012 event resulted in 1430 identified cases. Seventy-one people
96 developed peritonitis with perforation, and 17 people died in 2011-2012. The fatality rate
97 was 1.5% [6]. An initial descriptive epidemiological study recognised that contaminated
98 water supplies were likely responsible for most cases in 2011 [6], but did not elucidate on
99 transmission pathways or other risk factors for infection in the subsequent phases of the
100 outbreak. Patients in the 2006 and 2011 outbreaks appeared to come from the same areas
101 of the city, which led to suspicions that an infrastructure problem or spatial feature might
102 contribute to the risk of catching TF (S1 Protocol). It was observed that 2011 attack rates
103 were highest in military camps within the city, especially early in the outbreak. The
104 descriptive evidence suggested that the 2011 outbreak originated in the camps and
105 subsequently spread to the general population. Here, we provide a quantitative
106 investigation of exposure factors linked to infection in 2011, accompanied with
107 recommendations both for prevention and during emergency response.

108 **Methods**

109 We undertook both an ecological analysis and a case-control study in order to better
110 understand the primary transmission pathways of the epidemic and determine whether we
111 could identify other risk factors. Based on the descriptive epidemiology and general
112 knowledge of the epidemiology of typhoid, our primary hypotheses were 1) that the
113 outbreak was waterborne and/or 2) that the infection spread directly from person to
114 person.

115

116

117 **Study setting**

118 Kikwit is the largest city in the Bandundu province of DRC. The estimated population
119 in 2011 was 400,000 (Fig. 1). Kikwit is located in the south-west of DRC and is an important
120 commercial and administrative centre. In 2011, the city had one general referral hospital
121 and was administratively divided into two health zones, north and south. Each health zone
122 (“Zone de Santé”) was divided into health areas (“Aires de Santé”). There were 19 and 22
123 health areas respectively in north and south Kikwit at the time of the study. At the time of
124 the outbreak, there were three military camps in the city, which accommodated about 2400
125 people: staff and families. Ngubu camp and Nsinga camp were in close proximity to the
126 general population, while Ebeya camp was relatively separate from the city. The camp
127 residents could be considered a highly mobile population and are mostly not of local origin.
128 They had living conditions distinct from and mostly worse than the city’s settled residents.
129 The military staff and families originate from many regions of DRC and speak many
130 languages. Overall, living conditions in the three camps were poor and featured relatively
131 high population density and poor hygiene and sanitation conditions. As a result, this study
132 treated the three camps as three additional health areas which were analysed
133 independently of the Aire de Santé where they were situated [6].

134

135 **Fig. 1: Household density map of Kikwit city, DRC**, using homogenous areas visually classified as having
136 high, medium or low housing density based on recent high-resolution satellite imagery.

137

138 Kikwit is a hilly city with sandy soils and large erosion gullies. The city has a long wet
139 season with an average of about 200mm of rain per month, from early September through
140 end May. The city has centrally collected and distributed piped water, which is extracted
141 from artesian wells, inconsistently chlorinated (S1 Report) and distributed via an aging pipe

142 network to community tap points (standpipes, Fig. 2). Water pressure is usually low
143 throughout the network. Mains water is relatively expensive and difficult to access in parts
144 of the city (S1 Report). Some homes have access to private wells. Surface water sources are
145 widely available and generally free-of-charge. Surface sources are the river Kwilu, its
146 tributaries, as well as protected and unprotected springs. No changes to the water
147 infrastructure occurred in the time that elapsed between the 2011 outbreak and the survey
148 dates.

149 Sanitation in the city is privately managed. Latrines are typically dug out by hand,
150 and often are open air (no roof) and shared among multiple households. After a latrine fills
151 up it is typically covered with thin soil, a mango tree is planted and a replacement latrine is
152 dug nearby. Flooding of human waste out of active latrines is not unusual following
153 significant rainfall events.

154

155 **Fig. 2: Drinking water network and sources in Kikwit, 2011-2013.**

156

157 **Ecological analysis**

158 Study design and protocol for the spatial analysis is in S1 Protocol. During the 2011
159 typhoid outbreak, The Ministry of Public Health (MOPH) created a central register line list of
160 all cases, both suspected and confirmed. The case definitions were set by the Ministry of
161 Public Health (MOPH); “suspected case” was any person with fever $\geq 38^{\circ}\text{C}$ for more than
162 three days and digestive disorders, which were defined as diarrhoea, constipation or
163 abdominal pain, as well as a negative malaria test. A “confirmed case” was a suspected case
164 confirmed by isolation of *S. Typhi* from blood, bone marrow or duodenal fluid. The outbreak
165 was confirmed by the MOPH based on the results of cultures of 50 blood and stool samples,
166 which were tested in the University Hospital in Kinshasa. From the start of the outbreak, all
167 TF cases had their data entered into a central electronic line list developed by the MOPH.
168 The register included patient’s name, sex, age and address. By the end of the outbreak, the
169 line register had 1430 cases, with illness onset dates from 19 Nov 2011 to 5 January 2012.
170 The attack rate for each of the 41 health areas and three military camps was determined
171 using the central register line list and estimated 2011 population data. Environmental
172 elements were mapped [7] in early-mid 2013 for each health area in Kikwit using ArcGIS
173 software (ESRI, California, USA). Spatial attributes were assigned to an entire health area as
174 the predominate trait for that health area. A key environmental item of interest was the
175 predominant water distribution network that public water taps connected to in each area.
176 The network options were: northern pump, central pump, central gravity or off network.

177 Other spatial attributes for each health area were population density the (average) distances
178 from each health area to the nearest camp, market, school, springs, tarred road, water point
179 or health care clinic. Distances to these locations were of interest because these places tend
180 to be places where people cluster and thus might pass on infection. Data on socio-economic
181 status, sanitation statistics or water quality (such as via the WHO Joint Monitoring
182 Programme) were unavailable at the scale of health areas. Incident risk ratios were
183 estimated for each health area using negative binomial regression, testing for any
184 association between single or multiple exposures to environmental attributes in the health
185 area and attack rate. The outcome was set to be the number of cases within each health
186 area, the model offset was the (natural log transformation of the) total population in that
187 same area. Distances were modelled continuously under a Poisson functional form.
188 Categories were also available that described the water source in each health area (gravity
189 or pumped, north or central, see data in S1 Spreadsheet).

190

191

192 **Case-control study**

193 Study design and protocol is item S2 Protocol. The case-control study focused on
194 individual risk factors. The data were collected using structured household interviews
195 between February and May 2013. Descriptive results of the data collected about cases
196 during these interviews are described elsewhere [6]. Aspects of the structured survey as
197 reported previously will be briefly recapped here.

198 The survey was targeted at cases and controls living in the Aires de Santé with the
199 highest attack rates during the outbreak. The case-control study was done in only these
200 areas primarily for logistical reasons. The attack rate for each health area was estimated
201 using the denominator from population census data. For the case control study, we
202 surveyed residents in the eight most affected health areas (attack rates > 0.36%) while also
203 separating out and interviewing residents of the three military camps, because the camps all
204 had AR > 4% (Fig. 3).

205

206 **Fig. 3: The health areas of Kikwit city, DRC**, with low (<0.05%), intermediate (0.05% to 0.36%) or high
207 (>0.36%) total attack rates during the 2011 typhoid fever outbreak as well as the city's military camps
208 (labelled with name), markets, principal roads and points where water was tested.

209

210 To identify an odds ratio of at least 1.5 with a power of 80% for risk factors present in
211 30% of the control population, a sample size was set at 320 cases (25% of total), frequency
212 matched by age and sex to 640 controls.

213 A structured questionnaire was developed and piloted (S1 Questionnaire). Twelve
214 interviewers and one supervisor who spoke local languages were trained to verbally
215 administer the questionnaire. Interviewers exercised own judgement in how to translate the
216 questionnaire from French into other languages, when required. The interviewers were
217 trained how to record household location and its principal water source using GPS devices
218 (Garmin GPSMAP 76). Using the recorded addresses in the line list records, typhoid cases
219 (from the concurrent case definition) were chosen at random, using random numbers
220 generated in MS Excel. Cases were traced and then interviewed in the community. Serology
221 was not used to confirm recovered case status.

222 Two controls of the same sex, age class of 5 years interval (0-5, 6-10, 11-15, 16-20,
223 etc.) and health area were selected per case. Selection criterion for each control was not
224 having been suspected of typhoid during the outbreak period. After interviewing a case,
225 interviewers decided which end of their road to treat as the starting point, and then chose
226 two control households by rolling a die to select the n^{th} residences (alternating between left
227 and right side of the street). Posited controls were asked if they had TF or were ill during the
228 TF outbreak. Those who reported having TF or being ill were excluded as was their entire
229 household (assumed to share same latrine and water supply). The die was rolled again if
230 necessary to select a different household for a potential control. A maximum of one person
231 was interviewed per household. All respondents were requested to show their household
232 water storage containers, available soap and latrines. For children less than 13 years old, the
233 interview was conducted with the guardian or a family member who was living in the same
234 household and aware of the child's condition.

235 During the household survey, level of awareness about the 2011-12 outbreak was very
236 high and people claimed to be able to remember with accuracy if they had been ill during that
237 period. Interviewers were welcome in people's homes as representatives of MSF. No one
238 refused to be interviewed.

239 All GPS readings were recorded and visualized using ArcGIS software (ESRI, California,
240 USA). Conditional logistic regression (clogit) for risk factors were undertaken using STATA
241 version 14.2, with data grouped by health area. Odds ratios (OR) for most risk factors were
242 first estimated in single predictor models. Exposures and factors were excluded from
243 univariate analysis if < 5% of responses were different from the most popular answer to a

244 specific question. Any individual variable p-value <0.20 was carried forward into a multiple
245 predictor case-control analysis. We endeavoured to keep all categories in the model if a
246 variable had multiple levels. However, some variables were trialled by recoding them into
247 binary variables where exploratory analysis found a strong association (such as having any tap
248 water, as primary or secondary source or outside the home). Otherwise, risk factors were
249 retained in iterative modelling as long as they had p-value < 0.05 to produce the final
250 estimated adjusted odds ratios reported here. For purely categorical items, the reference
251 value was set at the value with greatest frequency; for ordinal items, the reference value was
252 set at the lowest rank answer. Using adjusted ORs and the fraction of cases receiving an
253 exposure, the population attributable risk [8] percentage was determined for key predictors
254 in the final model. Where few data were missing, those specific observations were excluded
255 in the final model; where many data were missing, the variable was not used in multivariate
256 analysis.

257

258 **Water quality testing**

259 Study design and protocol for the water quality analysis is within S2 Protocol. Water
260 samples of the principal source of drinking water of all interviewed cases were collected by
261 two trained water and sanitation community workers, on 18 distinct dates from 13.3.2013 to
262 10.4.2013. Replicate tests were done on water samples onsite for Free Residual Chlorine
263 (FRC) levels using the HANNA Photometer. Concentrations of ThermoTolerant Coliforms
264 (TTC) were measured using a Delagua field kit. Tests for *S. Typhi* specifically were not
265 undertaken – they seemed inappropriate so long (16 months) after the outbreak. A
266 pathogen-specific test (such as PCR for *S. Typhi*) also exceeded our research budget and
267 required equipment or laboratory facilities (for molecular biology) not available locally. In
268 contrast, tests for TTC were useful to indicate likelihood of ongoing faecal contamination
269 problems. Further details of the testing regime and results, including verification strategies,
270 are in Ali et al [6]. The water quality data were used to calculate what proportion of water
271 samples from each source could be considered high or low risk for transmission of human
272 disease, using categories adapted from UNHCR guidelines [9].

273

274 **Protocol deviations**

275 Members of the research team changed. The procedure for selecting controls as described
276 in the protocol was not used; a different set of procedures for selecting controls was
277 devised, as described previously. The minimum age of independent respondents was

278 changed from ten to 13 years old. Although specified in the protocol, the final study did not
279 assess risk factors for disease severity (as indicated by peritonitis or intestinal perforation).
280 No analysis of the 2006 outbreak was undertaken. Area-level education and income data
281 were not suitable or available, so not used in the ecological analysis. Data collection dates
282 were three months later than anticipated. The protocol also contains some factual errors
283 because it was written prior to data collection, such as stating there were 33 Aires de Santé
284 in Kikwit (actually there were 41, not 33), while the estimated population total was
285 misstated to be 350,000; actual population turned out to be higher. The number of water
286 quality tests per source was 1-4 (most often but not always 2). In the case-control study, we
287 decided to focus on the eight most affected Aires de Santé, not the seven most affected
288 areas as stated in the protocol. We don't believe that any of these deviations or factual
289 errors undermine our results or conclusions.

290

291

292 **Ethics**

293 Ethics approval was received from the Ethical committee of the School of Public
294 Health, University of Kinshasa (DRC) and Ministry of Higher Education, Academic and
295 Scientific Research. Written informed consent was sought and obtained for all respondents
296 or from their caretakers/guardians (children under 18).

297

298

299 **Results**

300

301 **Ecological analysis**

302 None of the spatial attributes could be linked with statistical significance ($p < 0.05$
303 for risk ratio) to the 2011 TF attack rate at health area level, except for water source. Table
304 1 shows findings (see supporting data in S1 Spreadsheet), which reports risk ratios and
305 attack rates for residents dependent on given water sources ($p < 0.001$). Residents who
306 were dependent on the central gravity system were five times more at risk compared to
307 residents on the northern (pumped) network (RR=6.20 vs. 1.21), and about three times
308 more at risk compared to those on the central pump system (RR=6.20 vs. 2.25).

309

310

311 **Table 1. Attack rates and Risk Ratios related to exposure to Kikwit water supplies**

312

Water Supply	Attack Rate/1000 (95%CI)	Risk Ratio (95% CI)
No mains water standpipes	9.73 (8.1-11.6)	1.00 (reference)
Central gravity system	55.94 (52.0-60.1)	6.20 (3.39-11.35)
Central pump system	21.8 (18.9-25.1)	2.25 (1.14-4.45)
Northern (pump) system	13.7 (11.1-16.7)	1.21 (0.59-2.49)

313

314

315

316

317 **Case-control study**

318 Data on occupational status of the head of household, demographic, sanitation and
319 water quality traits identified for camp and city populations are described in greater detail in
320 Ali et al [6]. Refer to the original survey questionnaire (S1 Questionnaire) and survey data,
321 both raw and derived variables, (S2 Spreadsheet) for more details. Out of the 320 cases
322 interviewed, 59 (18%) lived in the camps. Although the heads of households in the camps
323 had more secure employment (75% in camps vs. 25% in town had work contracts), the city
324 dwellers were more affluent, as indicated by greater access to electricity or a functioning TV.
325 Sharing latrines with other families is normal practice in Kikwit for both military and civilian
326 families. None of the observed latrines of the cases in camps and only 3% of cases in the
327 general population had materials to facilitate wash hands (eg., soap and water) at a close
328 distance (< 3 metres) from latrines. Upon request, 66% and 82% of cases in camps and
329 general population showed the available soap in the household. This suggests that although
330 respondents often said they *washed* their hands, many were in fact only rinsing their hands.
331 Age and education profiles were similar for both military and civilian families, but
332 households in the camps were more likely (77%) to live in a house of brick or concrete
333 construction; most non-brick homes were made from mud. Controls were 2:1 frequency
334 matched by age and sex to the recruited 320 cases.

335 The unadjusted odds ratios (OR) comparing cases and controls for individual risk
336 factors are in Table 2. The OR in Table 2 used the factors as coded in the original survey (S1
337 Questionnaire and S2 Spreadsheet), although some survey elements were excluded for
338 reasons described in the Methods, and for brevity, not all univariate results are listed in the
339 table. Age and sex associations with case status are shown to be insignificant in Table 2,
340 which indicates that frequency matching was successfully implemented. Seventeen
341 possible predictive factors had odds ratios with p-values ≥ 0.20 in single variate analysis.
342 Twenty factors were taken forward to be tried in multivariate estimations of OR (because
343 they had $p < 0.20$). Some hygiene, cooking customs, and indicators of socio-economic status

344 were among the risk factors that qualified for trial in multivariate OR estimations. At the
345 single variate stage, intake of any tap water (OR 3.41, 95%CI 1.88-6.19), whether tap water
346 was a primary or secondary source (OR 2.80, 95%CI 1.64-4.79), knowledge to wash hands
347 (OR 2.36, 95%CI 1.45-3.86), assertions that they know how to avoid typhoid (OR 0.44, 95%CI
348 0.31-0.61), and statement of habitual washing of hands before cooking (OR 5.12, 95%CI
349 3.11-8.44) had the strongest association with increased disease. Those who said that they
350 regularly shared their plates of food had reduced risk. All indicators suggestive of better
351 handwashing behaviour (more frequent handwashing or knowledge that handwashing
352 should reduce disease transmission), were positively associated with typhoid case status
353 (see data in Table 2). Aspects of the home environment (topography and home construction
354 materials) as well as habits of eating uncooked food were also significant enough to be
355 trialled in multivariate modelling. The number of water storage containers in the household
356 or claiming to have soap in the home did not reach the threshold to be tried in multivariate
357 analysis.
358

359 **Table 2. Unadjusted odds ratios (with 95% CI) for case status = recorded case of typhoid**
 360 fever, for responses collected using S1 Questionnaire. Response data for case-control study
 361 are available in S2 Spreadsheet.

Risk factor	individual matched OR (95% CI)	Risk factor	Individual matched OR (95% CI)
Odds ratios with p-value ≥ 0.20			
Kitchen is tiled	2.93 (0.63-13.63)	Respondent is literate	0.97 (0.57-1.66)
Household has functional electricity?	0.93 (0.68-1.28)	Household has a functional radio?	0.93 (0.71-1.24)
(females only) Did they live here in November 2011?	0.83 (0.52-1.32)	Main water storage vessel is covered	1.05 (0.75-1.46)
Stage of hygiene in household is reasonable	1.04 (0.79-1.37)	Stated there is soap in the house today	1.21 (0.78-1.87)
Number of water storage containers in household	1.02 (0.97-1.08)	Household has a functional TV	0.92 (0.68-1.24)
Number of persons in household	1.06 (1.00-1.10)	They said to treat water to avoid TF	0.94 (0.62-1.42)
		They said to use latrines to avoid TF	0.99 (0.71-1.37)
Do they eat raw fruit or vegetables?			
<i>Never</i>	1.0 (reference, n = 866)	What type is most used latrine?	
<i>Sometimes</i>	1.06 (0.63-1.79)	<i>Pit</i>	1.0 (reference, n=912)
<i>Regularly</i>	0.0001 (not estimable)	<i>Improved</i>	3.85 (0.36-44.23)
		<i>Flush</i>	0.62 (0.24-1.62)
Education levels (some or complete)		<i>Flush and septic</i>	1.54 (0.55-4.26)
<i>Primary</i>	1.0 (reference, n = 87)		
<i>Secondary</i>	0.93 (0.58-1.49)	Number of households using main latrine	
<i>Tertiary</i>	0.74 (0.42-1.31)	1	1.0 (reference) n = 495
		2-4	1.18 (0.88-1.58)
Male sex	1.03 (0.78-1.35)	5-7	0.83 (0.34-2.03)
Age	1.00 (0.99-1.01)	7+	0.59 (0.19-1.82)
Odds ratios with p-value < 0.20			
Respondent ever intakes tap water (primary, secondary source at home, or away from home)?	3.41 (1.88-6.19)	Principle water source chosen because it is protected	0.78 (0.58-1.05)
They say they know how to avoid TF	0.44 (0.31-0.61)	Tap water is a primary or secondary source of household water	2.80 (1.64-4.79)
They say wash hands to avoid TF	2.36 (1.45-3.86)	Household has a functional mobile phone	0.71 (0.46-1.09)
		Visible urine and/or faeces in latrine area	1.24 (0.94-1.65)
Do they eat uncooked food?			
<i>Never</i>	1.0 (reference, n = 694)	Do they share their plate with others?	
<i>Sometimes</i>	1.26 (0.91-1.73)	<i>Never</i>	1.0 (reference, n = 138)
<i>Regularly</i>	0.12 (0.04-0.39)	<i>Sometimes</i>	0.98 (0.66-1.45)
		<i>Regularly</i>	0.08 (0.04-0.16)

Do they wash hands after defecation?		What time of day do they collect water?	
<i>Never</i>	1.0 (reference, n=69)	<i>Morning</i>	1.0 (reference, n = 915)
<i>Sometimes</i>	1.48 (0.81-2.69)	<i>Midday</i>	1.56 (0.65-3.74)
<i>Always</i>	2.18 (1.19-4.00)	<i>Evening</i>	2.53 (1.04-6.18)
Do they wash hands after childcare?		What is the primary household water source?	
<i>Never</i>	1.0 (reference, n = 70)	<i>Tap</i>	1.0 (reference, n = 754)
<i>Sometimes</i>	3.16 (1.64-6.11)	<i>Protected spring</i>	0.13 (0.04-0.37)
<i>Always</i>	3.24 (1.63-6.45)	<i>Unprotected spring</i>	0.26 (0.09-0.71)
Do they wash hands before cooking?		<i>Well</i>	0.49 (0.10-2.34)
<i>Never</i>	1.0 (reference, n = 191)		
<i>Sometimes</i>	3.21 (2.00-5.16)		
<i>Always</i>	5.12 (3.11-8.44)		
Item shown when asked "Can you show me your soap?"			
<i>Nothing</i>	0.97 (0.59-1.61)		
<i>Laundry detergent</i>	0.49 (0.35-0.70)		
<i>Hand soap new in pack</i>	0.69 (0.33-1.44)		
<i>Used hand soap</i>	1.0 (reference, n = 412)	Topography of residence	
What level of education did they attain?		<i>Hilltop</i>	1.0 (reference, n = 620)
<i>Some primary</i>	1.0 (reference, n = 62)	<i>On a slope</i>	0.65 (0.46-0.91)
<i>Finished primary</i>	1.00 (0.38-2.64)	<i>In a dip</i>	0.37 (0.18-0.78)
<i>Some secondary</i>	0.77 (0.43-1.36)	What is their primary source for drinking water?	
<i>Finished secondary</i>	1.16 (0.66-2.06)	<i>Tap</i>	1.0 (reference, n = 750)
<i>Some tertiary</i>	1.67 (0.72-3.86)	<i>Protected spring</i>	0.10 (0.04-0.29)
<i>Finished tertiary</i>	0.53 (0.27-1.07)	<i>Unprotected spring</i>	0.57 (0.23-1.41)
What materials is their home made of?		<i>Well</i>	0.59 (0.14-2.37)
<i>Unconsolidated mud</i>	1.0 (reference, n = 121)		
<i>Consolidated mud</i>	0.90 (0.58-1.39)		
<i>Loose bricks</i>	1.67 (0.76-3.69)		
<i>Consolidated bricks or multilevel</i>	1.48 (0.93-2.36)		
Occupation of head of household:			
<i>Casual labourer</i>	1.65 (1.15-2.39)		
<i>Labourer with regular contract</i>	1.0 (reference, n = 380)		
<i>Own business</i>	2.31 (1.54-3.46)		
<i>Farmer</i>	1.78 (0.86-3.70)		
<i>Other</i>	2.92 (1.87-4.56)		

363

364 Notes: TF = Typhoid fever. For brevity, not all univariate calculations are shown. OR were
 365 not calculated for variables where < 5% of answers were different from the most popular
 366 answer.

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370 In all cases (OR data in Table 2), those who stated that they *always* wash hands after
371 defecation or before cooking and before infant care had significantly increased risk of
372 disease. Those who stated *never* in response to these questions, had strongly decreased risk.
373 Knowledge about handwashing was similarly correlated; those who said they knew they
374 should wash hands had much increased risk. Explanations for this unexpected finding are
375 explored in the Discussion.

376 To put multiple variables about washing hands behaviour or beliefs into the same
377 model could create collinearity problems. Moreover, the information about washing hands
378 before cooking or after childcare is incomplete because this question was only asked to
379 female heads of household, and hence there were missing data for 253 respondents.
380 Similarly, answers were missing for 264 respondents (56 cases and 208 controls), on
381 whether they mentioned washing hands when asked about ways to avoid catching TF.
382 However, there were no missing data about washing hands after defecation for any
383 respondent. To minimise collinearity and for ease of interpretation, only the variable about
384 handwashing habits after defecation was used to indicate handwashing knowledge or
385 behaviour, when generating the final model.

386 Table 3 shows our final predictive model with all final significant predictors with
387 adjusted odds ratios. Complete data were available for 320 cases and 636 controls. This
388 model adjusts for age and sex for completeness, but their coefficients are not shown
389 because their distribution was artificially imposed by the control recruitment method and
390 therefore cannot be interpreted as risk indicators. Regularly sharing food was also linked to
391 less illness (adj. OR 0.07, 95%CI 0.03-0.14). Contaminated mains water (adj. OR 4.25, 95%CI
392 2.18-8.28) was likely to be an important route for typhoid transmission in this population,
393 either via direct ingestion or additional exposure (hand washing habits). The population
394 attributable risk percentage (PAR%) for tap water consumption was estimated at 69.6%.
395 Choosing a water source for perceived protected status seemed to confer reduced risk (adj.
396 OR 0.68, 95%CI 0.48-0.95), while the indicator of visible urine/faeces in the respondent's
397 primary latrine area conferred increased risk of disease acquisition (adj. OR 1.43, 95%CI
398 1.05-1.95; PAR% = 17.3%). Other PAR values are reported in Table 3, although not for
399 exposures that reduced risk – the PAR was not developed for that purpose.

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Table 3. Adjusted odds ratios in typhoid fever outbreak, Kikwit DRC 2011.

Risk factor	Case n (%)	Control n (%)	Odds ratio	OR 95% CI	p	PAR
Plate sharing						
Never	56 (5.9)	82 (8.6)	1.0 (ref)		0.000	
Sometimes	254 (26.6)	388 (40.6)	1.29	0.84-1.98		---
Regularly	10 (1.0)	166 (17.4)	0.07	0.03-0.14		---
Occupation of head of household						
Casual labourer	86 (9.0)	169 (17.7)	2.12	1.41-3.17		14.1%
Labourer reg. contract	95 (9.9)	283 (29.6)	1.0 (ref)		0.000	
Own business	67 (7.0)	94 (9.8)	2.61	1.68-4.07		12.9%
Farmer	13 (1.4)	24 (2.5)	2.74	1.22-6.15		2.6%
Other	59 (6.2)	66 (6.9)	3.86	2.36-6.32		13.6%
Tap water is ever used						
No	29 (3.0)	107 (11.2)	1.0 (ref)		0.000	
Yes	291 (30.4)	529 (55.3)	4.29	2.20-8.38		69.6%
Wash hands after defecating						
Never	16 (1.7)	52 (5.4)	1.0 (ref)		0.000	
Sometimes	139 (14.5)	316 (33.0)	1.27	0.67-2.43		9.0%
Always	165 (17.3)	268 (28.0)	2.71	1.40-5.28		32.2%
Water source chosen because it is protected						
No	224 (23.4)	413 (43.2)	1.0 (ref)		0.028	
Yes	96 (10.0)	223 (23.3)	0.68	0.49-0.96		---
Visible urine/faeces at latrine						
No	136 (14.2)	302 (31.6)	1.0 (ref)		0.022	
Yes	184 (19.2)	334 (34.9)	1.44	1.06-1.97		17.3%

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Notes: Conditional (fixed-effects) logistic regression used to estimate adjusted odds ratios.
PAR = population-attributable risk. See text for model interpretation and variable coding.
Other model metrics: 956 observations, Log likelihood = -490.13, LR χ^2 = 187.91 (p = 0.000),
Pseudo R² = 0.1609.

411 +-

412 **Sources of drinking water**

413 According to the survey responses, the majority (90%) of cases in the general (not
414 camp) population used taps at communal distribution points as their principal source of
415 drinking water. Water sources for camp residents were more diverse. The most common
416 sources of drinking water for cases in each camp were (Table 4): an artesian well for Ngubu
417 camp (36%), taps at communal distribution points for Nsinga camp (34%), and an
418 unprotected source for Ebeya camp (30%). Overall, 34% of all camp cases used communal
419 taps as their principal source of drinking water.

420

421 **Table 4. Epidemiological and water quality data of the three military camps in**
422 **Kikwit, DRC, 2013.**

423

Camp	2011 Population	Total cases	Attack rate %	Main water source	TTC (CFU 100ml ⁻¹)
Ebeya	931	65	6.98	Unprotected spring	101-1000
Nsinga	681	40	5.87	Public water tap	101-1000
Ngubu	768	37	4.82	Artesian borehole	0

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425 Notes: TTC= Thermotolerant coliform colonies, CFU=colony forming units.

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428 Almost all the sources of tested principal drinking water were contaminated with
429 faecal coliforms to a very high degree (see S3 Spreadsheet for original data). Free residual
430 chlorine levels measured at the public water taps were insufficient (<0.2 mg.l⁻¹) to zero [6].
431 Figure 4 indicates the main types of water source tested and the proportion of each type of
432 each source that fell into risk categories to human health. There were 102 unique sources
433 identified by interviewees. Protected springs were most likely to be low risk. Only one of the
434 water taps conformed to published standards. Most respondents (892/960, 92.9%) who
435 were asked about possible treatment methods did not report that they treated their water
436 by chlorination, boiling or filtration (or another pathogen inactivation method); therefore,
437 we did not include water treatment factors when estimating odds ratios and exposures.

438

439 **Fig. 4: Health risks of water points in Kikwit, DRC, based on thermotolerant coliform colony**
440 **(TCC) counts:** 0 CFU.100ml⁻¹ (Minimal risk, according to concurrent UNHCR water pollution
441 guidelines [9]); 1-10 CFU.100ml⁻¹ (low risk), 11-100 CFU.100ml⁻¹ (intermediate risk), 101-1000
442 CFU.100ml⁻¹ (high risk) and >1000 CFU.100ml⁻¹ (very high risk).

443

444 Discussion

445 Multiple lines of investigation tied together here establish a strong association of
446 mains water with the spread of the outbreak from the camps to the rest of the population,
447 conforming with the multi-level evidence recommendations for surveillance of waterborne
448 infectious diseases made by Tillett et al. [10]. The ecological study strongly suggested that
449 the outbreak was linked to particular parts of the city water supply and showed that attack
450 rates for city residents were highest in the areas with a gravity-fed mains water distribution.
451 The case-control study confirmed that using mains water was most strongly and reliably
452 associated with the risk of disease acquisition. Descriptive epidemiological analysis found
453 that attack rates peaked earliest and were highest overall in the military camps [6]. The
454 outbreak appears to have started in the camps, likely due to more naïve population and
455 poorer living conditions, where a diversity of water sources were used, as shown in this
456 report. Microbiological analysis has repeatedly shown drinking water sources throughout
457 Kikwit to be mostly unsafe, due to high faecal coliform counts and inadequate chlorination
458 [6,11]. Ingress of faecal material during the outbreak period, due to low water pressure, was
459 very plausible to expect in many parts of the network concurrently observed to be in poor
460 repair (S1 Report). In 2011, the outdated mains water supply system in Kikwit probably
461 played an important role in disseminating typhoid from early cases in camps to the general
462 population.

463 This case-control study also adds to the descriptive epidemiology report, the
464 information that statements by respondents about regular handwashing were linked to
465 increased risk of disease. Handwashing with soap after toileting or prior to food preparation
466 and after infant care, should be best practice in all settings including where endemic
467 diseases are present, but this may not be true when the main water sources available for
468 washing are themselves contaminated and where soap may not be available. The case-
469 control survey did not ask questions about whether respondents used treated water for
470 handwashing, but 91% who were asked about treatment methods, did not mention any
471 method for treating household water. Moreover, it was observed [5] that most households
472 lacked any handwashing soap, and only three respondents had handwashing materials close
473 to their main latrine facility. It is very possible that many respondents said that they washed
474 hands, when in fact they merely rinsed their hands. Self-report is prone to another bias;
475 socially approved behaviours are usually self-reported more frequently than observed [12-
476 14]. Bias in favour of approved behaviours could explain our apparent finding that regularly
477 sharing food was protective. Moreover, the interview asked about current practice not

478 those at the time of the outbreak; it is certainly plausible that people who have had typhoid
479 would be more rigorous with handwashing practices after the outbreak, so the question
480 could not elicit accurate information about exposure at the time of outbreak. Inefficacy in
481 handwashing technique is an unexplored risk factor. In this resource scarce setting, it seems
482 likely that hand-drying materials are also limited; inadequate hand drying can leave
483 pathogens on the hands, too [14-16]. That so many respondents said they had soap but
484 then could not display used soap when asked, supports the suggestion of bias for answers
485 about handwashing behaviours. Consequently, given the potential biases in how the hand-
486 washing questions were answered, we conclude that the association between reported
487 handwashing and risk of typhoid acquisition cannot be taken as indicative of a real risk of
488 disease in people who do (properly) wash their hands.

489 As for occupation of head of household, labourer with a regular work contract had
490 the lowest quantifiable association with disease. The next lowest risk group was casual
491 labourers, adj. OR 2.12, 95%CI 1.41-3.17. The finding may be confounded because military
492 men fell into this occupational group with regular contracts. No further information was
493 available about the working environment for individuals with regular contracts. Possible
494 explanations are that this category (about one third of respondents) tended to indicate
495 households with more financial security due to regular work, or where the head of
496 household, because of long-term regular dirty work, had prior exposure and thus acquired
497 immunity to multiple *Salmonella* species and serovars [17-20].

498 Limitations: This study was undertaken 13-18 months after the end of the 2011
499 outbreak. Assuming that responses to questions asked in early 2013 can truly describe
500 behavioural practices in late 2011-early 2012 outbreak may be suspect. The questionnaire
501 did not ask about individual hygiene behaviour and practices during the outbreak to avoid
502 other types of recall bias. Local staff translated the questionnaire from French to other
503 languages as required during interviews; we did not monitor this process and it may have led
504 to inconsistencies in how questions were asked or answered; in the Kikwit area, French and
505 the Kituba language predominate but there are many regional languages and dialects
506 spoken plus interviewees could have come from anywhere in the DRC, which has over 200
507 recognised languages. We did not use serology to confirm that controls were negative or to
508 confirm cases. This means likely misclassification of some controls, which will have biased
509 the odds ratios downwards in Table 3; this means our evidence for implicating water and
510 sanitation in the spread of TF is understated. Ecological analysis was limited to only one
511 type of geography (health areas) and only in parts of the city, and only some spatial variables

512 (ones we could get data for). Water quality could have changed between 2011 and 2013.
513 We measured ThermoTolerant Coliforms about 16 months after the outbreak to gauge
514 ongoing contamination of city water supplies, rather than PCR amplification that specifically
515 looked for *S. Typhi* during the actual outbreak weeks. Heavy rainfall can cause latrine
516 overflows in Kikwit and could affect local supplies, changing preferred water sources;
517 however, the outbreak, survey and water quality testing all took place in wet months with
518 very similar levels of monthly rainfall (November-May period). We assumed that general
519 state of sanitation facilities (soap, latrines) did not change since the outbreak; however, we
520 do have considerable anecdotal information that this assumption is valid. Challenges in
521 tracing cases were encountered due to the 13 months elapsed time since creation of the line
522 list and survey start. Some civilian cases may have been misidentified as camp residents,
523 due to proximity of the camps to the general population, and vice versa.

524 Our recommendations address both prevention and emergency responses, and also
525 draw on observations and suggestions made by water sanitation engineers who visited
526 Kikwit in December 2011 (S1 Report). Our key recommendation to prevent or minimise
527 future outbreaks in Kikwit of typhoid and similar diseases, is improvement to the water
528 network. Descriptive, spatial and case-control studies all identify the water network as
529 instrumental in transmission of typhoid in 2011. Surveys of water supplies in Kikwit in both
530 July and November 2015 also found widespread unacceptable faecal contamination in all
531 drinking water sources tested; 97% of the isolated bacteria in surface waters had human
532 origin [21]. This finding was strongly linked to outbreaks of waterborne diseases thought to
533 affect up to 30% of the city's population annually. A full revamp of the city's water system
534 would clearly be very desirable. Work is arguably most urgent in those areas fed by gravity
535 supply, which are in the central area that also had the highest attack rates. Improvements
536 to the mains water network could include but should not be limited to: repairs to prevent
537 inundation (including replacing pipes and reversing soil erosion), consistent chlorination of
538 tap water, regular monitoring of the chlorination levels, rehabilitation of unprotected
539 springs, and closing latrines located uphill and in relative proximity to frequented water
540 sources or water mains pipes. Hygienic harvesting of rainwater in public places could be
541 implemented to make it easier to properly wash hands (S1 Report). It would also be
542 desirable to improve the overall sanitation and hygiene situation in Kikwit, especially within
543 the places that were hotspots for TF transmission in 2011 (military camps) [6].
544 Rehabilitating latrines, provision of ongoing resources to make handwashing safer and more

545 effective, to enable handwashing with soap and consistent household water treatment,
546 could be beneficial.

547 Recommendations as part of an immediate actionable response to an outbreak
548 should include: creation of minimum perimeters from latrines to water sources and rapid
549 drainage of runoff water around standpipes and hoses (S1 Report). Rapid testing of water
550 sources and rapid ascertainment of exposure risks during an outbreak would quickly
551 facilitate understanding how such disease was spreading. It is undesirable that the exposure
552 data in this study were collected as late as 14 months after the outbreak. Distribution of
553 handwashing materials with health campaigns to promote full washing, for users of all water
554 sources, would be desirable. Emergency distribution of chlorine, either in tablets or via
555 buckets, with usage instructions, to ensure more water treatment could be protective,
556 although work needs to be done to make the taste of chlorinated water more acceptable to
557 the local populace (S1 Report). Distribution of hygiene kits may well be appropriate,
558 especially to high risk groups [16]. Vaccine-based strategies for typhoid control are
559 recommended for school-age children in endemic countries – in this context, a targeted
560 vaccination in the camps might be effective emergency response or short-term prevention
561 measure [22,23].

562

563 **Conclusions**

564 Following high early transmission in military camps near the city of Kikwit, use of
565 contaminated mains water was consistently and reliably, strongly associated with typhoid
566 fever acquisition. A safer mains water network is the most valuable change that could
567 prevent future disease. Effective measures to better protect water supplies, include but are
568 not limited to: relocation of intake points, more consistent chlorination, preventing
569 inundation to the distribution network, and more convenient access to treated water. Safe
570 sources for the purposes of cooking and hand cleaning could reduce the size of TF and
571 similar disease outbreaks in future.

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660 **Supporting Information**

- 661 S1 Checklist. STROBE
- 662 S1 Report. Sanitation Engineers
- 663 S1 Protocol. Typhoid Fever spatial analysis
- 664 S2 Protocol. Typhoid Fever risk factors
- 665 S1 Questionnaire. Survey

- 666 S1 Spreadsheet. Health Area Attributes
- 667 S2 Spreadsheet. Survey Data and Derived Variables
- 668 S3 Spreadsheet. Results of Water Quality Tests
- 669