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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

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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.<sup>1</sup> Contact tracing and monitoring contact persons for 21 days were  
90 key measures to halt transmission.<sup>2</sup> 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.<sup>3</sup>

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%.<sup>4-9</sup>

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).<sup>10</sup>

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%).<sup>11,12</sup> 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.<sup>13</sup> 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;<sup>14</sup> 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.<sup>15</sup>  
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.<sup>14</sup>  
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.<sup>16</sup> 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.<sup>15</sup> 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.<sup>13</sup>

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.<sup>13</sup> 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.<sup>17,18</sup>

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).<sup>19</sup>

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.<sup>20</sup> 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.<sup>21,22</sup> 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.<sup>3,10</sup> Exposures to  
295 blood or vomit are other known risk factors for EVD transmission.<sup>12</sup> 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.<sup>21</sup>

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.<sup>23,24</sup> 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.<sup>25</sup> 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.<sup>26</sup> 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.<sup>10</sup> 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.<sup>27</sup>

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.<sup>28,29</sup>

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.<sup>30</sup>

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.

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## **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)
<b>Demographic information</b>			
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%)
<b>Exposure Information</b>			
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 <sup>a</sup>	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 <sup>b</sup>	626 (53·32%)	79 (36·57%)	705 (50·71%)
Provide care to EVD case <sup>c</sup>	659 (56·13%)	161 (74·54%)	820 (58·99%)
<b>Most reported symptoms after exposure to EVD case</b>			
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.**

<sup>a</sup> Burial rituals included washing, touching, dressing, kissing, and carrying the body of the deceased

<sup>b</sup> Other fluids included urine, sweat, saliva, tears and feces

<sup>c</sup> 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
<b>Age of contact person (per 10 years)</b>		1.15 (0.96–1.35)	0.11	1.15 (0.95–1.36)	0.13
<b>Sex of contact person</b>					
Female	3.53% (25/708)	1		–	
Male	4.69% (32/682)	1.34 (0.79–2.31)	0.36	–	
<b>Status of EVD case</b>					
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	–	
<b>Number of exposure to EVD cases<sup>†</sup></b>		0.98 (0.81–1.11)	0.76	–	
<b>Participation in a burial rituals</b>					
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
<b>Proximity with the EVD case</b>					
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
<b>Contact with EVD case body fluids</b>					
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
<b>Provide care to EVD case</b>					
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
<b>Paucisymptomatic</b>					
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.

<sup>†</sup> Effect for one supplementary exposure

	% seropositivity*	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
<b>Age of contact case (per 10 years)</b>		0.97 (0.75–1.21)	0.80	–	
<b>Sex of contact case</b>					
Female	2.74% (16/585)	1			
Male	3.90% (23/589)	1.45 (0.76–2.81)	0.26	–	
<b>Status of EVD case</b>					
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	–	
<b>Number of exposures to EVD case<sup>†</sup></b>		0.96 (0.73–1.14)	0.73	–	
<b>Participation in a burial rituals</b>					
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
<b>Proximity with the EVD case</b>					
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
<b>Contact with EVD case body fluids</b>					
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
<b>Provided care to EVD case</b>					
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.

<sup>†</sup>Effect for one supplementary exposure

	% seropositivity*	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
<b>Age of contact case (per 10 years)</b>		1.58 (1.16–2.17)	0.004	1.54 (1.12–2.12)	0.007
<b>Sex of contact case</b>					
Female	7.32% (9/123)	1		–	
Male	9.68% (9/93)	1.36 (0.51–3.62)	0.53	–	
<b>Status of EVD case</b>					
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	–	
<b>Number of exposures to EVD case†</b>		0.91 (0.65–1.12)	0.47	–	
<b>Participation in a burial rituals</b>					
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
<b>Proximity with the EVD case</b>					
In same household	8.33% (6/72)	1		–	
In same room	8.33% (12/144)	1.00 (0.37–2.98)	0.99	–	
<b>Contact with EVD case body fluids</b>					
Other fluids or no	8.86% (7/79)	1		–	
Blood or vomit	8.03% (11/137)	0.90 (0.34–2.54)	0.83	–	
<b>Provide care to EVD case</b>					
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

<b>Antigen</b>	<b>No positive</b>	<b>% positive</b>
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.

		<b>With Postebogui thresholds</b>	<b>By varying the thresholds</b>
		Estimate (95% CI)	Mean (min-max)
<b>Asymptomatic</b>	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)
<b>Paucisymptomatic</b>	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.