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Prevalence of infection among asymptomatic and paucisymptomatic contact persons exposed to Ebola virus in Guinea: a retrospective, cross-sectional observational study

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1 **Title:** Estimating the frequency of infection among asymptomatic and paucisymptomatic
2 contact persons exposed to Ebola in Guinea: a retrospective observational study

3 **Authors**

4 Mamadou Saliou Kalifa Diallo,*^{1,6,7} Muriel Rabilloud,*^{2,3,4,5} Ahidjo Ayouba,*¹ Abdoulaye
5 Toure,*^{1,6,7} Guillaume Thaurignac,¹ Alpha Kabinet Keita,^{1,7}Christelle Butel,¹ Cécé Kpamou,⁷
6 Alimou Thierno Barry,⁷ Mariama Dioulde Sall,⁷ Ibrahima Camara,⁷ Sandrine Leroy,¹ Philippe
7 Msellati,¹ René Ecochard,^{2,3,4,5} Martine Peeters,¹ Mamadou Saliou Sow,^{6,7,8} Eric Delaporte,^{1,9}
8 Jean-François Etard,¹ for the Contactebogui Study Group[§]

- 9 1. TransVIHMI, IRD, INSERM, Université de Montpellier, Montpellier, France
- 10 2. Hospices Civils de Lyon, Service de Biostatistique-Bioinformatique, Lyon, France
- 11 3. Université de Lyon, Lyon, France
- 12 4. Université Lyon 1, Villeurbanne, France
- 13 5. Laboratoire de Biométrie et Biologie Évolutive, Équipe Biostatistique Santé, Pierre-
14 Bénite, France.
- 15 6. Institut National de Santé Publique (INSP), Conakry, Guinée
- 16 7. Centre de Recherche et de Formation en Infectiologie de Guinée (CERFIG),
17 Université Gamal Abdel Nasser de Conakry, Conakry, Guinée
- 18 8. Service des maladies infectieuses et tropicales, Hôpital National de Donka, Conakry,
19 Guinée.
- 20 9. University Teaching Hospital, Montpellier, France

21 *Equal contributions

23 **First corresponding author:**

24 Jean-François Etard
25 TransVIHMI, IRD, INSERM, Université de Montpellier
26 34394 Montpellier, France
27 jean-francois.etard@ird.fr
28 +33467416297

29 **Alternate corresponding author:**

30 Eric Delaporte
31 TransVIHMI, IRD, INSERM, Université de Montpellier
32 34394 Montpellier, France
33 eric.delaporte@ird.fr
34 +33467416297

35 **Research in context**

36 **Evidence before this study**

37 We searched PubMed, Web of Sciences and Google Scholar for studies on frequency of Ebola
38 virus infection up to May 30, 2018 without language restrictions. We used the term *Ebola**
39 plus any of the following terms: *asymptom**, *paucisymptom**, *symptom**, *antibod**, *infect**,
40 *frequency*, *prev**, *seroprevalence*, *serosurvey*, *seropositivity*, *contact*.

41 Since the discovery of Ebola virus in 1976, assessment of anti-Ebola antibodies has been
42 carried out in different populations exposed to the virus: contact persons of EVD cases,
43 samples of the general population, and blood donors. The nature of the tests used as well as
44 the thresholds of positivity varied from one study to another. Most of these studies did not
45 distinguish asymptomatic from paucisymptomatic contact persons, and did not take level of
46 exposure to EVD cases into account. The frequencies of Ebola virus infection reported by
47 these studies ranged from 1% to 46%.

48 **Added value of this study**

49 Antibodies against three recombinant proteins, nucleoprotein (NP), glycoprotein (GP) and 40-
50 kDa viral protein (VP40) were assessed using a novel Luminex-based technology. We then
51 used a latent class model based on the result of the three antibody profiles to estimate the
52 frequency of Ebola virus infection in contact persons in Guinea during the 2013–2016
53 outbreak. We showed that seropositivity and the estimated frequency of Ebola virus infection
54 were correlated with the level of exposure and occurrence of symptoms.

55 **Implications of all the available evidence**

56 This study revealed a significant occurrence of a- or paucisymptomatic Ebola virus infections
57 among contact persons and contributes to a better knowledge of the clinical presentation of
58 Ebola virus infection. Their role in the chain of transmission remains to be evaluated. The
59 higher frequency of Ebola virus infection in contact persons who participated in burial rituals
60 confirms the importance of safe and dignified burials during Ebola outbreaks and the need to
61 systematically interview contact persons regarding participation in burial rituals.

62

63 **Summary**

64 **Background** The frequency of Ebola virus infection (EVI) among persons who have been in
65 contact with Ebola virus disease (EVD) remains unclear and is essential to understanding the
66 dynamics of the transmission. This study aimed to identify risk factors for seropositivity and
67 to estimate the frequency of EVI in Ebola-unvaccinated contact persons.

68 **Methods** Information on exposure, occurrence of symptoms after exposure and antibodies
69 response against glycoprotein, nucleoprotein, and VP40 from 1390 contact persons were
70 collected in a cross-sectional survey in Guinea. The frequency of EVI was estimated with a
71 latent class model.

72 **Findings** Symptoms were reported by 216 contact persons (15.50%). Seropositivity was
73 8.33% (18/216; 95% CI: 5.01% to 12.80%) among paucisymptomatic contact persons and
74 3.32% (39/1174; 95% CI: 2.37% to 4.51%) in asymptomatic ones ($p=0.002$). Seropositivity
75 increased with participation in burial rituals (aOR: 2.30; 95% CI: 1.21 to 4.17). Frequency of
76 EVI varied from 3.06% (95% CI: 1.84% to 5.05%) in asymptomatic contact persons who did
77 not participate in burial rituals to 5.98% (95% CI: 2.81% to 8.18%) among those who did,
78 and from 7.17% (95% CI: 3.94% to 9.09%) in paucisymptomatic contact persons who did not
79 participate to 17.16% (95% CI: 15.24% to 19.98%) among those who did.

80 **Interpretation** This study provides a new assessment of the frequency of EVI among contact
81 persons depending on exposure, provides evidence for the occurrence of paucisymptomatic
82 cases and reinforces the importance of closely monitoring at-risk contact persons.

83 **Funding** INSERM/Reacting, the French Ebola Task Force, IRD and MUSE/University of
84 Montpellier.

85

86 **Introduction**

87 The 2013–2016 Ebola virus disease outbreak in West Africa was larger than all prior
88 combined outbreaks, resulting in 28 616 confirmed, probable and suspected cases in Guinea,
89 Liberia and Sierra Leone.¹ Contact tracing and monitoring contact persons for 21 days were
90 key measures to halt transmission.² While it is essential to understand the transmission
91 dynamics of the disease and the clinical spectrum of EVD and its implication, the frequency
92 of asymptomatic or paucisymptomatic infections among contact persons remains unclear.³

93 The frequency of seropositivity against Ebola virus has been evaluated in several studies
94 during the previous outbreaks in Africa. These serological studies enrolled either contact

95 persons, samples of the general population or blood donors and the estimates varied widely,
96 between 1% and 46%.⁴⁻⁹

97 A recent study in Sierra Leone among 481 contact persons to EVD cases collected data on
98 exposure, symptoms after exposure and results of a glycoprotein IgG capture assay on oral
99 fluid. The seropositivity was 2·6% (95% CI, 1·2% to 4·7%) in asymptomatic household
100 contact persons of survivors (10 of 389) and 12·0% (95% CI, 6·1% to 20·4%) in symptomatic
101 ones (11 of 92).¹⁰

102 Two recent meta-analyses, based on data from serosurveys carried out between 1976 and
103 2015, yielded very different estimates of the proportion of asymptomatic infection among
104 contacts-persons: i.e. 3·3% (95% CI: 2·4% to 4·4%) and 27·1% (95% CI: 14·5% to
105 39·6%).^{11,12} These discrepancies might be explained by the heterogeneity of the assays used,
106 the heterogeneity of the study populations, the eventual cross-reactions with non-Ebola
107 viruses, or the absence of a clear gold standard assay or algorithm.

108 The present study aimed to 1) identify the risk factors associated with seropositivity and 2) to
109 estimate the frequency of Ebola virus infection in asymptomatic and paucisymptomatic
110 contact persons. It combined detailed information on exposure of contact persons and the
111 occurrence of symptoms after exposure, with quantitative data from a novel serological test
112 based on the response to three different antigens, initially validated on a large number of
113 samples from survivors of EVD.¹³ Data collection started two weeks after the last 2013–2016
114 EVD case was reported in Guinea.

115

116 **Methods**

117

118 **Study design and settings**

119 This cross-sectional study was conducted in Guinea, between May 2016 and September 2017.
120 Ethical approval was received from the National Ethics boards for Health Research
121 Committee (CNER, Guinea) and the Ethical Evaluation Committee of INSERM (CEEI,
122 France).

123 The study enrolled contact persons who were defined as people who had a physical contact
124 with 1) an EVD survivor case included in the Postebogui cohort study;¹⁴ or 2) other EVD
125 cases (alive or dead) not included in the Postebogui cohort but living in the same compound
126 as the survivor. According to the WHO definition, physical contact includes sharing the same
127 room/bed, caring for a patient, touching body fluids, or closely participating in a burial.¹⁵
128 Postebogui is a cohort study which enrolled more than 800 Ebola survivors from four sites to
129 study the long-term clinical, virological and psychosocial consequences of EVD in Guinea.¹⁴
130 All contact persons, aged at least seven years old and having not been diagnosed as EVD
131 cases during the Ebola outbreak were included. They were enrolled from the same four study
132 sites than the Postebogui survivors: Conakry, Forecariah, Macenta and N'Zerekore, located in
133 the main areas of the outbreak. Date of onset of the Postebogui EVD cases were excerpted
134 from the medical files of the Ebola Treatment Centers. Informed consent was obtained from
135 all contact persons, or from their parents or legal guardians for children. A questionnaire was
136 administered collecting age, sex, history of rVSV-ZEBOV vaccination, information on each
137 exposure to an EVD case and on occurrence of symptoms after exposure.¹⁶ The list of
138 symptoms was based on the symptoms of the Postebogui survivors and the WHO definition
139 for suspected case during an Ebola outbreak.¹⁵ All contact persons who reported symptoms
140 after exposure to an EVD case were categorized as paucisymptomatic and the others as
141 asymptomatic.

142 A total of 1721 contact persons were interviewed and tested to assess their immunological
143 response to three recombinant proteins against Zaire Ebola virus (EBOV): glycoprotein (GP),
144 nucleoprotein (NP) and 40-KDa viral protein (VP40). Among the contact persons 331 who
145 reported a history of vaccination were excluded from the analysis because the serological tests
146 were carried out after vaccination, resulting in a study population of 1390 contact persons
147 (Supplementary Figure 1).

148

149 **Procedures, outcomes and studied factors**

150 Blood samples from contact persons were collected on EDTA tubes to prepare dried blood
151 spots (DBS). For DBS preparation, 50 µl of whole blood were spotted on each of the five
152 circles of a 903 Whatman filter paper and dried at ambient temperature for 3 hours. The
153 remnant blood was aliquoted and stored as plasma at -20°C for backup. DBS were shipped to
154 the virology laboratory of IRD in Montpellier, France, for serology. The detection of
155 antibodies to recombinant GP, NP, and VP40 of Zaire Ebola virus was performed using a
156 Luminex-based assay. Reported sensitivity and specificity are well above 90% for each
157 antigen.¹³

158 To determine seropositivity, we used the thresholds defined previously using the Postebogui
159 samples: 501, 950, and 580 median fluorescence intensity (MFI) for GP (Kissoudougou
160 strain), NP, and VP40, respectively.¹³ Based on the serological responses, a contact person
161 was considered seropositive when their sample was reactive, i.e. above the threshold, for at
162 least two different antigens.

163 Potential predictive factors for seropositivity were socio-demographic characteristics,
164 exposure intensity, and presence of symptoms. Factors quantifying exposure intensity
165 included death of EVD case, number of symptomatic EVD cases encountered, participation in
166 burial rituals, residing in the same household or room than an EVD case, direct contact with
167 EVD case body fluids, and providing care to an EVD case.

168 In order to estimate the frequency of Ebola virus infection, we used the response to each of
169 the three antigens with a combination of different thresholds.

170

171 **Statistical and descriptive analysis**

172 Socio-demographic characteristics and exposure factors were described in all contact persons.
173 Categorical variables were described using absolute and relative frequencies, and continuous
174 variables using median, interquartile range, minimum and maximum values. Fisher's exact
175 test was used to compare percentages.

176

177 **Risk factors associated with seropositivity**

178 A set of logistic regressions were performed to identify the risk factors associated with
179 seropositivity and quantify their effect. The analysis was first performed on all contact
180 persons, then separately on asymptomatic and paucisymptomatic contact persons.

181 All factors with a p-value below or equal to 0.2 in univariate analyses were introduced in the
182 multivariate models. Goodness-of-fit of the models was assessed by the Hosmer-Lemeshow
183 test. The effects of the factors were quantified using unadjusted odds ratios (OR) for

184 univariate models and adjusted OR (aOR) for multivariate models with a 95% confidence
185 interval (95% CI).

186 The risk factors identified were then used to constitute two subgroups with different
187 seropositivity probabilities within the asymptomatic contact persons in the one hand and the
188 paucisymptomatic contact persons in the other hand, yielding four subpopulations. Probability
189 density curves of the MFI for each antigen were represented according to these
190 subpopulations. The distribution of the MFI values of each antigen was also described in all
191 the population and in the asymptomatic and paucisymptomatic subpopulations according to
192 exposure factors. The median, the 10th and 90th percentiles were used in order to be able to
193 compare in particular the distributions of the high values of MFI reflecting exposure intensity.

194

195 **Estimating Ebola virus infection**

196 None of the three antibody responses, performed as a single test, is a perfect reference (i.e.
197 “gold standard”) to confirm EVI status. Therefore, to estimate the frequency of EVI in the
198 four subpopulations identified above, we used a latent class model.^{17,18}

199 Briefly, the true status of contact persons regarding EVI was unknown and categorized as a
200 latent class (infected or non-infected). A probabilistic likelihood function-based model was
201 used to estimate the frequency of EVI in each subpopulation, under the assumptions that 1)
202 the tests are conditionally independent given EVI status and 2) the sensitivity and specificity
203 of each test does not vary across the subpopulations (see details in supplementary materials).¹⁹

204 We first estimated the frequency of EVI in the four subpopulations using the thresholds set
205 with the Postebogui study. Then, to evaluate the robustness of the estimates, we used several
206 possible combinations of thresholds (between 300 and 1200 MFI) for the three antibody
207 responses, resulting in 1000 estimates. All analyses were performed using R software version
208 3.4.

209

210 **Role of the funding source**

211 The study sponsors had no role in study design, data collection, data analysis, data
212 interpretation, or writing of the report. The corresponding author had full access to all the data
213 and had the final responsibility for the decision to submit for publication.

214

215 **Results**

216 Among the 1390 contact persons, 216 (15.5%) reported at least one symptom and 1174
217 remained asymptomatic (Supplementary Figure 1). The median age was 26 years (range 7–

218 88) and 682 (49%) were male (Table 2). Overall, they reported 2467 exposures to EVD cases
219 whose date of onset of symptoms span between 23/03/2014 and 19/10/2015 (1045 of 1390
220 dates available in medical records). The median time elapsed between onset of symptoms of
221 the Postebogui EVD and interview of contact persons was 844 days (IQR: 666-999). Half of
222 the contact persons were exposed to fewer than two EVD cases (range 1–17). The most
223 reported symptoms among the 216 paucisymptomatic contact persons were headache (81%;
224 174 of 216), fatigue (74%; 159 of 216) and fever (73%; 157 of 216). Following the WHO
225 definition for suspected case during an Ebola outbreak, 30 contact persons among the 216
226 paucisymptomatic reported a fever plus three symptoms from the following list: headache,
227 anorexia, vomiting, abdominal pain, diarrhea, muscle or joint pain, bleeding.

228 The proportion of individuals who reported being in contact with at least one EVD fatality
229 was higher in paucisymptomatic (45/99) than in asymptomatic contact persons (67/280) (45%
230 versus 24%, $p=0.0001$). The proportion of individuals who reported being in contact with
231 blood or vomit from an EVD case was higher in paucisymptomatic (137 of 216) than in
232 asymptomatic contact persons (548 of 1174) (63% versus 47%, $p=0.008$). Likewise, the
233 proportion of individuals who reported a participation in a burial ritual was higher in the
234 paucisymptomatic contact persons (44 of 216) than the asymptomatic contact persons (154 of
235 1174) (20% versus 13%, $p<10^{-3}$).

236 The observed seropositivity was 8.33% (95% CI: 5.01% to 12.80%) among
237 paucisymptomatic contact persons (18 of 216) versus 3.32% (95% CI: 2.37% to 4.51%) in
238 asymptomatic ones (39 of 1174) ($p=0.002$), for an overall seropositivity of 4.10% (95% CI:
239 3.12% to 5.28%; 39 of 1390) (Table 2). The adjusted OR for seropositivity of
240 paucisymptomatic compared to asymptomatic contact persons was 2.16 (95% CI: 1.17 to
241 3.85, $p=0.01$). Similarly, there was a greater than two-fold increase in seropositivity among
242 contact persons who participated in burial rituals (aOR: 2.30; 95% CI: 1.21 to 4.17;
243 $p=0.008$).

244 Among asymptomatic contact persons, univariate analysis allowed us to identify three risk
245 factors significantly associated with seropositivity: participation in burial rituals, contact with
246 blood or vomit, and living in the same room as an EVD case (Table 3). In multivariate
247 analysis, only participation in burial rituals (aOR: 2.30, 95% CI: 1.01 to 4.80, $p=0.03$) and
248 contact with blood or vomit (aOR: 2.37, 95% CI: 1.15 to 5.10, $p=0.03$) were independently
249 associated with seropositivity. Among paucisymptomatic contact persons, factors associated
250 with seropositivity were age of contact persons (aOR: 1.54 for an increase of 10 years of age,

251 95% CI: 1.12 to 2.12; $p < 0.01$) and participation in burial rituals (aOR: 2.40, 95% CI: 0.81 to
252 6.74, $p = 0.09$) (Table 4). In the subgroup of 30 contact persons meeting the suspected case
253 definition, the seropositivity reached 20.00% (95% CI: 7.71% to 38.56%) compared to
254 6.45% (95% CI: 3.38 to 10.99%) in the 186 paucisymptomatic not meeting the definition
255 ($p = 0.024$).

256 More details regarding different combinations of the antibodies against the three recombinant
257 proteins is provided in Table 5. For all the serological responses, the 90th percentile values
258 were always higher in paucisymptomatic than in asymptomatic contact persons
259 (Supplementary Table 1). In addition, the 90th percentile values for each antibody response
260 were higher in the most exposed individuals especially those who had participated in burial
261 rituals, or who had been in contact with blood or vomit.

262 For all antigens, the distribution in the four subpopulations was asymmetric and marked by a
263 small bump for the high values (Supplementary Figure 2). We can see that this bump was
264 more marked in contact persons who participated in burial rituals than in contact persons who
265 did not participate.

266 The results of estimating the frequency of Ebola virus infection using a latent class model are
267 shown in Table 6. In the asymptomatic contact persons, there was a two-fold increase in the
268 frequency of infection among participants in burial rituals compared to those who had not
269 participated in burial rituals, respectively 5.98% (95% IC: 2.81 to 8.18) versus 3.06% (95%
270 IC: 1.84 to 5.05). Similarly, among paucisymptomatic contact persons, the frequency of
271 Ebola infection in those who participated in burial rituals was 2.5 times higher than in those
272 who did not participate: 17.1% (95% IC: 15.24-19.98) versus 7.17 (3.94-9.09). The results
273 obtained by varying the combination of thresholds for the three antigens were comparable to
274 those obtained with the Postebogui thresholds. These results are illustrated in Supplementary
275 Figure 3.

276

277 **Discussion**

278 Our study shows that participation in burial rituals and contact with blood or vomit were
279 associated with Ebola virus seropositivity. Although there was no baseline assessment, the
280 very limited circulation of the Ebola virus before the outbreak and the high specificity of the
281 test used argue in favor of seroconversion being due to the exposures.²⁰ These results are
282 consistent with studies conducted after previous EVD outbreaks. Exposure during burials
283 rituals is a well-known risk factor for transmission of Ebola virus.^{21,22} We showed that, for all

284 tests, the bump in the high values of the MFI distribution was particularly marked among
285 those who participated in burial rituals. This particular shape is compatible with a mixture of
286 two distributions, one centered on a low value, below the thresholds, corresponding to the
287 most important part of the subpopulation and of a second one, centered on a high value,
288 corresponding to the seroconverted. These results underline once again the importance of the
289 safe and dignified burials in the context of an Ebola outbreak and the importance to closely
290 monitor those who participated in burial rituals. There was a two-fold increase in
291 seropositivity between asymptomatic and paucisymptomatic participants (8.33% versus
292 3.32%). These figures are close to the observed seropositivity in the Sierra Leone study (12%
293 [6.1% to 20.4%] in symptomatic cases versus 2.6% [1.2% to 4.8%] in asymptomatic),
294 delineating more clearly the frequency of asymptomatic or subclinical EVI.^{3,10} Exposures to
295 blood or vomit are other known risk factors for EVD transmission.¹² In our study, these risk
296 factors were statistically significant only in the asymptomatic contact persons but, given the
297 small number of paucisymptomatic contact persons, we probably lacked power to detect an
298 effect in that subpopulation. As in a previous study, sex did not appear to be a significant risk
299 factor for seropositivity.²¹

300 The latent class model, taking into account the results of the three antibody profiles in four
301 subgroups of the population with different EVI prevalences, allowed us to obtain robust and
302 unbiased estimates of EVI frequencies.^{23,24} EVI occurred in 3% to 17% of the contact
303 persons, depending on the presence of symptoms and exposure to burial rituals. This variation
304 based on level of exposure reveals a dose-response relationship between exposure and
305 seropositivity, providing additional confidence in the serological tests we used. The goodness-
306 of-fit test showed that our latent class model fits and we can assume that the tests results are
307 independent, conditional on the latent disease status.²⁵ An alternative that might take into
308 account a dependence between the tests would be a conditional dependence structure using a
309 Bayesian estimation method for example.²⁶ The gain in precision in estimating the frequency
310 of EVI using the latent class modeling approach in comparison to the observed seropositivity,
311 is very large. For example, the latent class model for the paucisymptomatic and burial sub-
312 group yields to an EVI frequency of 17.16% (95%CI 12.42 - 22.31) or 17.01% (min 15.24 –
313 max 19.98) while the observed seropositivity (7/44) was 15.9% (95%CI 6.64 - 30.06).

314 From a clinical perspective, the symptoms reported by 16% of the contact persons, while
315 unspecific, were all compatible with clinical manifestations of a mild EVI. The frequency of
316 the symptoms, their nature, and the proportion of seropositivity within the asymptomatic and
317 paucisymptomatic groups are in line with the Sierra Leone study's finding of 19.1% of

318 symptomatic contact persons.¹⁰ The latter study's results, along with ours, contribute to a
319 better knowledge of the clinical presentation of an EVI, running from an asymptomatic
320 infection, to a minimally symptomatic form, then an overt case, and finally a lethal form. The
321 elevated seropositivity observed in the subgroup of contact persons meeting the suspected
322 case definition argue in favor of missed cases during the contact tracing activities. Indeed,
323 several reports from Guinea underlined weaknesses of the contact tracing in evaluating
324 contacts as suspected cases.²⁷

325 Given the frequency of asymptomatic and paucisymptomatic cases revealed by our results, the
326 reported case fatality rate (62% in Guinea), based only on confirmed cases, overestimates the
327 true overall case fatality rate of Ebola virus infection. What are the drivers of such variability
328 in the clinical expression of the infection? The association shown by our data between
329 exposure to blood and vomit, participation in burial rituals, and the frequency of symptoms on
330 one hand, and between these exposures and the level of seropositivity on the other hand,
331 suggests that infectivity of the EVD source and the viral load played a role in the occurrence
332 of minimally symptomatic forms of EVI. However, other factors such as genetic determinants
333 of the host that limits virus spread cannot be excluded. Our study did not aim to explore the
334 mechanics of transmission chains but a detailed contact study in Sierra Leone failed to detect
335 any transmission from seropositive contact persons.^{28,29}

336

337 **Limitations**

338 The main limitation of our study relates to the retrospective and declarative nature of the
339 exposure data and symptoms, resulting in a likely recall bias. Participants were interviewed
340 long after their exposure to EVD cases and their answers to specific questions on
341 circumstances of a given exposure could have been inaccurate. Therefore, it is likely that the
342 paucisymptomatic group comprises EVD-related and EVD-unrelated symptomatic contact
343 persons, resulting in a mixture of the two populations. However, this measurement error could
344 only dilute the association since serological status was not known by the interviewers or
345 contact persons. In addition, persons in close contact with EVD cases could tend to report
346 more frequently symptoms. We controlled for that confounding effect by adjusting on proxy
347 of exposure and a strong statistical relation persisted, however we cannot rule out that residual
348 confounding may still be present. The study participants were sampled long after their
349 exposure to EVD cases and one could question the duration of the seropositivity after the
350 initial exposure. The documentation of a long and persistent antibody response against GP,
351 NP and VP40 antigens is reassuring.³⁰

352 Lastly, our study covered only contact persons residing in the compounds of the EVD
353 survivors who were included in the Postebogui study, and thus might not be representative of
354 all the contacts during the 2013–2016 EVD outbreak in Guinea. However, the Postebogui
355 cohort included two-thirds of the Guinean survivors and contact persons were recruited in
356 four different places. This suggests a reasonably good level of representation.

357

358 **Conclusion**

359 Our testing for Ebola virus antibodies targeting three antigens (GP, NP and VP40) in contact
360 persons showed that participation in burial rituals, along with body fluids, was the most
361 important risk factor associated with seropositivity, whether or not contact persons presented
362 symptoms. Contact persons who reported some symptoms after exposure were more likely to
363 be seropositive. This, again, reinforces the importance of identifying and closely monitoring
364 at-risk contact persons such as those who have participated in burial rituals or who are
365 exposed to blood or vomit. Using a latent class model, we showed a significant occurrence of
366 Ebola virus infection among contact persons, asymptomatic or paucisymptomatic, which
367 contributes to a better knowledge of the clinical spectrum of EVI. In addition, some
368 seropositive paucisymptomatic contact persons probably met the definition of suspected EVD
369 case, not previously evaluated as such. Whether or not these subclinical presentations of
370 minimally symptomatic infections confer some form of immunity against a subsequent
371 exposure, contribute to herd immunity, play a role in transmission, or if the infected person
372 harbors the virus in an immune privilege site, still remain open questions. Contacts tracers
373 should be trained to search and recognize minimally symptomatic EVI in order to take
374 appropriate conservative measures since, given our current knowledge, a risk of transmission
375 cannot be ruled out.

Contributors

SL, AT, JFE, ED, PM and MSS conceived and designed the study. AA, GT, CB, AKK, MP developed and performed the serological tests. AA, AT, GT, AKK, CB, CK, ATB, MDS, IC and JFE contributed to the data collection and curation. MSKD, MR, RE and JFE performed the data analysis, drafted the first version of the manuscript and wrote the final version. AA, AT, GT, AKK, CB, CK, ATB, MDS, IC, SL, PM, MP, MSS and ED revised the manuscript. All authors approved the final version. AW edited the manuscript.

§ Contactebogui Study Group

Abdoulaye Touré (Institut National de la Santé Publique, Conakry, Guinea) ; Ibrahima Balde, Alseny Balde, Amara Bamba, Thierno Alimou Barry, Ibrahima Camara, Amadou Camara, Aboubacar Mamy Conte, Diaby Aboubacar, Amadou Bailo Diallo, Mamadou Saliou Diallo, Saran Doumbouya, Emile Souro Kamano, Joel Balle Koivogui, Cece Kpamou, Jean Louis Monemou, Moriba Povogui, Maou Sakouvogui, Mariama Dioulde Sall, Abdoul Karim Soumah, Aboubacar Hawa Sylla (Centre de recherche et de formation en Infectiologie de Guinée, Conakry, Guinée) ; Ahidjo Ayouba, Christelle Butel, Eric Delaporte, Jean-François Etard, Alpha Kabinet Keita, Charlotte Laniece-Delaunay, Sandrine Leroy, Philippe Msellati, Martine Peeters, Bernard Taverne, Guillaume Thaurignac (TransVIHMI, IRD, INSERM, Université de Montpellier, Montpellier, France) ; Mamadou Saliou Sow (Donka National Hospital, Conakry, Guinea) ; René Ecochard, Muriel Rabilloud, Fabien Subtil (Hospices Civils de Lyon, Service de Biostatistique-Bioinformatique, Lyon, France) ; Yves Levy (INSERM, Paris, France) ; Jean-François Delfraissy, Yazdan Yazdanpanah (Reacting, INSERM, Paris, France);

Declaration of interests

We declare that we have no conflicts of interest.

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	Asymptomatic (n=1174)	Paucisymptomatic (n=216)	All (n=1390)
Demographic information			
Age of contact person median (min–max)	26 (7–88)	27 (7–69)	26 (7–88)
Sex of contact person (male)	589 (50·17%)	93 (43·05%)	682 (49%)
Exposure Information			
At least one exposure to a lethal case of EVD	280 (23·85%)	99 (45·83%)	379 (27·27%)
Number of exposure to EVD cases, median (min-max)	2 (1–17)	2 (1–17)	2 (1–17)
Participation in a burial rituals ^a	154 (13·12%)	44 (20·37%)	198 (14·24%)
Proximity with the EVD case			
In same household	615 (52·38%)	72 (33·33%)	687 (49·42%)
In same room	559 (47·61%)	144 (66·66%)	703 (50·58%)
Contact with body fluids from the EVD case			
Blood or vomit	548 (46·68%)	137 (63·43%)	685 (49·28%)
Other fluids or no ^b	626 (53·32%)	79 (36·57%)	705 (50·71%)
Provide care to EVD case ^c	659 (56·13%)	161 (74·54%)	820 (58·99%)
Most reported symptoms after exposure to EVD case			
Headache	–	174 (80·56%)	–
Fatigue	–	159 (73·61%)	–
Fever	–	157 (72·68%)	–
Vomiting	–	28 (12·96%)	–
Abdominal pain	–	19 (08·80%)	–
Diarrhoea	–	22 (10·18%)	–

Table 1: Socio-demographic and exposure information of 1390 contact persons with Ebola virus disease cases, Guinea, 2013-2016.

^a Burial rituals included washing, touching, dressing, kissing, and carrying the body of the deceased

^b Other fluids included urine, sweat, saliva, tears and feces

^c Providing care included helping to eat or drink, palpating, touching, and carrying the EVD case

	% seropositivity*	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age of contact person (per 10 years)		1.15 (0.96–1.35)	0.11	1.15 (0.95–1.36)	0.13
Sex of contact person					
Female	3.53% (25/708)	1		–	
Male	4.69% (32/682)	1.34 (0.79–2.31)	0.36	–	
Status of EVD case					
All alive	3.96% (40/1011)	1		–	
At least one exposure to a lethal case of EVD	4.49% (17/379)	1.14 (0.62–2.00)	0.65	–	
Number of exposure to EVD cases[†]		0.98 (0.81–1.11)	0.76	–	
Participation in a burial rituals					
No	3.44% (41/1192)	1		1	
Yes	8.08% (16/198)	2.47 (1.32–4.41)	0.003	2.30 (1.21–4.17)	0.008
Proximity with the EVD case					
In same household	2.91% (20/687)	1		1	
In same room	5.26% (37/703)	1.85 (1.07–3.28)	0.02	1.5 (0.82–2.80)	0.19
Contact with EVD case body fluids					
Other or no	2.70% (19/705)	1		1	
Blood or vomit	5.55% (38/685)	2.12 (1.23–3.79)	0.009	2.15 (1.23–3.91)	0.009
Provide care to EVD case					
No	2.81% (16/570)	1		1	
Yes	5.00% (41/820)	1.82 (1.03–3.37)	0.04	1.00 (0.51–2.02)	0.99
Paucisymptomatic					
No	3.32% (39/1174)	1		1	
Yes	8.33% (18/216)	2.65 (1.45–4.65)	0.001	2.16 (1.17–3.85)	0.01

Table 2: Risk factors associated with Ebolavirus seropositivity among 1390 contact persons, Guinea, 2013-2016.

* Using the definition of positivity with at least two reactive antigens. Thresholds are 501, 950, 580 MFI for GP, NP and VP40, respectively.

[†] Effect for one supplementary exposure

	% seropositivity*	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age of contact case (per 10 years)		0.97 (0.75–1.21)	0.80	–	
Sex of contact case					
Female	2.74% (16/585)	1			
Male	3.90% (23/589)	1.45 (0.76–2.81)	0.26	–	
Status of EVD case					
All alive	3.36% (30/894)	1			
At least one exposure to a lethal case of EVD	3.21% (9/280)	0.96 (0.42–1.96)	0.90	–	
Number of exposures to EVD case[†]		0.96 (0.73–1.14)	0.73	–	
Participation in a burial rituals					
No	2.94% (30/1020)	1		1	
Yes	5.84 % (9/154)	2.05 (0.90–4.23)	0.06	2.30 (1.01–4.80)	0.03
Proximity with the EVD case					
In same household	2.28% (14/615)	1		1	
In same room	4.47% (25/559)	2.01 (1.05–4.01)	0.03	1.54 (0.76–3.20)	0.23
Contact with EVD case body fluids					
Other fluids or no	1.92% (12/626)	1		1	
Blood or vomit	4.93% (27/548)	2.65 (1.36–5.48)	0.005	2.37 (1.15–5.10)	0.02
Provided care to EVD case					
No	2.33% (12/515)	1		1	
Yes	4.10% (27/659)	1.79 (0.92–3.70)	0.09	1.10 (0.52–2.42)	0.82

Table 3: Risk factors associated with Ebolavirus seropositivity in the 1174 asymptomatic contact persons, Guinea, 2013-2016.

* Using the definition of positivity with at least two reactive antigens. Thresholds are 501, 950, 580 MFI for GP, NP and VP40, respectively.

[†]Effect for one supplementary exposure

	% seropositivity*	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age of contact case (per 10 years)		1.58 (1.16–2.17)	0.004	1.54 (1.12–2.12)	0.007
Sex of contact case					
Female	7.32% (9/123)	1		–	
Male	9.68% (9/93)	1.36 (0.51–3.62)	0.53	–	
Status of EVD case					
All alive	8.55% (10/117)	1		–	
At least one exposure to a lethal case of EVD case	8.08% (8/99)	0.94 (0.35–2.48)	0.90	–	
Number of exposures to EVD case†		0.91 (0.65–1.12)	0.47	–	
Participation in a burial rituals					
No	6.39% (11/172)	1		–	
Yes	15.91% (7/44)	2.77 (1.00–7.53)	0.04	2.40 (0.81–6.74)	0.09
Proximity with the EVD case					
In same household	8.33% (6/72)	1		–	
In same room	8.33% (12/144)	1.00 (0.37–2.98)	0.99	–	
Contact with EVD case body fluids					
Other fluids or no	8.86% (7/79)	1		–	
Blood or vomit	8.03% (11/137)	0.90 (0.34–2.54)	0.83	–	
Provide care to EVD case					
No	7.27% (4/55)	1		–	
Yes	8.70% (14/161)	1.21 (0.41–4.43)	0.74	–	

Table 4: Risk factors associated with Ebolavirus seropositivity in the 216 paucisymptomatic contact persons, Guinea, 2013-2016.

* Using the definition of positivity with at least two reactive antigens. Thresholds are 501, 950, 580 MFI for GP, NP and VP40, respectively.

† Effect for one supplementary exposure

Antigen	No positive	% positive
GP	133	9.57
NP	47	3.38
VP40	138	9.93
At least one Ag	241	17.34
GP-NP	26	1.87
GP-VP40	45	3.24
NP-VP40	26	1.87
At least two Ag	57	4.10
Three Ag: GP-NP-VP40	20	1.44

Table 5. Antibody profile following different combinations of the antibody response to recombinant GP, NP, and VP40 of Zaire Ebola virus among 1390 contact persons with Ebola virus disease cases, Guinea, 2013-2016.

Thresholds are 501, 950, 580 MFI for GP, NP and VP40, respectively.

		With Postebogui thresholds	By varying the thresholds
		Estimate (95% CI)	Mean (min-max)
Asymptomatic	No burial	3.06% (1.84-5.05)	2.93% (1.92-3.84)
	Burial	5.98% (2.81-8.18)	6.16% (2.85-7.37)
Paucisymptomatic	No burial	7.17% (3.94-9.09)	6.75% (5.11-8.26)
	Burial	17.16% (12.42-22.31)	17.01% (15.24-19.98)

Table 6: Estimate of frequency of Ebola virus infection among 1390 contact persons according to occurrence of symptoms and participation or non-participation in burial rituals, using a latent class model, Guinea, 2013-2016.