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Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus

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Introduction

It is not known how frequently asymptomatic Ebola virus infection occurs, yet it could affect the course of epidemics. High rates of asymptomatic infection would reduce incidence through herd immunity, radically altering model predictions of epidemic spread.1 If those with asymptomatic infection are infectious it could explain re-emergences of Ebola virus disease (EVD) without known contact.

Methods

We validated a new oral fluid anti-glycoprotein IgG capture assay among survivors from Kerry Town Ebola Treatment Centre and controls from communities unaffected by EVD in Sierra Leone. We then assessed the seroprevalence of antibodies to Ebola virus in a cross-sectional study of household contacts of the survivors. All household members were interviewed. Two reactive tests were required for a positive result, with a third test to resolve any discrepancies.

Findings

The assay had a specificity of 100% (95% CI 98.9–100; 339 of 339 controls tested negative) and sensitivity of 95.9% (89.8–98.9; 93 of 97 PCR-confirmed survivors tested positive). Of household contacts not diagnosed with EVD, 47.6% (229 of 481) had high level exposure (direct contact with a corpse, body fluids, or a case with diarrhoea, vomiting, or bleeding). Among the contacts, 12.0% (95% CI 6.1–20.4; 11 of 92) with symptoms at the time other household members had EVD, and 2.6% (1.2–4.7; 10 of 388) with no symptoms tested positive. Among asymptomatic contacts, seropositivity was weakly correlated with exposure level.

Interpretation

This highly specific and sensitive assay showed asymptomatic infection with Ebola virus was uncommon despite high exposure. The low prevalence suggests asymptomatic infection contributes little to herd immunity in Ebola, and even if infectious, would account for few transmissions.

Funding

Wellcome Trust ERAES Programme, Save the Children.
(people eating from the same pot), were sought for this study. Interviews were done between July 3, 2015, and Sept 10, 2015, encouraging household members to tell their story as a group, as described elsewhere. For each person in the household who was ill or died of EVD we asked who had helped them and had contact with them. We also asked about exposures outside the household. With additional probing questions, we established the maximum exposure level for each person, including those who had not been ill and those who had died, using predefined levels. The highest level was touching the body of someone who died of EVD, then direct contact with body fluids of a wet case (ie, an EVD case infected in Sierra Leone) and four negative controls (plasma from UK donors) were included in each plate. Two positive controls (plasma from a UK EVD survivor Bioservices, Rockville, MD, USA) as described elsewhere. Two positive controls (plasma from a UK EVD survivor infected in Sierra Leone) and four negative controls (plasma from UK donors) were included in each plate. The cutoff for a reactive result was defined per plate as the mean optical density (OD) of the negative controls plus a fixed OD measure (0.1). Since the mean negative OD varied between 0.049 and 0.067 per plate, this is equivalent to 2.5–3 times the mean negative OD. We present the first field validation of a new assay. It had very high specificity and sensitivity and has the added advantage of being non-invasive so was well accepted. Using this assay we showed that the prevalence of seropositivity to Ebola virus in asymptomatic household contacts, many of whom were highly exposed, was only 2.6%. Additionally, 12% of contacts with some symptoms but never diagnosed with Ebola virus disease were seropositive. In these Ebola-affected households, asymptomatic infections accounted for 2.3% and missed symptomatic infections for 2.6% of all Ebola virus infections.

**Research in context**

**Evidence before this study**

We did a systematic review of studies of seroprevalence of antibodies to Ebola virus. We searched PubMed and Web of Science using the search string "ebola AND (asymptom* OR antibody* OR IgG OR immu*n* OR ELISA OR serol*) NOT vacc* NOT immuniz* AND (Humans[Mesh])", as well as reference lists (including those of previous reviews) and conference reports from the west Africa epidemic. We last updated the search on July 31, 2016, and used no language restrictions.

Different assays have been used and the specificity of the tests is frequently questioned. Of 50 studies, only six reported results for asymptomatic household contacts, with varying prevalence estimates: 2.5% in the first known Ebola virus outbreak using an immunofluorescence assay; and 1.0% in Uganda, 4.0% in the Democratic Republic of Congo, 6.5% in Sierra Leone, and 21.4% and 45.9% in Gabon, using different ELISAs.

**Added value of this study**

We present the first field validation of a new assay. It had very high specificity and sensitivity and has the added advantage of being non-invasive so was well accepted. Using this assay we showed that the prevalence of seropositivity to Ebola virus in asymptomatic household contacts, many of whom were highly exposed, was only 2.6%. Additionally, 12% of contacts with some symptoms but never diagnosed with Ebola virus disease were seropositive. In these Ebola-affected households, asymptomatic infections accounted for 2.3% and missed symptomatic infections for 2.6% of all Ebola virus infections.

**Implications of all the available evidence**

Asymptomatic infection with Ebola virus occurs but given the low seroprevalence seen even in highly exposed individuals, it would not be a major contributor to herd immunity. The availability of a reliable non-invasive assay that is easy to administer and highly acceptable in the field will greatly aid future investigations and interventions, including testing and targeting of vaccines.
unreactive samples from survivors, and a selection of other samples including those closer to the cutoff, were repeated. Samples with discrepant results were retested.

Using this assay, results from paired oral fluid and plasma samples have previously been shown to correlate well in 76 participants in an early phase Ebola vaccine trial in the UK ($r=0.68$, $p<0.0001$, two-tailed non-parametric Spearman’s correlation);13 ten EVD survivors tested in Connaught Blood Bank, Sierra Leone ($r^2=0.83$, linear regression); and 80 EVD survivors from Sierra Leone tested in the UK ($r^2=0.78$, linear regression) (Tedder RS, unpublished). Using the same cutoff as in our study, 78 of 80 samples from the EVD survivors were positive on serum, of which 76 were positive on oral fluid, giving a sensitivity compared with serum of 97.4% (76 of 78 samples). The two samples negative on both oral fluid and plasma were also negative on competitive and double-antigen bridging ELISAs. Additionally, 44 paired oral fluid and plasma samples from individuals not exposed to Ebola from The Gambia were negative on the capture ELISA using the same protocol (Tedder RS, unpublished).

Figure 1: Flow chart of study participants

Households were defined as those who ate from the same pot. They included everyone who stayed there at the time Ebola was in the household, including those who were not normally resident. Of those not swabbed, most were absent; eight refused (all had been asymptomatic) and four had died since Ebola. Of the six excluded swabs, three were miscoded and three were not found. EVD = Ebola virus disease.
Statistical analysis
We assessed the sensitivity and specificity of the assay under field conditions using samples from PCR-confirmed Kerry Town EVD survivors and from the community controls.

For further analyses, individuals were defined as having been infected if their sample was reactive on two or more tests, uninfected if their sample was unreactive on one or more tests, and indeterminate if their sample had an equal number of reactive and unreactive tests. Two reactive tests were required to define infection to maximise specificity and hence positive predictive value, which is important because the prevalence of asymptomatic infection could be low. The CIs for the proportion positive were calculated using exact methods because of small numbers.

We assessed risk factors for infection among asymptomatic and symptomatic household members using χ² or Fisher’s exact test as appropriate. We assessed confounding by age using logistic regression; further multivariable analysis was limited by the small number of events. Linear regression was used to assess the association of level of reactivity in the samples from survivors with time since admission and with age.

Data sharing
The raw data for this study are available online, by request.

Role of the funding source
The sponsor of the study 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 in the study and had final responsibility for the decision to submit for publication.

Results
The households of 123 of 151 Kerry Town survivors were included in the study. One survivor had subsequently died but the household included another survivor so was visited. Of the remaining survivors, eight lived outside Western Area, three had died, 16 were unavailable or uncontactable, and one refused to take part (figure 1).

The participating Kerry Town survivors lived in 91 households with 814 other household members, of whom 242 had died (227 from EVD, 11 from probable EVD, and four from unknown causes [who were excluded from further analyses]) and 45 were survivors from other facilities (figure 1). Of the 527 other household members, 96 had some symptoms around the time others in their household had EVD and 431 were asymptomatic. We collected 639 oral swabs from 153 survivors and 486 living household members, of which 633 (99·6%) could be analysed; only eight people (1·2%) refused to give a swab (figure 1).

The mean age of the household members was 16·7 years (SD 14·2, range <1–84); 57% were female. The age and sex distribution of participating survivors and household members was similar to non-participants.

Oral swabs were collected from 663 community controls. Three people with possible Ebola virus exposure (two Ebola intervention workers and one funeral attendee) were excluded. Due to availability of test kits, we analysed the first 113 samples from each setting giving a total of 339 (mean age 19·0 years [SD 15·6, range <1–76], 53% female).

The distribution of normalised ODs (NODs) in the Kerry Town survivors and the community controls in the first test is shown in figure 2. From the Kerry Town survivors, 113 (97·4%) of 116 samples were reactive on the first test. 97 samples were retested: the three unreactive samples remained unreactive; one reactive sample was unreactive on retesting and on a third test (NODs 1·59, 0·97, and 0·69), and was considered negative; another reactive sample was unreactive on retesting and reactive on the third test (NODs 2·50, 0·76, and 1·01), and considered positive. All remaining initially reactive samples were...
Among 389 asymptomatic contacts, ten (2.6%) of 388 were negative, and one indeterminate (table 1, appendix p 5). After subsequent tests, 21 were considered positive, compared with 11 (12.0%) of 92 symptomatic contacts without diagnosed EVD. Among those with duplicate tests, NODs were in good agreement in the different participant groups (appendix pp 2–4). Overall, comparison of the NODs of the first and second test using linear regression, gave an $r^2$ of 0.88.

Among the survivors from other treatment centres (for whom we did not have documented evidence of positive Ebola virus PCRs) 31 (86.1%) of 36 samples were positive for Ebola IgG. 40 (8.3%) of 481 samples from household contacts without diagnosed EVD were reactive on the first test. After subsequent tests, 21 were considered positive, 18 negative, and one indeterminate (table 1, appendix p 5). Among 389 asymptomatic contacts, ten (2.6%) of 388 were seropositive, compared with 11 (12.0%) of 92 symptomatic contacts ($p=0.004$). The asymptomatic infections were from different households, whereas two people with symptomatic undiagnosed infections were from the same household.

Asymptomatic infection was only seen in those older than 12 years. By contrast, among symptomatic contacts, seropositivity was highest in children younger than 5 years (four [26.7%] of 15) and in adults 30 years or older (six [35.3%] of 17) but undetected in teenagers and young adults (aged 10–29 years; table 3).

Level of exposure to Ebola correlated with seropositivity among asymptomatic and symptomatic contacts (table 3). Of the 12 individuals with direct contact with an EVD corpse who were not diagnosed with EVD themselves, four (33.3%) were infected, two asymptotically. Among the 229 without known EVD with the three highest exposure levels (contact with corpse, body fluids, or wet cases), 16 (7%) were infected, seven asymptotically. There were few socioeconomic factors associated with positivity (table 3). Associations with occupation and being household head were explained by age. 23 contacts had spouses who were EVD survivors so could potentially have been infected by sexual transmission after recovery. Two of these contacts were seropositive; both were male and had been symptomatic.

Among symptomatic contacts, neither the number of symptoms nor any individual symptom in the case definition, were associated with seropositivity, except for a non-significant correlation with red eyes ($p=0.07$; data not shown). The 11 seropositive undiagnosed symptomatic individuals were: a 1-year-old child with multiple symptoms who was not tested or admitted because of a nurses’ strike; a 2-year-old child and a 9-year-old child with multiple symptoms who were not taken to a facility; three people with two symptoms (headache plus fatigue, loss of appetite or muscle or joint pain); and five people with single symptoms (abdominal pain, red eyes, hiccups, fever, or headache).

Overall, in these households there were 168 survivors and 238 EVD deaths reported at interview (figure 1), so...
|                                | Asymptomatic | Any symptoms |
|--------------------------------|--------------|--------------|
|                                | Total (n)    | IgG positive | Total (n)    | IgG positive |
|                                | n            | % (95% CI)   | p            | n            | % (95% CI) | p    |
| Total                          | 389          | 10           | 2·6% (1·2–4·8) | 92           | 11          | 12·0% (8·1–20·4) |
| Sex                            |              |              |              |              |              |      |
| Male                           | 161          | 3            | 1·9% (0·0–5·3) | 46           | 6           | 13·0% (4·9–26·3) | 1·0 |
| Female                         | 228          | 7            | 3·1% (1·2–6·2) | 46           | 5           | 10·9% (3·6–23·6) |
| Age (years)                    |              |              |              |              |              |      |
| <2                             | 27           | 0            | 0·0% (0·0–12·8) | 3            | 1           | 33·3% (0·8–90·6) | 0·001 |
| 2–4                            | 43           | 0            | 0·0% (0·0–8·2) | 12           | 3           | 25·0% (5·5–57·2) |
| 5–9                            | 76           | 0            | 0·0% (0·0–4·7) | 18           | 1           | 5·6% (0·1–27·3) |
| 10–14                          | 73           | 4            | 5·5% (1·5–13·4) | 14           | 0           | 0·0% (0·0–4·1) |
| 15–19                          | 52           | 1            | 1·9% (0·0–10·3) | 7            | 0           | 0·0% (0·0–4·1) |
| 20–29                          | 67           | 2            | 3·0% (0·4–10·4) | 21           | 0           | 0·0% (0·0–16·1) |
| 30–39                          | 25           | 2            | 8·0% (1·0–26·0) | 9            | 2           | 22·2% (2·8–60·1) |
| 40–49                          | 11           | 1            | 9·1% (0·2–41·3) | 5            | 2           | 40·0% (5·3–85·3) |
| ≥50                            | 14           | 0            | 0·0% (0·0–23·2) | 3            | 2           | 66·7% (9·4–99·2) |
| Maximum exposure               |              |              |              |              |              |      |
| Handled corpse                 | 10           | 2            | 20·0% (2·5–55·6) | 0·003         | 2           | 100·0% (15·8–100) | 0·06 |
| Handled fluids                 | 39           | 4            | 10·3% (2·9–24·2) | 41           | 6           | 16·6% (5·6–29·2) |
| Direct wet contact             | 120          | 1            | 0·8% (0·0–4·6) | 17           | 1           | 5·9% (0·1–27·8) |
| Direct dry contact             | 68           | 0            | 0·0% (0·0–5·3) | 13           | 1           | 7·7% (0·2–32·6) |
| Indirect wet contact           | 11           | 0            | 0·0% (0·0–2·9) | 2            | 0           | 0·0% (0·0–64·2) |
| Indirect dry contact           | 52           | 1            | 1·9% (0·0–10·3) | 11           | 0           | 0·0% (0·0–28·5) |
| Minimal or no contact          | 89           | 2            | 2·2% (0·3–7·9) | 6            | 1           | 16·7% (0·4–64·1) |
| Occupation                     |              |              |              |              |              |      |
| Unemployed or child            | 282          | 4            | 1·4% (0·4–3·6) | 62           | 6           | 9·7% (3·6–19·9) | 0·004 |
| Health-care worker             | 9            | 0            | 0·0% (0·0–33·6) | 1            | 0           | 0·0% (0·0–97·5) |
| Manual work                    | 85           | 4            | 4·7% (1·3–11·6) | 22           | 1           | 45·0% (0·1–22·8) |
| Non-manual work                | 10           | 1            | 10·0% (0·3–44·5) | 6            | 4           | 66·7% (22·3–95·7) |
| Status in household            |              |              |              |              |              |      |
| Head                           | 23           | 1            | 4·3% (0·1–22·0) | 0·46         | 14          | 35·7% (12·8–64·9) | 0·01 |
| Member                         | 366          | 9            | 2·5% (1·1–4·6) | 78           | 6           | 7·7% (2·9–16·0) |
| Number of people in household  |              |              |              |              |              |      |
| 1–5                            | 24           | 0            | 0·0% (0·0–14·3) | 0·63         | 7           | 14·3% (0·4–57·9) | 0·90 |
| 6–10                           | 126          | 5            | 4·0% (1·3–9·0) | 39           | 5           | 12·8% (4·3–27·4) |
| 11–15                          | 163          | 3            | 1·8% (0·8–5·3) | 24           | 2           | 8·3% (1·0–27·0) |
| >16                            | 76           | 2            | 2·6% (2·2–9·2) | 22           | 3           | 13·6% (2·9–34·9) |
| Water available in household   |              |              |              |              |              |      |
| Sometimes                      | 78           | 0            | 0·0% (0·0–4·6) | 0·31         | 7           | 0·0% (0·0–41·0) | 0·89 |
| Most days                      | 125          | 3            | 2·4% (0·5–6·9) | 27           | 3           | 11·3% (2·4–29·2) |
| Every day                      | 183          | 6            | 3·1% (1·2–7·0) | 58           | 8           | 13·8% (6·2–25·4) |
| Soap available in household    |              |              |              |              |              |      |
| Sometimes                      | 117          | 2            | 1·7% (0·2–6·0) | 0·91         | 18          | 2·2% (0·3–4·7) | 1·0 |
| Most days                      | 72           | 2            | 2·8% (0·3–9·7) | 23           | 3           | 13·0% (2·8–32·6) |
| Every day                      | 197          | 5            | 2·5% (0·8–5·8) | 51           | 6           | 11·8% (4·4–23·9) |
| Latrine for household          |              |              |              |              |              |      |
| Shared or none                 | 228          | 5            | 2·2% (0·7–5·0) | 1·0          | 7           | 12·7% (6·0–22·7) | 0·52 |
| Household’s own                | 158          | 4            | 2·5% (0·7–6·4) | 21           | 2           | 9·5% (1·2–30·4) |
| Crowding (people per room)     |              |              |              |              |              |      |
| High                           | 89           | 3            | 3·4% (0·7–9·5) | 0·68         | 25          | 16·0% (4·5–30·1) | 0·16 |
| Medium                         | 253          | 5            | 2·0% (0·6–4·6) | 60           | 5           | 8·3% (2·8–18·4) |
| Low                            | 44           | 1            | 2·3% (0·1–12·0) | 7            | 2           | 28·6% (3·7–71·0) |

(Table 3 continues on next page)
assuming seropositivity is a marker of Ebola virus infection, the ten asymptomatic and 11 symptomatic seropositive participants contributed 2·3% (ten of 427) and 2·6% (11 of 427) to Ebola virus infections, respectively. The contribution by age and exposure level is shown in figure 3 and appendix (p 9). In all age groups the proportion of infections that were asymptomatic was low, but it was higher in those aged 5–14 years (four [6·3%] of 64) than in those younger than 5 years (none [0%] of 53) and people aged 15 years or older (six [2·0%] of 307; p=0·07). The proportion of undiagnosed symptomatic infections was higher in those younger than 5 years (four [7·5%] of 53) than in those aged 5–14 years (one [1·6%] of 64) and those aged 15 years or older (six [2·0%] of 307; p=0·07).

Among those with positive tests, the NOD was similar in survivors and in those with asymptomatic (p=0·9) or missed symptomatic infections (p=0·7) in Wilcoxon rank-sum tests (appendix p 6).

Among survivors, no relation was seen between the magnitude of the NOD and the length of time since admission (appendix p 7) but the NOD was higher at younger ages (r2=0·08, p<0·001; appendix p 8).

**Discussion**

The oral fluid IgG capture ELISA performed well in this field setting. The oral swabs were accepted by the population (only 1% refused) and were suitable for children and adults. The swabs required no processing before storage at –20°C, making them easy to use in field conditions. We optimised specificity by using a high cutoff (figure 2) and requiring two reactive results to confirm a positive; sensitivity remained high (95–96%).

Using this assay, 2·6% (ten of 388) of asymptomatic members of Ebola-affected households had evidence of Ebola virus infection. This result is lower than some household contact studies, but few such studies restricted examination to asymptomatic contacts, different assays were used, and the definition of contact varied. Excluding any symptomatic individuals, previous estimates were 2·5% (ten of 404) in Yambuku, Democratic Republic of the Congo, using an immunofluorescence assay;26 4·0% (four of 101) in Kikwit;27 21·4% (12 of 56) in Gabon;28 45·9% (11 of 24) among highly exposed contacts in Gabon;4 1·0% (two of 210) in Uganda;1 and 6·5% (12 of 185) in Kono, Sierra Leone,10 using different ELISAs. A preliminary report from Liberia studied 760 household members or sexual contacts; 13% were positive but it is not clear if all were asymptomatic or to which contact group they belonged.29

The higher proportion of asymptomatic infection in adolescents, and the higher reactivity levels in younger survivors are consistent with a lower risk of severe disease. Immunological differences between symptomatic and asymptotically infected individuals, and between adults and children, have been noted previously.22,23 The slight excess of missed symptomatic infections in children younger than 5 years is consistent with underdiagnosis in this group.21 There was no evidence that any of the seropositive results were due to late transmission via semen:25 only two spouses of EVD survivors were seropositive and both were male.

WHO guidelines for EVD survivor care25 suggest that a positive IgG test could help define survivors if certificates (issued on discharge from a treatment centre) are missing, so a highly specific test is essential. An acceptable, sensitive, and specific assay would also assist vaccine studies, where knowledge of pre-existing immunity is important, and in identifying previously undiagnosed EVD cases who might have played a crucial part in transmission.

Testing the sensitivity assumed the Kerry Town survivors were correctly diagnosed. All four seronegative Kerry Town survivors were documented PCR-positive before admission; after admission, two (including the one with reactivity near the cutoff) had high-level PCR results, one had two low-level PCR results, and for the

| Asymptomatic | Any symptoms |
|--------------|--------------|
| Total (n)    | Total (n)    |
|             | n  | % (95% CI) |   | n  | % (95% CI) |
| Spouse Ebola survivor |     |            |   |     |            |
| No           | 372 | 10 | 2·7% (1·3–4·0) |   | 86 | 9 | 10·5% (4·9–18·9) |
| Yes          | 17  | 0  | 0·0% (0·0–19·5) |   | 6  | 2 | 33·3% (4·3–77·7) |
| Household quarantined |     |            |   |     |            |
| No           | 62  | 1  | 1·6% (0·0–8·7) |   | 9  | 1 | 11·1% (0·3–48·2) |
| Yes          | 302 | 7  | 2·3% (0·9–4·7) |   | 71 | 10 | 14·1% (7·0–24·4) |
| Unknown      | 25  | 2  | 8·0% (1·0–26·0) |   | 12 | 0 | 0·0% (0·0–26·5) |

*p values from Fisher’s exact test for heterogeneity are presented for all variables. p values from a non-parametric test for trend across ordered groups (an extension of the Wilcoxon rank-sum test) are presented where the proportions suggest a trend. In this table, the one sample from an asymptomatic individual with an indeterminate result was taken as negative. Age was missing for one person, occupation for four, household characteristics (water, soap, latrine and crowding) for three, and quarantine for 37.

Table 3: Prevalence of Ebola IgG positivity in asymptomatic and symptomatic household members of Ebola virus disease survivors, Sierra Leone, 2015, by individual and household characteristics
one with the lowest reactivity (appendix p 5), who was aged in her 80s, we have no post-admission record of positive PCR results. Oral fluid containing insufficient IgG will fail to signal; this can only be checked by determining IgG concentrations, which was not available in this setting. We did not have paired serum samples from these individuals, though good correlation with oral fluid results has been shown previously.

The oral fluid samples were collected up to 10 months after exposure. A reduction in IgG concentrations is possible, though antibody persistence for several years has been noted previously, and we found no evidence of a reduction (appendix p 7). It is theoretically possible that low level infections might have led to low concentrations of IgG that were not detected, which would underestimate the proportion of asymptomatic infections. However, in our study, above the cutoff, the NOD was similar in those with asymptomatic infection and in survivors (appendix p 6). We did not have enough test kits to re-test all those with initially unreactive results, but all 119 tested in duplicate remained unreactive.

Because our initial contact was through the community re-integration team, we only investigated survivor households. Survivors might be less infectious than those who die, but 70% of households in the study had at least one EVD death and exposure levels were high: 47.6% (229 of 481) of household contacts without diagnosed EVD reported contact with corpses, body fluids, or wet cases, yet only 7.0% (16 of 229) of these were infected.

Accurate recall of symptoms is difficult. Forgetting or reluctance to admit previously unreported symptoms might overestimate the incidence of asymptomatic infection. Conversely, being in an EVD-affecte household might have led to over-reporting of symptoms. During interviews family members would contribute details of the exposure and health of others, probably increasing recall accuracy.

In conclusion, we have used a non-invasive assay to show that asymptomatic Ebola virus infection occurs, but accounted for only a small proportion of infections, so would have little effect on herd immunity. It is unknown whether those with asymptomatic Ebola virus infection are infectious, or could harbour virus in the long term, like some survivors. In that respect, the low proportion of asymptomatic infections is reassuring because these transmissions would be challenging to prevent. We also identified missed symptomatic cases, some of which were mild. Many questions remain, including why some people escape infection or disease despite high exposure, and whether those asymptotically infected will have any immunity in future outbreaks.

**Contributors**

JRG, HB, and FC designed the study with contributions from all other authors. RST and DS developed the assay, with SD, MGS, and [TS, HB and SJ led the fieldwork with MSB, AJK, OK, SHM, DS, and CT. CFH and CM led the laboratory work, with REGW and VC. HB and JRG did the analysis. JRG and HB led the writing with contributions from all other authors. All authors approved the final manuscript.

**Declaration of interests**

Save the Children International operated the Kerry Town Ebola Treatment Centre during the period under study, and employed SJ, MSB, AJK, OK, SHM, DS, and CT. FC was employed by Save the Children UK and was involved in commissioning the study and interpreting the findings. The authors declare no other competing interests.

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