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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Summary

Background The prevalence of Ebola virus infection among people who have been in contact with patients with Ebola virus disease remains unclear, but is essential to understand the dynamics of transmission. This study aimed to identify risk factors for seropositivity and to estimate the prevalence of Ebola virus infection in unvaccinated contact persons.

Methods In this retrospective, cross-sectional observational study, we recruited individuals between May 12, 2016, and Sept 8, 2017, who had been in physical contact with a patient with Ebola virus disease, from four medical centres in Guinea (Conakry, Macenta, N'zérékoré, and Forécariah). Contact persons had to be 7 years or older and not diagnosed with Ebola virus disease. Participants were selected through the Postebogui survivors' cohort. We collected self-reported information on exposure and occurrence of symptoms after exposure using a questionnaire, and tested antibody response against glycoprotein, nucleoprotein, and 40-kDa viral protein of Zaire Ebola virus by taking a blood sample. The prevalence of Ebola virus infection was estimated with a latent class model.

Findings 1721 contact persons were interviewed and given blood tests, 331 of whom reported a history of vaccination so were excluded, resulting in a study population of 1390. Symptoms were reported by 216 (16%) contact persons. The median age of participants was 26 years (range 7–88) and 682 (49%) were male. Seropositivity was identified in 18 (8·33%, 95% CI 5·01–12·80) of 216 paucisymptomatic contact persons and 39 (3·32%, 5·01–12·80) of 1174 (2–4) asymptomatic individuals (p=0·0021). Seropositivity increased with participation in burial rituals (adjusted odds ratio [aOR] 2·30, 95% CI 1·21–4·17; p=0·0079) and exposure to blood or vomit (aOR 2·15, 1·23–3·91; p=0·0090). Frequency of Ebola virus infection varied from 3·06% (95% CI 1·84–5·05) in asymptomatic contact persons who did not participate in burial rituals to 5·98% (2·81–8·18) in those who did, and from 7·17% (3·94–9·09) in paucisymptomatic contact persons who did not participate in burial rituals to 17·16% (12·42–22·31) among those who did.

Interpretation This study provides a new assessment of the prevalence of Ebola virus infection among contact persons according to exposure, provides evidence for the occurrence of paucisymptomatic cases, and reinforces the importance of closely monitoring at-risk contact persons.

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Introduction

The 2013–16 Ebola virus disease outbreak in west Africa affected more people and was wider spread than all previous outbreaks combined, resulting in 28 616 confirmed, probable, and suspected cases in Guinea, Liberia, and Sierra Leone.[1] Contact tracing and monitoring contact persons for 21 days were key measures to halt transmission.[2] Although knowing the prevalence of asymptomatic or paucisymptomatic infections among contact persons is essential to understand the transmission dynamics of the disease and the clinical spectrum of Ebola virus disease and its implications, this prevalence remains unclear.[3]

The prevalence of seropositivity against Ebola virus has been evaluated in several studies during previous outbreaks in central and eastern Africa. These serological studies enrolled contact persons, or used samples from the general population or blood donors, and the estimates varied widely, between 1% and 46%.[4–9]

A study in Sierra Leone of 481 contact persons of individuals with Ebola virus disease collected data for exposure, symptoms after exposure, and results of a glycoprotein IgG capture assay on oral fluid.[10] Seropositivity was identified in ten (2·6%, 95% CI 1·2–4·7) of 389 asymptomatic household contact persons of survivors and in 11 (12·0%, 6·1–20·4)

Research in context

Evidence before this study

We searched PubMed, Web of Sciences, and Google Scholar for studies on the prevalence of Ebola virus infection from database inception up to May 30, 2018, without language restrictions. We used the term "Ebola*" plus any of the following terms: "asymptom*", "paucisymptom*", "symptom*", "antibod*", "infect*", "frequency", "prev*", "seroprevalence", "serosurvey", "seropositivity", and "contact". Since the discovery of Ebola virus in 1976, assessment of anti-Ebola antibodies has been done in different populations exposed to the virus: contact persons of individuals with Ebola virus disease, samples of the general population, and blood donors. The nature of the tests used and the thresholds of positivity varied from one study to another. Most of these studies did not distinguish asymptomatic from paucisymptomatic contact persons and did not take the level of exposure to individuals with Ebola virus disease into account. The frequencies of Ebola virus infection reported by these studies ranged from 1% to 46%.

Added value of this study

We showed that seropositivity and the estimated prevalence of Ebola virus infection were associated with the level of exposure and occurrence of symptoms. A relationship between the degree of exposure and occurrence of symptoms on one hand and prevalence of infection on the other was shown. This study used a highly specific serological procedure and maximised the use of the information yielded by the tests. The study also provided a baseline estimation of the prevalence of the infection among contact persons, which is useful in the design of vaccine trials.

Implications of all the available evidence

This study revealed a significant occurrence of asymptomatic or paucisymptomatic Ebola virus infections among contact persons and contributes to a better knowledgve of the clinical presentation of Ebola virus infection. The role of contact persons in the chain of transmission remains to be evaluated. The higher prevalence of Ebola virus infection in contact persons who participated in burial rituals emphasises the importance of safe and dignified burials during Ebola outbreaks and the need to systematically interview contact persons regarding participation in burial rituals.

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of 92 symptomatic household contact persons of survivors.[10]

Two meta-analyses, based on data from serosurveys done between 1976 and 2015, yielded very different estimates of the proportion of asymptomatic infection among contact persons (ie, 3·3% [95% CI 2·4–4·4][11] and 27·1% [15–40]).[12] These discrepancies might be explained by the heterogeneity of the assays used and of the study populations, the eventual cross-reactions with non-Ebola viruses, or the absence of a clear gold-standard assay or algorithm.

The present study aimed to identify the risk factors associated with seropositivity and to estimate the prevalence of Ebola virus infection in asymptomatic and paucisymptomatic contact persons by combining detailed information on exposure of contact persons and the occurrence of symptoms after exposure with quantitative data from a novel serological test. The test was initially validated on a large number of samples from survivors of Ebola virus disease.[13]

Methods

Study design and participants

This retrospective, cross-sectional observational study was done in Guinea, between May 12, 2016, and Sept 8, 2017. During this period, we enrolled contact individuals and collected information. Data collection started 2 weeks after the last Ebola virus disease case from the 2013–16 outbreak was reported in Guinea.

The study enrolled contact persons, defined as people who had contact with an Ebola virus disease survivor included in the Postebogui cohort study[14] or contact with another individual or individuals diagnosed with Ebola virus disease (alive or dead) not included in the Postebogui cohort but living in the same compound as a survivor included in the Postebogui cohort. According to the WHO definition, which was closely adhered to during the study, exposure includes sharing the same room or bed as, caring for, touching body fluids of, or closely participating in a burial of someone with Ebola virus disease.[15] Postebogui is a cohort study that enrolled 802 survivors of Ebola virus disease from four sites to study the long-term clinical, virological, and psychosocial consequences of Ebola virus disease in Guinea.[14] All contact persons enrolled in our study were aged at least 7 years and had not been diagnosed with Ebola virus disease during the Ebola outbreak. They were enrolled at the same four study sites as the Postebogui survivors (Donka National Hospital, Conakry; Macenta Prefectoral Hospital, Macenta; N'zérékoré Regional Hospital, N'zérékoré; and Forécariah Prefectoral Hospital, Forécariah), located in the main areas of the outbreak. The initial identification and localisation of the potential contact persons was done by the Postebogui team. When the contact persons arrived at the study site to provide consent and enrolment, the interviewers evaluated them to decide if the contact definition was met or not. Dates of disease onset in the Postebogui cohort survivors were taken from the medical files of the Ebola treatment centres. A questionnaire was given to the contact persons assessing age, sex, history of recombinant vesicular stomatitis virus–Zaire Ebola virus vaccination, information on each exposure to an individual with Ebola virus disease, and on

occurrence of symptoms after exposure, accessed via the Voozanoo platform (Epiconcept, Paris, France).[16] The list of symptoms was based on the symptoms of the Postebogui survivors and the WHO definition for a suspected case during an Ebola virus disease outbreak.[15] All contact persons who reported symptoms after exposure to an individual with Ebola virus disease were categorised as paucisymptomatic, and contact persons who did not report symptoms were categorised as asymptomatic.

For more on **Voozanoo** see http://www2.voozanoo.net

Contact persons were given the questionnaire and interviewed and tested to assess their immunological response to three recombinant proteins against Zaire Ebola virus: glycoprotein, nucleoprotein, and 40-kDa viral protein. Test validation was based on samples from survivors of Ebola virus disease.[13] Contact persons who reported a history of vaccination for Ebola were excluded from the analysis because the serological tests were done after vaccination (appendix).

See Online for appendix

Informed consent was obtained from all contact persons, or from their parents or legal guardians for participants who were younger than 18 years. Ethical approval was received from the National Ethics boards for Health Research Committee (Guinea) and the Ethical Evaluation Committee of Institut National de la Santé et de la Recherche Médicale (France).

Procedures

Blood samples from contact persons were collected in EDTA (edetic acid) tubes to prepare dried blood spots. For dried blood spot preparation, 50 µL of whole blood was spotted on each of the five circles of a 903 Whatman filter paper and dried at ambient temperature for 3 h. The remaining blood was aliquoted and stored as plasma at –20°C as a back-up. Dried blood spots were shipped to the virology laboratory of the Institut de Recherche pour le Développement in Montpellier, France, for serology. The detection of antibodies to recombinant glycoprotein, nucleoprotein, and 40-kDa viral protein of Zaire Ebola virus was done with a Luminex-based assay (Luminex Corp, Austin, TX, USA). Reported sensitivity and specificity are well above 90% for each antigen.[13]

To determine seropositivity, we used the thresholds defined previously using the Postebogui samples: 501 median fluorescence intensity (MFI) for glycoprotein (Kissoudougou strain), 950 MFI for nucleoprotein, and 580 MFI for 40-kDa viral protein.[13] On the basis of the serological responses, a contact person was considered seropositive when their sample was reactive—ie, above the threshold for at least two different antigens.

Potential predictive factors for seropositivity were sociodemographic characteristics, exposure intensity, and presence of symptoms. Factors quantifying exposure intensity in a contact individual included death of the individual with Ebola virus disease with whom they had contact, number of individuals with symptomatic Ebola virus disease that they had contact with, and type of physical contact with an individual with Ebola virus disease (ie, participation in burial rituals, proximity with Ebola virus disease case, direct contact with body fluids, and providing care).

Statistical analysis

No a priori sample size was calculated. We enrolled as many contact persons as possible from the Postebogui survivors. We used descriptive statistics to present sociodemographic characteristics and exposure factors in all contact persons. Categorical variables are described with absolute and relative frequencies, and continuous variables with median, IQR, and minimum and maximum values (range). We used Fisher's exact test to compare percentages. A set of logistic regressions were done to identify the risk factors associated with seropositivity and quantify their effect. The analysis was first done on all contact persons, then separately on asymptomatic and paucisymptomatic contact persons.

All factors with a p value less than or equal to 0·2 in univariate analyses were introduced in the multivariate models. We assessed goodness-of-fit of the models with the Hosmer–Lemeshow test. The effects of the factors were quantified with unadjusted odds ratios (ORs) for univariate models and adjusted OR (aORs) for multivariate models, with a 95% CI.

The risk factors identified were then used to constitute two subgroups of contact individuals with different seropositivity probabilities, divided further into contact individuals who were asymptomatic and those who were paucisymptomatic, yielding four subpopulations: no burial participation and asymptomatic; burial participation and asymptomatic; no burial participation and paucisymptomatic; and burial participation and paucisymptomatic. Probability density curves of the MFI for each antigen are presented according to these subpopulations. We also describe the distribution of the MFI values of each antigen in the whole study population (appendix) and in the asymptomatic and paucisymptomatic subpopulations according to exposure factors. We used the median and the tenth and 90th percentiles to compare the distributions of MFI values, particularly the distributions of the high values of MFI, reflecting exposure intensity.

None of the three antibody responses, done as a single test, is a perfect reference (ie, gold standard) to confirm Ebola virus infection status. Therefore, to estimate the prevalence of Ebola virus infection in the four subpopulations identified, we used a latent class model.[17,18] Briefly, the true Ebola virus infection status of contact persons was unknown and categorised as a latent class (infected or non-infected). A probabilistic likelihood function-based model was used to estimate the prevalence of Ebola virus infection in each subpopulation, under the assumptions that the tests are conditionally independent given Ebola virus infection status, and the sensitivity and specificity of each test do not vary across the

subpopulations (appendix).[19] We first estimated the prevalence of Ebola virus infection in the four subpopulations using the thresholds from the Postebogui study. Then, to evaluate the robustness of the estimates, we used several possible combinations of thresholds (between 300 and 1200 MFI) for the three antibody responses, resulting in 1000 estimates. There were no missing data. All analyses were done using R software, version 3.4.

Role of the funding source

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

Results

1721 contact persons were interviewed and tested between May 12, 2016, and Sept 8, 2017. 331 of these individuals reported a history of vaccination so were excluded from the analysis, resulting in a study population of 1390 contact persons (appendix). Among the 1390 contact persons, 216 (16%) reported at least one symptom and 1174 (84%) remained asymptomatic (appendix). The median age of contact persons was 26 years (range 7–88) and 682 (49%) were male (table 1). Overall, the contact persons reported 2467 exposures to individuals with Ebola virus disease, whose date of onset of symptoms spanned between March 23, 2014, and Oct 19, 2015 (1045 [75%] of 1390 dates available in medical records). The median time that had elapsed between onset of symptoms in the Postebogui Ebola virus disease survivor and interview of the contact person was 844 days (IQR 666–999) for only those contact persons who had contact with a Postebogui survivor. Half of the contact persons were exposed to fewer than two individuals with Ebola virus disease (range 1–17). The most commonly reported symptoms among the 216 paucisymptomatic contact persons were headache (174 [81%]), fatigue (159 [74%]), and fever (157 [73%]). Following the WHO definition for a suspected case during an Ebola virus disease outbreak, 30 (14%) of the 216 contact persons who were paucisymptomatic reported a fever plus at least three symptoms from the following list: headache, anorexia, vomiting, abdominal pain, diarrhoea, muscle or joint pain, and bleeding.

The proportion of individuals who reported being in contact with at least one Ebola virus disease fatality was higher in paucisymptomatic (45 [45%] of 99) than in asymptomatic (67 [24%] of 280) contact persons (p=0·0001). The proportion of individuals who reported being in contact with blood or vomit from an individual with Ebola virus disease was higher in paucisymptomatic (137 [63%] of 216) than in asymptomatic (548 [47%] of 1174) contact persons (p=0·0001). Additionally, the proportion of individuals who reported participation in a burial ritual was higher in paucisymptomatic contact persons (44 [20%] of 216) than in asymptomatic (154 [13%] of 1174) contact persons (p<0·0077).

	Asymptomatic (n=1174)	Paucisymptomatic (n=216)	All (n=1390)
Demographic information			
Age of contact person, years	26 (7–88)	27 (7–69)	26 (7–88)
Sex			
Male	589 (50%)	93 (43%)	682 (49%)
Female	585 (50%)	123 (57%)	708 (51%)
Exposure information			
At least one exposure to a lethal case of Ebola virus disease	280 (24%)	99 (46%)	379 (27%)
Number of exposures to individuals with Ebola virus disease	2 (1–17)	2 (1–17)	2 (1–17)
Participation in a burial ritual*	154 (13%)	44 (20%)	198 (14%)
Proximity with the individual with Ebola virus disease			
In same household	615 (52%)	72 (33%)	687 (49%)
In same room	559 (48%)	144 (67%)	703 (51%)
Contact with body fluids from the individual with Ebola virus disease			
Blood or vomit	548 (47%)	137 (63%)	685 (49%)
Other fluids or none†	626 (53%)	79 (37%)	705 (51%)
Provided care to individual with Ebola virus disease‡	659 (56%)	161 (75%)	820 (59%)
Most commonly reported symptoms after exposure to individual with Ebola virus disease			
Headache	..	174 (81%)	..
Fatigue	..	159 (74%)	..
Fever	..	157 (73%)	..
Vomiting	..	28 (13%)	..
Abdominal pain	..	19 (9%)	..
Diarrhoea	..	22 (10%)	..

Data are median (range) or number (%). *Burial rituals included washing, touching, dressing, kissing, and carrying the body of the deceased. †Other fluids included urine, sweat, saliva, tears, and faeces. ‡Providing care included helping to eat or drink, palpating, touching, and carrying the individual.

Table 1: **Sociodemographic and exposure information of 1390 contact persons of individuals with Ebola virus disease**

18 (8·33%, 95% CI 5·01–12·80) of 216 paucisymptomatic contact persons were seropositive versus 39 (3·32%) of 1174 asymptomatic contact persons (p=0·0021), with an overall seropositivity of 4·10% (3·12–5·28; 39 of 1390; table 2). The aOR for seropositivity of paucisymptomatic compared with asymptomatic contact persons was 2·16 (1·17–3·85; p=0·0106). Similarly, seropositivity was higher among contact persons who participated in burial rituals than in those who did not (table 2).

Among asymptomatic contact persons, univariate analysis identified three risk factors significantly associated with seropositivity: participation in burial rituals, contact with blood or vomit, and living in the same room as a person with Ebola virus disease (table 3). In multivariate analysis, only participation in burial rituals and contact with blood or vomit (2·37, 1·15–5·10; p=0·0217) were independently associated with seropositivity. Among paucisymptomatic contact persons, factors associated with seropositivity were age of contact persons and, to a small

	Seropositivity*	Unadjusted OR (95% CI; p value)	Adjusted OR (95% CI; p value)
Age of contact person (per 10 year increase)	..	1·15 (0·96–1·35; p=0·11)	1·15 (0·95–1·36; p=0·13)
Sex of contact person			
Female	25/708 (4%)	1 (ref)	..
Male	32/682 (5%)	1·34 (0·79–2·31; p=0·36)	..
Status of individual(s) with Ebola virus disease to whom the contact was exposed			
All alive	40/1011 (4%)	1 (ref)	..
At least one exposure to a lethal case of Ebola virus disease	17/379 (4%)	1·14 (0·62–2·00; p=0·65)	..
Number of exposures to individuals with Ebola virus disease†	..	0·98 (0·81–1·11; p=0·76)	..
Participation in a burial ritual			
No	41/1192 (3%)	1 (ref)	1 (ref)
Yes	16/198 (8%)	2·47 (1·32–4·41; p=0·0031)	2·30 (1·21–4·17; p=0·0079)
Proximity to individual with Ebola virus disease			
In same household	20/687 (3%)	1 (ref)	1 (ref)
In same room	37/703 (5%)	1·85 (1·07–3·28; p=0·029)	1·50 (0·82–2·80; p=0·19)
Contact with body fluids of individual with Ebola virus disease			
Other or no contact	19/705 (3%)	1 (ref)	1 (ref)
Blood or vomit	38/685 (6%)	2·12 (1·23–3·79; p=0·0086)	2·15 (1·23–3·91; p=0·0090)
Provided care to individual with Ebola virus disease			
No	16/570 (3%)	1 (ref)	1 (ref)
Yes	41/820 (5%)	1·82 (1·03–3·37; p=0·0454)	1·00 (0·51–2·02; p=0·99)
Paucisymptomatic			
No	39/1174 (3%)	1 (ref)	1 (ref)
Yes	18/216 (8%)	2·65 (1·45–4·65; p=0·0009)	2·16 (1·17–3·85; p=0·0106)

OR=odds ratio. *Using the definition of positivity with at least two reactive antigens. Thresholds are 501, 950, and 580 median fluorescence intensity for glycoprotein, nucleoprotein, and 40-kDa viral protein, respectively. †Effect for one supplementary exposure.

***Table 2*: Risk factors associated with Ebola virus seropositivity among 1390 contact persons**

degree, participation in burial rituals (table 4). In the subgroup of 30 contact persons meeting the suspected case definition for Ebola virus disease, seropositivity reached 20·00% (7·71–38·56) compared with 6·45% (3·38–10·99) in the 186 paucisymptomatic individuals not meeting the definition of a suspected case (p=0·024).

Details regarding different combinations of the antibodies against the three recombinant proteins are provided in table 5. For all the serological responses, the 90th percentile values were always higher in paucisymptomatic than in asymptomatic contact persons (appendix). In addition, the 90th percentile values for each antibody response were higher in the most exposed individuals, especially those who had participated in burial rituals, or who had been in contact with blood or vomit.

For all antigens, the distribution of the probability density curves of fluorescence intensity in the four subpopulations: (no burial participation and asymptomatic; burial participation and asymptomatic; no burial participation and paucisymptomatic; and burial participation and paucisymptomatic) was asymmetric, and marked by a small bump in the curve at higher values, indicating a mixture of distributions of MFI within the participants exposed to burials (appendix). We can see that this bump in the curve seems to be more marked in contact persons who participated in burial rituals than in those who did not participate.

The results of estimating the prevalence of Ebola virus infection with a latent class model are shown in table 6. In asymptomatic contact persons, the prevalence of infection among participants of burial rituals was significantly higher than that of individuals who had not participated in burial rituals. Similarly, among paucisymptomatic contact persons, the prevalence of Ebola virus infection in individuals who participated in burial rituals was higher than in individuals who did not participate (table 6). The results obtained by varying the combination of thresholds for the three antigens were similar to those obtained with the Postebogui thresholds (appendix).

Discussion

Our study shows that participation in burial rituals and contact with blood or vomit from individuals who had Ebola virus disease were associated with Ebola virus seropositivity. Although no baseline assessment of seropositivity was done before exposure to Ebola virus disease, the limited evidence for circulation of the Ebola virus before the outbreak and the high specificity of the test used provide strong support for seroconversion being due to the exposures.[20] These results are consistent with studies done after previous Ebola virus disease outbreaks. Exposure during burial rituals is a well known risk factor for transmission of Ebola virus.[21,22] We showed that, for all antigen tests, the bump in the curve for high values of MFI distribution was particularly marked among individuals who participated in burial rituals. This particular shape of a probability density curve is compatible with a mixture of two distributions, one centred on a low MFI value, less than the thresholds, corresponding to the most important proportion of the subpopulation, and one centred on a high MFI value, corresponding to individuals who seroconverted. These results underline the importance of safe and dignified burials in the context of an Ebola virus outbreak and the importance of closely monitoring individuals who participate in burial rituals. The seropositivity between asymptomatic and paucisymptomatic participants more than doubled (8·33% *vs* 3·32%). These figures are close to the observed seropositivity in the Sierra Leone study (12% [95% CI 6·1–20·4] in symptomatic cases *vs* 2·6% [1·2–4·8] in asymptomatic cases), delineating more clearly the prevalence of asymptomatic or subclinical Ebola virus infection.[3,10] Exposures to blood or vomit are other known risk factors for Ebola virus disease transmission.[12] In our study, these risk factors were

	Seropositivity*	Unadjusted OR (95% CI; p value)	Adjusted OR (95% CI; p value)
Age of contact person (per 10 year increase)	..	0·97 (0·75–1·21; p=0·80)	..
Sex of contact person			
Female	16/585 (3%)	1 (ref)	..
Male	23/589 (4%)	1·45 (0·76–2·81; p=0·26)	..
Status of individual(s) with Ebola virus disease to whom the contact was exposed			
All alive	30/894 (3%)	1 (ref)	..
At least one exposure to a lethal case of Ebola virus disease	9/280 (3%)	0·96 (0·42–1·96; p=0·90)	..
Number of exposures to individuals with Ebola virus disease†	..	0·96 (0·73–1·14; p=0·73)	..
Participation in a burial ritual			
No	30/1020 (3%)	1 (ref)	1 (ref)
Yes	9/154 (6%)	2·05 (0·90–4·23; p=0·066)	2·30 (1·01–4·80; p=0·0356)
Proximity to the individual with Ebola virus disease			
In same household	14/615 (2%)	1 (ref)	1 (ref)
In same room	25/559 (4%)	2·01 (1·05–4·01; p=0·039)	1·54 (0·76–3·20; p=0·23)
Contact with body fluids of individual with Ebola virus disease			
Other fluids or none	12/626 (2%)	1 (ref)	1 (ref)
Blood or vomit	27/548 (5%)	2·65 (1·36–5·48; p=0·0056)	2·37 (1·15–5·10; p=0·022)
Provided care to individual with Ebola virus disease			
No	12/515 (2%)	1 (ref)	1 (ref)
Yes	27/659 (4%)	1·79 (0·92–3·70; p=0·098)	1·10 (0·52–2·42; p=0·82)

OR=odds ratio. *Using the definition of positivity with at least two reactive antigens. Thresholds are 501, 950, and 580 median fluorescence intensity for glycoprotein, nucleoprotein, and 40-kDa viral protein, respectively. †Effect for one supplementary exposure.

Table 3: **Risk factors associated with Ebola virus seropositivity in the 1174 asymptomatic contact persons**

	Seropositivity*	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age of contact person (per 10 year increase)	..	1·58 (1·16–2·17)	0·0040	1·54 (1·12–2·12)	0·0072
Sex of contact person					
Female	9/123 (7%)	1 (ref)	..	..	..
Male	9/93 (10%)	1·36 (0·51–3·62)	0·53	..	..
Status of individual(s) with Ebola virus disease to whom the contact was exposed					
All alive	10/117 (9%)	1 (ref)	..	..	..
At least one exposure to a lethal case of Ebola virus disease	8/99 (8%)	0·94 (0·35–2·48)	0·90	..	..
Number of exposures to individual with Ebola virus disease†	..	0·91 (0·65–1·12)	0·47	..	..
Participation in a burial ritual					
No	11/172 (6%)	1 (ref)	..	..	..
Yes	7/44 (16%)	2·77 (1·00–7·53)	0·049	2·40 (0·81–6·74)	0·099
Proximity to the individual with Ebola virus disease					
In same household	6/72 (8%)	1 (ref)	..	..	..
In same room	12/144 (8%)	1·00 (0·37–2·98)	0·99	..	..
Contact with body fluids of individual with Ebola virus disease					
Other fluids or none	7/79 (9%)	1 (ref)	..	..	..
Blood or vomit	11/137 (8%)	0·90 (0·34–2·54)	0·83	..	..
Provided care to individual with Ebola virus disease					
No	4/55 (7%)	1 (ref)	..	..	..
Yes	14/161 (9%)	1·21 (0·41–4·43)	0·74	..	..

OR=odds ratio. *Using the definition of positivity with at least two reactive antigens, thresholds are 501, 950, and 580 median fluorescence intensity for glycoprotein, nucleoprotein, and 40-kDa viral protein, respectively. †Effect for one supplementary exposure.

Table 4: **Risk factors associated with Ebola virus seropositivity in the 216 paucisymptomatic contact persons**

	Positive (n=1390)
Glycoprotein	133 (10%)
Nucleoprotein	47 (3%)
40-kDa viral protein	138 (10%)
At least one antigen	241 (17%)
Glycoprotein and nucleoprotein	26 (2%)
Glycoprotein and 40-kDa viral protein	45 (3%)
Nucleoprotein and 40-kDa viral protein	26 (2%)
At least two antigens	57 (4%)
All three antigens	20 (1%)

Thresholds are 501 median fluorescence intensity (MFI) for glycoprotein, 950 MFI for nucleoprotein, and 580 MFI for 40-kDa viral protein.

Table 5: **Antibody profile following different combinations of the antibody response of Zaire Ebola virus among 1390 contact persons**

	With Postebogui thresholds*	With varying thresholds†
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)

*Data are estimate (95% CI). †Data are mean (range).

Table 6: **Estimate of prevalence 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**

statistically significant only in asymptomatic contact persons but, given the small number of paucisymptomatic contact persons, we probably lacked power to detect an effect in that subpopulation. The aOR associated with participation in burials are of the same order in the asymptomatic and paucisymptomatic groups (2·30 *vs* 2·40) but the small sample size of paucisymptomatic individuals (n=216) limited the power. As in a previous study,[21] sex did not appear to be a significant risk factor for seropositivity. There is no obvious difference in the age distribution between asymptomatic and paucisymptomatic contact persons (median 26 *vs* 27). The age effect, introduced as a continuous variable, is likely to be less diluted in the paucisymptomatic group compared with the asymptomatic one.

The latent class model, taking into account the results of the three antibody profiles in four subgroups of the population with different Ebola virus infection prevalences, allowed us to obtain robust and unbiased estimates of Ebola virus infection frequencies.[23,24] Ebola virus infection occurred in 3–17% of the contact persons, depending on the presence of symptoms among contact persons and exposure to burial rituals. This variation based on level of exposure reveals a dose–response relationship between exposure and seropositivity, providing additional confidence in the serological tests we used. The goodness-of-fit test showed that our latent class model fits and we can assume that the tests results are independent, conditional on the latent disease status.[25] An alternative that might take into account a dependence between the tests would be a conditional dependence structure with, for example, a Bayesian estimation method.[26] The gain in precision in estimating the prevalence of Ebola virus infection with the latent class modelling approach in comparison with the observed seropositivity is very large. For example, the latent class model for the paucisymptomatic and burial subgroup yields an Ebola virus infection prevalence of 17·16% (95% CI 12·42–22·31) or 17·01% (range 15·24–19·98), whereas the observed seropositivity (occurring in seven of 44 individuals) was 15·9% (95% CI 6·64–30·06; table 4).

From a clinical perspective, the symptoms reported by 16% of the contact persons, although unspecific, were all compatible with clinical manifestations of a mild Ebola virus infection. The prevalence of the symptoms, their nature, and the proportion of seropositivity within the asymptomatic and paucisymptomatic groups are in line with the finding of the study in Sierra Leone[10] of 19·1% for symptomatic contact persons. The Sierra Leone study's results, along with ours, contribute to a better knowledge of the clinical presentation of an Ebola virus infection, running from an asymptomatic infection, to a minimally symptomatic form, then an overt case, and finally a lethal form. The elevated seropositivity observed in the subgroup of contact persons meeting the criteria for suspected Ebola virus disease supports the argument that some cases were missed during the contact-tracing activities. Indeed, several reports from Guinea underlined weaknesses of the contact tracing with regard to the evaluation of contacts for suspected Ebola virus disease.[27]

Given the prevalence of asymptomatic and paucisymptomatic individuals revealed by our results, the reported case fatality rate (67% in Guinea),[1] based only on confirmed cases, overestimates the true overall case fatality rate of Ebola virus infection. What are the drivers of such variability in the clinical expression of the infection? The associations shown by our data between exposure to blood and vomit and participation in burial rituals, and the prevalence of symptoms on the one hand, and between these exposures and the level of seropositivity on the other hand, suggests that infectivity of the Ebola virus disease source and the viral load played a role in the occurrence of minimally symptomatic forms of Ebola virus infection. However, other factors such as genetic determinants of the host that restrict virus spread cannot be excluded. Our study did not aim to explore the mechanics of transmission chains, but a detailed contact study in Sierra Leone did not detect any transmission from seropositive contact persons.[28,29]

The main limitation of our study relates to the retrospective and declarative nature of the exposure data and symptoms, resulting in a probable recall bias.

Participants were interviewed long after their exposure to individuals with Ebola virus disease and their answers to specific questions on circumstances of a given exposure could have been inaccurate. Therefore, the paucisymptomatic group probably comprised symptomatic contact persons whose symptoms were either related or unrelated to Ebola virus disease, resulting in a mixture of the two populations. However, this measurement error would only have weakened the observed association, because serological status was not known by the interviewers or contact persons. Additionally, people in close contact with individuals with Ebola virus disease might have tended to report symptoms more frequently than those who were not. We controlled for this confounding effect by adjusting on proxy of exposure, and a strong statistical relation persisted; however, we cannot rule out that residual confounding could still be present. The study participants were sampled long after their exposure to individuals with Ebola virus disease; however, research supporting a long and persistent antibody response against glycoprotein, nucleoprotein, and 40-kDa viral protein antigens is reassuring.[30]

Our study covered only contact persons residing in the compounds of the Ebola virus disease survivors who were included in the Postebogui study, and thus might not be representative of all the contacts during the 2013–16 Ebola virus disease outbreak in Guinea. However, the Postebogui cohort included two-thirds of the Guinean survivors, and contact persons were recruited from four different places. This coverage suggests a reasonably good level of representation.

Our testing for Ebola virus antibodies targeting three antigens (glycoprotein, nucleoprotein, and 40-kDa viral protein) in contact persons showed that participation in burial rituals and exposure to body fluids of individuals who had Ebola virus disease were the most important risk factors associated with seropositivity, whether or not contact persons presented with symptoms. Contact persons who reported some symptoms after exposure were more likely to be seropositive than those who were asymptomatic. This finding again reinforces the importance of identifying and closely monitoring at-risk contact persons, such as those who have participated in burial rituals or who have been exposed to the blood or vomit of individuals with Ebola virus disease. Using a latent class model, we showed a significant occurrence of Ebola virus infection among contact persons, both asymptomatic or paucisymptomatic, which contributes to a better knowledge of the clinical spectrum of Ebola virus infection. In addition, some seropositive paucisymptomatic contact persons probably met the definition of a suspected Ebola virus disease case, not previously evaluated as such. Whether or not these subclinical presentations of minimally symptomatic infections confer some form of immunity against a subsequent exposure, contribute to herd immunity, or play a role in transmission, or whether the infected person harbours the virus in an immune-privileged site, still remain open questions. Contact tracers should be trained to search for and recognise minimally symptomatic Ebola virus infection and to take appropriate conservative measures because, given the current knowledge, a risk of transmission cannot be ruled out. Lastly, this study provides baseline estimations of the seroprevalence of the infection, which is useful information in the design of vaccine trials.

Contributors

SL, AT, J-FE, ED, PM, and MSS conceived and designed the study. AA, GT, CB, AKK, and MP designed the biological component of the study (serology) and did the serological tests. AA, AT, GT, AKK, CB, CK, TAB, MDS, IC, and J-FE contributed to the data collection and curation. MSKD, MR, RE, and J-FE did the data analysis, drafted the first version of the manuscript, and wrote the final version. AA, AT, GT, AKK, CB, CK, TAB, MDS, IC, SL, PM, MP, MSS, and ED revised the manuscript. All authors approved the final version.

Declaration of interests

We declare no competing interests.

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Data sharing

Data collected for the study that underlie the results reported in this Article, including deidentified participant data and a data dictionary, will be made available following publication for 5 years at http://dx.doi.org/10.17632/vzzrzj8g2s.1 for researchers who provide a methodologically sound proposal (to be directed to jean-francois.etard@ird.fr) with a signed data access agreement.

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