EBOLA VIRUS TRANSMISSION AND DISEASE SEVERITY IN SIERRA LEONE 2013-2016

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Declaration

I, Hilary Louise Bower, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

BACKGROUND

The 2013-2016 Ebolavirus Disease (EVD) epidemic in West Africa emphasised critical gaps in the knowledge of Ebolavirus, including the age distribution, true burden, transmission dynamics and risk factors in households. Against this background this research, undertaken during the epidemic, worked with a large cohort of Sierra Leonean survivors and their households to ask:

1. What is the true age distribution of Ebola virus infection and case/infection fatality?
2. To what extent do asymptomatic and unrecognised 'mild' Ebola virus infections exist?
3. What impact do age, type of exposure and other factors have on risk of contracting and dying from Ebola infection?
4. What characterises transmission in households and what is the extent of household secondary transmission?

METHODS

The study population were survivors discharged from Kerrytown Ebola Treatment Centre, Sierra Leone between November 2014 and March 2015 and their households. Semi-structured household interviews and a novel exposure hierarchy were used to obtain detailed information on individual and household-level risk factors, exposures, transmission and outcomes, and uncover unnotified cases and deaths. A new IgG immunoassay using non-invasive oral fluid samples was used to detect unreported infection: sensitivity and specificity of the novel assay was established using PCR-confirmed survivor samples and community controls. Risk of EVD was calculated by age, sex and exposure level. adjusted for confounding and clustering, and relative risks estimated using logistic regression. Likely transmissions chains were constructed from the detailed exposure narratives and household secondary attack rates were estimated: negative binomial regression was used to assess the determinants of intrahousehold spread. All survivors were followed-up 6-13 months after discharge and verbal autopsy of subsequent deaths conducted to assess the frequency of fatal recrudescence and 'late' deaths related to EVD.

RESULTS

The study involved 933 people, including 168 survivors and 238 deaths, from 94 households, and 339 community controls.

Risk of ebolavirus infection was strongly correlated with exposure and with age, with the lowest risk in children aged 5 to 19, even after adjustment for exposure. There was no consistent trend between case fatality rate and increasing exposure. Based on complete follow-up of all survivors over a mean

10 months post-discharge, the first estimates of frequency of viral recrudescence (0.7%) and of 'late' deaths (those associated with EVD occurring after discharge, 2.6%) were established.

The oral fluid assay was found to be highly sensitive (95.9%) and specific (100%), well-accepted by participants. It showed that 2.6% of asymptomatic household members had been infected, as were 12.0% of household members who recalled experiencing any symptoms but who were not investigated or diagnosed.

The estimated household attack rate was 28% and the household secondary attack rate 18%, giving a reproductive number (R) of 1.2. Larger household size, cases aged over 45 years, and cases with more severe disease were important drivers of household transmission.

CONCLUSION

The extent of undiagnosed symptomatic (and potentially infectious) cases represent a substantial risk to epidemic control and indicate the needed to improve diagnosis. The relatively low household secondary attack rate and R, plus the finding that only one third of cases caused onward transmission suggest it should be possible to curtail spread faster with interventions which empower households to prevent transmission Immunological research is needed to identify what protects young children and teenagers from infection – information which may assist development of vaccine and therapeutics.

Acknowledgements

The studies described here were carried out in collaboration with the Ministry of Health & Sanitation of Sierra Leone, staff from the Kerrytown Ebola Treatment Centre, a large cohort of Ebola survivors and their households in Freetown and the Western Area, and a cast of essential colleagues including the Sierra Leone study team of Sembia Johnson, Mohammad Bangura, Cecilia Turay, Osman Kamara, Saidu Mansaray and Alie Kamara then of Save the Children International and now working in different health-related capacities in Sierra Leone; Dr Catherine Houlihan, Dr Elizabeth Smout, Professor Francesco Checchi, and Professor Tim Clayton from the London School of Hygiene & Tropical Medicine; and the laboratory experts Professor Richard Tedder, Steve Dicks, Dhan Samuel (sadly deceased) then of Public Health England, and Raoul Guetiya Wadoum of the University of Makeni Infectious Disease Research Laboratory.

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Abbreviations

AR	Attack Rate
CFR	Case Fatality Rate
CI	Confidence Intervals
DRC	Democratic Republic of Congo
ETC	Ebola Treatment Centre
EV	Ebolavirus
EBOV	Ebolavirus Zaire strain
EVD	Ebolavirus disease
GP	Glycoprotein
IgG	Immunoglobulin
LSHTM	London School of Hygiene & Tropical Medicine
NP	Nucleoprotein
OD	Optical density
R	Reproduction number
SAR	Secondary Attack Rate
SUDV	Ebola Virus Sudan strain
VP40	An ebolavirus protein
WHO	World Health Organisation

SECTION 1: ANALYTICAL COMMENTARY

1. PREAMBLE

The field work for this PhD by Prior Publication took place from June to August 2015 in Sierra Leone during the 2013-2016 Ebola epidemic. The project, initiated by LSHTM Professor Judith Glynn as a component of an extensive longitudinal research project into Ebola Virus Disease (EVD) sequelae, aimed to address key gaps in Ebola epidemiology with a large rigorous and timely community-level study.

Professor Glynn, who was my MSc Epidemiology supervisor, had noted early in the epidemic anomalies in age-specific EVD incidence and case fatality reporting, raising her concerns in the *Lancet* in December 2014.[1] At that time, I was working as a field epidemiologist with the NGO Médecins Sans Frontières in Bo, Sierra Leone, seeing at first hand the gaps in surveillance and response, and becoming aware of cases that didn't seem to fit the accepted spectrum of Ebola virus disease, including one, an apparently asymptomatic pregnant woman who suffered the stillbirth of highly viraemic child, which led to my first EVD publication.[2]

These experiences sparked discussions between myself and Professor Glynn, particularly about how to better understand the age distribution and spectrum of Ebola infection, and how this might impact understanding of Ebola transmission and risk, and in June 2015 I joined Professor Glynn as co-principal investigator and field research lead to develop, implement and analyse the study described here.

A PhD thesis is intended to demonstrate the intellectual and research capacity of an individual, and while the outline of the study was in place when I joined, my expertise in leading the detailed development and implementation of the protocol, particularly the quantitative-qualitative and virtual autopsy methods, and the team training and management, contributed substantially to the success of the field work. Intellectually I worked to elaborate, extend and refine how we approached the research questions and methods, and I performed >90% of the study analysis, drafting and finalising all papers bar one. I also instigated and carried out the systematic review and meta-analysis of Ebola serology.

However, I want to highlight that the work was collaborative from the outset. I was challenged and advised by Professor Glynn throughout; laboratory colleagues such as Professor Richard Tedder and Dr Catherine Houlihan were essential to my understanding and interpretation of the serology; and I lent substantially on the knowledge of Sierra Leonean colleagues to improve and implement the study activities. As a result, the reader will see often in this commentary reference to 'we' rather than I. I firmly believe public health research is a collaborative act, and that understanding, ideas and solutions to problems stem from interaction of expertise and knowledge. I hope that my acknowledgement that this PhD project was not developed and performed solely by me will not be read as absence of contribution on my part, but rather of honestly co-produced work.

2. INTRODUCTION

Since its recognition in 1976 in Yambuku, Zaire (now Democratic Republic of Congo DRC),[3] a small cadre of specialist doctors, virologists and researchers have worked to build knowledge of Ebolavirus, to identify the zoonotic host(s), describe routes of transmission, and explore the mechanisms by which the virus both triggers and damages the immune system. But extensive knowledge of the disease had been hard to capture from the small, relatively rapidly contained outbreaks often occurring in hard-to-reach places, and in 2013 many key epidemiological and clinical characteristics remained obscure. The unprecedented size of the 2013-16 West Africa epidemic – 28,616 reported cases, 11,361 reported deaths – brought human, social and economic tragedy. But it also raised the possibility of investigating epidemiological questions that have dogged the planning and implementation of effective Ebola responses for years. This commentary describes a community-based research project, undertaken in Sierra Leone in the second year of the epidemic, to address a number of these outstanding questions, namely:

1. What is the true age distribution of Ebola virus infection and case/infection fatality?
2. To what extent do asymptomatic and unrecognised 'mild' Ebola virus infections exist?
3. What impact do age, type of exposure and other factors have on risk of contracting Ebola infection, and on the risk of dying from it?
4. What characterises transmission in households and what is the extent of secondary transmission there?

In the following pages, I will explain how different elements of the study were designed to address these questions, how the findings have contributed to the knowledge base of Ebolavirus epidemiology, and what they mean for prevention and control. I will examine the extent to which the methods used were successful in gaining good quality measurable information in the epidemic context, including the technical performance and contextual value of a novel assay using oral fluid sampling as a non-invasive alternative to phlebotomy in this sensitive and high-risk setting.

3. BACKGROUND

Ebola Virus Disease

Ebola virus disease is a severe, often fatal, zoonotic illness caused by infection with an RNA virus of the Filoviridae family, genus e*bolavirus (*EV*)*. Six distinct species have been identified of which five are associated with human infection: Zaire, Sudan, Bundibugyo, Taï Forest, and Reston. The majority of outbreaks (21 of 31), reported cases (33,790 of 34,754) and deaths (14,853 of 15,315) have been caused by Zaire ebolavirus (EBOV).[4] Recent genetic sequencing shows the EBOV that infected patients in West Africa had barely mutated, being 97% similar to the virus which first emerged in 1976.[5]

Historically EVD outbreaks have been triggered by a single case of zoonotic transmission, referred to as a spillover event, often linked to activities such as hunting and butchering, or unwitting exposure to infected animal body fluids. Fruit bats are the commonly-suspected reservoir though to date there is no definitive proof.[6] Once spillover has occurred, however, Ebola spreads through direct, non-aerosol, human-to-human contact with the infected tissues or body fluids of a person who is sick with or has died from Ebola, or through contaminated fomites and surfaces.

In addition to blood, EV has been detected in semen, breast milk, sweat, saliva, urine, tears, cerebrospinal fluid, aqueous humour, amniotic fluid, skin swabs and stool of survivors.[7, 8] It has been documented in the semen of a small number of male survivors up to 700 days after discharge from an Ebola Treatment Centre (ETC) but extent of infectivity remains unclear.[9] [10] [11] Transmission after recovery appears rare but, as larger outbreaks create a greater pool of survivors, there is concern that outbreaks may be triggered more frequently by transmission from persistently-infected survivors. Clusters epidemiologically linked to persistently-infected survivors of the West Africa and 2018-2020 DRC epidemics have been documented,[12, 13] and the 2021 outbreaks in Guinea and DRC have both been genetically-linked to survivors infected respectively five and two years previously.[14, 15] Infection through sexual transmission has been strongly suspected in a small number of cases.[14, 16, 17] and two cases of suspected transmission through breastmilk have been reported.[18, 19]

EVD typically starts as a non-specific viral syndrome of rapid onset. Most common initial symptoms are high fever, fatigue, myalgia, abdominal pain and rash – the so-called 'dry' phase of Ebola – followed in some cases by 'wet' symptoms including vomiting and diarrhoea often with heavy fluid loss and dehydration which are important factors in outcome.[7, 20] Although previously described as 'Ebola haemorrhagic fever' due to the frequency of bleeding observed during early outbreaks, in the West Africa epidemic, haemorrhage was seen in under half of admitted cases[21, 22] and a wider spectrum of disease became evident.

Treatment

Until recently, treatment for Ebola was limited to supportive care with even symptomatic treatment constrained by isolation measures. However there is increasing optimism that mortality-reducing treatments will be found: the first well-controlled multi-drug randomised trial of Ebola therapies was carried out during the 2018-20 epidemic in DRC, finding two monoclonal antibody treatments significantly increased chance of survival.[23] [24] Two vaccines have been licensed which, to date, have been used as prophylaxis for health workers and for direct and indirect contacts in a ring vaccination strategy.[25-28] Duration of protection is not yet known either for vaccine or natural immunity. Recovery from EVD can be complicated by long-lasting clinical sequelae.[29]

Outbreak Response

The main objective of Ebola outbreak response is to provide care as early as possible to the infected and to remove cases from the community in order to break chains of transmission. Essential interventions include alert systems at community and facility level, active case search, daily contact tracing, support for quarantined households and survivors, infection prevention and control measures and personal protective equipment in health facilities, and Ebola Treatment Centres where patients can be isolated and appropriately cared for, all of which need to be underpinned by strong communication and community involvement.

Challenges to effective response include weak surveillance and diagnostic systems, deficits of trust between communities and authorities, and non-specific early symptoms which mean public health intervention may not start until more infectious severe cases or deaths start to be detected. The longer the delay in raising the alert and establishing control interventions, the higher the chance that the virus will spread.

The epidemic in Sierra Leone

Sierra Leone experienced the largest number of confirmed cases and deaths of the three countries in the 2013-2016 epidemic. Cases began in the remote district of Kailahun in May 2014, two months after Guinea had declared its first case in the bordering prefecture of Gueckedou. By 15 October 2014 all districts were affected, intense transmission was occurring in the capital and large towns, and health facilities were overwhelmed. By the time the country declared the epidemic over on 13 April 2016, 14,124 confirmed and probable cases and 3,956 deaths had been reported.[30] Given the extent of underreporting (estimated at 17%-300%[31-33]) both figures are likely underestimates. Nevertheless, the magnitude of the epidemic, its duration, and its penetration into the population raised the possibility of exploring long-outstanding questions that could only be addressed through community-level research.

4. KNOWLEDGE GAPS IN 2013

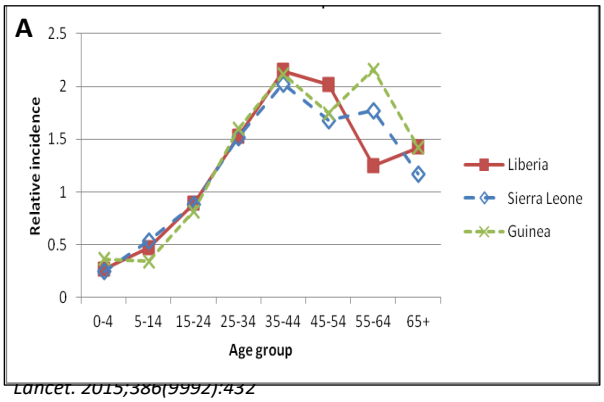
Prior to 2013 Ebola outbreaks were mostly small (median 52 cases, range 1 to 425) and occurred in relatively isolated rural settings in Africa. Only five outbreaks – in DRC and Uganda – had generated more than 100 cases. Reported case fatality rates (CFR) varied widely within and between species ranging from 47%-89% for EBOV and 36%-65% for Sudan ebolavirus (SUDV).[4] All epidemiological parameters were uncertain, including associations related to age, exposure and disease, and the variation in severity of disease. Much of what was known about risk of EV transmission came from anecdotal reports or hospitalised case series, both likely to miss non-severe infection.[34] Analytical studies had tended to focus on measuring the impact of certain high risk exposures. Few community-

level studies had been done and none of the size that could tease out risk-factor specific characteristics or examine biases such as under-reporting and severity of disease with any precision. The possibility of asymptomatic and 'mild' EV infection had been suspected since the first outbreaks, but evidence varied widely. In the following section I review what was known about Ebolavirus infection and disease in 2013 and highlight the gaps in knowledge that these studies sought to address.

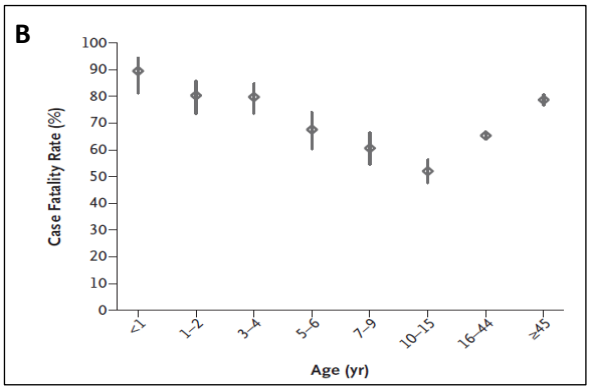
Age distribution of infection and disease

The unclear age distribution of EVD in West Africa was the starting point for the studies described here. Early WHO notification data from the West Africa epidemic showed incidence increasing almost linearly with age (Figure 1A).[1, 35] But case fatality from the same dataset showed a different pattern with the lowest CFRs in people aged 10-15 years old and the highest in young children and older adults,[36] suggesting that young children are particularly susceptible to Ebola (Figure 1B). Given the difficulty of reducing exposure of young children who need to be cared for and held, these two patterns seemed incongruous.

Figure 1: A. Relative age-specific incidence of Ebola Virus Disease 2013 to 2014 per 100,000 population, based on WHO notification data: all probable and confirmed cases. B. Case Fatality Rate based on notified deaths.



Lancet. 2015;386(9992):432



WHO Ebola Response Team. Ebola Virus Disease among Children in West Africa. New England Journal of Medicine. 2015;372(13):1274-7

Studies of earlier outbreaks had not observed this linear trend in incidence, rather they reported that children appeared to contract Ebola infection less and survive more frequently than other age groups.[37, 38] In one of the few studies to report detailed age groups, Breman *et al* investigating the first identified Ebola outbreak in Yambuku, DRC showed a clear 'sparing' of children aged 1-14 years (2 cases/1000 persons) compared to those aged under one (14/1000) and aged 15-49 years (11/1000), with incidence falling again after age 50 (7/1000).[3] As the main mode of transmission was contaminated syringes in the mission hospital, followed by contact with contaminated body fluids, the low incidence in children was tentatively attributed to low exposure, although the authors also noted that girls aged 5-14 had taken on the death rites duties normally performed by older women who were severely affected.

Some 20 years later, and despite classifying age only by under and over 18 years, Dowell *et al* again highlighted the sparing of children in the 1995 outbreak in Kikwit, DRC, noting that case-contacts over 18 had three times the risk of infection of those under 18.[37] Dowell argued that even an immunologically-naïve population could not explain the unusual age distribution of EV infection in Kikwit, and hypothesised that children must either be less exposed, or less susceptible to the pathogen, or experiencing a different, possibly subclinical, form of infection. Based on the Kikwit investigation, he concluded that lower exposure was the most likely explanation, since much of the elevated risk of infection in adults was associated with their more frequent exposure to ill family members and because there was, in his opinion, no serological evidence of sub-clinical Ebola illness.[*] But, he added, as adults still had elevated risk even after adjusting for increased exposure, there remained unexplained factor(s) and one of them could be that children were less susceptible.[39]

Other evidence of sparing of children followed: Mupere *et al* reported 5-15-year-olds were less represented than under-5s or 15–18-year-olds in a cohort of 90 children hospitalised for EVD in Gulu, Uganda in 2000. He speculated that younger children were more at risk due to prolonged contact with sick and dying parents with whom they were often admitted, and older children were more at risk due to caring for parents and relatives and attending burial ceremonies.[38] Borchert *et al,* retrospectively investigating an index case and 73 contacts from the Masindi, Uganda, outbreak in 2000, calculated a secondary attack rate of 53% among 15–49 year-old contacts compared to 7% among all other ages, attributing this to the tradition of keeping children away from ill family members.[40]

A systematic review of transmission risk factors in 2015, however, concluded that no previous study had been large enough, nor employed sufficiently detailed or consistent age categories to accurately differentiate or compare attack rates by age.[41]

Reviewing these findings in 2015, we considered one important possible explanation had been overlooked: missed cases. Official incidence and mortality data in the West Africa epidemic were known to be inaccurate.[31-33] Reporting occurred through health facilities, community alert systems or contact tracers, but all these were negatively affected by the population's fear of quarantine and stigma, by suspicion around the benefit of admission, and by the actual and perceived availability of healthcare. As the epidemic developed, many health facilities closed due to fear of transmission while the public shunned formal facilities, seeking care from more trusted, but no less risky, informal providers. In addition, early symptoms of Ebola are similar to other common childhood illnesses for which families may not usually seek care, increasing reluctance to risk taking a child to a health facility.

[*] In fact the Yambuku team identified 2.5% (95% CI 1.2-4.5) of asymptomatic contacts had serological evidence of EBOV infection. [77]

Outcome reporting was also impacted by the strain on systems: in Sierra Leone under 40% of confirmed cases had a recorded outcome, leading to inaccurate CFR calculation.[42] Missed cases and deaths are also problematic for accurate assessment of burden of disease, and for estimations of risk and exposure, as are missed asymptomatic or 'mild' infections. Data collection in previous smaller outbreaks might have been somewhat better, but cases and deaths are still likely to have been missed due to the focus on severe cases and hospitalisation.

This fundamental gap in understanding of Ebolavirus distribution led us to postulate that a large community-level study using methods that could uncover un-reported cases and deaths and unrecognised infections would have potential to overcome the biases and gain clarity on the true distribution of Ebola infection, disease and fatality. It would need to include measures of exposure in those who did not get Ebola as well as those who did and, if possible, capture exposure in such a way as allow it to be recorded as a single highest-level variable rather than a number of separate risk factors. This would improve adjustment by lessening small number effects and collinearity, as well as reducing the need to probe for details of every, possibly lower risk, exposure. The ability to adjust more precisely would be important in better understanding the relationship between age and exposure. Our sense was that in over-crowded households in a society where caring for elders even when young is a family duty, the possibility of children, even those small enough to need caring and holding, being less exposed enough to prevent infection seemed unlikely.

Exposure and risk of infection

A second area of uncertainty in 2015 related to the relative risks of exposures in relation to infection and disease. Associations between risk of infection and factors such as direct contact with body fluids, caring for individuals in later stages of infection especially those close to death, preparing deceased for burial, and nosocomial spread due to inadequate PPE had been identified by 2013.[41, 43] Ebola virus had been cultured from multiple body fluids[44-47] so it was considered possible that touching a person or materials or surfaces they had touched could result in transmission.[35] But this information was based on limited, often anecdotal, datasets from small outbreaks and hospitalised cases, and studies lacked detail to separate out fomite transmission from direct contact. A systematic review and meta-analysis by Brainard *et al* [41] of all published evidence on risk factors for community acquisition of EV infection between 1976 to 2014 found only 26 eligible studies, of which only eight could calculate risk ratios for developing EVD, and only two adjusted estimates for confounding. With the exception of the investigation of the first Ebola outbreak in Yambuku (318 cases, >1000 contacts[3]), most studies covered fewer than 45 cases and, where included at all, under 200 contacts and/or controls.

The review identified over 30 studied exposures but concluded it was not possible to specify with any degree of certainty how risky specific behaviours were. Risk factors were too inconsistently defined to

allow pooled analysis, were largely unadjusted, and/or used incomparable measures of association. Apparently similar exposures in different studies produced widely varying estimations of risk. For example, studies examining risk of touching a dead body yielded odds ratios ranging from 1.07 to 38.5.[48, 49] While one study carefully broke down the exposure of caring for a patient into 'early care (not death)', 'in home until death', and 'at hospital until death' and showed a significant positive trend,[35] most used a simple binary variable (caring versus not caring for a case)with ORs varying from 1 to 8.9. Only one study evaluated difference in risk between exposure to cases in wet or dry stages of EVD.[37] Assessment of age as a risk factor was confounded by different categorisations of age, e.g. >18, >30, >40, 41-60 years.

In 2013, therefore, understanding of how factors such as age, exposure intensity, and disease severity, interact and influence risk of infection was uncertain at best.

Household transmission

Few studies prior to 2013 had assessed Ebola transmission patterns within households nor the determinants for whether infection is spread or contained in a household. Two systematic reviews, which attempted to consolidate existing information on household risk factors and secondary attack rates, identified key problems in the studies including: small numbers (<400 household contacts across all studies); not distinguishing between primary and secondary cases; including non-household contacts; and estimating secondary attack rates (i.e. the proportion of all contacts infected by a primary case) rather than household secondary attack rates (i.e. the proportion of susceptible household members infected by a household primary case).[41, 50]

Brainard *et al*[41] concluded no past study allowed estimation of a household secondary attack rate. But Dean *et* al [50] took a different view. They considered that seven studies, including five that had been reviewed by Brainard *et al,* had sufficient data to permit calculation of a pooled household secondary attack rate (SAR), which they estimated to be 15.4% with significant heterogeneity due to variable definition of households and contacts. Dean also estimated a household SAR related to direct contact (22.9%, using data from five studies including one that they had previously excluded for unclear household definition) and to indirect case contact (0.8% using data from three of the five studies). Finally, they reported SAR among household members who provided care was almost 17 times higher than those who did not, after adjusting for direct contact.

While these reviews and studies testified to the efforts to quantify transmission within households, knowledge was constrained by insufficiently detailed and well-defined data and based only on notified cases and deaths, and there was little or no information on what might drive intrahousehold transmission beyond case-contact.

Spectrum of disease

In 2013, confirmation of the true spectrum of EV infection was an important knowledge gap, with the existence of asymptomatic and/or pauci-symptomatic EV infection still strongly contested by some. Various estimates had been reported, some from populations where no case of symptomatic Ebola had ever been seen, and critics highlighted poor specificity of assays and potential cross-reactivity.[51-53] To gain a clearer understanding, I carried out a systematic review of EV seroprevalence surveys from 1961 (samples taken 15 years before official identification of EV) to 2016, and confirmed that estimates of "asymptomatic" seropositivity varied from 1%-46%.[54] On closer scrutiny, however, I found only 13 of the 51 studies identified analysed asymptomatic contacts in groups with a clearly-defined risk of exposure. The others included people with potentially very different exposures, such as healthcare workers, non-household contacts, even sometimes symptomatic or pre-symptomatic individuals. In addition, studies used different thresholds for positivity and different assay formats. In the final analysis we found only eight study populations were comparable, and these produced a pooled estimate of seroprevalence among asymptomatic people with known case contact of 3.3%, far lower than many studies up to that time. The fragmented nature of the evidence made it clear that a fresh look at the possibility of subclinical EV infection was overdue if the contribution (or not) of this to the burden of disease was to be understood.

5. RESEARCH QUESTIONS

Against this background, we designed a study to address the following four research questions:

1. What is the true age distribution of Ebola virus infection and case/infection fatality?
2. To what extent do asymptomatic and unrecognised 'mild' Ebola virus infections exist?
3. What impact do age, type of exposure and other factors have on risk of contracting Ebola infection, and on the risk of dying from it?
4. What characterises transmission in households and what is the extent of household secondary transmission?

And, as we decided to use a novel immunoassay assay using oral fluid to identify past EBOV exposure, we would also assess the performance, feasibility and acceptability of this minimally invasive method of sampling.

In the following chapters, I will describe the methodologies and approaches used to address the study questions, summarise the study results (reported in full in the accompanying publications), review how these findings contributed to understanding of Ebolavirus infection, how they relate to and have informed subsequent research, and highlight where I believe they can inform efforts to improve response to Ebola outbreaks.

6. METHODOLOGY

To overcome the bias in diagnosis and notification described above, the study was designed to:

- Identify un-notified cases and deaths within a defined target population including any with unrecognised asymptomatic or pauci-symptomatic infection,
- Involve a population with known and measurable levels of exposure to infection, and create a method of assigning a single risk level for each individual rather than recording multiple discrete risk factors,
- Include a large enough cohort of cases and contacts to permit analysis of age-specific risk,
- Approach study participants in such a way to allow the gathering of in-depth and detailed information.

To address all these factors, it was clear we needed to recruit households that had had at least one confirmed EV case to ensure that every household contact had had the possibility of exposure to infection. Due to previous research activities with the Save the Children Kerry Town ETC survivor follow-up programme, in which all survivors discharged from the ETC were registered, we were able to access a cohort of households containing at least one survivor, and therefore a cohort of potential household contacts who we could be sure had had the possibility of exposure to infection. This contact with the survivor programme would facilitate the initial invitation to participate, and additionally, I was able to recruit to the study team six former ETC staff, including two Ebola survivors, who helped to create an atmosphere of trust from the beginning.

This approach, however, excluded households which had only experienced EV deaths, as only households with survivors were registered to the follow-up programme. However, while this clearly introduced a bias, there were pragmatic and human reasons to focus on the survivor households and find ways to manage the bias created. Firstly, at the time there appeared to be no efficient way to locate households where all those with recognised EVD had died since outcome registration was not consistently completed. Secondly we were concerned that approaching families who had only fatal EV experience, in some cases very recently, could be more sensitive and potentially traumatic for both participants and staff. We were also already aware that fatal experiences were sadly not in short supply among the survivor households: 70% had experienced at least one death and many multiple deaths. Against this background, we decided that the best approach would be to assess and adjust parameters for bias using statistical methods (see Analytical Methods).

In addition to ensuing known exposure, our research questions required the identification of the asymptomatic and undiagnosed as well as the symptomatic and diagnosed. For this we needed to measure seroprevalence of anti-Ebola IgG, but our systematic review had shown there was no reliable commercial assay and, although neutralising assays performed better, their use was impractical in a

field study. Plus, in the ongoing epidemic environment, taking blood samples was going to be difficult if not impossible due to biosafety and to community concerns around blood-taking. We needed a non-invasive assay. Fortunately, a group from Public Health England had a novel immunoassay which used minimally invasive, low biohazard 'oral fluid' as the analyte, and which had performed well in pilot studies. In collaboration with them, we integrated field validation of this new assay into the study protocol.

To integrate the above considerations, we designed a mixed methods cross-sectional study supported by three novel components: an exposure scale which we hoped would allow us to better quantify exposure risk, a qualitative interview approach which we anticipated would help affected families feel comfortable to describe the detail of their lived experiences with Ebola, and lastly as noted above, seroprevalence testing by self-administered oral fluid swab.

Finally, in October 2015 after completing the main data collection, news that a Scottish nurse who contracted Ebola in Sierra Leone had been re-hospitalised nine months later in the UK with near-fatal recrudescence of disease[55] led us to question whether similar cases were occurring in Sierra Leone where weak health services might see 'late' EVD-related deaths go unreported. In response, we added a follow-up study of survivors using a modified version of the WHO verbal autopsy tool[56] to evaluate the extent to which any deaths in survivors after recovery could be linked to recrudescence or other EVD sequelae.

Study population

The study population included 151 confirmed survivors cared for in the Save the Children International-operated ETC in Kerrytown between November 2014 and March 2015 (when the ETC was closed) and their household members.

In total, 123 of the 151 survivors agreed to take part; consent was also sought from each household member. Of the 28 survivors not included, one refused to participate, eight lived outside the Western Area, three had no family in the Western Area or bad relations with them, three had died, and 13 could not be contacted. One household withdrew during the interview for unclear reasons. Control participants for the assay validation were recruited from three Western Area Rural District villages which at that time had reported no cases of Ebola and had instigated strict self-quarantine. For this group we sought only minimal demographics and excluded anyone with risk of exposure e.g. health work, travel.

Qualitative method for quantitative data

Previous studies had focussed on trying to identify exposure risk through information on individual activities. This approach poses a number of problems. For data collection, it means using either short

questionnaires likely to elicit narrow information, or a long detailed individual questionnaire that can sap participant cooperation. Assessing multiple exposures can also lead to small subgroup numbers and difficulty in adjusting for confounding. Plus yes/no questions reduce the opportunity for interviewers to develop rapport with respondents, potentially leading to false denials.

To avoid these issues, we decided to identify only the highest risk exposure experienced by each household member with and without reported Ebola infection, including survivors and, by proxy, those who died, and locate this on an overall risk scale, thus removing the need to record every type of exposure experienced by each household member. Through literature review, discussion with ETC staff, and back and forth between myself and Professor Glynn, we estimated the likely exposure risk of different types of contact, differentiating also when these were associated with wet or dry cases, and aggregated exposures of similar risk into levels, from which we created an 8-level hierarchical scale (Figure 2). We were able to validate this hierarchy as part of the study and consequently used it as a categorical variable in analysis enabling better adjustment for exposure.

Figure 2: Exposure classification tool for EVD risk in households

Level 1:	Contact with the **body** of an EVD patient after death / prepared the body for burial
Level 2:	**Direct** contact with **body fluids**, e.g. blood, diarrhoea, vomit, urine, or a baby breastfed by an EVD-positive woman
Level 3:	**Direct** close contact with **wet case** i.e. with diarrhoea/vomiting/ bleeding), e.g. person helped dress, embraced, carried, helped care for, or shared the bed of an EVD patients with wet symptoms; or a mother who breastfed an EVD-positive child
Level 4:	**Direct** close contact with **dry case** i.e. without wet symptoms at the time. e.g. person helped dress, embraced, carried, helped care for, or shared bed of an infected person without wet symptoms
Level 5:	**Indirect** close contact with **wet case**, e.g. washed clothes or bed linen of an infected with wet symptoms, or slept in the same room but not the same bed as the person
Level 6:	**Indirect** close contact with **dry case**, e.g. person washed clothes, bed linen of an infected person without wet symptoms. Formal/informal **health workers** without known contact with a case; ETC workers in PPE; Ebola Intervention workers [outside household only]. Attended **funeral without contact** with the body [outside household only]
Level 7:	**Minimal** contact, e.g. shared meals, shared utensils, sat in the same room. Children placed in observation centres [outside household only]
Level 8:	**No actual contact**, e.g. person kept distance once person was symptomatic

***Examples**:*

- *Husband of person later confirmed to be EV positive reports caring for her while she was ill, carrying her to the toilet, helping her clean herself after being sick and incontinent:* ***Level 2***
- *Three sons <10 years A, B & C slept in the same room. B & C shared a same bed. B confirmed Ebola positive on presentation with fever, headache, body ache. C is* ***Level 4****; A is* ***Level 5***
- *Contact tracer with no household exposure:* ***Level 6***

Next we needed to create an environment in which affected families would feel able to describe in detail their experiences during the time when Ebola was in their household, which would facilitate recall and honesty as well as mitigate the experience of reliving what were likely to be painful experiences of illness and death. The environment would need to be supportive and encouraging since, even though we would not use a long questionnaire, we still needed to elicit in-depth information about all household members, surviving, deceased and apparently non-infected. We also needed to reduce the tendency for respondents to forget, or be reluctant to admit, existence of unreported cases or deaths, certain types of exposure and other sensitive experiences.

To achieve this, we invited the whole household of each survivor to come together as a group and describe in their own words what occurred during the time they were affected by Ebola. In this model, the study staff worked in teams of three: one as 'lead interviewer, focussed on building rapport with the household, using probing questions to guide the conversation, and encouraging each person to contribute their experience, while the two others were 'observers', concentrated on listening for mention of risks in the pre-defined levels, recording the maximum exposure level for each person, and making notes to support the level assigned in post-meeting review.

Any interview process risks provoking feelings of stress or duress even if the person gives informed consent. By intensively preparing the study team for this approach (see Training below), using staff who already had rapport with many of the participants, supervising carefully, and letting survivors and their families tell their own story, we believed this risk could be reduced to a minimum.

Safe spaces: physical and mental

As we were unable to enter family homes due to epidemic restrictions, and in consideration of potential issues of stigma and negative community perception around 'Ebola responder' teams, I needed to find places where participants and study team could meet and talk safely and confidentially outside the household. Spaces needed to be large enough to seat a large family (participating members ranged from 3-15), within reasonable distance of the households, and located so that the study team didn't have to move between sites during the working day, log-jam traffic in Freetown being routine. Many schools were closed at this time and through local contacts, I was able to meet with head teachers and arrange use of classrooms in conveniently-located schools. In all cases access to premises was given free of charge after discussion of the study and its goals.

In the control villages, as our request was simply for as many people as possible to give an oral swab, on the advice of community and religious leaders, we installed ourselves in the village halls (and once, a spacious bus stop), and made use of the village megaphone systems to alert the community to our request. This was followed by half-hourly information sessions to ensure that anyone arriving to donate understood the study and gave informed consent for their sample donation.

Infection control measures were important for safety and confidence. Household members were asked not to attend if experiencing any symptoms; we used calibrated infrared thermometers at entry; and staff used gloves when packing swab tubes (though as described below, self administration meant staff only handled the tube after it was in a Ziploc bag). All waste was collected and burned by arrangement in the hospital incinerator. The latter was through excess of caution as no bio-hazard waste was created, only gloves, swab packaging, biscuits wrappers and 'rubber' (plastic) drinks bottles.

Given the continuing epidemic and the harsh experiences of the communities we were visiting, I was acutely aware of the need to avoid provoking stigma or insecurity. In this, again, I was fortunate to work with Sierra Leonean colleagues who were both embedded in the community and brought personal experience of the epidemic, who helped me negotiate the twin demands of community mores and perceptions and ethical research. Two team members had been trained in psychosocial support methods during their work with the ETC but the whole team were impressive in their humanity and sensitive support to the households participating.

While I did not set up formal debriefs for team members, each household session ended with the team sitting together to consolidate their findings on exposure levels which also formed a level of debrief. In addition, as I attended ~80% of interview sessions, together with the Sierra Leonean team lead Sembia Johnson who accompanied the teams daily, there was opportunity to talk individually to team members, while the often slow return car journeys due to traffic also gave plenty of time to discuss the day.

Minimally invasive sampling

Oral fluid is the liquid that pools in the gingival crevice between teeth and gums. It is obtained by rubbing the gums with a swab and, unlike saliva, contains traces of serum (usually 1-2µL/100 µL) allowing the detection of antibodies.[57] In May 2015, a study of therapeutic use of convalescent plasma, which used the novel Ebola IgG Capture Assay on 10 paired donor serum/oral fluid samples, demonstrated clear correlation in the sero-reactivity of the two analytes.[58] Deploying this assay in our study not only fulfilled our need for a low bio-hazard method of sampling that was more community-acceptable than drawing blood, it was also an opportunity to further validate the assay in a large population at risk of infection. If proven sensitive and specific, oral fluid testing for Ebola could potentially be an important research and operational tool in low resource field settings.

The interview process

The household interview brought together the components described above and took two to four hours, depending on family size and the number of cases. The study team started by discussing the study and its objectives using patient information sheets I had refined with the team. Krio was the main language of discussions but as the study team spoke the other participant languages (Mende

and Temne), no translators were needed. Consent was taken individually with parents or guardians consenting for under 18s and children over 12 years were asked to assent. We ensured that participants understood that there was no direct benefit to them from the study, and that they would not receive individual serology results because it was not possible to say what these would mean for individual immunity or protection and we did not want to create a false sense of security, especially in the context of the ongoing epidemic.

After this, the interview process opened with participants creating an inventory of age, sex, relationship, and Ebola status and symptoms for every household member, alive or dead, who was in the household at the time Ebola struck and completing a short (1-page) individual questionnaire about testing and symptoms. This gave the study team early information to ensure the later discussion covered all household members. A brief socio-economic questionnaire was also completed with the household head.

Next, study staff demonstrated the oral fluid swab on themselves. To reduce any suspicion a household member was invited to pick a swab for the staff member to use. Afterwards each household member chose their own swab from a large box and self-administered it. In many interviews, the swabbing period was quite performative: family members were asked to do their swab one at a time including children while other family members guessed or timed the 90-seconds ideal for a good quality sample. The swabs were well accepted with usually only a request for water to rinse their mouths afterwards. Followed by soft drink and biscuits, the activity set a pleasant atmosphere for the discussion to follow.

After sampling, the lead interviewer focussed the household's attention on telling their story and, using probing questions, encouraged them to talk about each person who had been living in the household at the time Ebola stuck, whether still alive or dead and describe who had had Ebola, what kind of symptoms they had had, who looked after them, who helped them with which activities, who shared a bed or a room, who prepared their body if they had died. Family members were encouraged to remind, discuss or add detail to each others' accounts. Adults spoke for young children and corroborated information from older children. Although we did not ask for onset dates – we considered they were likely to be unreliable and would break the flow - the interviewer prompted to establish the order in which members became ill. This was achieved in such detail that we were later able to construct transmission chains from the team notes.

At the end of the session, families were given a bucket and laundry and personal soap for the family (it had been made clear during the consent process that the household would receive this regardless of withdrawal or refusal to consent). After saying goodbye to the family, the study team consolidated their observations and, using the exposure scale, recorded a single highest exposure level for each

household member. Discrepancies between team members were rare and resolved by reviewing observations. Generally each 3-person team performed 1 - 3 household interviews per day depending on the availability and size of households.

Staff training

A full 7 days was allocated to training because of the novelty of non-directive qualitative interviewing for the study team, and the importance of using the method correctly, not leading or influencing participants. There was also a need to absorb both the interview guide and the exposure scale, so as to be able to concentrate fully on the participants and refer to documents as little as possible.

The training included multiple periods of role play both in English and in Krio, with specific focus on avoiding leading questions and on engaging without indicating preferred response and was extended beyond the originally planned three days. All team members were trained in and performed both lead and observer roles to reduce fatigue.

Study Team reviewing, revising & role-playing the participant information and interview guide in Freetown, May 2015

One of the risks of group approaches is that discussion may be dominated by one or more person, or influenced by 'groupthink', where family members who don't agree may simply not speak so as not to contradict others in front of strangers. However, although it is normal for Sierra Leonean households to clearly identify a household head who would usually speak for the family, intensive practice during training helped the study team members create a relaxed atmosphere which fostered often quite exuberant discussion between family members and space for all to speak.

Data collection

All interviews were supervised by the senior Sierra Leonean team leader. I attended ~80% of interviews myself to supervise the 'tone' and to hear stories first-hand to enhance my understanding (many conversations were mixed Krio/English). I read all participant forms and team notes each evening, reviewed exposure level decisions, and followed up with individual interviewers if I had queries or concerns. I created the questionnaire and study data base in Epi-Info and trained the data entry clerk. I checked entries in batches, often daily, and highlighted any issues to the team and the data clerk. Only rarely did we need to re-contact a household to clarify a point. I did all subsequent data management.

Analytical methods

Analysis involved both pre-planned work in response to the study research questions and *post hoc* work to take advantage of the large database collected. Statistical methods included χ^2 or Fischer's exact tests for association, multivariable logistic regression (adjusted for household clustering using random effects) to assess effect of age and other factors, linear regression to assess level of antibody reactivity with time since admission and by age, and negative binomial regression to analyse household transmission as the data was over-dispersed. As odds ratios were very large, we used marginal standardisation to present results as risk ratios in all analyses, and the Delta method to estimate 95% Confidence Intervals(CI). In the analysis of the effects of mother's illness and breast feeding on risk of EVD in infants, we repeated the calculation of risk ratios using Poisson regression with robust error variance to confirm findings.

First analyses were done before serology was available and used data on confirmed survivors and fatal cases and collected from household members. To identify potentially missed cases and deaths, we divided apparently un-infected household members into four groups by relating the symptom information they shared to the WHO EVD case definition[59] as follows:

1. Those who reported being well throughout (asymptomatic)
2. Those who reported some symptoms but who did not fulfil the case definition
3. Those with case definition symptoms and a negative test
4. Those with case definition symptoms who had not been tested.

Only group 4 were counted as probable cases and sensitivity analyses were done to test the effect of including and excluding this group. Although this categorization was based on participant recall, we reasoned that the relatively short time since Ebola had struck and the intensity of the experience were likely to encourage memory, especially in a supportive environment, but we remained alert to the possibility of participant bias. Findings using this classification were later compared to those using serology.

As noted above, we did not collect onset dates. However, as all households clearly identified the first person to become ill, non-primary cases could be defined, and detailed narrative notes allowed these to be ordered and household transmission chains to be reconstructed. From these, we calculated a reproductive number, a household secondary attack rate (the proportion of exposed household members infected by the primary case) and household attack rate (the overall proportion of household members infected), and analysed characteristics of primary and subsequent cases.

To counter the bias incurred by studying only survivor households, we adjusted parameter estimates by assuming that, given the estimated case fatality rate of 66% for this outbreak, two households with

only fatal cases would have been missed for each of the households in the cohort with only survivor cases, and that the households with only fatal cases would be the same size as the households with only surviving cases. As households were selected by presence of a survivor (i.e. someone with 0% chance of EVD death), we adjusted the CFR by excluded one index survivor per household to ensure that all others in the household had the same chance of dying.

Samples from PCR-confirmed survivors and community controls were used to assess sensitivity and specificity of the oral fluid assay. Two reactive tests were specified *a priori* as the requirement for a positive result and the cut-off per plate was defined as the mean optical density (OD) of the negative control plus 0.1, as this is the standard method used in field laboratories. Other methods of setting the cut-off were explored (e.g. using multiples of the mean negative control) to confirm this was appropriate. Samples with discrepant results were retested. To simplify presentation, we normalised results, taking the ratio of the test OD to the cut-off so that results greater than 1 indicated reactivity.

* Note on study publications

The papers addressing the study questions[†] were drafted consecutively, starting with the systematic review of Ebola seroprevalence surveys described above (Paper 1).[54] Papers 2 and 3 addressed study questions 1 and 3, describing age and exposure-specific attack rates, the validation of our exposure classification scale, and the effect of mother's illness on very young children.[60, 61] Paper 4 also addressed study question 1 examining case fatality in the study population, the potential for late deaths and recrudescence, and the role of infecting dose in outcome.[62]

Paper 5 integrated the serology results, describing the frequency and impact of asymptomatic and unrecognised EVD, and reporting on the performance of the oral fluid assay.[63] Paper 6 addressed question 4, exploring individual and household factors associated with household transmission and estimating a household secondary attack rate.[64] Paper 7 in the Appendix is the report of the asymptomatic case that first led me into this research work.[2]

It is important to note that Papers 2 to 5 were published before serology results were available and used reported symptoms to identify probable cases. We did this in order to make timely contributions to the knowledge base, and because sensitivity analyses gave us confidence in the validity of our findings. In January 2016, the findings were confirmed by serology.

[†] 1. What is the true age distribution of Ebola virus infection and case/infection fatality?
2. To what extent do asymptomatic and unrecognised 'mild' Ebola virus infections exist?
3. What impact do age, type of exposure and other factors have on risk of contracting Ebola infection, and on the risk of dying from it?
4. What characterises transmission in households and what is the extent of household secondary transmission?
5. Performance, feasibility and acceptability of this minimally invasive method of sampling.

7. RESULTS

The study involved 933 people in 94 households, including 123 Kerrytown survivors, 45 survivors of other ETCs, and 238 deaths. Exposure histories were collected from 905 people and oral swabs from 633 of 695 living household members and 339 community controls. The missing data is explained by 56 household members unable to attend interview (we were able to collect individual-level data via family members for 28 of these) and four survivors who had died since ETC discharge. With this dataset, the study was one of the largest investigations of Ebola virus infection and disease using primary community-collected data at the time.

Risk and exposure (Papers 2 & 5)

Risk of developing EVD, whether diagnosed, asymptomatic or unrecognised, was strongly correlated with level of exposure ($p < 0.001$) rising steeply and linearly in line with our pre-defined exposure classification hierarchy (Figure 3A). This was confirmed when serology was integrated (Figure 3B).

Figure 3: Risk of contracting Ebola virus by exposure level among households of Kerrytown survivors: validation of the exposure classification tool

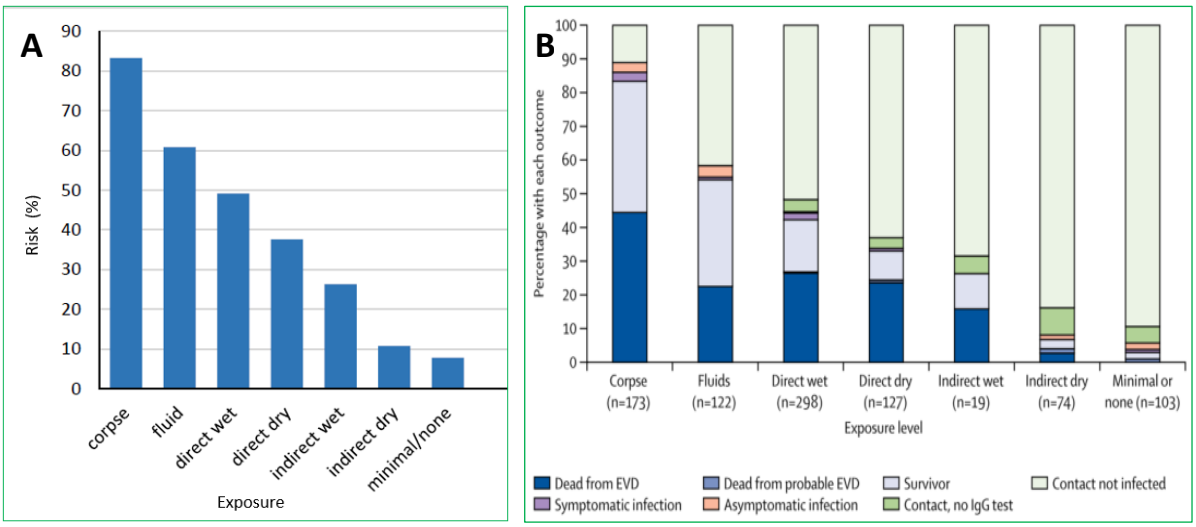
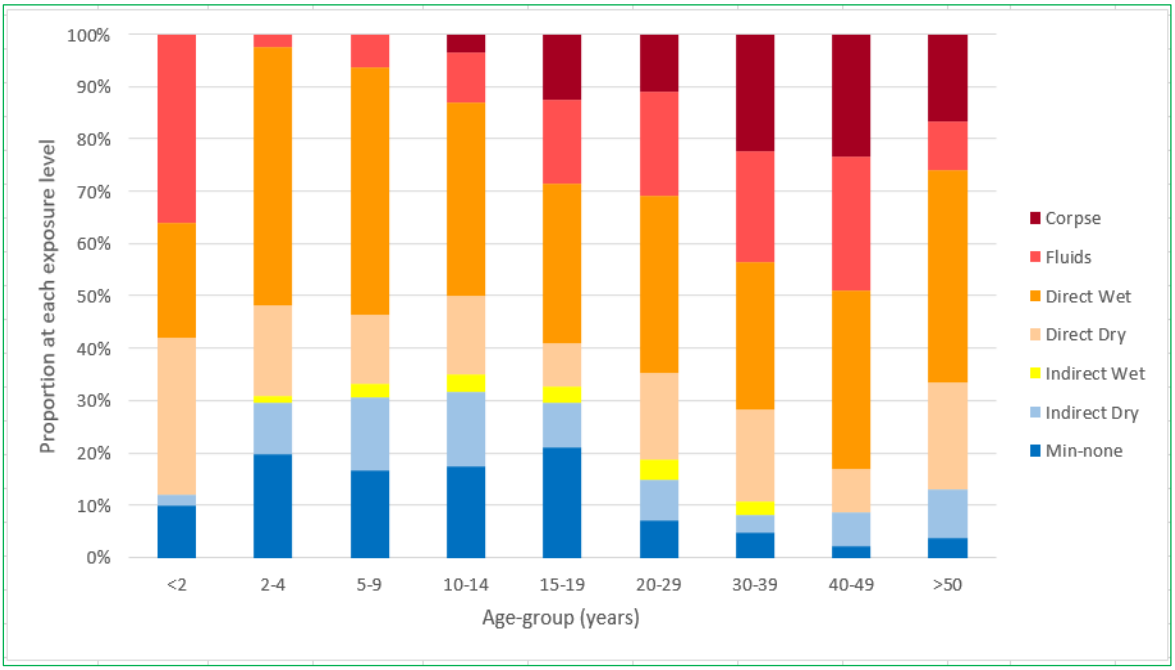


***Figure 3A**: risk of Ebola virus disease by exposure level among study participants excluding primary cases, based on reported symptoms and probable deaths.*
***Figure 3B**: risk of Ebola virus infection and disease by exposure level and by serological result excluding primary cases.*
Levels of exposure in both A & B correspond to those shown in Figure 2

Children had lower exposure than adults but exposure levels in the household studied were high overall with over 50% of all members regardless of age having at least direct exposure to a wet patient (Figure 4). As others had reported,[35, 37, 41] we found the highest risks in the top three categories of the scale: namely contact with a corpse, direct contact with body fluids, and with 'wet' patients. Risks from direct contact with dry patients and indirect contact with wet patients were still considerable -— 5-fold higher than minimal or no contact with a case -— but there was no discernible increase from indirect contact with dry patients compared to minimal or no contact.

Figure 4: Levels of exposure by age in Kerry Town survivor households (excluding primary cases)

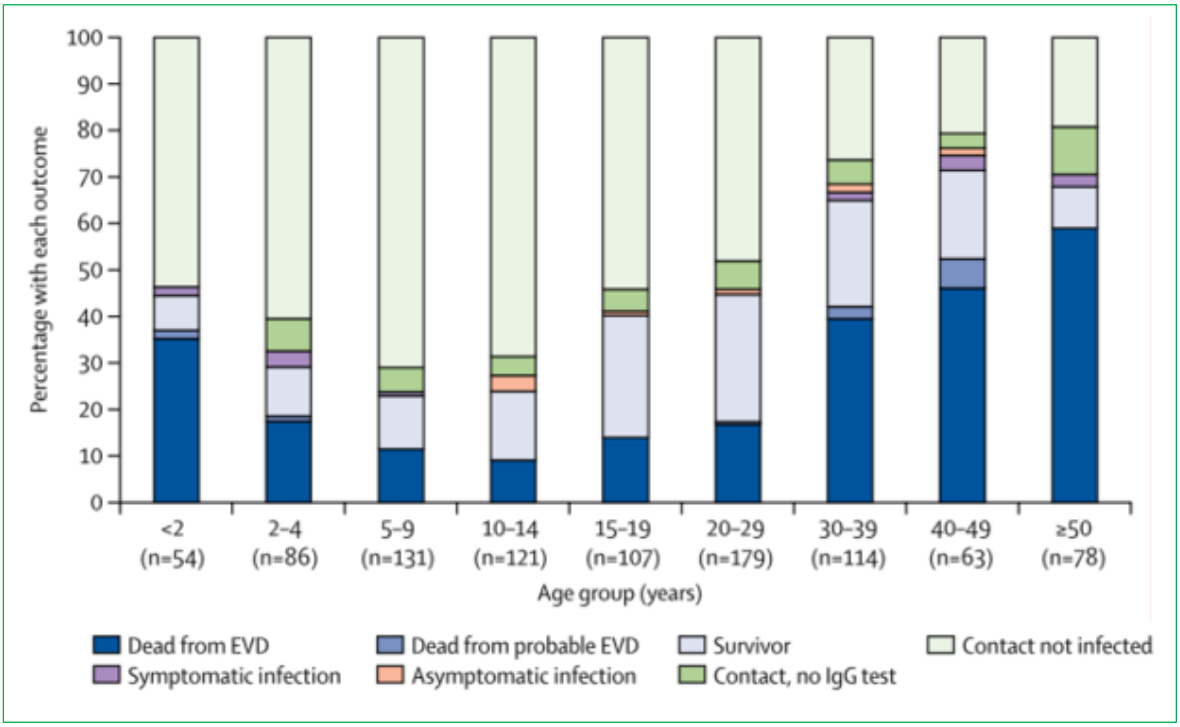


Despite the strong associations, the highest risk exposures described above did not definitively predict infection: 49.8% (229/460) of household members exposed at these levels did not become infected. Overall there was little difference in risk of EVD by sex, regardless of age, and having a spouse that contracted EVD first was not a risk factor after adjustment for age and sex. Type of household sanitation facilities was also not associated with EVD risk.

Age-specific risk of developing EVD (Paper 2)

With detailed information on exposure and inclusion of un-notified cases, we were able to show that risk of developing EVD varied significantly by age even when adjusted for exposure ($p < 0.004$) and, similar to published case fatality rates, indicated a sparing of children and teenagers but not of very young children. Risk of EVD in children aged 5 to 14 years was at least 50% lower than those aged over 30. After adjustment for exposure, occupational and household variables, this pattern remained strong. Contrary to the linear rise in EVD incidence by age seen in published notification data,[1, 35] children aged under 5 years in this study had 26% higher risk of developing EVD than 5-14 year olds, similar to the pattern seen in case fatality, suggesting this age-group was disproportionately missed in notification data. Among adults the pattern of risk was similar to the published notification data rising steeply to plateau in age groups over 30. This "J-shaped" curve (Figure 5) remained in multivariable analysis adjusted for level of exposure, sex, household size and household clustering. In sensitivity analysis that excluded probable cases, association with exposure level strengthened and the "J-shaped" association with age became more marked.

Figure 5: Risk of Ebola virus infection and disease in Kerry Town survivor households, by age and outcome



Mother to child transmission (Paper 3)

After adjustment for age, sex, exposure and household factors such as crowding and sanitation, under 3-year-olds in this cohort were seven times more likely to contract Ebola if their mothers had EVD than if she remained un-infected, and risk increased if the mother died. But it was also notable that more than a quarter of under 3s whose mothers developed Ebola did not become infected (27%, 15/40), and half of infants with direct contact with mothers with wet symptom EVD remained well. Breastfeeding did not appear to confer any additional risk regardless of infection status of the mother, but the study had limited power to detect an association due to the small numbers and already high risks in the group. Both risk of EVD and case fatality decreased with increasing age of the child.

Case fatality and 'late deaths' (Paper 4)

This study was the first large investigation to integrate information on unnotified cases and deaths. Based on notified cases only, the CFR in this cohort was 68.5%. When unnotified probable cases and deaths were included, however, this dropped to 57.5%, and remained at 57.9% when serology confirming asymptomatic and unrecognised symptomatic infection were integrated, suggesting our qualitative interview method successfully captured the true distribution of cases and deaths. When adjusted to take into account of the study's survivor household bias, CFR increased to 67.0%.

Complete follow-up of all survivor participants a mean of 10 months after ETC discharge revealed four deaths giving a risk of 'late death' potentially related to EVD of 2.6%. As only one of these cases died

after full recovery from EVD, the maximum estimate for risk of viral recrudescence leading to death in this cohort was 0.7%. When these late deaths are included, the unadjusted and adjusted CFRs for the cohort increased slightly, to 58.9% and 68.3% respectively. It must be noted that these estimations include all ETC-confirmed survivors, however nine of these were antibody-negative. ETC case notes suggested some may have been erroneously diagnosed but were not available for all. Therefore underestimation of CFR of ~1.5% cannot be ruled out. This group also reduces the estimate of assay sensitivity.

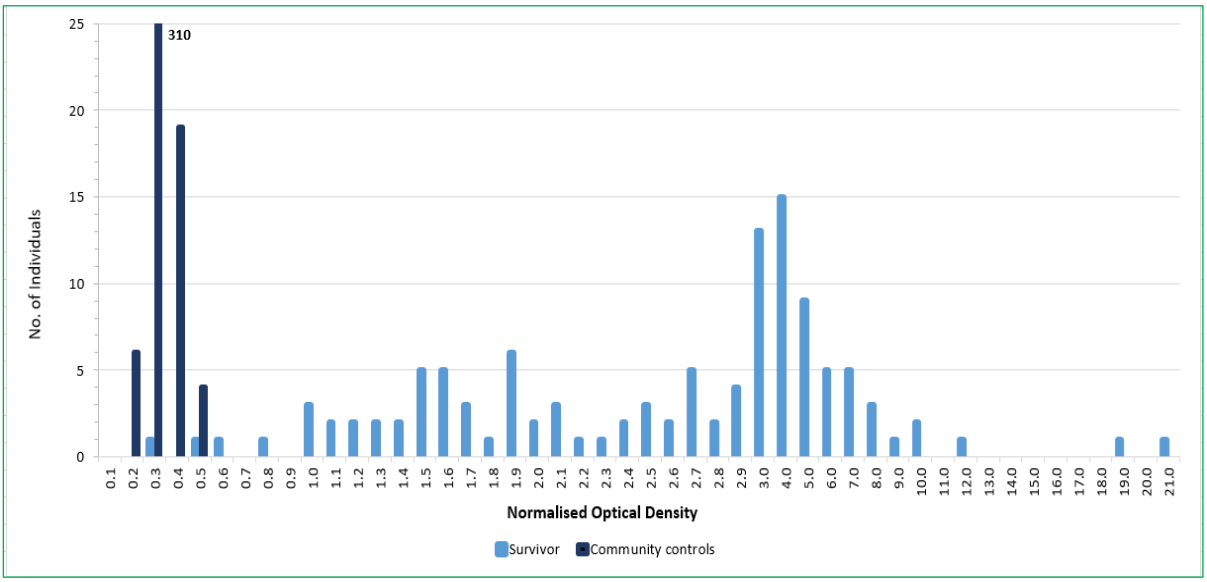
Similar to risk of infection, CFR was highest in the youngest and oldest age groups and lowest in children aged 10-14 years, but unlike risk of infection there was no consistent trend between CFR and increasing exposure. Beyond age, factors most strongly correlated with mortality were increasing household size, possibly explained by the difficulty of managing many affected members, and being infected earlier in the epidemic when services were most overstretched and confidence in the response was at its lowest.

Health work and 'non-manual' occupations were associated with higher CFR even when adjusted for age, though religious or community leaders, all of whom died, made up a third of the non-manual group and so the association may reflect activities other than occupation. There was no association between socio-economic status and outcome, suggesting that once ill, living conditions had little effect. It is worth noting that as there was no public/private provision of Ebola care, there was no differential advantage to being better off.

Novel oral fluid assay validation (Paper 5)

The oral fluid anti-glycoprotein IgG capture assay proved to be highly sensitive (95.9%, 95% CI 89.8 – 98.9%) and specific (100%, 98.9 - 100.0%) for Ebola virus antibodies (Figure 3), and well accepted by participants. Only 1.2%. refused sampling.

Figure 6: Serology results using the Oral fluid IgG capture assay in 116 Kerry Town survivors and 339 community controls



** Mean of all tests results per individual*

Asymptomatic and pauci-symptomatic infection (Paper 5)

Using the oral fluid assay, we identified 21 additional Ebola infections among household contacts not previously diagnosed with EVD: 10 who reported experiencing no symptoms during the time Ebola was in their household, and 11 who recalled having symptoms but were not tested or notified. Among all asymptomatic household contacts, 2.6% (95% CI 1.2-4.7, 10/388) were EBOV IgG positive, while 12% (6.1-20.4, 11/92) of household contacts who recalled any symptoms had had unrecognised EVD: four with case definition symptoms and seven with only one or two 2 symptoms. No individual symptom or number of symptoms were associated with seropositivity in the unrecognised cases, but numbers were small.

These 21 unrecognised infections in living household members increased the total number of people infected with Ebola in the studied population by 4.9% with asymptomatic and unrecognised symptomatic infections contributing 2.3% and 2.6% respectively. All asymptomatic infections occurred in people aged 12 and over, countering the hypothesis that this may account for low EVD incidence in young children. By contrast, children aged under 5 and adults over 30 years were most likely to experience unrecognised symptomatic EVD, consistent with under-diagnosis in these age groups.

Household transmission (Paper 6)

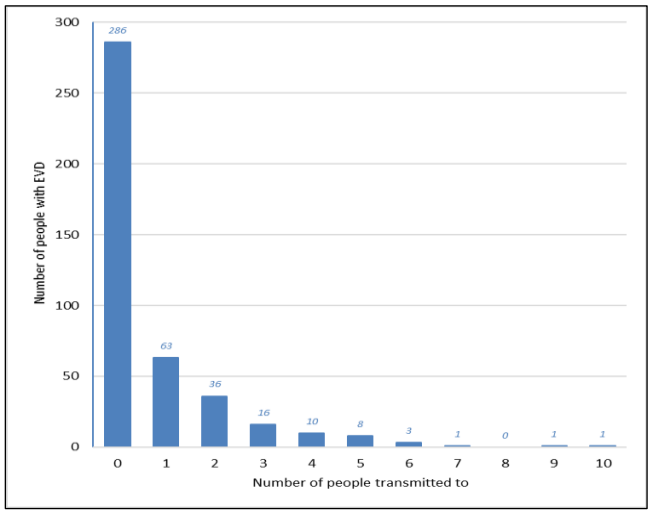
Excluding primary cases, the household attack rate in this cohort was 38%, and the secondary attack rate based on the first generation of intra-household transmission was 24%, leading to a reproduction number (R) of 1.8. After adjustment to take account of excluding households with only fatal cases, the household attack rate reduced to 28%, the household secondary attack rate to 18%, and R to 1.2.

Study households had a wide range of EVD experiences, from a single case and no subsequent spread to all household members being affected. Household size and the age and severity of illness of the primary case were important determinants of initial spread. Households of ≥6 people were more likely than smaller ones to have any secondary spread. Children were least likely to be a primary case, least likely to further transmit and, if they did, rarely to more than one person.

Once spread occurred, however, the main drivers of the proportion infected (the household attack rate) were older age (>45) and severity of illness of the primary, particularly if they died whether at home or in a facility, older mean age of other household members, and more crowded households. Overall household size did not influence the proportion infected once adjusted for all other factors. Proportions infected were lower if the household included a health worker or was affected later in the epidemic, possibly due to earlier symptom awareness or more rapid transfer of cases to an ETC.

As far as we could disentangle the likely transmission chains from the narratives, spread of infection in these households was over-dispersed: only a small proportion of those infected were responsible for most transmission (Figure 7).

Figure 7: Number of intra-household transmissions per EVD case in households of Ebola survivors



In total only one third of all those infected transmitted to someone else in their household; 33% of primary cases and 80% of non-primary cases did not transmit further. Of those who did transmit, 77% died. Excess risk of transmission from fatality remained even if the person did not die at home suggesting it was more likely related to presence of wet symptoms than to contact with the corpse. Risk of transmission from cases with only dry symptoms was less but not negligible: one-third of the households with 'dry' primary cases had subsequent cases.

While some primary cases were linked to well-known high-risk activities such as funeral rites or frontline work, more often the probable source of their exposure was visiting and/or nursing sick friends and relatives or bringing sick people into the household for care.

8. DISCUSSION

My research was designed to improve understanding of key aspects of Ebola virus epidemiology, particularly the relationship between age and exposure, the true burden of infection, and what influences spread between individuals and within households where the majority of transmission takes place. It also aimed to establish a method of researching Ebola infection during outbreaks that did not require drawing of blood. The study I implemented was the first large community-based study to integrate information on unnotified cases and deaths, to collect information on exposures in such a way as to allow rigorous adjustment, and to assess non-clinical risk factors for death from EVD. It was also the first cohort study with active follow-up for death after discharge from an ETC over an extended period (mean 10 months).

In this chapter I review how our findings have contributed to the knowledge base, how they relate to and have informed subsequent research, and where these findings may inform efforts to improve response to Ebola outbreaks.

Understanding age and Ebola

After minimising selection bias and adjusting for exposure, this study has demonstrated that inherent differences in susceptibility most likely underlie the age distribution of Ebola and better explain lower incidence and case fatality in children than the commonly-cited hypothesis of lower exposure alone.

School-age children have been shown to have the lowest severity of disease in multiple infectious diseases including measles, cholera, yellow fever, tuberculosis, influenza, meningococcal meningitis.[65] The COVID-19 pandemic also shows distinct age-specific patterns including lower rates of infection, severity and mortality in young children.[66, 67] Our use of household questioning to uncover missed cases and overcome the under-notification in official statistics showed that children aged under 5 had far higher incidence than officially reported, mirroring their high case fatality, suggesting opportunities had been missed to detect and provide care for these children who, when infected, are at high risk of death. Conversely children aged 5-14 years had much lower case fatality (30-40% compared to the 50-60% in official statistics[68]) paralleling the sparing seen in their risk of contracting EVD. Similar results were reported by Cherif *et al* in Guinea (82.9% CFR in under 5s, 65.4% in 5-10-year-olds, 48.9% in 10-14 year-olds).[69] We also showed that 5-14 year-olds were not less exposed but similarly exposed to other age groups, with more than half of 5-14 year olds experiencing exposures in the top three risk categories. We also found no child under 12 infected asymptomatically, countering the hypothesis that sparing may be linked to non-symptomatic response to infection.

What mechanisms drive this sparing from infection in the 5-14-year age group is still unclear. No statistically significant differences in viral load have been found between hospitalised adults and children who do become ill, but different cytokine and chemokine responses and faster speed of

immune reaction have been seen in children compared to adults.[70] Disentangling whether these only come into play to combat infection once it has entered the body, or whether they play a role in protecting this age group from infection itself needs further biological investigation. One possible source of detailed immunological information may be age-specific antibody response data collected during the recent Ebola vaccination trials, which could also elucidate the role of age in the effectiveness of vaccines.

Spectrum of EBOV disease

Understanding the full spectrum of how EBOV infection manifests is important for measuring impact of and controlling Ebola outbreaks. Recent debate over the extent to which truly asymptomatic and pre-symptomatic SARSCoV2 infection might drive transmission of COVID-19 offers a timely illustration of how critical these states can be for public health response and for modelling likely epidemic spread.[71] Our study expanded knowledge of three key aspects of the EV spectrum: asymptomatic infection, 'mild' infection, and undiagnosed symptomatic cases – all of which have ramifications for control and for measurement of epidemiological parameters.

Asymptomatic infection

Given the completeness of our cohort and high performance of the novel assay, our finding that 2.6% (95% CI 1.2-4.7%) of reportedly asymptomatic contacts were infected, and contributed 2.3% to overall cases, supports the existence of asymptomatic EV infection but not in the high proportions postulated by some previous studies.[51, 54] This estimate is statistically similar to the pooled estimate of 3.3% (95% CI 2.4-4.4%) asymptomatic infection among well-defined case-contacts identified in my systematic review of Ebola virus serosurveys to 2016.[54]

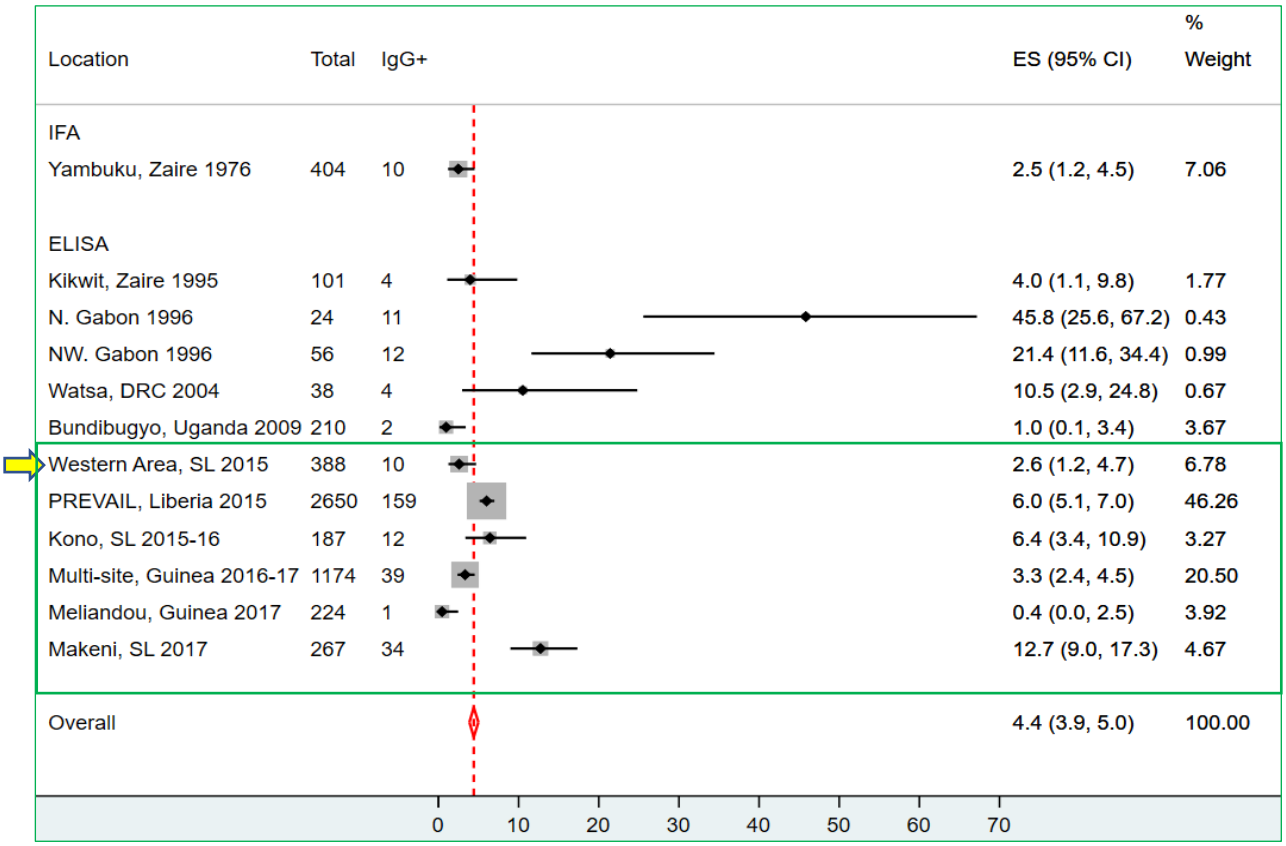
Further evidence has accumulated since that review. A study by Timothy *et a*l, on which I was technical advisor and a co-author, re-investigated the 2013 outbreak in Meliandou, the origin village of the West Africa epidemic, using the same oral fluid assay and a similar exposure hierarchy, and found 0.45% (1/224) infection among asymptomatic household contacts, contributing 5.5% (1/18) to the overall burden of adult infection.[72] Diallo *et al,* also in Guinea, using a latent class model that combined results from serum assays targeting the three main EBOV antigens (GP, NP and VP4) to counter suggestions that low rates of asymptomatic infection may be due to investigating only GP, reported asymptomatic infection of 3.3% (39/1174) among a large cohort of household contacts.[73]

Others have reported higher rates, but still relatively low compared to some pre-2013 studies. Sneller *et al* reported 6.0% of a large cohort (159/2,650) of asymptomatic close contacts recruited during the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) trial were EBOV GP-positive on serum ELISA.[74] Richardson *et al,* also using a serum ELISA, found 6.4% (12/187) of asymptomatic members of quarantined households in a village in Kono, Sierra Leone, had been infected.[75]

Halfmann *et al* in Makeni, Sierra Leone, again using assays for GP, NP and VP4 reported that 40% of undiagnosed close contacts were seropositivity to at least one protein, but that only the 12.7% (34/267) reactive to GP could be confidently defined as asymptomatic infection due to the differing rate of neutralising antibodies seen between the antigens (>90% in GP-reactive samples; <10% in NP/VP4-reactive samples). They also stated that this estimate was likely inflated as 21% of the contacts were healthcare workers with a very different level of exposure – illustrating the importance of precision in defining the contact groups.[76]

Revised to include the above studies, the pooled estimate of asymptomatic infection in seroprevalence surveys with confirmed case contact is 4.4% (95% CI 3.9-5.0%) (Figure 8).

Figure 8: Seroprevalence of Ebola IgG antibodies in asymptomatic populations with confirmed case contact revised to include estimates from the West Africa epidemic



Revised forest plot and meta-analysis from Systematic Review and Meta-analysis of Seroprevalence Surveys of Ebolavirus Infection 1961-2016, now including seroprevalence estimates from the West Africa outbreak (green box). Red dotted line and diamond indicated pooled estimate. Yellow arrow indicates the estimate of the Kerrytown survivors study described in this PhD. ES = Effect size. IFA = Immunofluorescence Assay. ELISA = Enzyme-Linked Immunosorbent Assay. References: Yambuku[77]; Kikwit[47]; N Gabon[78]; NW Gabon[79]; Watsa[80]; Bundibugyo[81]; Western Area[63]; PREVAIL[74]; Kono[75]; Multisite Guinea[73]; Meliandou[72]; Makeni[76].

It must be remembered that all these studies, including our own, assigned symptom status based on household member recall. One possible explanation for higher estimates of asymptomatic infection

in some 2017 studies could be greater reluctance to admit experiencing symptoms in some settings, for example during clinical trial recruitment. Timothy *et al* extended the qualitative interview process by adding key informant interviews to triangulate participant statements and found a higher proportion of the 'confirmed' symptomatic were seropositive than those not confirmed (60% versus 11%).[72] Including the one unconfirmed 'symptomatic' seropositive person as asymptomatic increases the proportion of asymptomatic household members infected to 1%.

Antibodies have now been detected in survivors up to 40 years after infection with some able to neutralise live virus.[82, 83, 84] The extent of protection this affords is yet to be determined, as are what constitutes a protective antibody titre, and the roles of other immune system mechanisms and phenomena such as viral persistence or unrecognised re-exposure which may boost immune response.[85, 86]

The infectiousness of pauci-symptomatic and asymptomatic infections is also unclear. It has been suggested that sub-clinical infection could explain the emergence of new chains of transmission.[87 88] As we recreated transmission chains by ordering people based on their contact with symptomatic cases, we were not able to assess risk of transmission from asymptomatic cases, but our findings regarding the positive association between severity of disease and onward transmission, and the link established by others between high viral loads and severity of disease,[89, 90] suggest a low probability that asymptomatic individuals would contribute substantially, if at all, to spread. Given that 90% of infected participants with only dry symptoms (61/70) did not create any onward transmission in the household, it seems reasonable to assume that force of transmission by asymptomatic individuals would be even less. Control efforts are, therefore, likely to be more effective if focussed on identifying symptomatic individuals (including reactivated survivors) as early as possible and initiating the appropriate infection control measures in the home prior to reporting, rather than attempting to detect asymptomatic cases.

Regarding association between level of exposure and how EBOV infection manifests, i.e. a potential 'dose' response, there is little, and contradictory, evidence. Our study found only weak correlation between level of exposure and whether any symptoms were experienced, and no association with case fatality rate. By contrast, Diallo *et al* did postulate a dose-response based on findings that, in a cohort of pauci- and asymptomatically-infected individuals, the former were more likely than the latter to have been exposed to the highest risk activities.[73] More research is needed to understand these relationships.

Despite low prevalence and potentially low risk of transmission, presence of asymptomatic seroconversion does have some policy implications, such as how to define a 'survivor'? In some countries, registration as an EVD survivor gives access to extended programmes of social and medical

support. If ETC records were missing, it has been suggested that a reliable IgG test could identify survivors for these programmes.[75] Now policymakers may need to decide if all those found EBOV IgG positive, regardless of illness experience, will be considered survivors. The answer needs to balance potential strain on resources of providing benefit to those who may not need it (at least for reasons related to Ebola infection) and the unknown extent to which the mildly or asymptomatically-infected escape negative sequalae, another area which requires further research.

It has been suggested that integrating new knowledge on the different manifestations of infection will change predictions from mathematical models and therefore alter response decisions.[91] Certainly more reliable estimates of the asymptomatic fraction, the mildly symptomatic, and unreported fully symptomatic cases, allow modellers to move away from more extreme scenarios (e.g. 50% of infections being asymptomatic[92]) and may improve predictions of epidemic size and severity. But key unknowns remain, particularly the extent that natural immunity is induced by sub-clinical or mild infection. A rare study which investigated median antibody level in serum samples taken 12 months after infection using the Filovirus Animal Nonclinical Group (FANG) GP ELISA found confirmed survivors had antibody levels five times higher than antibody-positive asymptomatic contacts.[74] By contrast, our study, using a GP ELISA in oral fluid, found similar antibody response in both groups. More research is needed to understand the contribution of asymptomatic/pauci-symptomatic infection to protective immunity, which will be important not only for estimations of herd immunity, but also to allow vaccine efficacy studies to factor in realistic levels of pre-existing immunity and to inform strategies for use of vaccines.

Undiagnosed symptomatic cases

The extent of undiagnosed symptomatic (and potentially infectious) cases represents a more substantial risk to epidemic control. That 20% (92/481) of sampled household contacts in our cohort recalled having some of the symptoms included in the Ebola case definition at the time when others in their family were diagnosed with Ebola is not surprising: most listed symptoms were common in Sierra Leone. What is concerning for epidemic response is that almost half of this group (40/92) matched the case definition and were not tested, with four (10%) subsequently confirmed infected to have been infected by serology. A nurses' strike prevented one of these cases from being diagnosed, but the others simply did not want to engage with the response, the same explanation given by most who did not report symptoms at the time. Among those reporting some symptoms but who did not fit the complete case definition, an even greater proportion (21.2%, 7/33) were antibody positive. Overall 12.0% (11/92) of people who experienced Ebola-like symptoms were unrecognised cases.

Subsequent studies have uncovered similar proportions of unrecognised cases. Diallo *et al,* who classified missed cases in a similar way to our study in a large Guinean cohort found 6.5% (12/186) of

pauci-symptomatics and 20.0% (6/30) of case definition-positives had been infected with EBOV, meaning 8.3% of all true symptomatic cases were unrecognised (18/216).[73] Also in Guinea, Timothy *et al* reported six undiagnosed seropositive household contacts who had had at least three 'EVD-like' symptoms during the outbreak and one with minimal symptoms, representing 38.9% (7/18) of all true symptomatic cases.[72] In Liberia, Sneller *et al* found 7% (102/601) of close contacts reporting symptoms were EBOV antibody-positive but did not differentiate between those that met case definition and those that only experienced some symptoms. This team also compared frequency of symptoms in contacts, finding antibody-positive contacts had twice the rate of most symptoms of antibody-negative contacts, and that there was positive correlation between antibody level and experiencing at least one EVD listed symptom, suggesting mild symptoms might provoke more immunity than asymptomatic infections.[74]

In our study, the people most likely to experience unrecognised symptomatic EVD were children under 5 and adults over 30 years. This is consistent with under-diagnosis in young children in whom febrile illness for other reasons is common meaning initial Ebola symptoms might have been less remarked. However, this was compounded by lack of trust in the care available and lack of support during quarantine which led many households to fear the consequences of disclosure, and participants often reported not wanting to risk approaching a health facility for something that could be a normal illness. The extent of unrecognised symptomatic disease confirmed by our studies and others is a serious risk to effective control activities and response managers need to work on ways to overcome these barriers to detection early in an outbreak.

Prevention and control

Although super-spreading events such as unsafe funerals and nosocomial incidents were known to be important drivers of Ebola epidemics prior to 2013, our work and that of subsequent studies confirm that household transmission is a key driver of spread, continuing throughout an outbreak while other drivers are mitigated by interventions.[93-96] Nevertheless, the adjusted first generation reproduction number (R) in our large cohort of households of 1.2, the finding that the proportion of households infected reduced later in the epidemic, and the over-dispersed pattern of transmission (where only a small proportion of those infected are responsible for most transmission), also seen by others,[97-99] suggests that it should be possible to curtail transmission in many households faster.

Faye *et al* in Guinea highlighted the importance of intervening in household spread, noting that onward infection from non-healthcare-worker EVD cases was three times higher (1.4) in the community than in hospitals (0.4) and via funerals (0·5) and that while Infection Prevention and Control measures introduced in April 2014 reduced R in hospitals and funerals to under 0.1, it

remained unchanged in the community: 72% of all transmission occurred between household members.[94]

Some studies reported lower household transmission rates: Skrip *et al* in Liberia and Reichler *et al* in Sierra Leone reported household SARs of 4.1%[96] and 9.9%[98] respectively, most likely because these studies covered periods when the epidemic was slowing. Fang *et a*l reported that household SAR in Sierra Leone dropped from 9.3% to 1.7% after 23 October 2014, linking this to improved case isolation and safe burial, but both figures are likely to be underestimated as households in this study were defined by linking names and addresses from case report forms which were frequently inaccurate.[100] A rigorous systematic review of all studies that estimated a household SAR since 1978 reported a mean of 15.4% (95% CI, 10.0%–20.9%), similar to our adjusted estimate of 18%.[50]

Earlier take-up and acceptance of interventions by households will be critical to any effort to curtail household transmission more quickly, and this relies on early 'real' involvement of communities in planning and tailoring the interventions. My experience is that, under the pressure to respond, public health workers often feel challenged by the way communities want to deal with EVD which may not conform to standard guidelines.[101] Listening to these ideas and helping communities work out what they need to do and why is time-consuming, and the time when it is crucial to do it is the very same time when responders can feel that 'action' i.e. installing known public health measures, must be the first priority. But, as highlighted in the multiple evaluations of the West Africa epidemic,[102-105] standard transmission control measures founder badly if they do not chime with the community. Recently, authors of the after-action review of the 2018-20 outbreak in Eastern DRC again highlighted that this 'lesson' is proving hard to learn, writing that the lack of subtlety, absence of integrated social and public health analysis, and late timing of community engagement was as responsible for slowing control of that epidemic as the substantial security and political challenges.[106, 107]

Communities need to have as clear as possible understanding of why certain public health interventions are promoted so that they can assess this alongside their understanding and experience,[108] and responders need to have as clear as possible understanding of how the community is perceiving the disease, and be willing to listen and co-create tailored approaches.[109, 110] For example, in the cohort we studied, many people took relatives and friends into their homes even though they knew they were ill. Other studies show that cases occurred in households because families were quarantined in close quarters without means to protect themselves, or because they lacked clear understanding of how transmission could occur or be prevented.[95] Real engagement with households on acceptable ways to reduce risk (examples might include household disinfection kits,[111] community-led case-finding,[112, 113] practical messaging[114, 115] and "safe while you wait approaches"[116]) rather than simple exhortations not to do these things, has potential to reduce the excess transmission.

In a detailed study of a single, early, and highly-affected village in Kailahun, Sierra Leone, Caleo *et al* noted community compliance coincided with the return of a survivor whose first-hand testimony caused a shift in belief that Ebola existed and increased acceptance of strict control measures. The change occurred "when the health messages given to the community started to mirror their reality" – sadly only after seven weeks of transmission and many deaths.[95]

Targeting intensified control efforts

Our study identified certain characteristics among households and household members that made onward transmission more likely, and which may help prioritise response efforts when cases outpace resources, or to indicate households at higher risk for intensified attention. Firstly, large households (≥ 6 members) were more likely to have any secondary spread, and cases in very large households (≥16) had three times the risk of dying of those in smaller households. Once infection was established in a household, however, the drivers of higher onward transmission were source cases with more severe disease, particularly if they died, regardless of where, and older cases (>45 years). Children (< 5 and 5-14 years) were least likely to transmit and, if they did, rarely to more than one person.

The association of increased transmission from older individuals was independent of severity of illness and may be related to the respect given to older people in Sierra Leonean households and the additional care and visitors that might engender. Higher case fatality in very large households may be due to a greater number of older members, to crowding, or to the difficulty of caring for more ill members. All these factors suggest that where resources are limited, intensifying attention and support to larger affected households could be justified, particularly for example in infection prevention and control measures which may help households themselves act to prevent transmission.

Subsequent studies have reported similar associations between household size and older age and increased household transmission. Caleo *et al* reported transmission occurred exclusively through a small number of large households, stoked by the need to provide care for sick relatives.[95] Robert *et al,* analysing risk factors for transmission in 129 chains from the latter half of the outbreak in Guinea, and Lindblade *et al* in Liberia concurred with the positive association with older adults and that young children were least likely to transmit.[97, 117] Two studies have contradicted these observations: Skrip *et al* in Liberia reported higher risk of household transmission from under 15 year-olds but noted that their definition household was limited by lack of information on addresses, and that no difference in transmission was seen by age overall.[96] Reichler *et al* reported transmission was more likely in households with younger index cases but validity of the finding was limited by the small number of child index cases.[98]

Others have highlighted similar findings to ours regarding the importance of how sick a case becomes in the household and contacts' involvement in providing care. Investigating a Sierra Leonean cohort

from later in the epidemic than ours, Reichler *et al* reported that death of an index case in the household, length of time a wet case spent in the household, household members providing care to a case, and absence of reported fever in the source case as significant factors in transmission, while behaviours adopted to avoid contact with body fluids of the source were protective.[98] Faye *et al* in Guinea,[94] and WHO's West Africa International Ebola Response Team analysing ~6500 cases across the three affected countries,[118] both reported reduced transmission from cases admitted early to hospital compared to those who remained in the community. Dean's systematic review highlighted higher secondary attack rates in contacts who provided direct nursing care than those that did not.[50] However, in contrast to our findings that source fatality increased risk regardless of location of death, Lindblade *et al* found that in rural Liberia and Guinea more secondary infections resulted from people who died at home than those who died in ETCs, possibly related to delay in transfer to care.[99]

All these studies support the importance of a response approach which combines increased knowledge and capacity in families to protect themselves while caring for a sick household member with building community trust in ETCs to encourage early isolation. Such an approach has the potential to reduce transmission related to household exposure to wet symptoms[119] as well as improving chance of survival for the case.[120]

Lastly, several studies have suggested very low cycle threshold (CT) (the PCR parameter that indicates viral presence: low CT indicates high viral load) in the primary case is predictive of high risk households and could be used to prioritise investigation, contact tracing and control efforts if needed.[94, 121] However as having a CT value relies on case willingness to present as well as efficient sharing of laboratory results, scrutinising larger households may be more effective in providing an earlier alert if resource constraints require prioritisation.

Understanding case fatality

The adjusted CFR of 67.0% in our study is much higher that the 28% originally cited by WHO for Sierra Leone.[122] However, it is very similar to the 62.9% reported by Garske *et al* after their 2017 reanalysis of data officially reported to WHO from the three most-affected countries, in which they included 'suspected cases' and excluded those with no recorded outcome.[42] Using modelling and imputation Garske also found age had a profound effect on survival probability, as have other studies,[120, 123] and concluded that as age trends were similar across all three countries there is good reason to believe that the sparing of children and teenagers is a real biological effect.

Aside from increasing age as an alerting factor, our finding of higher CFRs in larger households mirroring increased risk of transmission described above, provides another reason for increased focus on these households during response. Studies subsequent to ours [42, 124] also concurred on the importance of time period in the epidemic in relation to CFR, highlighting the significance of both

availability of and trust in ETC care. Away from the community, ETC-based studies in West Africa and Uganda have identified CT value at admission as an important indicator of CFR and suggest the value could be used to prioritise enhanced medical support.[120, 125]

As noted above, there is little evidence to suggest a 'dose' response related to outcome: people exposed to sources with more severe disease do not appear to develop more severe disease. Biological mechanisms driving outcome remain to be clarified, though different immune responses and genetic signatures have been reported in survivors and fatalities from early in disease course.[126-128]

Risk of death post-discharge

Our study was the first to quantify the risk of dying from illness potentially associated with EVD after discharge from ETC care. Previous studies of sequelae reported only on patients seen in follow-up clinics so missed any later deaths. Our finding of a relatively low risk of 'late death' – 2.6% over a mean of 10 months after discharge – has been reinforced by Keita *et al*'s larger study in Guinea which found a similar estimate of 5.2% late deaths among 1130 survivors over a median of 22 months from discharge[129] Due to its size, this study was also able to assess excess mortality in survivors post-discharge, finding it five times higher than expected for the general population during the first year of the epidemic, and only dropping to a similar level after December 2015, two years after the beginning of the epidemic. Post-discharge mortality was higher in those who spent longer in an ETC and, unexpectedly, the highest post-discharge excess deaths in the early outbreak period were seen in children aged 5-14 years, the opposite of fatality during illness. No study has assessed frequency of post-discharge deaths in Liberia, but one that enrolled antibody-confirmed survivors and uninfected contacts 12 months after the survivor's discharge found similar death rates in both groups, consistent with the Guinea study at 12 months.[74]

Recrudescence and persistence

The understanding that ebolavirus RNA can be detected in immune-privileged sites in the body in some cases for many months,[16] strong suspicion of cases of sexual transmission, and case reports of a small number of suspected recrudescent cases[14 13, 15, 17, 18, 55, 130, 131] indicate that survivors are likely to be an important source of future outbreaks.

Our study was the first to document potential for reactivated illness in a large cohort of survivors and our finding that <1% experienced recrudescence of active viral illness leading to death, suggests this is unlikely to be an important cause of fatality among survivors. But viral persistence as an outbreak trigger is another matter. Prior to 2013, new Ebola outbreaks rarely occurred in the same location, a pattern indicative of zoonotic spillover. But these earlier outbreaks had relatively few survivors, compared to the unprecedented numbers now in existence. A 2019 investigation of all reported cases in the West Africa epidemic identified only 13 possible events of viral persistence-derived transmission

of Ebola,[132] but by 2020-21 three out of the four new outbreaks in DRC and Guinea were linked to viral persistence in survivors,[14, 15, 17] and the fourth in Mbandaka, DRC showed evidence of both zoonotic spillover and genetic links to the 2018 outbreak there.[133] The unexpected finding that the 2021 outbreak of EBOV in Guinea (16 cases, 12 deaths) was caused by a reactivation of infection dormant in a survivor without symptoms for at least 5 years[14] means the possibility of outbreaks triggered by survivors cannot be ignored in preparedness and surveillance planning for some time to come, nor the implications for care of survivors. Other researchers have suggested that naturally acquired immunity to EBOV may decay and spike in waves prompted by reactivation of sequestered virus or by re-exposure (zoonotic or human), adding to possibility of sudden survivor-triggered outbreaks.[85]

Though survivors are a major concern, more reliable means of predicting risk of zoonotic spillover are also needed. As environmental and land use changes open up new opportunities for contact, better understanding of the animal–human interface, including clarity regarding the EBOV reservoir host(s) and what drives risk of spillover, could help predict locations at high risk of spillover and facilitate surveillance and preparedness. Current indicators are too broad and at-risk countries have too limited resources and too many infectious threats to focus attention on possible Ebola spillover based on them, however studies exploring methods of increasing the reliability of predictions are underway.

Minimally-invasive alternative

The EBOV oral fluid G-capture assay proved to be high performance minimally-invasive alternative to phlebotomy. It was well accepted in the community, can be self-applied, and carries minimal biohazard for staff – all of which provide significant advantages for undertaking research during an Ebola outbreak or in any region suspected of having Ebola virus circulation where taking blood is difficult for social, cultural, or biohazard reasons. Oral fluid samples are also easier for participants to give and for researchers to work with. The assay is kit-based and can be analysed at the bench in low resource laboratory settings unlike blood samples which require Biosecurity Level 4 containment when viral haemorrhagic fever infections are possible. Swabs can be kept without processing in normal vaccine cool boxes with icepacks and stored in a -20°C freezer.

In our study, only eight participants (1.2%) refused to swab, six from one family. A ongoing study in Guinea using the same assay, on which I am a co-investigator, found 77% of community participants preferred to give an oral fluid sample than a blood sample and 21% had no preference.[134] Furthermore, 80% of participants in a community-based study to validate a similar oral swab-based assay for Lassa Fever, of which I was principal investigator, said they would be happy to give an oral fluid sample regularly for research and preferred oral fluid donation to blood because it was easier and pain-free.[135] Other researchers have shown close correlation between oral fluid and serum EBOV

antibody results, including an investigation in Guinea of paired blood and oral fluid samples in the largest cohort tested the longest time after EBOV infection (61 subjects, 6-7 years, R^2 = 0.70, p <0.0001). [57, 136]

As the Ebola oral fluid assay identifies IgG, which is detectable only from 8-10 days after onset of symptoms,[137] it does not have a role in acute diagnosis or contact tracing. But its attributes can facilitate the mass community research that has been so difficult to attempt using high-risk phlebotomy.[51] With good community communication, samples could be collected and analysed much closer to the time of infection when presence or not of symptoms and related characteristics can be more accurately captured.

Oral fluid sampling may also be able to improve surveillance and preparedness. The ongoing Guinean study mentioned above is currently testing whether the oral fluid assay can be used to search for un-noticed spillover events in high-risk communities which could then inform enhanced surveillance activities and preparedness. If proven effective, such screening would be a resource-sparing way of putting in place the early alert essential for authorities to act rapidly to prevent spread. Oral fluid assays could also be used to assess exposure to infection prior to vaccine trials, and vaccine coverage through pre/post surveys (differentiating between vaccine and natural immunity cannot yet be done as both assay and current vaccines target GP), reducing both costs and participant burden.

Finally, the assay used in our studies was by no means the first oral fluid assay, but our demonstration of its value in the field in a viral haemorrhagic fever environment and the high performance achieved has contributed to development of new oral fluid assays for Lassa Fever[135], Zika[138] and SARSCoV2.[139]

9. STUDY LIMITATIONS AND REFLECTIONS

Missing households

The most important limitation of this study is that it was built on ETC survivor support links and therefore did not include households in which all EVD cases died. This may have led to under-representation of small households, as there would be less chance that they would include a survivor, and missed households which did not seek care, both of which may have had different exposure and outcome experiences. Although, we were able to address the survivor bias by adjusting parameters using an estimation of the overall CFR, and relative risks by age and exposure should not be biased, on reflection I believe that we could have found ways to address the challenges that led to the decision not to try to recruit households that had suffered only fatal EVD cases and inclusion of such households would have reinforced our findings. The reasons for excluding these households were two-fold: it would be more difficult to locate them as they were not registered in the survivors' programme, and

we feared that families with only fatal cases might be more distressed by our approach than those who experienced a combination of survival and death, especially given the ongoing epidemic.

In hindsight, having witnessed the extent of loss experienced by the participating survivor households and having explored some of literature regarding research involving bereaved families in other disease fields, I have come to see that it was unlikely that those experiencing only fatality at that time would have been more traumatised by our approach than those experiencing a combination of fatal and surviving cases. While it has been a commonly-expressed concern that involving newly bereaved households in research by households could provoke further distress or even physical trauma,[140 141] recent research suggests this is not a given, that risk of negative events is linked to methods used and, indeed, that well-designed qualitative research can even have therapeutic effects for bereaved particpants.[142-144]

Much learning in Africa around methodologies for working with the bereaved stems from the field of HIV research, where in-depth household case studies are often the central tool. One study from Zimbabwe on the impact of AIDS-related parental loss on children, for example, highlights the importance of spending time in households and using participatory techniques to allow stories to emerge in participants' own way and time.[145] Other researchers have emphasised that an initial mode of approach that allows potential subjects time to consider their participation (through personalised letters or calls from third parties for example) can make research even with very recently bereaved families feasible and acceptable.[143, 144] Many participants in these studies report gaining solace from altruistic reasons for taking part in research, while others found it personally therapeutic to have the opportunity to tell their story in their own time and own way.

In hindsight, I believe the method of engagement we developed, using un-time-bound family conversations would have been able to manage and mitigate distress felt by only bereaved households, just as it did with participant households, 70% of whom had also suffered bereavement. And while it may not have been possible to locate all families that suffered only deaths, especially given the incompleteness of information collected on patients during the height of the epidemic, we could have attempted to do so through interrogating the ETC medical records. Therefore, should I be in a similar position in the future, I would make all efforts to include all households.

Reliance on recall

The study relied on the recall of live survivors and household members, both for their own experience and to provide proxy information for those who had died. Although the period between the Ebola event and the interview was relatively short (4-9 months), accurate recounting especially of the kind of detail we requested is difficult: people may forget or be reluctant to disclose, or may be hyper-sensitised to such aspects as symptoms and exposure and so over-report. However, the strong linear

association between exposure and disease in the study was reassuring and we believe our whole-household qualitative method mitigated this bias to a large extent.

Sample and assay limitations

The absence of paired serum/oral fluid samples was a potential limitation for assay validation, but this was mitigated by using survivors confirmed PCR-positive in a high quality laboratory as the standard, and unexposed controls. This had the additional advantage of allowing validation to take place in an endemic country and demonstrating feasibility of deploying a non-blood-based assay during a viral haemorrhagic fever outbreak. It has been suggested that sub-clinical infection may produce a larger response to the EBOV protein VP40 while survivors respond more strongly to EBOV anti-glycoprotein (GP),[146] meaning our findings on pauci- and asymptomatic could be underestimated as the oral fluid assay targets only GP. Due to our in-country focus, we could not use sophisticated laboratory work such as neutralisation or multi-protein assays. However, based on two large studies which found minimal differences in proportion of asymptomatic infection identified by the three key EBOV protein assays (GP, NP, VP40) and neutralisation,[73] [147] we believe this bias is unlikely.

Finally, we were limited in the analysis of exposures in pauci-symptomatic and asymptomatic participants due to the small number of events. But we did see close correlation between level of exposure and seropositivity in both asymptomatic and symptomatic contacts supporting the interpretation that these are true manifestations of Ebolavirus infection.

10. CONCLUSION

The scientific effort that occurred during the 2013–16 epidemic has considerably advanced the evidence base for Ebolavirus infection. I believe the work I have done in collaboration with colleagues has contributed to these advances, providing robust information about the manifestations and risks of Ebolavirus infection, adding levels of understanding regarding the patterns of Ebolavirus transmission, highlighting the critical importance for prevention and control of gaining complete information beyond hospitalised cases, and demonstrating new tools and approaches that make working with households and communities to identify people at risk of infection more feasible.

Many questions remain and, unfortunately, some answers will likely only be found with further outbreaks. But with enhanced knowledge of transmission routes encouraging more effective reduction of exposure, in addition to new vaccines and treatment approaches, we hope the human toll will be considerably diminished.

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SECTION 2: RESEARCH PUBLICATIONS

Paper 1: A systematic review and meta-analysis of seroprevalence surveys of Ebolavirus infection

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Student ID Number	021367	**Title**	Ms
First Name(s)	Hilary		
Surname/Family Name	Bower		
Thesis Title	Ebola virus transmission and disease severity in Sierra Leone 2013-16		
Primary Supervisor	Professor Jimmy Whitworth		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where was the work published?	Nature Scientific Data		
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Analysis: A systematic review and meta-analysis of seroprevalence surveys of ebolavirus infection

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Asymptomatic ebolavirus infection could greatly influence transmission dynamics, but there is little consensus on how frequently it occurs or even if it exists. This paper summarises the available evidence on seroprevalence of Ebola, Sudan and Bundibugyo virus IgG in people without known ebolavirus disease. Through systematic review, we identified 51 studies with seroprevalence results in sera collected from 1961 to 2016. We tabulated findings by study population, contact, assay, antigen and positivity threshold used, and present seroprevalence point estimates and 95% confidence intervals. We classified sampled populations in three groups: those with household or known case-contact; those living in outbreak or epidemic areas but without reported case-contact; and those living in areas with no recorded cases of ebolavirus disease. We performed meta-analysis only in the known case-contact group since this is the only group with comparable exposures between studies. Eight contact studies fitted our inclusion criteria, giving an overall estimate of seroprevalence in contacts with no reported symptoms of 3.3% (95% CI 2.4–4.4, $P < 0.001$), but with substantial heterogeneity.

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Introduction

Knowing if ebolavirus infection manifests asymptomatically is critical to understanding its spread and to estimating the role herd immunity could have in reducing transmission. Investigating unrecognised infections could also help in the development and targeting of vaccines. However, despite a surprisingly large number of investigations into the seroprevalence of ebolavirus IgG since the first outbreak in Yambuku, Zaire (now Democratic Republic of Congo)[1–51], consensus on results has proved elusive. The main reasons for this are the range of findings, positive results in unexpected locations, and a lack of confidence in immunofluorescence antibody (IFA) tests used in early studies.

Concerns about IFA specificity stem largely from studies showing positive results in populations expected to be negative, although the most frequently cited—in 200 Panamanian Indians with no known exposure—found only one Ebola virus IgG positive on a high cut-off giving a specificity of 99.5%[4]. Unexpected seropositivity has also been seen in African countries without reported cases of ebolavirus disease (EVD) such as the Central African Republic, Cameroon and Zimbabwe, only some of which can be attributed to using low test cut-offs. But, as some ELISA-based studies have produced similar findings[37,38], these positive results may indicate zoonotic exposure with filoviruses or unrecognised human-to-human transmission rather than poor specificity.

'Asymptomatic' status can only be defined for a certain period, such as during an outbreak, though excluding mild symptoms is difficult. In outbreak areas asymptomatic subjects could have experienced unrecognised symptomatic EVD in the past so, even apart from problems with the test, ebolavirus antibody seropositivity does not necessarily mean asymptomatic infection.

We aimed to provide an up-to-date and easily accessible overview of serological findings to date, to help researchers contextualise studies prompted by the 2014–16 West Africa epidemic. The most comprehensive review of ebolavirus serology—Kuhn's Filoviruses: A Compendium of 40 years of Epidemiological, Clinical and Laboratory Studies[52]—covers work to 2008. In addition to reviewing this key reference, we carried out a systematic review of serosurveys in people without symptoms of EVD up to July 2016.

Results

Characterisation of seroprevalence surveys of IgG antibodies to ebolavirus

We identified 51 studies covering 84 sample populations reported to have had no symptoms of EVD during the outbreak period, or to have come from populations with no known outbreaks. In total these studies investigated the presence of ebolavirus IgG in 44,147 subjects using samples collected since 1961.

Thirteen studies reported 16 study populations involving 2,664 participants with household or known case-contact[5–7,9,12,36,41,42,45,47,49–51]. Eleven studies reported 17 study populations covering 5,327 participants living in outbreak areas but without reported case-contact[5–7,9,14,33,39,40,42,43,46]. The remaining studies reported on 51 groups involving 36,156 subjects from general populations, often in settings ecologically similar to ebolavirus outbreak areas but without known cases of EVD[1–3,5,8,10,11,13,15–35,37,38,44–46,48,51].

Table 1 (available online only) gives a detailed breakdown of the study populations, test methods and results.

Overall estimates of ebolavirus seroprevalence in asymptomatic individuals

Only the group with known case-contact had exposures that are comparable across studies and are therefore appropriate to combine by meta-analysis. In this group eight study populations fulfilled the inclusion criteria of testing by ELISA or using a IFA cut-off ≥1:64 (ref. 5,36,41,42,47,49–51). Pooling these results gave an overall estimate of seroprevalence in asymptomatic people with known case-contact of 3.3% (95% CI 2.4–4.4, $P < 0.001$), but with substantial heterogeneity due to three small studies with higher estimates.

In the other two categories—participants living in outbreak areas but without reported case-contact exposure and general populations in areas without known cases of EVD—exposure was either not well characterised or not well known. Even where EVD cases had not been reported, zoonotic exposure or different forms of disease manifestation could not be ruled out. The highly heterogeneous nature of these study populations makes any single summary estimate inappropriate. In outbreak areas estimates ranged from 0.9 to 17%, and in general populations described as unexposed estimates ranged from 0 to 24%.

Evidence of assay validation

Few teams reported any validation of the assays used. Some studies repeated analyses with the same technique, usually in a US or European laboratory, but only seven of the 51 studies reported validation work through a different diagnostic platform. Of these, two retested a proportion of IFA positives against ELISA, finding close to 100% consensus[26,30]. Three tested ELISA against western blot of which two found 100% specificity[38,46,53]; the third did not report results[41]. Another found 77 and 75% specificity for ELISA against western Blot and IFA respectively[34], and a further study confirmed IFA results by western blot but did not report results[33].

Two studies in Sierra Leone included field testing of ELISA assays in PCR-confirmed positive samples from EVD survivors and community controls with no known exposure to EVD cases from the research area. One, using a novel IgG-capture ELISA[54], found 95.9% (95%CI 89.9–98.9%) sensitivity and 100%

specificity (95%CI 98.9–100%) using oral fluid samples from 97 survivors and 339 community controls[51]. The other, using the commercially available ALPHA Diagnostics assay, selected a cut-off that gave 96.7% sensitivity and 97.7% specificity in serum samples from 30 survivors and 132 community controls[50].

Discussion

We identified 51 studies covering 84 sample populations of 44,147 subjects reported to have had no symptoms of EVD during the outbreak period or to come from populations with no known outbreaks. Most data originated from Western and Middle Africa, and were collected during epidemiological investigations around outbreaks, or in serosurveys in countries without outbreaks but with similar ecology and animal hosts, which aimed to map the geographical extent of the virus. Some studies reported retrospective analysis of samples collected for other reasons prior to the first known outbreak in 1976.

An important finding of our review is the extreme heterogeneity of the studied populations and the lack of clarity in describing their exposure levels. We found that while some studies characterised their sample population clearly by level of contact and presence of symptoms, in many the level of contact/exposure was less clear, and some did not separate results for symptomatic and asymptomatic subjects. This makes comparison of results difficult, and combining results from the majority of the studies impossible. It may also explain the wide variation of findings which have perplexed investigators over time.

Many studies also employed very different cut-offs to define seropositivity meaning a simple review of results can be misleading. For our analysis, we excluded any study that used a cut off below $\geq$ 1:64 for the studies using IFA, based on the advice in the literature, but there is no definitive evidence that this is an appropriate threshold. The cause of low IFA titre and whether it reflects false positives, or waning antibody response resulting from historical infection which may or may not have been symptomatic, has been frequently discussed. Recently 10 of 12 survivors from Yambuku were reported to have varying degrees of EBOV GP and NP reactivity by ELISA, 40 years after the outbreak[55]. Other studies have shown positive ELISA results in survivors up to 11 years after infection, but neither reported IFA results for comparison[56].

There is no international reference measurement procedure for ebolavirus antibodies and the World Health Organisation has acknowledged the urgent need for one. Interestingly, given the scepticism often expressed regarding the specificity of IFA techniques in ebolavirus serology, a WHO collaborative study undertaken in 2015 to identify an interim reference standard found IFA no less specific or sensitive than the other methods employed, but only a few samples were tested[57].

There are several limitations to the work presented here. The full information necessary for precision or clear interpretation was often not available. To pursue as high quality research as possible, we have focussed on publications that have undergone peer review and did not search grey literature. With the exception of Kuhn *et al.*[52], which has been the standard reference on filovirus seroprevalence surveys to date, we did not search books. In addition to the limitations of the studies themselves noted above and in Table 1, we also note that the distinction of symptomatic and asymptomatic in the papers relied on self-reported health status, which may not be reliable.

To conclude, we present here a comprehensive updated review of seroprevalence surveys for ebolavirus infection in order to better understand the variation in rates found. We highlight the urgent need for validated standardised assays and for detailed characterisation of study population exposures to enable more generalizable estimates of the extent of asymptomatic ebolavirus infection to be made.

Methods

Search strategy and systematic review

A systematic search was done in PubMed to identify peer-reviewed papers presenting original data on ebolavirus infection seroprevalence using the following search string:

ebola AND (asymptom* OR antibod* OR IgG OR immun* OR ELISA OR serol*) NOT vacc* NOT immuniz* AND (Humans[Mesh])

No limitations were placed on language or location of study. Reference lists of the most comprehensive review to date[52] and other papers were also reviewed. Although the focus of interest was data on subjects reported not to have symptoms at the time of an outbreak, we included papers reporting seroprevalence in all populations apart from those with diagnosed EVD in the initial review to ensure relevant studies were not missed.

The search produced 355 citations which were reviewed by title and abstract. Inclusion criteria were: investigation of any African species of ebolavirus immunoglobulin G (ie. not Reston) in individuals without ebolavirus symptoms or in general population groups, with information on denominators and seropositivity and description of those tested. The same search but limited to 2008 to 2016 was rerun on Web of Science; references prior to 2008 were checked against Kuhn *et al*'s list[52]. Four additional citations were found on Web of Science but none were retained for detailed reading. Six citations for papers not already included were identified from reference lists and retained for detailed reading.

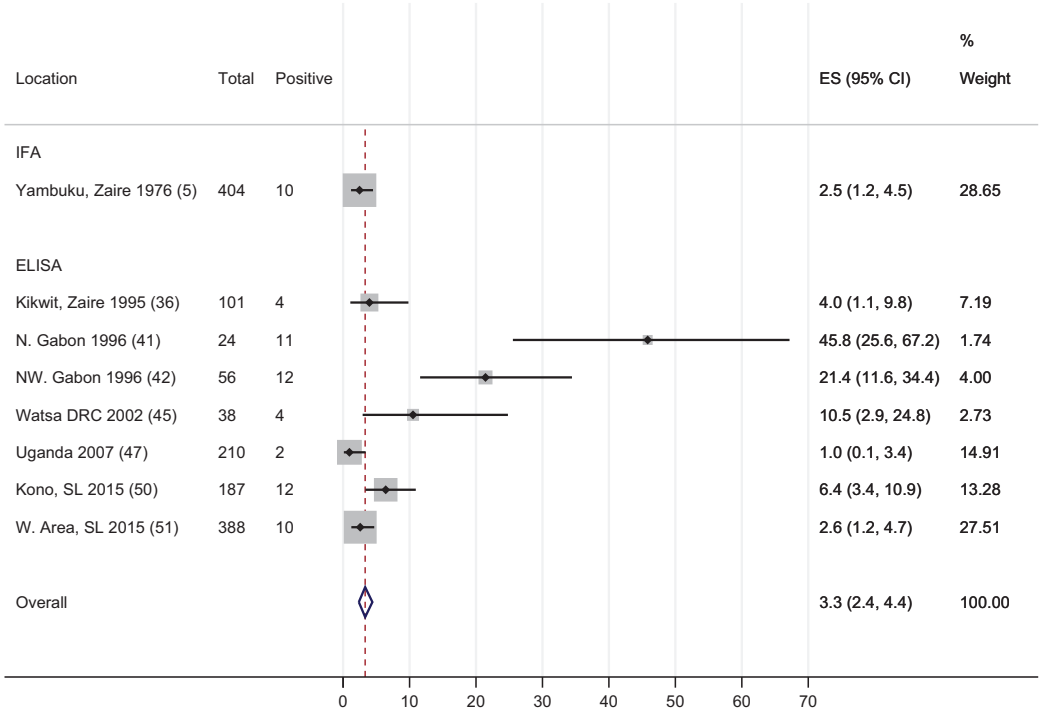

Figure 1. Forest plot and meta-analysis of seroprevalence of ebolavirus IgG among contacts of EVD cases reported to be asymptomatic during the outbreak period. Further details of each included study are given in Table 1. Legend: Ref: reference number; IFA: Immunofluorescence Assay; ELISA: Enzyme-linked immunosorbent assay; ES: Estimated proportion; N, NW: North, Northwestern; SL: Sierra Leone; W. Area: Western Area Province. Note: Zaire now Democratic Republic of Congo; Rhodesia now Zimbabwe.

Total citations: 365 of which 297 (81%) discarded for the following reasons:

- Detailed immunology or genetics with no relevant data collection for seroprevalence
- Description of acute phase diagnosis and/or investigation of convalescent subjects
- Epidemiology and/or treatment of symptomatic confirmed cases without investigation of non-case populations
- Investigations on sample populations without identifiable non-symptomatic individuals
- Studies examining immune response related to vaccination trials
- Review/comment articles without original data
- Modelling papers without original data
- Preliminary or duplicate reports of the same research study/data.

Sixty-eight papers were read in detail after which a further 20 were discarded for the reasons above. Data extracted from the remaining 48 papers included date of sera collection, composition of study population(s) in terms of exposure, location, selection process and any other defining characteristics, assay type, technique and antigens used, positivity threshold, number of participants per population type, number/proportion of IgG positive individuals, and any information on repeatability or test validity. All selected papers were scrutinised by both authors independently and results discussed and reconciled.

The last search was made on 31 July 2016. Two presentations from the 2016 Conference on Retroviruses and Opportunistic Infections (CROI, Feb. 2016) and one from the 8th International Symposium on Filoviruses (Sept. 2016) describing findings from the 2014–2016 outbreak were also included. A paper reporting one of the CROI presentations has subsequently been published (Nov 2016) and is referenced.

Categorisation of exposure

Many of the studies reported results on sub-populations with different exposures. To reduce heterogeneity for analysis we categorised these sub-populations under three broad headings according to the extent of exposure: household or known case-contact; living in outbreak areas but without reported case-contact; and subjects drawn from general populations in locations without known EVD. Where study populations were reported to include symptomatic cases and gave enough information to identify these cases, we removed them and recalculated results.

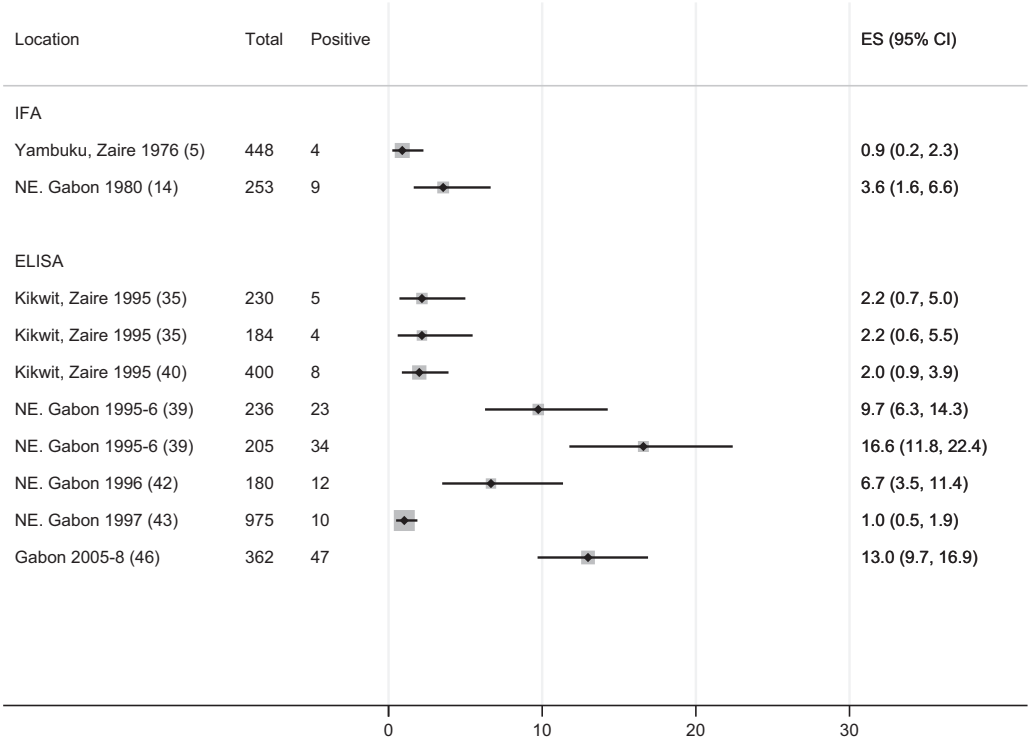

Figure 2. Forest plot of seroprevalence of ebolavirus IgG in individuals reported to be asymptomatic during the outbreak period, recruited in areas with known EVD cases, excluding direct contacts of EVD cases. Further details of each included study are given in Table 1. Legend: Ref: reference number; ES: Estimated proportion; IFA: Immunofluorescence Assay ELISA: Enzyme-linked immunosorbent assay; DRC: Democratic Republic of Congo; N, NE: North, Northeastern.

We excluded one study of PCR negative 'suspects' with close, no or unknown contact exposure due to lack of information on symptom status[58]. In two other studies, sub-groups were not included in the table because they were reported to include symptomatic cases but gave insufficient information to allow recalculation of the seroprevalence estimate excluding those with symptoms[1,18].

Interpretation of seropositivity

We have recorded seropositivity results by antigen species where reported; where results were not reported by species, we record positivity to 'ebolaviruses'. 'Overall' positivity is noted where it was reported or where it was possible to rule out double-counting.

To expose the problem of the different positivity thresholds used, we have recorded all studies and their reported cut-off in Table 1. Study characteristics and results have also been formatted as a machine-readable open access dataset (Data Citation 1).

Data visualisation

To summarise the data visually and present 95% confidence intervals, we created Forest plots for each of the three exposure categories (Figs 1,2,3) which allow results to be compared in the different contact groups. To address the problem of varying thresholds, we included only those IFA studies that reported results according to the 1:64 titre cut-off cited as more stringent by WHO and others[5,18,21,59], or which reported enough detail for this threshold to be applied. For ELISA studies, the range of methods used to define positivity was too wide to assign a common threshold so all have been included in the Forest plots, with their method of defining the cut-off detailed in Table 1 (available online only).

Statistical analyses

We performed a meta-analysis using the Freeman Tukey arcsine square root transformation method and 'fixed effects' (weighted average) inverse variance (*metaprop*, STATA[60]) on the eight study populations with known-case contact. We chose a 'fixed effects' (weighted average) model as contact should give

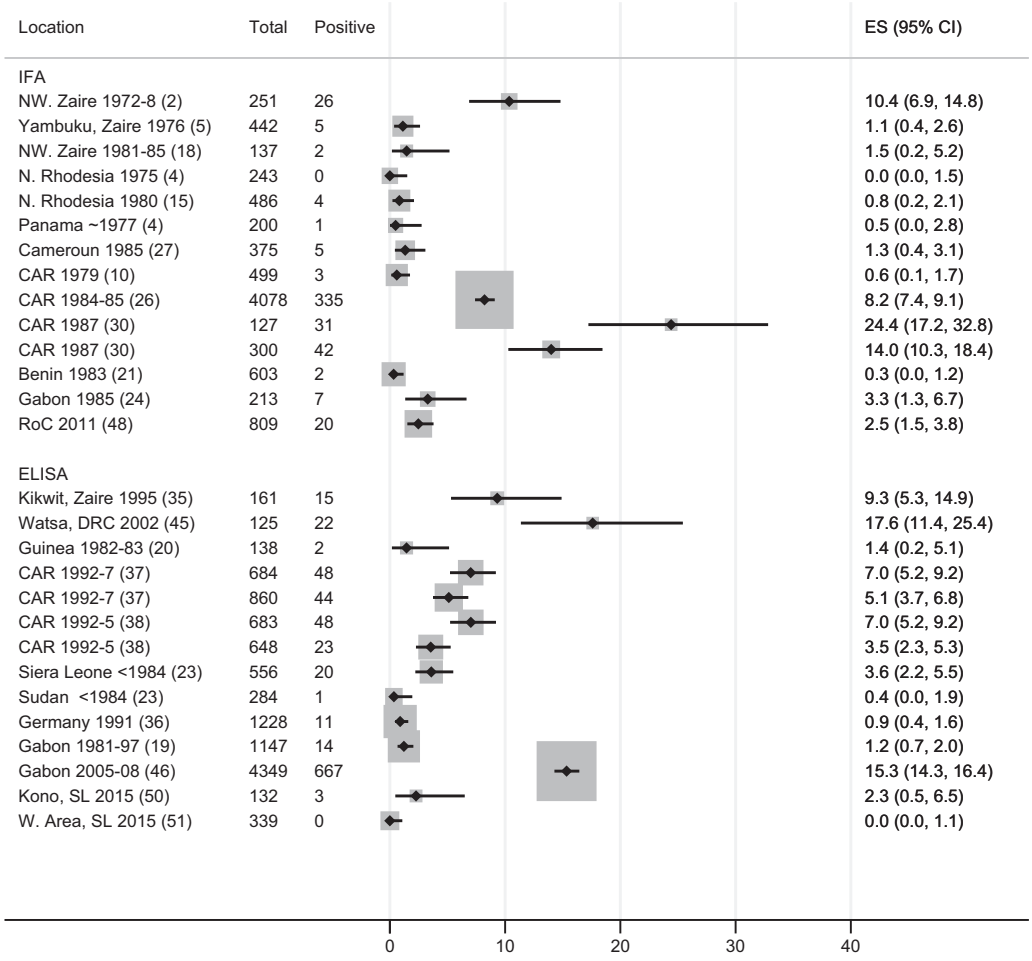

Figure 3. Forest plot of seroprevalence of ebolavirus IgG in general populations living in areas without reported EVD cases. Further details of each included study are given in Table 1. Legend: Ref: reference number; IFA: Immunofluorescence Assay; ELISA: Enzyme-linked immunosorbent assay; ES: Estimated proportion; IFA: Immunofluorescence Assay; ELISA: Enzyme-linked immunosorbent assay; DRC: Democratic Republic of Congo; RoC: Republic of Congo; CAR: Central African Republic; N, NW: North, Northwestern. Note: Zaire now Democratic Republic of Congo; Rhodesia now Zimbabwe.

similar risks in different contexts, and because random effects models give too much weight to small studies[61]. We present an pooled summary estimate for the group with known contact exposure (Fig. 1). We do not show summary estimates for the groups covering subjects living in outbreak areas but without reported case-contact, or drawn from general populations in locations without known EVD (Figs 2 and 3) as these populations are likely to have very different exposure levels so an overall summary estimate of prevalence would be meaningless.

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Data Citations

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Author Contributions

H.B. conceived the review, created the search strategy, retrieved and screened the papers, extracted and compiled the data, created the graphics, carried out the analysis and drafted the paper. J.R.G. reviewed the strategy and analysis, checked all data extraction, and contributed to subsequent drafts of the paper.

Additional Information

Table 1 is only available in the online version of this paper.

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Table 1: Findings of seroprevalence studies investigating presence of ebolavirus immunoglobulin G antibodies in 'asymptomatic' populations, 1961 - 2016

A = populations with household contact or known case contact

B = populations without known contact but in outbreak areas or villages with cases

C = general population – no known outbreak exposure or contact

Location	**Year sera collected**	**Population type** (as described in paper)	**Group**	**No. of samples**	**# IgG +ve**	**Species % +ve**	**Assay type**	**Cut-off** (as described in paper	**Antigen & validation information**	**Notes**
Assab, Awash, Blue Nile, Illubor, & Ogaden regions, Ethiopia[1]	1961/62	Asymptomatic individuals from area not affected by ongoing Yellow Fever epidemic	C	178	42	EBOV 24%	IFA(w)	≥1:16	Antigens: Polyvalent ELM (Ebola-Lassa-Marburg viruses) & monovalent EBOV (Mayinga): all sera reacting with ELM also reacted with monovalent EBOV antigen but not with monovalent Marburg and Lassa.	Samples from symptomatic Yellow Fever-negative individuals also tested: 12% positive.
N.W. Zaire (now Democratic Republic of Congo DRC)[2]	1972-78	General population from area west of Yambuku (site of 1st recorded outbreak in 1976)	C	251	43 26	EBOV 17.1% 10.4%	IFA(w)	≥1:16 ≥1:64	Antigen: EBOV (May. 80826)	
Harbel, Bong town, Yekepa, Liberia[3]	1973	Staff & family members of rubber and mining companies	C	592	83	Ebolavirus 14.0%	ELISA/ WB	Not stated	Antigen not stated	Sera taken in 1973 and investigated for Lassa and ebolavirus in ~1986. Ebola statistic reported without further information.
Northern Rhodesia (now Zimbabwe)[4]	1975	"Control group"	C	243	2 0	EBOV 0.8% 0.0%	IFA(w)	≥1:8 ≥1:64	Antigen not specified in report: Kuhn[52] notes antigen as EBOV	Areas sampled had no known outbreak
Yambuku, Zaire (DRC)[5]	1976	Asymptomatic contacts of cases	A	404	10	EBOV 2.5%	IFA(w)	≥1:64	Antigen: EBOV Validation: Repeat testing of the 4 antibody positive with no known contact gave the same results. 32/33 (97%) positive samples (including samples from cases) confirmed positive by CDC (US)[4] (p141)	
		Residents from villages with cases but no known contact	B	448	4	EBOV 0.9				
		Residents from 4 neighbouring villages with no cases	C	442	5	EBOV 1.1%				
Maridi, Sudan (now South Sudan)[6]	1976	Close family contacts	A	93	13	SUDV 14.0%	IFA(w)	≥1:8	Antigen: SUDV Validation: 42 of 48 clinically diagnosed survivors from Nzara (87%) were considered positive using the same IFA protocol. Several samples from Nzara were retested and confirmed positive by CDC (US) using the same protocol.	9 antibody positive family contacts had symptoms and have been excluded from these figures. Not clear if all subjects in this group were interviewed about symptoms.
		Asymptomatic Maridi schoolboys with no known contact	B	29	3	SUDV 10.3%				
		Asymptomatic hospital staff with probable/possible contact	A	64	7	SUDV 10.9%				4 nurses; 1 cleaner, 1 toilet cleaner, 1 water carrier were positive

Nzara, Sudan (now South Sudan)[6]	1976	Asymptomatic cotton factory workers (site of index case but reportedly no direct contact)	B	109	7	SUDV 6.4%	IFA(w)	≥1:8		Among factory workers titre range was 1:16 - 1:32
		Close family contacts of clinically diagnosed cases	A	78	1	SUDV 1.3%				Only 6/31 (19.4%) of clinically diagnosed subjects were antibody positive, and none had levels > 1:32
San Blas Islands, Panama[4]	1977	San Blas Indians	C	200	1	EBOV 0.5%	IFA(w)	≥1:64	Antigen: not specified in report: Kuhn[52] records antigen as EBOV	Areas sampled had no known outbreak. Method of selection not known
Tandala, Zaire (DRC)[7]	1977-78	Missionaries and 'a few' hospital staff with case contact (1977)	A	50	0	EBOV 0%	IFA(w)	≥1:16	Antigen: EBOV	One doctor, who tested positive in 1977 and 1978 and had history of severe illness after attending the autopsy of a haemorrhagic fever victim in 1972, is excluded. Some individuals gave samples in both the 1977 and 1978 groups
		Hospital staff with case contact (1978)	A	71	0	EBOV 0%				
		Residents of villages with confirmed /suspect cases	B	346	21	EBOV 6.1%				
		Residents of other villages in same area	B	750	58	EBOV 7.7%				
Liberia [8]	1978-79	Random rural general population in multiple counties	C	433	26	Ebolavirus 6.0%	IFA(w)	≥1:16	Antigens: unspecified ebolavirus, Marburg & Lassa viruses. No sera positive for ebolavirus was positive for MARV or LASV.	No known outbreak. Titre range: 1:16 to 1:1024
Nzara/Yambio, (South) Sudan[9]	1979	Asymptomatic adult family members of cases with known physical contact	A	38	12	Ebolavirus 32%	IFA(w)	≥1:16	Antigen: unspecified	
		Asymptomatic adult family members of cases who denied physical contact	B	23	3	Ebolavirus 13%				
		Adults from families without known cases in same area	B	45	8	Ebolavirus 18%				Unknown if these people were exposed in 1976 outbreak, which could explain the high prevalence
Bangassou, Central African Republic (CAR)[10]	1979	General population in forest and semi-forest zones	C	499	10 3	Ebolavirus 2.0% 0.6%	IFA(w)	≥1:16 ≥1:64	Antigen: Polyvalent of unspecified ebolavirus, MARV & LASV, followed by monovalent test for positive samples. Positive sera sent to CDC (US) for repeat testing; results not reported.	Areas sampled had no known outbreak
Moloundou, Lolodorf Bipindi, Lomie, Yaounde & Pete, Cameroon[11]	1980	General population in five regions (forest, pre Sahelian savannah and the capital) and different ethnic groups	C	1517	147	Ebolavirus 9.7%	IFA(w)	≥1:16	Antigens: unspecified ebolavirus provided by CDC (US)	No known outbreak. Positives in all areas, range 3%-23%. Highest in Pygmies and rain forest farmers. 6% in the capital, Yaoundé. Report to OCEAC in the same year gave positivity of 6.2% (51/821) in Moloundou, compared to 13.2% for the same location in this study, and 29% (20/70) in Mbatika, but positivity threshold used is not reported.[63]

Lugulu, western Kenya[12]	1980	Family and close neighbours of an IFA confirmed case (asymptomatic?)	A	84	4	Ebolavirus 4.8%	IFA (?)	≥1:16	Antigens: monospecific, triple (unspecified ebolavirus, MARV, LASV) and poly-antigen (CCHFV, RVFV, ebolavirus, LASV, MARV). Validation: sera examined at National Institute of Virology, Johannesburg and CDC(US): labs used different thresholds, so positive confirmed only where both found ≥1:16	Area in Western Kenya, close to Nzoia. Samples collected during investigation of 2 MARV suspect cases who were later shown to be ebolavirus positive.
Kenya[13]	1980	Different studies in 5 regions of Kenya: - Lodwar, Laisamis, Masia, Malindi/Kilifi - Nzoia	C	1058 841	18 9	EBOV/SUDV 1.7% 1.1%	IFA(w)	≥ 1:16	Antigens: inactivated unspecified ebolavirus, MARV, CCHFV, RVFV, & LASV; positives tested against EBOV(May) & SUDV (Boniface & Maleo). Authors report 'most' of the Nzoia samples were only tested against EBOV(May)	No known outbreak but Nzoia cohort reported to include suspected cases and their contacts. Highest prevalence: Lodwar 7.8% (north-west Kenya). Note referenced paper includes some sera reported on in other papers.[72]
Haute Ogooue, Gabon[14]	1980	General population in outbreak area but no known contact	B	253	16 8 5 1 21 8	EBOV 6.3% 3.2% SUDV 2.0% 0.4% EBOV/SUDV 8.3% 3.1%	IFA(w)	≥ 1:16 ≥1:64 ≥ 1:16 ≥ 1:64 ≥ 1:16 ≥1:64	Antigens: inactivated polyvalent unspecified ebolavirus/LASV MARV: positives tested against EBOV(802850) & SUDV(802681).	Samples from the Occupational Health Services, plus 28 women & their newborns. One sample was positive ≥1:64 on both EBOV & SUDV
Northern Rhodesia (now Zimbabwe)[15]	1980	Asymptomatic schoolboys (8-10y): no known outbreak	C	486	9 4	EBOV 1.9% 0.8%	IFA(g)	≥ 1:8 ≥ 1:128	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, unspecified ebolavirus slides provided by CDC (US): positives tested against individual antigens (EBOV & SUDV) Validation: 4 of 5 positive samples sent to CDC (US) were ≥1:128 in repeat IFA testing.	None were positive for SUDV.
Pool, Congo-Brazzaville (now Republic of Congo)[16]	1981	Children from 20 villages aged 3-15 years and unvaccinated for smallpox	C	790	119	Ebolavirus 15.0%	IFA(?)	Not stated	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, unspecified ebolavirus; also tested against monovalent antigens	Pool region is on the border with DRC. Areas sampled had no known outbreak but populations were selected for close contact with animals
Grand Bassa, Liberia[17]	1981-82	Individuals asymptomatic for EVD consisting of 106 epilepsy patients; 87 healthy relatives of these patients; 32 unrelated geographically matched controls.	C	225	26 4 29	EBOV 11.6% SUDV 1.8% Overall 12.9%	IFA(w)	unclear	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon) (known as CRE_2LM); positives retested against individual antigens. Validation: Difficulties with non-specific binding led researchers to replicate and use blinded observers to read results. Only samples with unequivocally positive results by 2 observers were considered positive.	No known outbreak. Similar proportion positive in each participant group. 38% epilepsy patients had a febrile illness 1-4 weeks before onset of epilepsy, but no significant difference in seroprevalence with & without febrile history. Paper says 30 positives in total but one counted twice (positive for EBOV and SUDV). Titres ranged from 32-128 for EBOV; 6/29 had antibodies to more than 1 virus.

Sud-Ubangi sub-region, DRC (includes Tandala)[18]	1981-85	Age/sex matched controls from the same villages as reported cases	C	137	2	Ebolavirus 1.5%	IFA(w)	≥1:64	Antigen: polyvalent CRE_2LM; positives tested against unspecified ebolavirus-specific antigens.	In addition 188 contacts of possible and probable cases were tested; 28 were positive at ≥1:64 but all had had symptoms fitting the definition of a possible or clinical case. It is not clear how many of the other contacts had symptoms.
Northeastern, southeastern & western Gabon[19]	1981-1997	Six rural communities (Makokou, Doussala, Doussieousou, Matadi-Ngoussa, Moukoro, Latoursville): sera gathered during onchocerciasis research	C	1147	14	EBOV 1.2%	ELISA (k)	Mean +3 SD of OD of negative controls	Antigen: EBOV Validation: In 2003, 6 of original 14 positives were re-bled (others unavailable): 2 were still positive. 14 controls (relatives and 'cohorts') were unreactive	6 seropositives were from north-eastern Gabon where outbreaks had occurred; 8 were from western communities more than 500km from known epidemics. Authors also investigate and correlate animal with human outbreaks. Conclude that less virulent strains of EBOV affected western areas.
Madina-Ula, Guinea[20]	1982-83	Healthy adults sampled during an outbreak of an unknown disease	C	138	11 4 2	EBOV 7.8% 2.9% 2.2%	ELISA(r) ELISA(r) IFA(b) ELISA(r) IFA (b)	≥ 1:8 ≥ 1:512 ≥ 1:16 ≥ 1:512 ≥ 1:64	Antigen: EBOV	Areas sampled had no known outbreak
Benin[21]	1983	General population, non-outbreak country	C	603	2	EBOV or SUDV? 0.3%	IFA (?)	≥ 1:64	Antigen: EBOV, SUDV	Unpublished data cited by Gonzalez *et al* (2005): no further information, not specified which ebolavirus antigen samples were reactive to.
Ethiopia, Awash valley[1]	1983	Unexposed children	C	250	0	EBOV 0.0%	IFA(w)	≥ 1:16	Antigens: Polyvalent ELM (Ebola-Lassa-Marburg viruses) & monovalent EBOV (May)	Areas sampled had no known outbreak
Karamoja, Uganda[22]	1984	'Healthy' adults 20-40y recruited during visits to a health centre, excluding any with current or recent fever	C	132	4 4	EBOV 3.0% SUDV 3.0%	IFA(w)	unclear	Antigen: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon) (CRE_2LM); positives retested against individual antigens.	No known outbreak. Not clear if some samples had antibodies to both EBOV & SUDV. Not specified how many > 1:64. All samples < 1: 128.
Mobai, Sierra Leone & unspecified location Sudan[23]	<1984	No information on population type: general population assumed from description - Mobai, Sierra Leone - Sudan	C	556 284	10 10 1 0	EBOV (a) 1.8% (b) 1.8% (a) 0.35 (b) 0	(a) ELISA +ve/IFA+ ve (b) ELISA +ve/ IFA - ve or unclear	ELISA: +ve within 2 SD of +ve ref. sera; -ve within 1 SD of negative ref sera IFA ≥ 1:100	Antigen: EBOV Validation: All? samples were tested using both assays	Year of sample collection is not recorded. Paper reports a high level of cross reactivity with MARV, lasting a number a number of years after infection.

Haute Ogooue, Gabon[24]	1985	Inhabitants of Ambinda village	C	213	20 6 2 1 22 7	EBOV 9.4 % 1.4% SUDV 0.9 % 0.5% Overall 10.3% 3.3%	IFA (j)	≥ 1:16 ≥ 1:64 ≥ 1:16 ≥ 1:64 ≥ 1:16 ≥ 1:64	Antigen: polyvalent CCHFV (IB-AR-10200 Nigeria), RVFV (ZH-501 Egypt), LASV (Josiah), MARV (Musoki), EBOV (May), SUDV(Bon) (CRE_2LM); positives retested against individual antigens.	No known outbreak
Nola,Ikaumba, Bozo, Bangassou, Mbre & Birao, Central African Republic [25]	1984-85	General population from 5 ecological regions including one close to Zaire/DRC outbreak area	C	836	152 63	EBOV 18.2 % SUDV 7.5 %	IFA(j)	≥ 1:16	Antigens: EBOV (May), SUDV (Bon), MARV (Mus)	No known outbreak but Zemio which borders DRC accounted for 43% of EBOV positives
Nola,Ikaumba, Bozo, Bangassou, Mbre & Birao, Central African Republic [26]	1984-85	Asymptomatic general population from 5 ecological distinct zones selected on accessibility: additional villages wer chosen where multiple ethnic groups coexisted.	C	4295* 4078*	681 209 853 259 914 335	EBOV 15.9% 5.1% SUDV 19.8% 6.4% Overall 21.3% 8.2%	IFA (j)	≥ 1:16 ≥ 1:128 ≥ 1:16 ≥ 1:128 ≥ 1:16 ≥ 1:128	Antigens: polyvalent EBOV (May), SUDV (Bon), MARV (Mus), LASV (Jos), CCHFV (10200), RVFV(ZH501); positives (≥ 1:16) retested against monovalent antigen. Validation: 185 samples were reanalysed by ELISA in 1996: results confirmed original analysis.[21]	Study linked to one above in CAR[25] but using different set of sera sampled in the same period. * Two different denominators are cited: 4296 people are reported for all titre levels; 4078 people are reported in the table describing only samples showing titres ≥ 1:128. Highest prevalence in woods and forest regions. 86% of titres ≥ 1:64 but reached as high as 1:2048
Nkongsamba, Cameroon[27]	1985	Randomly selected urban general population (15-44 years)	C	375	7 5	Ebolavirus 1.9% 1.3%	IFA (?)	≥ 1:16 ≥ 1:64	Antigens: polyvalent unspecified ebolavirus, CCHRV, RVFV, MARV)	These samples were included in the following multi-country study which used a different threshold.[28] One sample was positive for both ebolavirus and RVFV
Central Africa[28] (now Middle Africa)	1985-87	Randomly selected sera collected in: Cameroon (Mora, Maroua, Nkongsamba) Central African Republic (Bangui) Chad (N'djamena) Republic of Congo (Pointe Noire, Brazzaville) Equatorial Guinea (Bioco Island, Nsork) Gabon (Libreville, Port-Gentil, Ogooue-Ivindo, Haut Ogooue, Ngounie)	C	1152 327 334 728 688 1841	89 107 334 51 111 259	EBOV/SUDV 7.7% 32.7% 3.6% 7.0% 16.1% 14.0%	IFA (w)	≥ 1:16	Antigens: polyvalent EBOV (May), SUDV (Bon), MARV (Mus), LASV (Jos), CCHFV (10200), RVFV(ZH501); positives (≥ 1:16) retested against monovalent antigen.	Areas sampled had no known outbreak

Chobe, Northern Botswana[29]	1984-86	1984: 52 asymptomatic villagers) 1985: 25 villagers with non-specific or ictero-haemorrhagic symptoms 1986: 77 asymptomatic villagers	C	154	0	EBOV/SUDV 0%	IFA (J)	≥ 1:16	Antigens: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon): positives re-tested against monovalent antigens.	Areas sampled had no known outbreak. Unable to separate results for the symptomatic group. Only reaction found was against RVFV. Testing performed in Paris.
Lobaye, Central African Republic[30]	1987	Asymptomatic general population, Lobaye district: Pygmy hunter-gathers	C	127	31	EBOV/SUDV 24.4%	IFA (J)	≥ 1:128	Antigens: polyvalent EBOV (May), SUDV (Bon), MARV (Mus), LASV (Jos), CCHFV (10200), RVFV(ZH501); positives (≥ 1:16) retested against monovalent antigen, considered reactive if ≥ 1:128. Validation: 296 samples from this study and 185 samples from the CAR 1984-85 study above were re-analysed in 1996 using ELISA (≥1:400 & sum of 4 ODs ≥ 1.000). 6.2% were Ebola IgG positive (30/481) compared to 6.4% in these samples previously by IFA.[21,26]	Area with no known outbreak. Of the positives, 45 reacted to both EBOV & SUDV: it is not possible to identify how this splits between the groups.
		Asymptomatic general population Lobaye district: Mozombo/Mbati subsistence farmers	C	300	42	EBOV/SUDV 14.4%				
Nigeria[31]	1988	Asymptomatic general population in different locations	C	1677	30 22	SUDV 1.8% EBOV/SUDV 1.3%	IFA(w)	≥ 1:10	Antigens: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon): positives with titre ≥ 1:10 retested against monovalent antigens. Known positive/negative controls used	Areas sampled had no known outbreak. All positive samples came from savannah areas (Benue/Gongola) Of the positives, none reacted to EBOV alone.
Antanarivo, Mandoto, andasibe, Tsiroanomandidy & Ampijoroa, Madagascar[32]	1989	Asymptomatic adults from 5 different areas (urban & rural, cattle-lands, forested)	C	381	17	EBOV 4.5%	IFA (j)	≥ 1:16	Antigens: polyvalent CCHFV, RVFV, LASV, MARV, EBOV (May), SUDV(Bon): positives retested against monovalent antigens	Areas sampled had no known outbreak. Range of titres: 1:16 to 1:512; highest prevalence in the capital Antanarivo 13.3%.
United States[33]	1990	CDC (US) employees with current or previous occupational exposure to monkeys. None ill.	B	550	42	EBOV/SUDV/ RESTV/MARV 7.6%	IFA (?)	≥ 1:16	Antigens: EBOV, SUDV, Reston ebolavirus (RESTV), MARV. Validation: confirmed by western Blot	This paper summarises 2 others [73,74] Results are for positivity to at least one of the four antigens, which include Marburg.
		Adult primary care outpatients in US	C	449	12	2.7%				
Germany[34]	1991	Various groups of healthy individuals, blood donors and routine diagnostic samples, plus 56 individuals who had had contact with Marburg patients in 1972.	C	1288	11 44	EBOV 0.85% RESTV 3.4%	ELISA IFA or WB	ELISA: 1:100 IFA: 1:40 WB: +ve if stained ≥ 2 viral proteins)	Antigens: EBOV (May), RESTV, Marv(Mus) Validation: Considered positive if ELISA confirmed by IFA or WB. Confirmation: ELISA vs IFA 75%; ELISA vs Western Blot 77%.	Authors state that the sample groups showed no significant differences in the prevalence of antibody against the 3 filoviruses and so they treated as one group for analysis and only overall results reported. WB results: “most” sera reacted with the NP protein, “less” with VP40, VP35 & VP30, and ‘few’ with VP24. None reacted to GP or L proteins.

Kikwit, DRC[35]	1995	Four forest site populations near Kikwit town, site of outbreak	B	230	5	EBOV 2.2%	ELISA (k)	≥ 1:400 & OD sum ≥ 1.25	Antigen: unspecified but Kuhn[52] reports EBOV; sera tested in CDC (US) Special Pathogens Lab.	Differentiation between forest and city workers was difficult: publicity brought people out of their areas: self identified occupations. 95% of participants including all 9 positives said they knew someone with Ebola.
		City workers, Kikwit	B	184	4	EBOV 2.2%				
		Asymptomatic volunteers from unaffected villages near Kikwit	C	161	15	EBOV 9.3%				5/15 positives knew someone who had had Ebola
Kikwit, DRC[36]	1995	Household contacts aged 3m-58y	A	101	4	Ebolavirus 4.0%	ELISA (k)	≥ 1:400 & OD sum ≥ 1.25	Antigen: unspecified but probably EBOV; sera tested in CDC (US) Special Pathogens Lab.	Paper cites 5 positive sera but 1 miscarried 3 days before giving her positive specimen so fits case definition for Ebola. One of the remaining 4 may have acquired Ebola by sexual transmission from a convalescent. Out of 81 sero-negative household contacts, 15 had episodes of illness fitting case definition at some point during follow-up.
Central African Republic [37]	1992-97	Pygmy general population: southern regions of CAR (Lobaye, Belemboke)	C	684	48	EBOV 7.0%	ELISA (n)	≥ 1: 400	Antigens: EBOV, MARV, RVFV, LASV, Yellow fever (YF) Hantaviruses (Seoul, Puumala and Thottapalayam) Validation: 244 sera taken in Lobaye in 1995 (11.6% ELISA positive to EBOV) were retested with IFA to EBOV (May) & SUDV (Bon): 34% were positive.	Prevalence of EBOV seropositivity varied between 2% and 13% in different participant groups.
		Bantu villagers : southern region of CAR (Lobaye, Belemboke, Nola, Bangassou)	C	860	44	EBOV 5.1%				
Central African Republic[38]	1992-95	Pygmy subgroup (Lobaye, Belemboke: all sites no known outbreaks)	C	683	48	EBOV 7.0%	ELISA (k)	Mean + 2 SD of negative controls ≥1:400 & OD sum of 4 dilutions > 1.0	Antigens: EBOV (May) Marv(Mus); tests performed by Institut Pasteur, Bangui. Validation: 14 positive & 54 negative samples sent to CDC (US) to be tested against strain antigens: all results confirmed.	Primary or secondary forest areas with some agricultural activities
		Non-pygmy subgroup (Lobaye, Belemboke, Bangassou, Nola: all sites no known outbreaks)	C	648	23	EBOV 3.5%				
Ogooue Ivindo, Gabon[39]	1995-96	Residents of 3 encampments (Andock, Minkebe, Mekoua) in the area where the epidemic occurred (including some contacts)	B	236	23	EBOV/SUDV /RESTV 9.7%	ELISA (k)	mean + 3 SD of negative controls	Antigens: EBOV, SUDV, RESTV with known positive/negative controls	1 positive serum from a survivor excluded from encampment group; unclear how many known case contacts are included in this group.
		Residents of 3 outbreak villages (Mayibout 1 & 2, Mvadi) where cases were reported during the outbreak	B	205	34	EBOV/SUDV /RESTV 16.6%				

Kikwit[40]	1995	Healthcare workers in outbreak area (70% hospital; 30% health centre) who did not have known EVD	B	400	8	EBOV 2.0%	ELISA (k)	Sum of adjusted OD >1.25	Antigen: EBOV	The 8 positives were from a group of 12 samples which were "borderline positive" on 1st test. Only 4 of these samples were retested: all were negative and have been excluded. 129 of the 402 subjects reported being ill during Ebola period. Two with fever and haemorrhage (tested EBOV negative) have been excluded.
Gabon [41]	1996	Selected asymptomatic family members directly exposed to body fluids during outbreaks in 1996	A	24	11	EBOV 45.9%	ELISA (k)	Mean adjusted OD for 10 control samples	Antigen: EBOV Validation : confirmed with western blot on NP and VP40 proteins	Subjects were asymptomatic throughout and were sampled several times. 1st samples showed no antibodies suggesting no prior immunity; IgG appeared 15-18 days after first possible exposure. Paper also describes results of viral RNA detection after 2 rounds of RT-PCR, finding positive results in 7/11 antibody-positive individuals tested and 0/13 antibody-negative individuals.
Nouna River, Ogooue-Ivindo, Gabon[42]	1996	Residents in gold-mining villages with contact exposure in 1995 epidemic	A	56	12	EBOV 21.4%	ELISA (?)	OD > mean +2 SD of 3 known negative controls	Antigen: ebolavirus Gabon 95-39/3 (Centre International de Recherches Medicales de Franceville)	All subjects reported fever and diarrhoea at least once in 1-year period of study, but not haemorrhagic symptoms. IgG positive titre range (OD 310-2,666). Age, sex, ethnic group not associated with seropositivity. Non- significant difference in seropositivity in people on site during 1995 epidemic (8.2%) and not on site (3.7%), among those with no reported contact
		Residents in same villages without contact exposure	B	180	12	EBOV 6.7%				
Upper Ivindo River, Ogooue-Ivindo, Gabon[43]	1997	Individuals from 8 permanent villages in outbreak-prone region (4 survivors excluded)	B	975	10	EBOV 1.0%	ELISA (k)	Mean OD negative controls +3 SD	Antigen: EBOV, performed in National Institute for Communicable Diseases South Africa. Validation: All positives plus a random selection of 28 negatives were retested with same protocol in CDC (US) – all were confirmed with response mainly directed to NP, VP40, VP35 and sGP viral proteins.	Serosurvey done in 1997; questionnaires done in 1999 on 10 positives: only 1 had contact, none were ill.

Belarus & Ukraine[44]	1997	"Foreign visitors" mostly from Africa: unclear if any had history of EVD symptoms	C	562	30	EBOV 5.3%	IFA (w)	Not specified	Antigens: EBOV (May), MARV (Voege) LASV (Jos)	Authors suggest positive results among foreign visitors reflect historic infection/ recovered cases, and unexpected results reflect cross-reactivity with infections such as malaria, HIV and influenza. Other observers suggest the results are just as likely to be artifact.[63]
		Belarus/Ukraine residents "at risk of HIV"	C	506	20	EBOV 4.0%				
		Blood donors from the Blood Transfusion Institute, MoH Belarus & workers at the Belorussian Scientific Research Institute of Epidemiology & Microbiology	C	131	21	EBOV 16.0%				
Watsa region, DRC[45]	2002	Efe tribe pygmies exposed to a possible case at some time in their lives in household, occupation or funeral setting; no history of haemorrhagic fever symptoms	A	38	4	EBOV 10.5%	ELISA (k)	2 × mean +3 SD of negative controls value	Antigen: EBOV ODs were expressed as percent positivity of a confirmed EBOV-positive sample; negative controls were from 60 South African subjects 'almost certain' to be seronegative.	A total of 300 people were sampled from 39 communities. 137 who reported experiencing haemorrhagic fever symptoms sometime in their life are excluded from this summary. 22% of those reporting symptoms were IgG positive.
		Efe pygmies no reported exposure to possible cases; no history of haemorrhagic fever symptoms	C	125	22	EBOV 17.6%				
Gabon[46]	2005-08	Random sample of asymptomatic people aged >16 years without exposure, over all 9 provinces of Gabon	C	4349	667	EBOV 15.3%	ELISA (k) & WB	Cut-off based on negative controls from a French population	Antigen: EBOV Validation: Random sample of 138 positives were tested by western blot in 2008 and all were positive to at least one EBOV antigen.[53]	Gabon experienced 7 outbreaks between 1994 & 2002 affecting >20 villages and towns; in total there were 208 cases and 151 deaths.
		Random sample of asymptomatic children from 6 villages in outbreak-prone province (Ogooue-Ivindo)	B	362	47	EBOV 12.9%				
Bundibugyo, Uganda[47]	2007	Adult contacts of survivors >18 y. Samples taken ~29 months after outbreak	A	210	2	EBOV/SUDV/ TAFV/BDBV 1.0%	ELISA (s)	Mean OD of negative controls plus 3 SD	Antigens: EBOV, SUDV, Tai Forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV), MARV(Mus) Validation: 28/36 confirmed cases tested positive to BDBV, 20/36 to EBOV, 10/36 to SUDV, 12/36 to TAFV & 29/39 to any of the 4 strains.	15/223 contacts positive but 13 were symptomatic at the time of the outbreak, therefore only the 2 asymptomatic contacts are included in the table.
Republic of Congo[48]	2011	Healthy blood donors 18-65y, no known case exposure	C	809	20	EBOV 2.5%	Double IFA	Reciprocal endpoint titres ≥20	Antigens: EBOV (ATCC 1978), MARV (Popp 1967) Authors state: 'double IFA' technique has higher specificity than 'regular' IFA because only antibodies that detect filoviral antigens in co-localisation with a monoclonal antibody are considered.	Seropositivity ranged from 1.6% - 4% depending on city/rural location; 4% in Pointe Noire.
Liberia [PREVAIL][49]	2015	Close contacts of cases. NB 126 of the contacts were sexual partners of survivors after discharge.	A	760	98	EBOV 12.9%	ELISA (Alpha)	unspecified	Antigen: EBOV	Preliminary results. Study excluded from meta-analysis of known case contact group (A) because unclear what proportion of participants were symptomatic.

Kono, Sierra Leone[50]	2015-16	Asymptomatic close contacts of cases aged ≥ 4 years who had been resident in quarantined houses during the period of active Ebola transmission	A	185s	12	EBOV 6.4%	ELISA (Alpha)	4.7 U/ml	Antigen: EBOV GP Validation: 29/30 PCR-confirmed EVD survivors, and 3/132 community controls were positive: 96.7% sensitivity, 97.7% specificity	2 other positives had fever. Not clear if negatives were asked about symptoms
		Individuals from 3 villages without reported cases	C	132	3	2.3				
Western Area, Sierra Leone[51]	2015	Household contacts of cases, asymptomatic at the time EVD was in the household	A	388	10	EBOV 2.6%	ELISA (PHE)	Mean OD of negative controls + fixed OD measure (0.1)	Antigen: EBOV GP. "Positive" only if repeat test was positive Validation: 93/97 PCR-confirmed EVD survivors and 0/339 community controls were positive: sensitivity 95.9% (95%CI 89.9 – 98.9%); specificity 100% (95%CI 98.9 – 100%)	Tests were done on oral fluid.
		Individuals from 3 villages in Western Area without reported EVD cases	C	339	0	EBOV 0%				

Abbreviations

CDC (US): Centers for Disease Control and Prevention, Atlanta, USA; PHE: Public Health England; EBOV: Zaire ebolavirus; SUDV: Sudan ebolavirus; BDBV: Bundibugyo ebolavirus; TAFV: Tai Forest Fever Virus; MARV: Marburg Fever Virus; CCHFV: Crimean Congo Haemorrhagic Fever Virus; RVFV: Rift Valley Fever Virus; LASV: Lassa Fever Virus; IFA: immunofluorescence assays; ELISA: Enzyme-linked immunosorbent assay; WB: Western Blot; OD: optical density; SD: standard deviation

Assay technique notation

IFA (w): Wulff & Lange[62]
IFA(j): Johnson[63]
IFA (g): Gardner[64]
IFA (b) Baskirtsev[65]
Double IFA: Emmerich[66]
IFA (?) ELISA (?): technique not referenced
ELISA (k): Ksiazek[67]
ELISA (s): Schoepp[68]
ELISA (v): Viral Haemorrhagic Fever Consortium (SL)[69]
ELISA (n): Nicklasson[70]
ELISA(r) : Rezapkin[71]
ELISA(PHE): Lambe[54]
ELISA(Alpha): ADI[75]

Paper 2: Exposure-specific and age-specific attack rates for Ebola virus disease in Ebola-affected households, Sierra Leone

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Surname/Family Name	Bower		
Thesis Title	Ebola virus transmission and disease severity in Sierra Leone 2013-16		
Primary Supervisor	Professor Jimmy Whitworth		

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Exposure-Specific and Age-Specific Attack Rates for Ebola Virus Disease in Ebola-Affected Households, Sierra Leone

Hilary Bower, Sembia Johnson, Mohamed S. Bangura, Alie Joshua Kamara, Osman Kamara, Saidu H. Mansaray, Daniel Sesay, Cecilia Turay, Francesco Checchi, Judith R. Glynn

Using histories of household members of Ebola virus disease (EVD) survivors in Sierra Leone, we calculated risk of EVD by age and exposure level, adjusting for confounding and clustering, and estimated relative risks. Of 937 household members in 94 households, 448 (48%) had had EVD. Highly correlated with exposure, EVD risk ranged from 83% for touching a corpse to 8% for minimal contact and varied by age group: 43% for children <2 years of age; 30% for those 5–14 years of age; and >60% for adults ≥30 years of age. Compared with risk for persons 20–29 years of age, exposure-adjusted relative risks were lower for those 5–9 (0.70), 10–14 (0.64), and 15–19 (0.71) years of age but not for children <2 (0.92) or 2–4 (0.97) years of age. Lower risk for 5–19-year-olds, after adjustment for exposure, suggests decreased susceptibility in this group.

In Ebola epidemics in West Africa and elsewhere, children appear to have been relatively spared (*1–5*). Published notification data for the West Africa outbreak that began in 2013 show a linear increase in incidence of Ebola virus disease (EVD) with age in persons up to ≈35 years of age, followed by a plateau in incidence for older age groups (*6*). Among children, the World Health Organization has reported a slightly increasing incidence with increasing age in Liberia and Sierra Leone but no clear pattern in Guinea (*4*). In contrast, published case-fatality rates for EVD are lowest for persons 10–15 years of age and highest for young children and older adults (*4,7*).

These age patterns could result from bias in recognizing, diagnosing, or reporting cases; differences in exposure; or differences in susceptibility to disease. Official data from the West Africa outbreak are known to be inaccurate (*8,9*). In previous, smaller outbreaks, case ascertainment could have been more complete because of the smaller scale, but EVD cases might have been missed, especially mild cases; deaths may also have been missed because the elderly and very young are more likely to sicken and die from other causes. Children with fever are less likely than adults to visit health facilities for care, and children may be underreported as contacts (*10*).

Exposure patterns are likely to differ by age and sex. Women may be more at risk from caring for the sick and men from carrying sick persons to the hospital. Children may be deliberately kept away from sick persons and funeral rites, and lower incidence among children has been attributed to these factors (*1,11*). However, preventing exposure of young children in Ebola-affected households is difficult. Children need to be held, fed, and cared for and often share beds with adults or other children; they may also be exposed through breastfeeding (*12*).

The high case-fatality rate observed in children <5 years of age and especially in those <1 year of age (*4*) suggests that young children are particularly susceptible to Ebola; consequently, low incidence in young children may reflect low exposure or low ascertainment. In a study of 27 Ebola-affected households after the Kikwit outbreak in 1995, children <18 years of age had lower risks of disease than adults, after adjustment for reported exposures (*13*).

Assessing whether risk by age depends on exposure or susceptibility requires a comparison of exposures in persons with and without EVD. A recent systematic review of risk factors for transmission of Ebola virus found few studies reporting data on risks (*14*) and no previous study large enough to stratify in detail by age (*3,13,15–19*). We interviewed a large cohort of EVD survivors and their household members to determine exposure levels of all members, living and dead, and to calculate attack rates and relative risks by age, sex, and type of exposure. The Sierra Leone Ethics and Scientific Review Committee and the Ethics Committee of the London School of Hygiene and Tropical Medicine approved the study.

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Methods

All survivors who were discharged from Kerry Town Ebola Treatment Centre (ETC), Sierra Leone, during November 2014–March 2015 and who lived in the Western Area were eligible for the study. During July–August 2015, members of the study team, which had assisted in survivor reintegration into the community, contacted survivors or their parents or guardians and asked them to bring all household members who were present at the time Ebola was affecting their household to an interview to be conducted at 1 of various locations. To make contact, the field team went to addresses of survivors when addresses were available and complete enough to locate or used telephone numbers when available. Team members were university graduates, nurses, and paramedics and included Ebola survivors; they received extensive training in interview techniques and were supervised by the first 2 authors (H.B. and S.J.), 1 or both of whom attended all interviews.

After obtaining individual written informed consent from each participant or parents or guardians of participants <18 years of age, the interviewers compiled a list of all members in each household and included information on age, sex, and household members who had had EVD and those who had died of EVD. Households were defined as persons eating from the same pot at the time EVD was in the household, regardless of how much time had been spent in the household, and included persons who joined the household to assist someone who was ill.

We asked household members to describe in their own words what had occurred in the household. For each person reported as having had EVD, we asked what symptoms occurred at home and which persons had helped that person during his or her EVD illness, shared a bed or had contact with the person, or had contact with the body if the person died. Adults spoke for young children and corroborated information from older children. Using probing questions and predefined exposure levels, we assigned a maximum exposure for persons who had been present in the household. The levels, which we developed on the basis of the literature and discussion with ETC staff, included touching the corpse of someone who died from EVD; direct contact with body fluids of a wet patient (i.e., with diarrhea, vomiting, or bleeding); direct contact with a wet patient; direct contact with a dry patient (i.e., without diarrhea, vomiting, or bleeding); indirect contact with a wet patient (e.g., washing clothes); indirect contact with a dry patient; minimal contact (e.g., shared meals); or no known contact (Table 1). We also asked about exposures outside the home and classified these exposures by using the same scale. For those reported as not having had EVD, we asked about any symptoms at the same time that others in the household had EVD. Study team members, all of whom are multilingual, conducted interviews in the participants' language and recorded key outcomes in English.

Definitions

Laboratory-confirmed EVD survivors who were reported from Kerry Town ETC, survivors from other ETCs, and all persons reported by the family as having died of EVD were counted as EVD case-patients. Deaths for which the family was unsure of the cause and symptomatic persons who were not tested or did not receive a diagnosis of EVD were classified as probable EVD case-patients if they fit the Sierra Leone case definition for probable cases (*20*).

For each household, the first person who became ill was identified as the likely primary case-patient. Some households reported 2 people who became ill at the same time, and they are counted as co-primary case-patients. No household described >1 period when Ebola occurred in the household. To avoid overburdening participants, we did not collect time sequences or dates and defined all nonprimary case-patients in a household as subsequent case-patients.

Analysis

Our initial descriptive analysis of outcomes by age and sex included all household members. We subsequently analyzed primary case-patients separately because their exposure occurred outside the household, and we compared their characteristics with those of all other household members.

Table 1. Classification of level of exposure to EVD patients in study of EVD risk for household members, Sierra Leone, 2014–2015*

Level	Definition
1	Contact with the body of EVD patient after death/prepared the body for burial
2	Direct contact with body fluids (e.g., blood, diarrhea, vomit, urine, or a baby breastfed by an EVD-positive woman)
3	Direct close contact with wet case; i.e., with diarrhea/vomiting/bleeding (e.g., person helped dress, embraced, carried, helped care for, or shared bed of an EVD patient with wet symptoms; or mother breastfed an EVD-positive child)
4	Direct close contact with dry case (i.e., without wet symptoms at the time) (e.g., person helped dress, embraced, carried, helped care for, or shared bed with an EVD patient without wet symptoms)
5	Indirect close contact with wet case (e.g., washed clothes or bed linen of an EVD patient with wet symptoms, or slept in the same room but not the same bed)
6	Indirect close contact with dry case (e.g., person washed clothes or bed linen of EVD patient without wet symptoms); formal/informal health workers without known contact with an EVD patient; ETC workers in PPE; Ebola Intervention workers (outside household only); person attended funeral without contact with the body (outside household only)
7	Minimal contact (e.g., person shared meals or utensils or sat In the same room; children placed in observation centers [outside household only])
8	No actual contact (e.g., person kept distance once EVD patient was symptomatic)

*ETC, Ebola Treatment Centre; EVD, Ebola virus disease; PPE, personal protective equipment.

In the analysis of risks for disease by age, sex, and exposure level, we excluded primary case-patients and household members who were alive but not present for the interview and unable to consent to individual data collection. We explored the following variables for their effects on disease risk and as confounders of the associations of other variables and disease risk: having a spouse who contracted EVD first; occupation; being household head versus household member; and household-level variables (i.e., household size; crowding [number of persons/number of rooms]; and access to water, soap, and latrine). Our analysis used logistic regression and adjusted for household clustering by using random effects. Because risks were large, we used marginal standardization to estimate risk ratios (RRs) and the delta method to estimate 95% CIs (*21,22*). All analyses used Stata 14 (http://www.stata.com). We also performed a sensitivity analysis that excluded case-patients and deaths classified as probable EVD cases.

Results

Study Population

Of 151 EVD survivors discharged from Kerry Town ETC, we included 123 survivors from 94 households in the study. The other 28 survivors had a similar age distribution to those included (39% of survivors not included vs. 36% of those included were <15 years of age) and a slightly higher proportion of males (54% of those not included vs. 38% of included survivors). We collected detailed information for 937 persons, including exposure histories for 909 (Figure 1).

Overall, 448 persons were reported as having had EVD or probable EVD, of whom 238 (53%) died; 227 deaths were reported as caused by EVD, and the 11 other deaths fit the EVD case definition. Among survivors, 123 were EVD patients at the Kerry Town ETC, and 45 were at other ETCs. An additional 42 household members had probable EVD; the remaining 485 household members had no evidence of EVD.

Risk for EVD was lowest for children 5–14 years of age but higher for children <2 years of age and for adults (Table 2). Risk increased with age for adults up to ≈35 years of age and then plateaued for older adults (Figure 2, panel A). Because most probable case-patients were children, the lower risk for children was more extreme when probable case-patients were excluded (Table 2). EVD risk was similar for male and female study participants, even when results were stratified by age (Figure 2, panel B).

Primary Case-Patients

Primary case-patients were identified for 91 households and co-primary case-patients in 3 households. Compared with all other household members, primary case-patients were older, usually ≥30 years of age; slightly more likely to be male; and more likely to be household heads, healthcare or EVD front-line workers, or religious or community leaders (Table 3). Children or students were least likely to be primary case-patients. In 5 households,

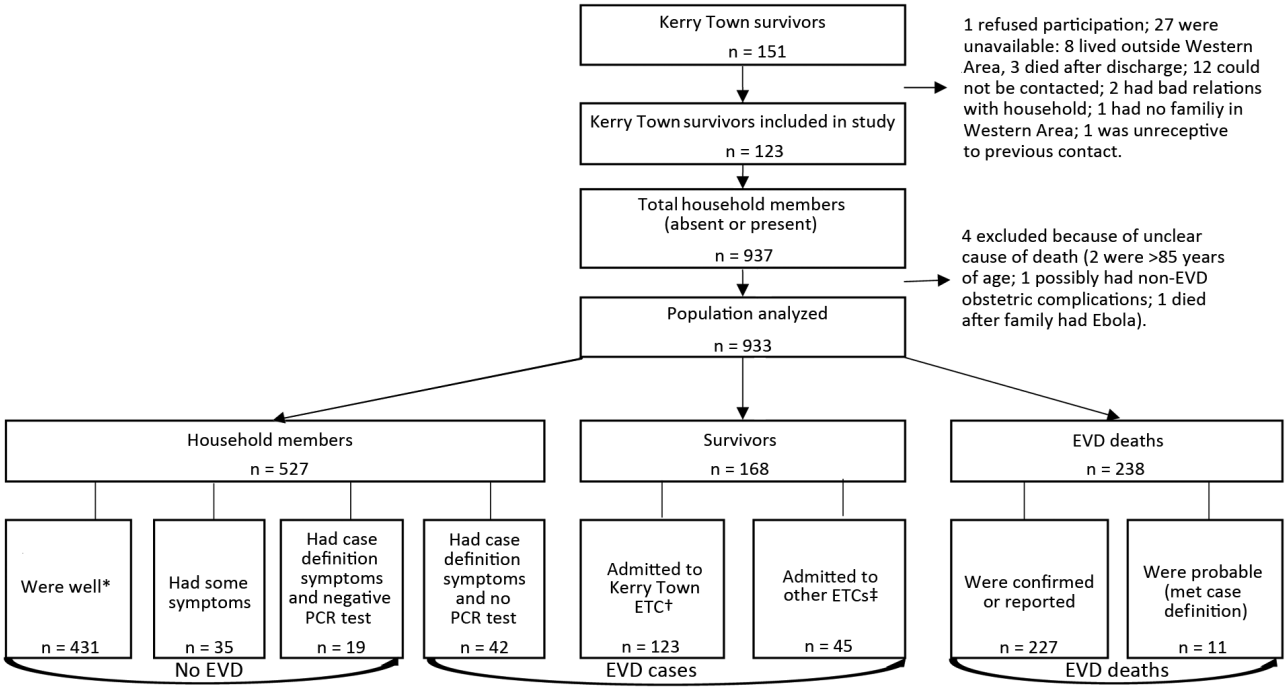

Figure 1. Flow diagram showing the population composition for study of Ebola-affected households related to survivors from the Kerry Town Ebola Treatment Centre (ETC), Sierra Leone, 2014–2015. EVD, Ebola virus disease. *Includes 23 not present for interview. †Includes 1 who died after discharge. ‡Includes 5 not present for interview.

Table 2. Distribution of outcomes by age and sex among Kerry Town Ebola Treatment Centre survivors and their household members, Sierra Leone, 2014–2015*

Characteristic	Total	No. (%)							
		Persons with no symptoms	Persons with some symptoms†	SympCD and neg test‡	SympCD and no test§	EVD survivors	Probable EVD deaths¶	EVD deaths	Overall % EVD#
Total	933	431 (46.2)	35 (3.8)	19 (2.0)	42 (4.5)	168 (18.0)	11 (1.2)	227 (24.3)	48
Sex									
M	399	184 (46.1)	19 (4.8)	11 (2.8)	20 (5.0)	62 (15.5)	5 (1.3)	98 (24.6)	46
F	534	247 (46.3)	16 (3.0)	8 (1.5)	22 (4.1)	106 (19.9)	6 (1.1)	129 (24.2)	49
Age, y**									
<2	54	27 (50.0)	2 (3.7)	0	1 (1.9)	4 (7.4)	1 (1.9)	19 (35.2)	46
2–4	86	49 (57.0)	2 (2.3)	2 (2.3)	8 (9.3)	9 (10.5)	1 (1.2)	15 (17.4)	38
5–9	131	82 (62.6)	4 (3.1)	4 (3.1)	11 (8.4)	15 (11.5)	0	15 (11.5)	31
10–14	121	78 (64.5)	3 (2.5)	3 (2.5)	8 (6.6)	18 (14.9)	0	11 (9.1)	31
15–19	107	57 (53.3)	4 (3.7)	2 (1.9)	1 (0.9)	28 (26.2)	0	15 (14.0)	41
20–29	178	76 (42.7)	8 (4.5)	4 (2.2)	10 (5.6)	49 (27.5)	1 (0.6)	30 (16.9)	51
30–39	114	31 (27.2)	3 (2.6)	3 (2.6)	3 (2.6)	26 (22.8)	3 (2.6)	45 (39.5)	68
40–49	62	12 (19.4)	4 (6.5)	1 (1.6)	0	12 (19.4)	4 (6.5)	29 (46.8)	73
≥50	76	18 (23.7)	5 (6.6)	0	0	7 (9.2)	0	46 (60.5)	70

*Excluded are 4 persons who died with uncertain cause (3 females, 1 male). EVD, Ebola virus disease.
†Persons had some EVD symptoms but did not fulfill case definition.
‡Persons met EVD case definition on interview but reported a negative PCR test for Ebola.
§Persons met EVD case definition on interview but were never tested.
¶Description of symptoms leading to death were compatible with Ebola, but EVD was not diagnosed at the time.
#Overall % EVD includes EVD cases and deaths, SympCD and no test, and Probable EVD deaths as case-patients.
**Age missing for 4 persons (2 reported EVD deaths, 1 probable EVD death, 1 with no symptoms).

primary case-patients joined the household when they were already ill.

Likely sources of infection were identified for 68 (70%) of 97 primary case-patients. When >1 source of infection was possible, we selected the highest exposure level (Table 1). Thirty primary case-patients visited a household with an EVD patient; 16 of those 30 went to help the ill patient. Eight prepared bodies for burial or touched the corpse; 6 attended funerals; 4 carried a person with EVD symptoms; 8 attended healthcare facilities; and 12 worked as healthcare or front-line workers, 5 of whom were known to have treated an EVD patient.

Subsequent Case-Patients

The overall risk for acquiring EVD was 43% and was similar for male and female participants (Table 4); the risk by age was J-shaped, as for the full study population. Among household members, 60% reported direct contact with a wet patient or their fluids or with a person who died of EVD (Table 4). Only 10 (1.2%) household members had a substantially higher level of exposure outside the household than inside.

Attack rates increased steeply and linearly with the predefined exposure levels. Exposure levels were high at all ages and for males and females (Figure 3), but exposure to EVD corpses increased with age, and direct exposure to fluids was higher for children <2 years of age, largely because of breastfeeding, and for older adults. After adjustment for age and sex, attack rates varied by occupation and were higher in larger and more crowded households. We found no clear associations with household-level measures of sanitation nor with having a spouse who developed EVD first (Table 4).

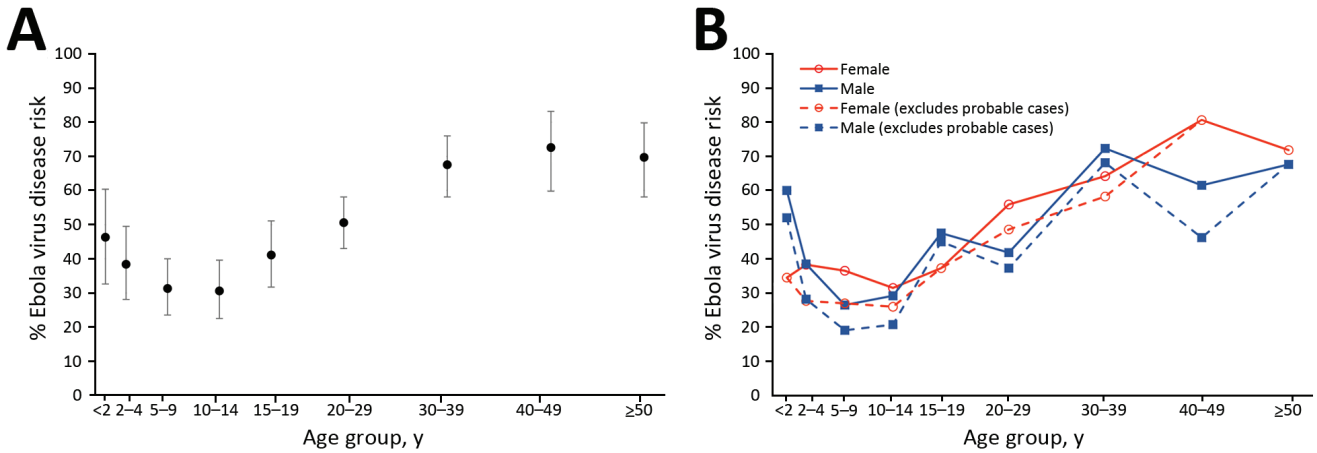

Figure 2. Risk for Ebola virus disease in Ebola-affected households of Kerry Town Ebola Treatment Centre survivors, by age and sex, Sierra Leone, 2014–2015. A) Risk by age group; bars indicate 95% CIs. B) Risk by sex and age group with and without probable cases.

Table 3. Risk factors associated with being the first EVD case in a household, compared with all other household members in households of Ebola Treatment Centre survivors, Kerry Town, Sierra Leone, 2014–2015*

Risk factor	Total population	No. primary cases	Risk,%	Adjusted RR* (95% CI)	p value†
Sex					
M	400	47	11.8	1.3 (0.93–1.9)	
F	537	50	9.3	1	0.1
Age, y‡					
<2	54	3	5.6	0.57 (0.17–1.9)	
2–4	86	2	2.3	0.24 (0.06–1.0)	
5–9	131	2	1.5	0.15 (0.04–0.65)	
10–14	121	3	2.5	0.26 (0.08–0.86)	
15–19	107	7	6.5	0.69 (0.30–1.6)	
20–29	179	17	9.5	1	
30–39	114	26	22.8	2.4 (1.4–4.2)	
40–49	63	15	23.8	2.5 (1.3–4.7)	
≥50	78	20	25.6	2.6 (1.5–4.7)	<0.001
Occupation§					
Heathcare worker, formal and informal	21	10	47.6	3.9 (2.0–7.5)	
Ebola front-line worker	11	3	27.3	2.5 (0.86–7.1)	
Driver	23	6	26.1	1.7 (0.67–4.1)	
Religious leader/chief/teacher	12	5	41.7	2.7 (1.1–6.9)	
Farmer/fisherman/unskilled	54	12	22.2	1.7 (0.83–3.3)	
Office/business	47	8	17.0	1.3 (0.62–2.8)	
Child/student	511	15	2.9	0.41 (0.12–1.4)	
Trader/tailor/service	205	25	12.2	1	0.01
Position in household					
Household head	87	32	36.8	2.3 (1.5–3.8)	
Household member	850	65	7.6	1	<0.001

*EVD, Ebola virus disease; RR, risk ratio. Adjusted for age, sex, and household clustering.
†p values calculated from likelihood ratio test in logistic regression model.
‡Age missing for 4 persons.
§No occupation recorded for 29 persons, including 28 with no individual-level data.

A multivariable analysis (Table 4) showed that developing EVD as a subsequent case-patient was strongly associated with age (p = 0.004), level of exposure (p<0.001), not being a household head (p = 0.03), and household size (p = 0.01). Sex was kept in the model a priori but was not associated with EVD risk. Occupation was not associated with EVD risk after adjustment for exposure level (p = 0.2). In the full model, the association with age was still J-shaped. The lowest risk was for persons 5–19 years of age, and risks were higher for older than younger adults. Additional adjustment for other available variables had little effect on associations. In the sensitivity analysis that excluded probable EVD cases, associations with exposure levels were stronger, and the J-shaped association with age was more marked (Table 5).

Discussion

In Ebola-affected households in our study, the age pattern for EVD incidence in children differed from that reported for the overall epidemic by the World Health Organization (*4*) and was closer to the age pattern of reported case-fatality rates; children <5 years of age had higher risks than older children (*4,7*). Among adults, the pattern was similar to previous findings (*6*), with

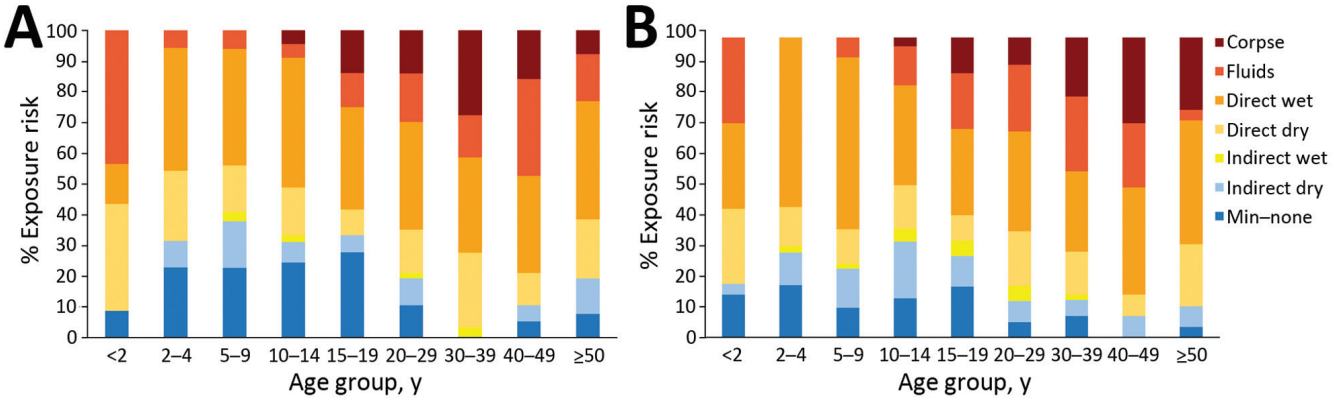

Figure 3. Levels of exposure to Ebola virus disease among households of Kerry Town survivors, excluding primary case-patients, by age and sex, Sierra Leone, 2014–2015. A) Male participants; B) female participants. Levels of exposure correspond to those shown in Table 1. Min–none, minimum or no exposure.

Table 4. Risk factors associated with development of EVD in subsequent case-patients in Ebola-affected households, Kerry Town, Sierra Leone, 2014–2015*

Risk factor	No. patients/no. total (%),† N = 809	Adjusted RR‡ (95% CI)	Adjusted RR§ (95% CI)	Multivariable RR¶ (95%CI)	p value#
Sex					
M	136/337 (40.4)	1.1 (0.87–1.4)	1.06 (0.85–1.3)	1.03 (0.87–1.2)	
F	211/472 (44.7)	1	1	1	0.7
Age, y					
<2	22/51 (43.1)	0.79 (0.49–1.3)	0.80 (0.49–1.3)	0.92 (0.64–1.3)	
2–4	31/81 (38.3)	0.70 (0.45–1.1)	0.70 (0.46–1.1)	0.97 (0.72–1.3)	
5–9	38/127 (29.9)	0.44 (0.28–0.68)	0.44 (0.28–0.69)	0.70 (0.50–0.97)	
10–14	34/114 (29.8)	0.41 (0.25–0.67)	0.41 (0.25–0.67)	0.64 (0.45–0.93)	
15–19	35/95 (36.8)	0.53 (0.33–0.84)	0.53 (0.33–0.84)	0.71 (0.49–1.02)	
20–29	72/155 (46.5)	1	1	1	
30–39	51/85 (60.0)	1.2 (0.82–1.6)	1.2 (0.82–1.6)	1.1 (0.83–1.4)	
40–49	30/46 (65.2)	1.2 (0.82–1.8)	1.2 (0.83–1.8)	1.1 (0.80–1.6)	
≥50	33/53 (62.3)	1.3 (0.87–1.8)	1.3 (0.88–1.8)	1.3 (0.97–1.8)	0.004
Maximum exposure					
Handled corpse	60/72 (83.3)	18.1 (7.4–44.1)	13.5 (5.4–33.5)	11.1 (4.5–27.4)	
Handled fluids	73/120 (60.8)	13.1 (5.4–31.9)	9.7 (3.9–24.1)	8.5 (3.5–20.6)	
Direct wet contact	146/297 (49.2)	10.4 (4.3–25.1)	8.3 (3.4–20.1)	7.1 (3.0–17.1)	
Direct dry contact	47/125 (37.6)	7.1 (2.9–17.7)	5.6 (2.3–13.9)	5.3 (2.2–12.9)	
Indirect wet contact	5/19 (26.3)	5.7 (1.6–20.1)	4.9 (1.4–16.8)	4.7 (1.5–14.6)	
Indirect dry contact	8/74 (10.8)	1.4 (0.43–4.6)	1.3 (0.40–4.2)	1.3 (0.41– 4.0)	
Minimal/no contact**	8/102 (7.8)	1	1	1	<0.001
Position in household					
Household head	24/52 (46.2)	1.2 (0.79–1.79)	0.62 (0.35–1.1)	0.62 (0.39–1.0)	
Household member	323/757 (42.7)	1	1	1	0.03
Household size					
≥16	120/209 (57.4)	5.2 (1.6–16.9)	5.0 (1.5–16.8)	2.9 (1.1–7.8)	
11–15	98/290 (33.8)	2.4 (0.70–7.9)	2.4 (0.70–8.3)	1.7 (0.63–4.6)	
6–10	121/270 (44.8)	3.7 (1.2–12.0)	3.9 (1.2–12.8)	2.7 (1.04–7.0)	
1–5	8/40 (20.0)	1	1	1	0.01
Occupation					
HCW (formal and informal)	8/11 (72.7)	1.6 (1.1–2.5)	1.8 (0.91–3.6)		
Ebola front-line worker	1/8 (12.5)	0.16 (0.02–1.5)	0.14 (0.02–1.3)		
Driver	11/17 (64.7)	1.2 (0.67–2.0)	1.1 (0.55–2.3)		
Religious leader/chief/teacher	5/7 (71.4)	1.6 (0.95–2.8)	1.8 (0.70–4.4)		
Farmer/fisherman/unskilled	18/41 (43.9)	0.81 (0.49–1.4)	0.85 (0.48–1.5)		
Office/business	26/39 (66.7)	1.3 (0.89–1.8)	1.4 (0.87–2.2)		
Child/student	173/484 (35.7)	0.52 (0.40–0.69)	1.0 (0.63–1.6)		
Trader/tailor/service	96/177 (54.2)	1	1		
Water available					
Sometimes	45/131 (34.4)	0.58 (0.2-–1.3)	0.59 (0.28–1.3)		
Most days	118/265 (44.5)	1.0 (0.59–1.59)	0.96 (0.60–1.5)		
Every day	182/408 (44.6)	1	1		
Soap available					
Sometimes	64/194 (33.0)	0.78 (0.42–1.4)	0.84 (0.47–1.5)		
Most days	113/200 (56.5)	1.5 (0.90–5.5)	1.4 (0.84–2.3)		
Every day	168/410 (41.0)	1	1		
Latrine					
Household's own	107/286 (37.4)	0.7(0.43–1.2)	0.72 (0.43–1.2)		
Shared/none	238/518 (45.9)	1	1		
Crowding					
High	126/238 (52.9)	2.1 (0.89–4.7)	2.4 (1.0–5.4)		
Medium	189/483 (39.1)	1.4 (0.63–3.2)	1.6 (0.71–3.6)		
Low	30/83 (36.1)	1	1		
Spouse with Ebola first					
Yes	45/77 (58.4)	1.6 (1.2–2.1)	1.0 (0.68–1.5)		
No	302/732 (41.3)	1	1		

*Subsequent case-patients were any household members who contracted Ebola virus disease after the first (primary) case-patient. Data were excluded for 4 deaths from uncertain cause, 27 persons with no individual-level data, and 97 primary cases. Data were missing for 2 persons with no recorded age, 6 persons with no recorded occupation, and 5 persons (in 1 household) with no recorded information about water, soap, latrine, and crowding. EVD, Ebola virus disease; HCW, healthcare worker; RR, risk ratio.
†Number of subsequent case-patients/total number of household members in study, excluding primary case-patients.
‡Adjusted for household clustering.
§Adjusted for age, sex, and household clustering.
¶Adjusted for clustering and all other factors included in the model.
#p values for multivariable model calculated from likelihood ratio test in logistic regression model.
**Only 7 persons reported no contact, so these 2 categories are combined.

a plateau occurring >35 years of age. This pattern was similar whether probable case-patients were included or not. Children were less likely than adults to be primary case-patients, and among child primary case-patients, no particular trend by age was observed (Table 3). The higher risk for EVD among children <5 years of age than among older children may suggest that very young children have been disproportionately missed in notification data.

Our study included only survivor households because it was conducted by building on survivor-support links; consequently, it missed households with only fatal cases and those in which no one sought care. Compared with all Ebola-affected households, households in our study were likely to be larger; to include more EVD patients, which increases the chance that ≥1 household member survived; and to include more children ≥5 years of age, who have a lower case-fatality rate than younger children. These characteristics would tend to increase attack rates and may explain the high attack rate overall and the association of attack rate with household size. These characteristics might also increase the proportion of cases among children, although children ≥5 years of age had a relatively low incidence of EVD.

After excluding primary case-patients, we examined the extent to which age patterns could be explained by exposure levels. After we adjusted age-specific incidence data by exposure, children 5–19 years of age still had a lower risk for EVD, although the lower risk was less marked, and the increased risk with age for adults no longer plateaued but continued upward. If we measured exposure accurately, these findings suggest that some of the variation in risk by age within households results from differences in susceptibility. In the interviews, we avoided lengthy questionnaires with each person to try to reduce questionnaire fatigue, respondents' forgetting or denying types of exposure, and possibly overburdening already traumatized households. Instead, we encouraged families to tell their stories, ensuring that we learned which household members had contact with each EVD patient and what type of contact. Consequently, the conversation flowed naturally, with different household members contributing and providing details, helping to minimize recall bias. This approach also enabled us to acquire details for

Table 5. Sensitivity analysis excluding probable cases showing risk factors associated with development of EVD as a subsequent case-patient in Ebola-affected households, Kerry Town, Sierra Leone, 2014–2015*

	Total, excluding probable cases, N = 764		
Risk factor	No. patients/no. total (%)†	Adjusted RR‡ (95% CI)	p value§
Sex			
M	114/315 (36.2)	1.0 (0.83–1.2)	
F	188/449 (41.9)	1	1.0
Age, y			
<2	21/50 (42.0)	0.99 (0.66–1.5)	
2–4	22/72 (30.6)	0.98 (0.68–1.4)	
5–9	27/116 (23.3)	0.69 (0.47–1.0)	
10–14	26/106 (24.5)	0.60 (0.39–0.93)	
15–19	34/94 (36.2)	0.77 (0.52–1.1)	
20–29	63/146 (43.2)	1	
30–39	47/81 (58.0)	1.2 (0.86–1.6)	
40–49	28/44 (63.6)	1.2 (0.82–1.8)	
≥50	33/53 (62.3)	1.5 (1.1–2.0)	0.002
Maximum exposure			
Handled corpse	60/72 (83.3)	40.6 (8.5–194.5)	
Handled fluids	65/112 (58.0)	30.5 (6.4–144.8)	
Direct wet contact	125/276 (45.3)	24.1 (5.2–113.2)	
Direct dry contact	41/119 (34.5)	16.7 (3.6–78.1)	
Indirect wet contact	5/19 (26.3)	17.2 (3.1–94.7)	
Indirect dry contact	4/70 (5.7)	2.3 (0.37–14.3)	
Minimal/no contact	2/96 (2.1)	1	<0.001
Position in household			
Household head	22/50 (44.0)	0.58 (0.35–0.98)	
Household member	280/714 (39.2)	1	0.02
Household size			
≥16	108/197 (54.8)	2.6 (0.98–6.7)	
11–15	90/282 (31.9)	1.5 (0.57–3.9)	
6–10	96/245 (39.2)	2.3 (0.89–5.7)	
1–5	8/40 (20.0)	1	0.04

*Subsequent case-patients were any household members who contracted EVD after the first (primary) case-patient. EVD, Ebola virus disease; RR, risk ratio.
†Excluded data: 4 deaths from uncertain cause; 27 persons with no individual-level data, 97 primary case-patients; 45 case-patients were classified as having EVD on the basis of their histories but had no diagnosis of EVD at the time. Missing data: 2 persons with age unknown.
‡Adjusted for clustering and all variables in the model.
§p values calculated from likelihood ratio test in logistic regression model.

children and for persons who had died, although use of proxy respondents may have limited accuracy of exposure measurement. We conducted the interviews 4–9 months after the illness, but participants provided considerable detail in their responses. Inaccuracies in recall would lead to a failure to adjust completely for exposure level, whereas any tendency to recall greater exposures for household members with EVD would increase the association with exposure and result in the association between age and EVD being overadjusted for exposure level.

We predefined exposure levels so that we could record only the highest level and not probe for details for possible lower levels. This approach differed from that of other studies (*13,14,16*), which recorded several exposures and adjusted during analysis. Our hierarchy of exposure appears to be accurate; we found strong correlations between EVD risk and each increase in exposure level.

As others have reported (*13,14,16*), the highest risk for EVD exposure was from contact with dead bodies. Risk was also high from direct contact with fluids and with wet patients and was lower but still considerable (5-fold [17-fold in the sensitivity analysis], compared with minimal risk) from direct contact with dry patients and indirect contact with wet patients (Tables 4,5). We found no discernible increase in risk from indirect contact with dry patients compared with exposures classified as minimal risk (Table 1). Overall, after exclusion of primary and co-primary case-patients, we found a high household attack rate, higher than found in previous studies (*23*), perhaps reflecting the urban setting and the bias toward households with multiple cases.

Children had lower exposure than adults, but exposure levels in these households were high overall; >50% of each age group had at least direct exposure to a wet patient. In the sensitivity analysis, correlation between exposure levels and outcome was stronger, suggesting misclassification of some case-patients included as probable EVD cases; this analysis also showed a markedly lower EVD risk in children ≥5 years of age.

A lower susceptibility to EVD among children is possible. Lower attack rates or case-fatality rates in children have been found for other viral diseases, including varicella (*24*), smallpox (*25*), and West Nile virus disease (*26*). For EVD, different cytokine and chemokine responses related to survival have been noted for adults and children (*27*).

We found little difference in risk by sex, even when stratified by age. Household-level measures of sanitation had surprisingly little effect on the outcome (*28*). Having a spouse who contracted EVD first was not a risk factor after we adjusted for age; consequently, sexual transmission did not appear to be an important factor in the acute phase.

We established likely sources of infection for 70% of primary case-patients. Although some were linked to high-risk activities, more were related to visits to friends and relatives, including some visits to nurse sick relatives. Other households were infected by taking in sick relatives. These activities show remarkable altruism at a stage of the epidemic when Ebola was well known. More support to families to protect themselves in the home when they helped those not known to have EVD might have prevented these transmissions.

Much of what we know about risks for Ebola virus transmission comes from anecdotal reports or case series (*29*). Few studies have measured risk associated with particular exposures directly (*13,15,16,18,23*), and none have been large enough to examine risk by age in detail. This study collected information on >800 contacts, enabling estimates of exposure-specific and age-specific attack rates. After we adjusted for exposure, age patterns for Ebola attack rates were similar to those for case-fatality rates. Inherent differences in susceptibility, which warrant further investigation, likely underlie both distributions.

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Paper 3: Effects of mother's illness and breastfeeding on risk of Ebola virus disease in a cohort of very young children

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RESEARCH ARTICLE

Effects of Mother's Illness and Breastfeeding on Risk of Ebola Virus Disease in a Cohort of Very Young Children

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Abstract

Background

Young children who contract Ebola Virus Disease (EVD) have a high case fatality rate, but their sources of infection and the role of breastfeeding are unclear.

Methods/Principal Findings

Household members of EVD survivors from the Kerry Town Ebola Treatment Centre in Sierra Leone were interviewed four to 10 months after discharge to establish exposure levels for all members of the household, whether or not they became ill, and including those who died. We analysed a cohort of children under three years to examine associations between maternal illness, survival and breastfeeding, and the child's outcome. Of 77 children aged zero to two years in the households we surveyed, 43% contracted EVD. 64 children and mothers could be linked: 25/40 (63%) of those whose mother had EVD developed EVD, compared to 2/24 (8%) whose mother did not have EVD, relative risk adjusted for age, sex and other exposures (aRR) 7·6, 95%CI 2·0–29·1. Among those with mothers with EVD, the risk of EVD in the child was higher if the mother died (aRR 1·5, 0·99–2·4), but there was no increased risk associated with breast-feeding (aRR 0·75, 0·46–1·2). Excluding those breastfed by infected mothers, half (11/22) of the children with direct contact with EVD cases with wet symptoms (diarrhoea, vomiting or haemorrhage) remained well.

Conclusion/Significance

This is the largest study of mother-child pairs with EVD to date, and the first attempt at assessing excess risk from breastfeeding. For young children the key exposure associated with contracting EVD was mother's illness with EVD, with a higher risk if the mother died. Breast feeding did not confer any additional risk in this study but high risk from proximity to a

author (FC), employed by Save the Children UK, was involved in commissioning the study and interpreting findings. Full responsibility for study design, data collection and analysis, decision to publish, & preparation of the manuscript was with JRG. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: Save the Children International (SCI) operated the Kerry Town ETC and employed the local field work team members (SJ, MSB, AJK, OK, SHM, DS, CT). One author (FC) is employed by SCI and was involved in commissioning the study and interpreting findings. No SCI or Wellcome Trust staff were involved in the analysis or the decision to submit for publication. The corresponding author (JRG) had full access to the study data and was responsible for the decision to submit for publication. HB and JRG declare that they have no conflicts of interests.

sick mother supports WHO recommendations for separation. This study also found that many children did not become ill despite high exposures.

Author Summary

Our study is the first to quantify sources of infection and describe risk of transmission of Ebola to young children. We found that the risk of a child under three developing Ebola disease was low unless their mother had EVD, and that the risk was particularly high if their mother died of EVD. But we found no additional risk from breastfeeding. WHO recommends separating asymptomatic breast-fed infants from their mothers if they develop Ebola, and using formula feeding. We support the need for separation because of the high risk related to proximity, but more research is needed to more fully understand this, particularly given the importance of breast-feeding in preventing other childhood illnesses. We also found young children in Ebola-affected households whose mothers were not ill had a surprisingly low risk of developing EVD which was not all explained by low exposure to the virus. Many children stayed well despite having direct contact with EVD patients with diarrhoea, vomiting or bleeding who are considered the most infectious. We hope these findings will provide impetus for more detailed studies into age-related response to the Ebola virus.

Introduction

Young children experience a high case fatality rate from Ebola, but the incidence of Ebola Virus Disease (EVD) in children appears to be lower than in adults.[1–4] Young children may have limited exposure outside the home, but within the household maintaining hygiene in young children is difficult, although efforts may be made to keep children away from those who are sick. For very young children who need to be fed and held, contact with sick caregivers may be unavoidable.

Breastfeeding is a possible additional source of infection for young children: Ebola has been found in breast milk, but the risk to breastfed babies and the contribution of breastfeeding to transmission is poorly understood.[5,6] An investigation of household contacts following the Ebola outbreak in Gulu, Uganda in 2000 included five infants whose mother had EVD: three of four infants who were breastfed developed EVD.[7] The other infant was reported to have been separated from his mother early in the course of her illness and remained well; it is not clear if he was breastfed. Two recent systematic reviews of transmission of Ebola did not did not mention risks associated with breastfeeding.[8,9]

As part of a study of transmission patterns in Sierra Leone we collected data on exposure patterns and outcomes of all individuals present in the households of EVD survivors. In this analysis we sought to identify likely sources of infection and characterise risk of transmission to young children, including those breastfed by mothers with EVD.

Methods

In July-September 2015, interviews were sought with the household members of all individuals who were discharged from the Ebola Treatment Centre in Kerry Town, Sierra Leone ("Ebola survivors") from November 2014 to March 2015. Contact was made through members of the survivor support team who were involved in their reintegration into the

community. An initial approach was made to explain the study. If the household head agreed, an interview was arranged at a community centre or other meeting place and all who were in the household at the time that members of the households had Ebola were encouraged to attend.

At the interview, individual informed written consent to participate in the study was sought from all adults, and from parents or guardians for children (< 18 years), with assent from children of 12 years or older. An inventory was drawn up of all household members who had been present in the household at the time that one or more household members were ill with EVD, including any who had died or were not present at the interview. For each member we asked whether they had had Ebola. We asked relatives whether any deceased had died of Ebola.

Household members were asked to describe what happened when Ebola came to their household, including who became ill first, whether those with Ebola had any diarrhoea, vomiting or bleeding while they were at home, and who looked after them. They were encouraged to tell the narrative in their own words, with probing questions to clarify who had been exposed and how. For each household member (including those who had died, but excluding any absent members or those who refused consent) we sought to establish the highest-risk exposure. Reported exposures were ranked a priori from highest to lowest as: contact with the body of someone who died of Ebola; direct contact with body fluids of someone with Ebola, including breastfeeding, or other direct contact with "wet" cases (i.e. those with diarrhoea, vomiting or bleeding); direct contact with "dry" cases (i.e. those without diarrhoea, vomiting or bleeding); indirect contact with a wet case (e.g. washing their clothes); indirect contact with a dry case; minimal contact (e.g. shared utensils); and no known contact. For each mother-baby pair who both had EVD we attempted to ascertain from the narratives who was affected first.

All survivors from the Kerry Town Ebola Treatment Centre had EVD confirmed by PCR. We did not have laboratory data for those from other treatment centres or for those who died, so have relied on the families' reports. For individuals who were not reported as having had Ebola we asked about symptoms at the time that Ebola was in the household. For the analysis they were classified as not having had Ebola if they were asymptomatic or had symptoms that did not fulfil the Sierra Leone Ministry of Health and Sanitation case definition for "probable" Ebola,[10] or had had a negative test; and as having had Ebola if they were symptomatic and fulfilled the case definition for probable Ebola and were not tested. The case definition was contact with a case plus fever or miscarriage or unexplained bleeding; or contact plus three or more symptoms (of fatigue, headache, loss of appetite, nausea or vomiting, abdominal pain, diarrhoea, muscle or joint pain, sore throat or pain on swallowing, hiccups).

In this analysis we concentrate on risks to children aged less than three years at the time Ebola reached their household in order to include all those who were breast fed, and examine attack rates, case fatality rates and the role of breast feeding. Proportions were compared using X^2 or Fisher's exact test. Analyses used multivariable logistic regression. Because the outcome is very common we have presented the results as risk ratios (RR) using marginal standardization to estimate RRs, and the delta method to estimate 95% confidence intervals (95%CI).[11–13] We repeated the analysis calculating risk ratios using Poisson regression with robust error variance.[14] Crowding (number of people per room) and sanitation (access to water, soap and latrine) were considered as possible confounders, in addition to age, sex and the exposure variables. The effects of clustering by household were explored using generalised estimation equations in logistic regression: the results were very similar to analyses ignoring clustering so clustering is not included in the models. Analyses used STATA 14.

Ethics Statement

The study was approved by the Sierra Leone Ethics and Scientific Review Committee and the Ethics Committee of the London School of Hygiene & Tropical Medicine. At the interview, individual written informed consent to participate in the study was sought from all adults, and from parents or guardians for children (< 18 years), with assent from children of 12 years or older.

Results

One hundred and fifty one survivors were discharged from Kerry Town Ebola Treatment Centre from November 2014 through March 2015, of whom 138 were still living in the Western Area of Sierra Leone when sought for interview in July-September 2015. Twelve were uncontactable and a further two were known to have bad relationships with their households so were not approached. We contacted and interviewed 123 Kerry Town survivors, living in 94 households. Only one contacted survivor refused to be interviewed, and only two of 526 household members refused to participate. A further 37 members were not available to attend the interview. Some households also included survivors who had been treated in other facilities.

The households contained 77 children aged less than three years: 43% (33/77) got EVD, including four who fitted the case definition but were not diagnosed at the time. The risk of EVD was 54% (13/24) in those under one year; 40% (12/30) in those aged one year and 35% (8/23) in those aged two years (p-value for trend = 0.2). The risk was slightly higher in males than in females: 51·4% (18/35) vs 35·7% (15/42), p = 0.2. Three of the children were primary or co-primary cases in their household. Overall, 24 children under three years died of EVD, giving a case fatality rate of 73%: 85% (11/13), 75% (9/12) and 50% (4/8) at ages under-one, one, and two respectively (p-value for trend = 0·1).

Among the 77 children were 13 whose mothers were not present (including two mothers who had died in other households), or were not clearly identified: six (46%) of these children developed EVD and five died compared to 27 cases (42%) and 19 deaths among the 64 children who could be linked to their mothers.

Details of the mother-child pairs for whom the outcome of both mother and child are known are shown in Table 1 for the 40 whose mothers had EVD, in Table 2 for the 24 whose mothers had no symptoms, and in summary for all 64 in Table 3. The highest level of exposure is shown, in terms of direct or indirect exposure to those with EVD in the home or outside. None of the children had direct contact with dead bodies. Breastfeeding was taken as the highest exposure if the mother had EVD unless the child developed symptoms before or at the same time as the mother.

EVD in the children was much more likely among those whose mother had EVD (25/40, 63%) than among those whose mother did not get EVD (2/24, 8%, risk ratio (RR) 7·5, 95% confidence interval (CI) 1.9–28.9, p<0.0001, Table 3). The RR remained high after adjusting for age and sex of the child (RR 9·4, 95% CI 2·6–34·0), and after additionally adjusting for maximum exposure level (RR 7·6, 95%CI 2·0–29·1). Household crowding and sanitation were not associated with EVD in the child, and adjusting for them made little difference to the results. After adjusting for mother's EVD status and exposure levels, the risk of EVD in the child decreased with age (Table 3). After adjusting for mother's EVD, age, and sex, there was no effect of exposure level.

Among those whose mother had EVD, excluding the two pairs in which the children were ill first, the risk of EVD in the child was higher if the mother died (79% vs 50%, Table 3), giving a relative risk of 1·6 (95% CI 0·97–2·6). This association was similar after adjusting for the child's age and sex and additionally for exposure level. Of the 13 children who did not get EVD

Table 1. Details of exposure and outcomes in mother-child pairs in which the mother had Ebola.

Child's age in years	Mother's outcome	Child's exposure	Other Ebola cases	No. of people in household	Child's outcome	Timing
Breast fed						
<1	Survivor	breastfed	4	10	Well	mother first
<1	Survivor	breastfed	6	14	Survivor	mother first
<1	Survivor	breastfed	0	12	Survivor	unclear
<1	Survivor	breastfed	15	26	Death EVD	mother first
<1	Survivor	breastfed	2	6	Death EVD	mother first
<1	Survivor	breastfed	9	15	Death EVD	mother first
<1	Death EVD	breastfed	4	10	Death EVD[1]	mother first
<1	Death EVD	breastfed	11	17	Death EVD	mother first
<1	Death EVD	breastfed	18	26	Death EVD	unclear
<1	Death EVD	breastfed	10	13	Death EVD	mother first
1	Survivor	breastfed	3	15	Well	mother first
1	Survivor	breastfed	0	3	Well	mother first
1	Survivor	breastfed	1	9	Well	mother first
1	Survivor	breastfed	9	13	Death EVD	mother first
1	Death EVD	breastfed	3	11	Well	mother first
1	Death EVD	breastfed	13	27	Survivor	mother first
Breast fed, child ill before or same time as mother						
1	Survivor	indirect contact wet case	1	9	Death EVD	child first
2	Survivor	direct contact wet case	4	7	Death EVD	same time
Not breast fed						
<1	Death EVD	direct contact wet case	12	16	Death EVD	unclear
1	SymCD/ NoTest[2]	direct contact body fluids	3	11	Well	unclear
1	Survivor	direct contact body fluids	4	13	Death EVD	unclear
1	Survivor	direct contact wet case	9	15	Death EVD	mother first
1	Survivor	direct contact wet case	4	10	Well	mother first
1	Survivor	direct contact wet case	4	9	SymCD/NoTest[3]	mother first
1	Survivor	direct contact dry case	15	26	Death EVD	unclear

(*Continued*)

Table 1. (*Continued*)

Child's age in years	Mother's outcome	Child's exposure	Other Ebola cases	No. of people in household	Child's outcome	Timing
1	Survivor	direct contact dry case	4	12	Death EVD	unclear
1	Death EVD	direct contact dry case	15	19	Headache/ cough only	unclear
1	Death EVD	direct contact dry case	15	26	Death EVD	unclear
2	SymCD/ NoTest[4]	direct contact dry case	5	15	Well	unclear
2	Survivor	direct contact wet case	3	11	Well	unclear
2	Survivor	direct contact wet case	19	26	Headache only	unclear
2	Survivor	direct contact wet case	4	7	Survivor	mother first
2	Survivor	direct contact dry case	9	14	Well	unclear
2	Survivor	direct contact dry case	1	5	SymCD/NegTest[2]	unclear
2	Survivor	indirect contact dry case	16	26	Well[5]	unclear
2	Death EVD	direct contact body fluids	5	8	SymCD/NoTest[6]	mother first
2	Death EVD	direct contact body fluids	5	8	SymCD/NoTest[6]	mother first
2	Death EVD	direct contact wet case	3	6	Death EVD	mother first
2	Death EVD	minimal contact	4	19	Well[5]	unclear
2	Death EVD	minimal contact	2	6	SymCD/NoTest[2]	mother first

Death EVD = death from Ebola; Survivor = Survived Ebola; SymCD/NoTest = fulfilled case definition for Ebola, not tested; SymCD/NegTest = fulfilled case definition for Ebola but tested negative

[1] Possibly infected in utero or perinatally.

[2] Fever only

[3] Multiple symptoms, not tested because of nurses' strike

[4] Fever and headache

[5] Moved out of household after first case

[6] Multiple symptoms

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whose mother survived, five had contact with the mother when she was a wet case and five only when she was a dry case (unknown for three).

As the only child over two years who was breastfed got ill at the same time as the mother and was therefore excluded, the analysis of breastfeeding was restricted to the under two's. We also excluded the child who became ill first (Table 1), leaving 26 children. The proportion of children with EVD was very similar in those who were or were not breast fed (69% vs 70%, Table 3), RR 0·98, 0·58–1·7. There was no evidence of increased risk from breastfeeding after adjusting for age and sex (RR 0·76, 0·46–1·2) or for whether the mother died (Table 3).

The analyses were re-run excluding the six mother-child pairs for which either the mother or the child was classified as having EVD on the basis of symptoms (Table 1). The associations

Table 2. Details of exposures and outcomes in mother-child pairs in which the mother did not have Ebola.

Child's age (years)	Child's exposure	Other Ebola cases in household	No. of people in household	Child's outcome
<1	direct contact wet case	19	26	Death EVD
<1	direct contact wet case	14	15	Death EVD
<1	direct contact wet case	6	12	Well
<1	direct contact wet case	1	13	Well
<1	direct contact dry case	6	18	Well
<1	direct contact dry case	5	13	Well
<1	direct contact dry case	2	14	Well
<1	direct contact dry case	1	9	Well
<1	minimal contact	6	18	Well
<1	minimal contact	3	11	Well
<1	minimal contact	2	8	Well
1	direct contact wet case	6	12	Well
1	direct contact wet case	1	13	Well
1	direct contact dry case	2	11	Well
1	direct contact dry case	1	12	Well
1	direct contact dry case	1	3	Well
1	direct contact dry case	1	4	Headache only
1	minimal contact	2	7	Well
1	minimal contact	6	8	Well[1]
2	direct contact wet case	1	6	Well
2	direct contact wet case	1	13	Well
2	direct contact wet case	1	13	Well
2	direct contact dry case	2	11	Well
2	minimal contact	3	11	Well

Death EVD = death from Ebola

[1] Moved out of household after first case

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with having a mother with EVD (fully adjusted RR 6.5, 1·6–26·0) and with breastfeeding (fully adjusted RR 0·74 (0·47–1·2) were similar to the main analysis, but the effect of having a mother who died of Ebola was lost (fully adjusted RR 1·3, 0·76–2·1). The analyses were also rerun using Poisson regression. The results were similar to the main analysis.

Among the children under three years whose mother did not get EVD, only two children got EVD. Both were aged under one year, from households with many EVD cases (Table 2), and both were reported to have had close contact with wet cases in the household. Seven other children whose mother did not have EVD and 4 whose mother had EVD but were not breastfed, had direct contact with wet cases and did not get ill. Overall, excluding children breastfed by mothers with EVD, half (11/22) of the children who had direct contact with wet cases or fluids remained well. These contacts included sharing beds with and embracing close relatives who suffered from vomiting and/or diarrhoea.

Discussion

Among the very young children in this study the risk of EVD depended largely on whether their mother developed EVD, with an additional risk for those whose mothers died of Ebola. The high risk in those with sick mothers is expected, and the higher risk in those with mothers who died may reflect higher viral loads and/or viral shedding in these mothers. The low risk in

Table 3. Associations with Ebola in children under three years.

	Child's outcome					
	Ebola /Total	%	RR (95% CI)	RR adjusted age and sex	Full model	P (*full model*)
All mother-baby pairs						
Mother had Ebola						
No	2/24	8.3	1	1	1	<0.001
Yes	25/40	62.5	7.5 (1.9–28.9)	9.4 (2.6–34.0)	7.6 (2.0–29.1)[1]	
Child's age						
< 1yr	12/22	54.6	1	1	1	0.03
1 yr	9/24	37.5	0.69 (0.36–1.3)	0.67 (0.35–1.3)	0.54 (0.33–0.88)[1]	
2 yrs	6/18	33.3	0.61 (0.29–1.3)	0.59 (0.28–1.3)	0.51 (0.27–0.96)[1]	
Child's sex						
Female	11/31	35.5	1	1	1	0.2
Male	16/33	48.5	1.4 (0.76–2.5)	1.4 (0.80–2.6)	1.4(0.87–2.2)[1]	
Child exposure level						
breastfeeding	11/16	68.8				
direct wet	11/22	50.0				
direct dry	3/16	18.8	0.68 (0.50–0.94)[2]	0.70 (0.51–0.95)	0.93 (0.76–1.1)[1]	0.5
indirect wet	1/1	100				
indirect dry	0/1	0.0				
minimal	1/8	12.5				
Among those with mothers with Ebola[3]						
All						
Mother survived	12/24	50.0	1	1	1	
Mother died	11/14	78.6	1. 6 (0.97–2.6)	1.5 (0.98–2.3)	1.5 (0.99–2.4)[4]	0.06
Under 2s						
Not breastfed	7/10	70.0	1	1	1	
Breastfed	11/16	68.8	0.98 (0.58–1.7)	0.76 (0.46–1.2)	0.75 (0.46–1.2)[5]	0.3

[1] Model included age, sex, mother's Ebola, and exposure level

[2] Modelled as a linear term across categories

[3] Excluding two in which the child was ill first/at the same time

[4] Adjusted for age, sex, and exposure level

[5] Adjusted for age, sex, and mother's death

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children in Ebola-affected households when the mother was not ill is surprising, and cannot all be explained by low exposure in the children. Overall, nearly two thirds of under-three year olds had direct contact with wet cases in the household or their body fluids. While the risk of disease decreased with decreasing exposure, half of the young children with direct exposure to wet cases remained well.

Only three children were deliberately sent out of the household to reduce exposure, and for all three there was some exposure before they left. The opportunities for households to protect children from exposure are limited, particularly as more and more cases arise, and young children share beds with sick relatives. While a 'no touch policy' may be understood by older children, it is impossible to explain to an infant.

Among children whose mothers had EVD, being breastfed did not appear to increase the risk. Numbers were small and risks were already high in this group so there was limited power to detect an association. Current WHO guidelines recommend that asymptomatic breastfed

infants of Ebola-infected mothers should be separated from their mothers and replacement fed.[15] Although we found no excess risk from breastfeeding, further studies, ideally with larger, pooled datasets, are needed to assess this further before suggesting any changes to the recommendation. The high risk from proximity to a sick mother supports the need for separation.

The children in this study all came from households with at least one survivor. This may mean small households and households with fewer cases are underrepresented, as there would be a lower chance for small households to include a survivor, and households in which all cases of EVD died are missed. This might underestimate the case fatality rate and overestimate attack rates, but should not bias the relative risks by age and exposure.

This study shows the remarkable resilience of some young children despite apparent exposure to Ebola. This could be dose-related—we do not know the actual viral exposure through contact or breastfeeding—but in other contexts some people seem to be infected from minimal exposures. Relative resistance to Ebola could be influenced by genetic factors,[16] though the correlation between infections in mothers and children is more likely to reflect exposure patterns than shared genes. It is possible that there is some protection through maternal antibody from breastfeeding (perhaps more in mothers who survive) that counteracts any increased risk from transmission via breastmilk.

This is much the largest study of mother-child pairs with EVD to date, and the first attempt to assess any excess risk from breastfeeding. By visiting households after transmission had ceased and talking to all members we were able to determine exposure in much more detail than is possible in an acute epidemic situation. And because we included all children in these households, including those who were not sick, we have been able to calculate age and exposure-specific attack rates. In these households the risk to young children was largely dependent on whether their mother had EVD, regardless of whether they were breastfed.

Supporting Information

S1 File. STROBE checklist. Completed STROBE checklist.
(DOCX)

Author Contributions

Conceived and designed the experiments: JRG HB FC. Performed the experiments: HB SJ MSB AJK OK SHM DS CT. Analyzed the data: JRG HB. Wrote the paper: HB JRG FC SJ MSB AJK OK SHM DS CT.

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Paper 4: Deaths, late deaths, and role of infecting dose in Ebola virus disease in Sierra Leone: retrospective cohort study

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Deaths, late deaths, and role of infecting dose in Ebola virus disease in Sierra Leone: retrospective cohort study

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ABSTRACT

OBJECTIVES
To assess the frequency of fatal recrudescence from Ebola virus disease after discharge from treatment centres, and explore the influence of infecting dose on case fatality rates.

DESIGN
Retrospective cohort study.

SETTING
Western Area, Sierra Leone.

PARTICIPANTS
151 survivors treated for Ebola virus disease at the Kerry Town treatment centre and discharged. Survivors were followed up for a vital status check at four to nine months after discharge, and again at six to 13 months after discharge. Verbal autopsies were conducted for four survivors who had died since discharge (that is, late deaths). Survivors still living in Western Area were interviewed together with their household members. Exposure level to Ebola virus disease was ascertained as a proxy of infecting dose, including for those who died.

MAIN OUTCOME MEASURES
Risks and causes of late death; case fatality rates; odds ratios of death from Ebola virus disease by age, sex, exposure level, date, occupation, and household risk factors.

RESULTS
Follow-up information was obtained on all 151 survivors of Ebola virus disease, a mean of 10 months after discharge. Four deaths occurred after discharge, all within six weeks: two probably due to late complications, one to prior tuberculosis, and only one after apparent full recovery, giving a maximum estimate of recrudescence leading to death of 0.7%. In these households, 395 people were reported to have had Ebola virus disease, of whom 227 died. A further 53 people fulfilled the case definition for probable disease, of whom 11 died. Therefore, the case fatality rate was 57.5% (227/395) for reported Ebola virus disease, or 53.1% (238/448) including probable disease. Case fatality rates were higher in children aged under 2 years and adults older than 30 years, in larger households, and in infections occurring earlier in the epidemic in Sierra Leone. There was no consistent trend of case fatality rate with exposure level, although increasing exposure increased the risk of Ebola virus disease.

CONCLUSIONS
In this study of survivors in Western Area, Sierra Leone, late recrudescence of severe Ebola virus disease appears to be rare. There was no evidence for an effect of infecting dose (as measured by exposure level) on the severity of disease.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Understanding who dies from Ebola virus disease, and when, is crucial for determining the effect of interventions and planning the public health response

Case fatality rates vary by age and viral load on admission to treatment centres, but it is not known if they vary by infecting dose

Frequency of recrudescence and late deaths from Ebola virus disease after discharge from treatment centres is unknown

WHAT THIS STUDY ADDS

This is the first cohort study of Ebola virus disease with active follow-up for late deaths, and the first large community based study to investigate risk factors for death from the disease

Recrudescence of severe Ebola virus disease appears to be rare up to 10 months after discharge

Infecting dose, as measured by extent of exposure to body fluids, strongly correlated with risk of developing the disease, but there was no consistent trend with case fatality rate

Introduction

Understanding who dies from Ebola virus disease is crucial for determining the effect of interventions and planning the public health response. The case fatality rate for the disease is high but estimates have varied between outbreaks and in reports describing the west African outbreak. In previous outbreaks, case fatality rates have been between 34% and 88%, with generally lower rates for the *Sudan* and *Bundibugyo ebolavirus* species than for *Zaire ebolavirus*.[1-3] In the west African outbreak of *Zaire ebolavirus*, the case fatality rate based on the notification data for certain and probable cases was 65%, slightly lower in Guinea and higher in Liberia than in Sierra Leone, but some cases may not have been notified.[4] Estimates from west African treatment centres have ranged from 31%[5] to more than 70%,[6] but patients who die or recover without reaching the centres are not included and variation reflects admission policies and delays, and patient mix as well as care. Community level data should give the best estimates but there are few such studies,[7 8] and to ensure unbiased estimates they would need to include any unreported mild cases and assessment of any unreported deaths.

Late deaths due to Ebola complications or recrudescence of the virus would also be excluded from estimated case fatality rates. The recrudescence of Ebola virus disease in a nurse in the United Kingdom, nine months after the original episode, raised the possibility that similar events are occurring but are being missed in west Africa, where they might be fatal.[9] In Liberia,

a nine year old child was readmitted with meningoencephalitis and a polymerase chain reaction (PCR) test that was positive for Ebola virus one day after discharge with negative blood tests.[9] Recrudescences are important not only for the individuals but also as a possible source of further outbreaks. The frequency of severe recrudescence leading to late deaths is not known, although it has been noted that the virus can persist in protected body sites for at least nine months.[10] Studies of post-Ebola sequelae have so far concentrated on survivors attending clinics,[11 12] or have not managed to contact all survivors:[13] unless intensive follow-up is conducted, deaths could be missed.

Several studies have looked at risk factors for death from Ebola virus disease. There is a clear association with age in the larger studies, with the lowest case fatality rates in children aged over 4 years and high rates in children aged under 2 years and older adults, and little difference by sex.[2 4] Some studies have found case fatality rates decreased over the course of an outbreak,[2 8] perhaps reflecting improved care. Survival in those in treatment centres is better than overall survival,[8 14] but whether this reflects the treatment or the selection of those surviving long enough to get to centres is not clear. Among patients in the treatment centres, the key clinical predictor of mortality is the estimated viral load on arrival.[14-16]

In the first known Ebola outbreak, in Yambuku, Democratic Republic of Congo, case fatality rates were higher in patients who acquired the infection following injection (100%, 85/85) than by contact (80%, 119/149, P<0.001).[17] This comparison was not adjusted for other factors but the age distribution of patients infected by injection and by contact was similar.[17] The association between route of infection and mortality, and the strong correlation between viral load on admission to treatment centres and mortality, suggest an effect of infecting dose on severity of disease—as found, for example, for measles.[18] A dose effect could also explain the lower case fatality rate in older children, if they are less exposed. The effect of infectious dose on severity of disease has not been investigated previously for Ebola virus disease.

In this retrospective cohort study, we assess risk factors for death from Ebola virus disease, including level of exposure to individuals with Ebola virus disease and their body fluids as a proxy of infecting dose. We also assess the frequency of late deaths in those patients discharged as survivors.

Methods

As part of a retrospective cohort study of transmission patterns, all survivors (or their parents or guardians) who were discharged from the Kerry Town Ebola treatment centre between November 2014 and March 2015 were sought and asked to attend an interview, together with anyone who was living with them at the time that anyone in their household had Ebola virus disease. All the people living in the household at that time were enumerated, and their age, sex, and whether they had had or died from Ebola virus disease was recorded. For those household members who were not said to have had Ebola virus disease, we asked about symptoms at that time. For those discharged as survivors following negative PCR tests for the virus, but who were subsequently found to have died, a verbal autopsy with family members was conducted by a physician, and we examined medical notes and sought information from the treating physicians, where available. The verbal autopsies used a modified version of the World Health Organization's 2014 verbal autopsy instrument. Households were sought for interview between June and September 2015, and again between December 2015 and January 2016 to confirm vital status and conduct verbal autopsies. Individual, written informed consent was sought before interviews, with consent from parents or guardians for those aged under 18 years.

To estimate the level of exposure to Ebola virus, we asked household members to describe in their own words what happened when the Ebola infection struck. For each person with Ebola virus disease, we asked what symptoms they had had, who had taken care of them, who helped them with different activities, who shared a bed with them, among other details. We also asked about any external contacts. The aim was to identify the extent of contact with possibly infective body fluids. Using an eight level scale, we assigned the maximum contact level for each person in the household. We predefined this scale on the basis of the available literature and in discussion with frontline health workers working with individuals with Ebola virus disease. Exposure, from the highest to lowest levels, was defined as follows:

- Direct contact with, or touching, the body of a person who died of Ebola virus disease
- Direct contact with the body fluids of a patient who has Ebola virus disease with wet symptoms (that is, diarrhoea, vomiting, or bleeding)
- Direct contact with a patient with wet symptoms (eg, sharing a bed, providing care, embracing, carrying)
- Direct contact with a patient with dry symptoms (that is, without wet symptoms)
- Indirect contact with a patient with wet symptoms (eg, washing their clothes)
- Indirect contact with a patient with dry symptoms
- Minimal contact (eg, shared meals)
- No known contact

We defined individuals with Ebola virus disease as those already known as survivors from the Kerry Town treatment centre, reported by their families to be survivors from other treatment centres, or reported to have died of the disease. In addition, we included individuals (living or dead) not reported as having had Ebola virus disease but who had symptoms fitting the Sierra Leone case definition of probable disease,[19] unless they had had a negative PCR test at the time. We assessed the effect of including people with probable disease in a sensitivity analysis. Recrudescence of Ebola virus disease was defined as illness or death that could not be attributed to a non-Ebola related cause after a period of full recovery from confirmed Ebola virus disease.

Analyses of risk factors for death used multiple logistic regression, adjusting for clustering by household using random effects. In addition to age, sex, and exposure level, we assessed other risk factors for associations with the outcome of Ebola virus disease. These factors included first or subsequent case in the household, date of Ebola virus infection in household, position in household (head or member), occupation, number of people in the household, and household living conditions (as a score based on measures of crowding and sanitation (access to water, soap, and latrine)). Age, sex, and exposure level were kept in the multivariable model a priori. We added other variables one by one and retained them in the model if they were associated with mortality. We repeated the analyses excluding those cases and deaths classified as probable Ebola virus disease but not reported as Ebola virus disease by the family. We used Stata 14 for analysis.

Patient involvement

Two survivors of Ebola virus disease were involved in the development of the questionnaire and the implementation of the study and were asked to advise on interpretation and writing up of results. There are no plans to disseminate the results of the research directly to the study participants or the relevant patient community, but we will disseminate results to the Ministry of Health and Sanitation and the Ministry of Social Welfare, Gender and Children's Affairs in Sierra Leone, who have responsibility for Ebola survivors.

Results

Late deaths

We obtained follow-up information on all 151 survivors who had been discharged from the Kerry Town Ebola treatment centre with negative blood tests, either from direct contact, or from families or other informants. Four of these survivors had died. The others were known to be alive for a mean of 10 months (range six to 13) after discharge. Details of the four late deaths are as follows:

- Patient A: a 25 year old woman who died 15 days after discharge. During admission, she showed signs of hepatitis. Her liver function tests had greatly improved before discharge but her amylase level was very high. At discharge, her family reported that she was unable to walk but could crawl. She "felt fine" for two days but then developed abdominal swelling, diarrhoea, and swelling of the legs and face, and she looked pale and jaundiced. A postmortem swab by the burial team was found to be negative for Ebola virus by PCR.
- Patient B: a 32 year old woman who died one day after discharge. She was very confused on admission, then improved but continued to act strangely. She had high blood pressure on some days but not consistently. Her platelet count was normal. At discharge, she was unable to walk. The following day, she had a sudden severe headache and was unable to talk or use her limbs. She died that evening. A postmortem swab by the burial team was found to be negative for Ebola virus by PCR.
- Patient C: a 17 year old boy who died five weeks after discharge. His health was reported to have returned to normal after discharge. He then developed weight loss, night sweats, and a productive cough that started after discharge. One week before death, he had pain and difficulty swallowing solids but no other specific symptoms. He died in his sleep. A postmortem swab by the burial team was found to be negative for Ebola virus by PCR.
- Patient D: a 6 year old boy who died one week after discharge. He had had a cough for several months before having Ebola virus disease. On recovery, he remained short of breath with a productive cough and fluctuating pyrexia that did not respond to antibiotics. He was transferred to a paediatric hospital for investigation of possible tuberculosis. A chest radiograph was compatible with miliary tuberculosis. A postmortem PCR test was borderline positive for Ebola virus.

Household members

Of the 151 Kerry Town survivors sought for interview in June to September 2015, eight were living outside Western Area. We did not seek to interview households of survivors known to have died after discharge, except for patient A (because there was another survivor in the household). One survivor refused to take part and 16 were unavailable or not contactable at that time. Therefore, the remaining 123 survivors (including patient A) were included in the study.

The 123 survivors lived in 94 households with 816 household members. We excluded four household members whose cause of death was unclear (fig 1). Overall, 395 people were reported to have had Ebola virus disease in these households (including patients treated at other facilities), of whom 227 died (excluding patient A). A further 53 people fulfilled the case definition for probable Ebola virus disease, of whom 11 died. Therefore, the case fatality rate was 57.5% (227/395) for reported Ebola virus disease, or 53.1% (238/448) including probable disease.

Figure 2 shows the case fatality rate by age, and table 1 shows the associations with death among individuals with Ebola virus disease. The case fatality rate was highest in children under 2 years old and older adults, and lowest at ages 10 to 14 years. The case fatality rate was higher in larger households, with little difference by sex, and varied by exposure level, occupation group, time period, and position in household.

In the full multivariable analysis, only age, household size, date, occupation, and exposure level were associated with death (table 2). Results were similar in a sensitivity analysis after excluding probable disease (table 2). Despite variation in the outcome by exposure level, there was no consistent trend with increasing exposure. For comparison, figure 3 shows the association of exposure level with risk of Ebola virus disease among household contacts (excluding primary cases) in these households.

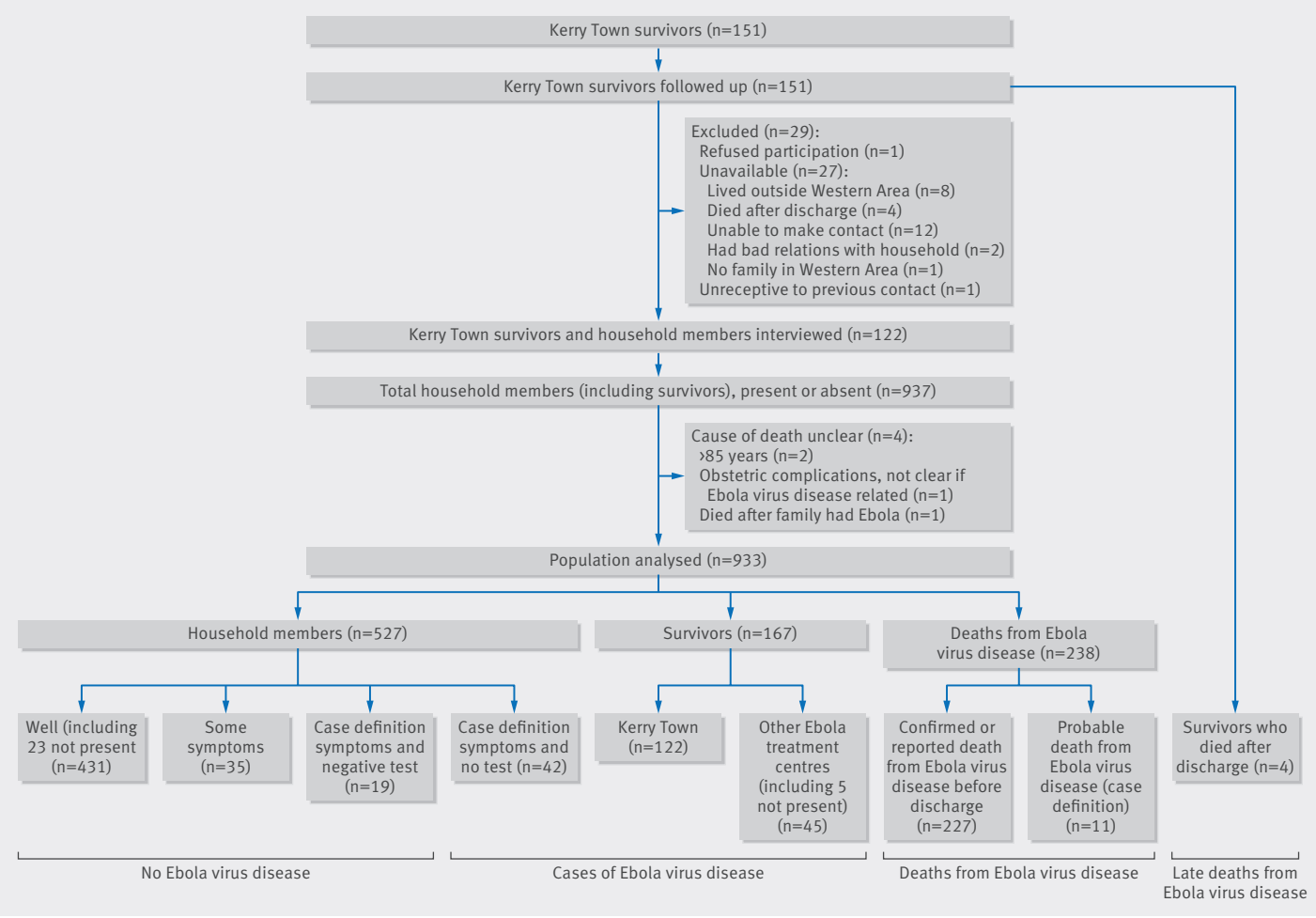

Fig 1 | **Flow diagram showing study composition of participants, from households of survivors of Ebola virus disease**

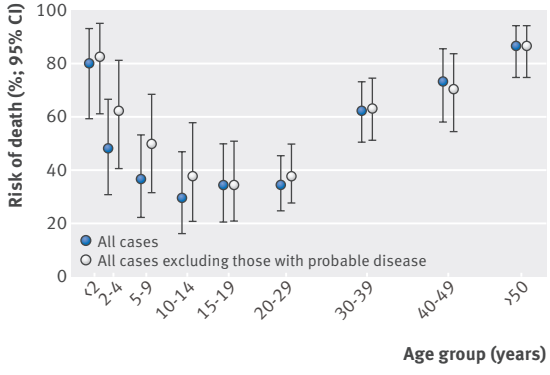

Fig 2 | **Case fatality rates by age among people with Ebola virus disease**

Discussion

Principal findings

In this study, we identified four survivors of Ebola virus disease who died after discharge. All four late deaths could have been caused by Ebola virus disease and its sequelae, although only one patient had a positive PCR result in the postmortem swab. Patient A might have had pancreatitis as a direct effect of the Ebola virus disease.[20] Patient B appears to have had a stroke.[21] Patient C could have had an unrelated chest infection, perhaps tuberculosis, although the duration was short. Patient D could have died of tuberculosis and with, rather than of, Ebola virus disease. If all these deaths were due to Ebola virus disease, this would give a risk of late death of 2.6% (four of 151), but only patient C could be considered a recrudescence because only he had a period of full recovery and so fulfilled the case definition. However, patient C had a negative PCR result postmortem. Bearing in mind the limitations of assigning cause of death by verbal autopsy, particularly with non-medical informants, this would give a maximum estimate of 0.7% recrudescence within a mean follow-up of 10 months.

Among the individuals with Ebola virus disease in this study, we found a U shaped pattern of death by age with a high case fatality rate in the youngest and oldest age groups. We found no association with household level socioeconomic factors other than number of people in the household. The date of Ebola virus infection in the household strongly correlated with mortality. Earlier cases of the disease occurred at the height of the epidemic in Sierra Leone when services were most stretched. By mid-January, case numbers had fallen considerably,[4] treatment centre beds had increased,

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Table 1 | Univariable associations between individual level and household level factors and mortality among individuals with Ebola virus disease

	No of deaths/cases	Proportion (%)	Odds ratio (95% CI) adjusted for clustering, age, and sex	P
Age (years)				
<2	20/25	80.0	7.5 (2.3 to 24.2)	<0.001
2-4	16 /33	48.5	1.9 (0.78 to 4.7)	
5-9	15/41	36.6	1.1 (0.47 to 2.5)	
10-14	11/37	29.7	0.75 (0.30 to 1.9)	
15-19	15/44	34.1	1.0 (0.44 to 2.4)	
20-29	31/90	34.4	1	
30-39	48/77	62.3	3.7 (1.8 to 7.6)	
40-49	33/45	73.3	5.8 (2.4 to 13.8)	
≥50	46/53	86.8	15.9 (5.9 to 42.7)	
Sex				
Female	135/263	51.3	1	0.82
Male	103/185	55.7	1.1 (0.67 to 1.7)	
Primary case				
Yes	61/97	62.9	1	0.10
No	177/351	50.4	0.58 (0.30 to 1.1)	
Exposure level†				
Corpse	39/69	56.5	1	0.009
Fluid	30/80	37.5	0.44 (0.19 to 1.0)	
Direct wet	89/163	54.6	1.7 (0.79 to 3.6)	
Direct dry	37/56	66.1	2.2 (0.89 to 5.5)	
Indirect wet	4/11	36.4	0.80 (0.16 to 3.9)	
Indirect dry	18/24	75.0	2.1 (0.58 to 7.4)	
Minimal/none	21/40	52.5	1.2 (0.45 to 3.3)	
Month of illness				
November	43/63	68.3	1	0.11
December	152/297	51.2	0.45 (0.19 to 1.1)	
January	31/55	56.4	0.67 (0.23 to 2.0)	
February/March	12/33	36.4	0.24 (0.067 to 0.83)	
Position in household				
Head	34/56	60.7	1	0.03
Member	204/392	52.0	2.4 (1.1 to 5.6)	
Occupation				
Manual	91/168	54.2	1	0.005*
Non-manual	34/44	77.3	4.3 (1.6 to 11.6)	
Healthcare worker	18/22	81.8	4.2 (1.0 to 17.9)	
Child/student	89/202	44.1	1.9 (0.76 to 4.8)	
Unknown	6/12	50.0	0.52 (0.096 to 2.8)	
Household size (no of people)				
1-5	6/23	26.1	1	<0.001
6-10	68/163	41.7	1.9 (0.62 to 5.7)	
11-15	66/129	51.2	2.6 (0.87 to 8.0)	
≥16	98/133	73.7	7.4 (2.4 to 23.1)	
Living conditions‡				
Low	29/67	43.3	1	0.56
Medium	14/251	57.4	1.6 (0.69 to 3.5)	
High	64/127	50.4	1.3 (0.56 to 3.2)	
Area of residence				
Rural	54/97	55.7	1	0.60
Urban	183/348	52.6	0.83 (0.41 to 1.7)	

*Excluding unknown category.
†Corpse=direct contact with body of a person who died of Ebola virus disease; fluid=direct contact with body fluids of patient with wet symptoms; direct wet=direct contact with patient with wet symptoms; direct dry=direct contact with patient with dry symptoms; indirect wet=indirect contact with patient with wet symptoms; indirect dry=indirect contact with patient with dry symptoms; minimal/none=minimal or no known contact.
‡Household living conditions based on measures of crowding and sanitation (access to water, soap, and latrine). Possible scores were 0-10. More than half the population had scores of 6 or 7. Low was taken as <6, medium 6-7, high >7.

and staff members were more experienced. The variation by occupation group might reflect the benefits of prompter action, if some groups were more reluctant to seek admission. The non-manual group included 10 religious leaders and chiefs, who all died. Place of residence had no effect on mortality, but was not a good proxy for access to treatment centres because availability of places at different centres varied over time, and household members were often sent to different treatment centres.

Table 2 | Multivariable analysis of association between individual and household level factors and mortality among individuals with Ebola virus disease, overall and after excluding probable disease

	All cases		All cases excluding probable disease	
	Odds ratio (95%CI)*	P	Odds ratio (95%CI)*	P
Age (years)				
<2	10.2 (2.5 to 41.0)		8.0 (1.7 to 37.3)	
2-4	1.3 (0.42 to 3.8)		1.4 (0.41 to 4.9)	
5-9	0.92 (0.31 to 2.8)		1.2 (0.39 to 4.0)	
10-14	0.70 (0.22 to 2.2)		0.74 (0.23 to 2.4)	
15-19	1.1 (0.40 to 3.0)	<0.001	0.87 (0.30 to 2.5)	<0.001
20-29	1		1	
30-39	4.1 (1.9 to 9.0)		3.7 (1.6 to 8.1)	
40-49	6.1 (2.4 to 15.6)		5.1 (2.0 to 13.5)	
≥50	10.1 (3.6 to 28.4)		8.4 (3.0 to 23.6)	
Sex				
Female	1	0.80	1	0.44
Male	1.1 (0.67 to 1.7)		1.2 (0.74 to 2.0)	
Exposure level†				
Corpse	1		1	
Fluid	0.38 (0.16 to 0.89)		0.46 (0.19 to 1.1)	
Direct wet	1.1 (0.52 to 2.4)		1.3 (0.60 to 2.6)	
Direct dry	2.1 (0.83 to 5.0)	0.01	2.7 (1.1 to 7.0)	0.01
Indirect wet	0.48 (0.096 to 2.4)		0.52 (0.10 to 2.6)	
Indirect dry	1.2 (0.33 to 4.2)		2.1 (0.44 to 9.9)	
Minimal/none	1.2 (0.44 to 3.2)		1.2 (0.43 to 3.3)	
Month of illness				
November	1		1	
December	0.65 (0.30 to 1.4)	0.04	0.48 (0.21 to 1.1)	0.01
January	1.2 (0.44 to 3.3)		0.69 (0.23 to 2.0)	
February/March	0.21 (0.064 to 0.72)		0.14 (0.041 to 0.49)	
Occupation				
Manual	1		1	
Non-manual	2.7 (1.0 to 7.2)		2.8 (1.1 to 7.5)	
Child/student	1.4 (0.55 to 3.5)	0.04‡	1.6 (0.64 to 4.2)	0.07‡
Healthcare worker	5.2 (1.1 to 25.2)		3.8 (0.79 to 18.4)	
Unknown	1.4 (0.20 to 10.4)		0.59 (0.070 to 5.1)	
Household size (no of people)				
1-5	1		1	
6-10	2.1 (0.63 to 7.1)	0.003	2.4 (0.71 to 7.9)	<0.001
11-15	3.1 (0.93 to 10.4)		3.6 (1.1 to 12.2)	
≥16	7.0 (2.0 to 24.5)		8.4 (2.4 to 29.2)	

*Odds ratios adjusted for all other factors in the table.
†Corpse=direct contact with body of a person who died of Ebola virus disease; fluid=direct contact with body fluids of patient with wet symptoms; direct wet=direct contact with patient with wet symptoms; direct dry=direct contact with patient with dry symptoms; indirect wet=indirect contact with patient with wet symptoms; indirect dry=indirect contact with patient with dry symptoms; minimal/none=minimal or no known contact.
‡Excluding unknown category.

We found no evidence of a consistent association between case fatality and the extent of exposure to body fluids. Given the strong correlation between these measured exposure levels and risk of Ebola virus disease (fig 3), our predefined exposure scale seemed to be a reasonable measure of infecting dose.

Strengths and limitations of the study

This large study had a complete follow-up at six to 13 months after discharge, so late deaths will not have been missed. The case fatality rate in this study underestimated the overall case fatality rate, because our starting point was survivor households (because they could be approached through the treatment centre outreach team). Excluding one index survivor per household would give a case fatality rate of 75% (227/301) for reported Ebola virus disease, or a rate of 67% (238/354) including probable disease.

We did not include households in which all individuals with Ebola virus disease died; therefore, small households could have been under-represented in our sample. This exclusion might partly explain the association found between case fatality rate and household size, but it is also possible that large households with many affected members found it particularly difficult to provide care.

Associations between the other risk factors and death should not be biased. We were able to include probable cases and deaths that might have been missed from notification data, and assess their influence on the results. In our study, inclusion of probable disease lowered the case fatality rate, but had little effect on the

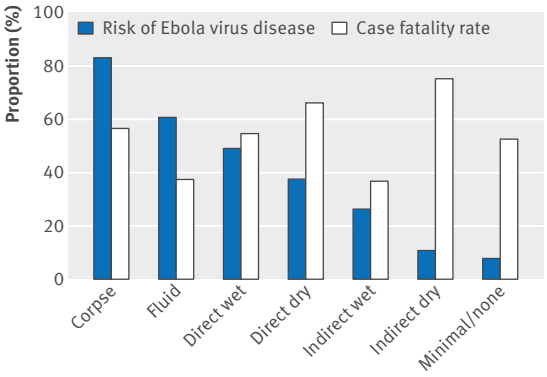

Fig 3 | Relation of exposure level with risk of Ebola virus disease and with case fatality rate. To assess risk of disease by exposure level, primary cases in each household were excluded. Probable Ebola virus disease and deaths are included. Corpse=direct contact with body of a person who died of Ebola virus disease; fluid=direct contact with body fluids of patient with wet symptoms; direct wet=direct contact with patient with wet symptoms; direct dry=direct contact with patient with dry symptoms; indirect wet=indirect contact with patient with wet symptoms; indirect dry=indirect contact with patient with dry symptoms; minimal/none=minimal or no known contact

associations with mortality. We did not know which of the deaths occurred in treatment centres, so could not assess the benefit of admission directly.

Comparison with other studies

To our knowledge, this is the first cohort study of Ebola virus disease with active follow-up for late deaths, and the first large community based study to look at risk factors for death from the disease. Previous studies of sequelae have reported on patients seen in clinics so would have missed any deaths.[11 13]

The U shaped pattern of death by age was similar to that seen in the WHO notification data[4] and, as in these notification data,[4] we found a marginally higher case fatality rate in males. The lack of association between socioeconomic status and case fatality suggests that although socioeconomic status has been associated with the risk of individuals having Ebola virus disease,[22] once ill, living conditions had little effect on the outcome. The variation in case fatality rate by occupation group could reflect different responses to illness; delays in coming forward for treatment by healthcare workers have been reported previously.[23]

This study also looks at the association between exposure level (as a proxy of dose) and case fatality rate in Ebola virus disease. A lack of association between infecting dose and severity of disease suggests that symptomatic illness can be established by one or very few organisms.[24] Dose can therefore affect the probability of contracting disease without influencing severity and risk of death once a person becomes ill. This is consistent with animal challenge studies which find that animals receiving low doses of Ebola virus either died or remained asymptomatic,[25 26] although higher doses were associated with a shorter time to death.[27] It is also compatible with the association between Ebola viral load on admission to treatment centres and outcome, since a high viral load at this stage suggests a failure to control viral multiplication rather than a high initial infecting dose. However, deep sequencing of viruses has found the same minority variants in different patients, suggesting that the transmission bottleneck allows through more than one virus.[28 29] Whatever the mechanism, it appears that once a person becomes ill, factors other than infectious dose determine the outcome, and different immune responses have been noted in survivors and fatalities from early on in the disease.[30]

Conclusions and policy implications

The age pattern of the case fatality rate suggests that differences in susceptibility are important in determining the outcome of Ebola virus disease. However, the associations with time period, occupation, and household size suggest that care given was crucial in reducing mortality, emphasising the importance of Ebola treatment centres. Infecting dose of the virus did not appear to have a role. All deaths after discharge occurred within a few weeks, and we have follow-up information six to 13 months later on all survivors. Recrudescence of severe active disease leading to death appears to be rare, which should be reassuring for Ebola survivors and their contacts, but does not remove the need for continued monitoring of survivors' health.

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Contributors: JRG, HB, and FC designed the study with contributions from all the other authors. HB, SJ, and ES led the fieldwork with MSB, OK, and CT. SO maintains the survivor database. JRG and HB did the analysis. JRG led the writing with contributions from all the other authors. All authors have approved the final manuscript. JRG is the study guarantor, had full access to all the data, and is responsible for the decision to submit for publication.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: support from Save the Children and the Wellcome Trust for the submitted work; Save the Children International operated the Kerry Town Ebola treatment centre during the study, and employed the field team members; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: Permission for the study was granted by the Sierra Leone ethics and scientific review committee and the ethics committee of the London School of Hygiene & Tropical Medicine. All living participating household members gave individual, written informed consent (parents or guardians gave consent for those aged under 18 years).

Data sharing: A full dataset will be available with restrictions to ensure confidentiality and prevent deductive disclosure in the London School of Hygiene and Tropical Medicine's data repository, DataCompass. The consent mentioned publication but did not explicitly mention data sharing; the presented data are anonymised and risk of identification is low.

The guarantor affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Paper 5: 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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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

Judith R Glynn, Hilary Bower, Sembia Johnson, Catherine F Houlihan, Carla Montesano, Janet T Scott, Malcolm G Semple, Mohammed S Bangura, Alie Joshua Kamara, Osman Kamara, Saidu H Mansaray, Daniel Sesay, Cecilia Turay, Steven Dicks, Raoul E Guetiya Wadoum, Vittorio Colizzi, Francesco Checchi, Dhan Samuel, Richard S Tedder*

Summary

Background The frequency of asymptomatic infection with Ebola virus is unclear: previous estimates vary and there is no standard test. Asymptomatic infection with Ebola virus could contribute to population immunity, reducing spread. If people 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 new 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.

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*Dr Dhan Samuel died in April, 2016.

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, perhaps with persistent viral shedding, it would help explain some failures in control and the emergence of new chains of transmission.[2]

The extent of asymptomatic infection is unclear because previous findings have varied widely (eg, from 1% to 46% of household contacts),[3,4] with positive results reported in some populations unlikely to have been exposed to filoviruses.[5–7] This finding has led to questions about assay specificity and cross-reactivity for ELISAs as well as for the older immunofluorescence antibody techniques. There is no assay approved by the US Food and Drug Administration, and the need for caution in interpreting Ebola virus antibody serosurveys continues to be emphasised.[8]

A reliable serological test could also help identify missed cases with minor symptoms. Asymptomatic infections and missed symptomatic cases might explain the apparent lower incidence of Ebola virus disease (EVD) in children.[9,10] Diagnosis might be missed in young children,[11] and older children could be less susceptible to developing EVD if infected.[12]

A test for Ebola virus antibodies with high sensitivity and specificity is needed. Taking blood is difficult in an Ebola epidemic, due to both the infection risk and population suspicion. We describe the field validation of a new capture ELISA that detects IgG to Ebola virus glycoprotein in oral fluid,[13] and the results of a large seroprevalence study in Ebola-affected households.

Methods

Participants and data collection

All survivors from Kerry Town Ebola Treatment Centre, Sierra Leone, who were discharged between Nov 22, 2014, and March 27, 2015, and their household members

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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 antibod* OR IgG OR immun* 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.

(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.[12] 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.[12] 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 with diarrhoea, vomiting, or bleeding); direct contact with a wet case (including nursing and personal care, sharing a bed); direct contact with a dry case (ie, an EVD case without wet symptoms); indirect contact with a wet case (eg, washing clothes or bed linen); indirect contact with a dry case; minimal contact (eg, shared meals); and no known contact.

Individuals who did not report EVD were asked about symptoms at the time that others in the household had EVD. Those reporting symptoms were classified using the Sierra Leone case definition for probable EVD[14] (ie, either contact plus fever or miscarriage or unexplained bleeding, or contact plus three or more symptoms [of fatigue, headache, loss of appetite, nausea or vomiting, abdominal pain, diarrhoea, muscle or joint pain, sore throat or pain on swallowing, and hiccups]).

Swabs (Oracol, Malvern Medical Developments, Worcester, UK) for oral fluid collection were demonstrated by the field staff and then self-administered, with adults helping children. Each swab was rubbed firmly on the gums for 90 s, sealed, put in a cool box, and transferred daily to a –20°C freezer for storage before processing.

Additionally, we recruited community controls in three neighbourhoods of rural Western Area, Sierra Leone, without known EVD cases (Kent, Tokeh, and York). Community leaders with megaphones asked for volunteers of all ages, and we then excluded any with exposure to Ebola and collected oral swabs as described above.

Individual written informed consent was obtained from all participants (or their parents or guardians for those younger than 18 years) before interview and sample collection. Permission for the study was granted by the Sierra Leone Ethics and Scientific Review Committee and the Ethics Committee of the London School of Hygiene & Tropical Medicine.

Procedures

Oral fluid samples were tested for Ebola virus glycoprotein IgG using a new IgG capture assay based on the EBOV Mayinga GP antigen (rGPδTM [catalogue 0501-016]; IBT Bioservices, Rockville, MD, USA) as described elsewhere.[13] 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 normalised ODs (ie, the ratio of the test OD to the cutoff), so results greater than 1 were reactive. All reactive samples from household members and controls, all

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).

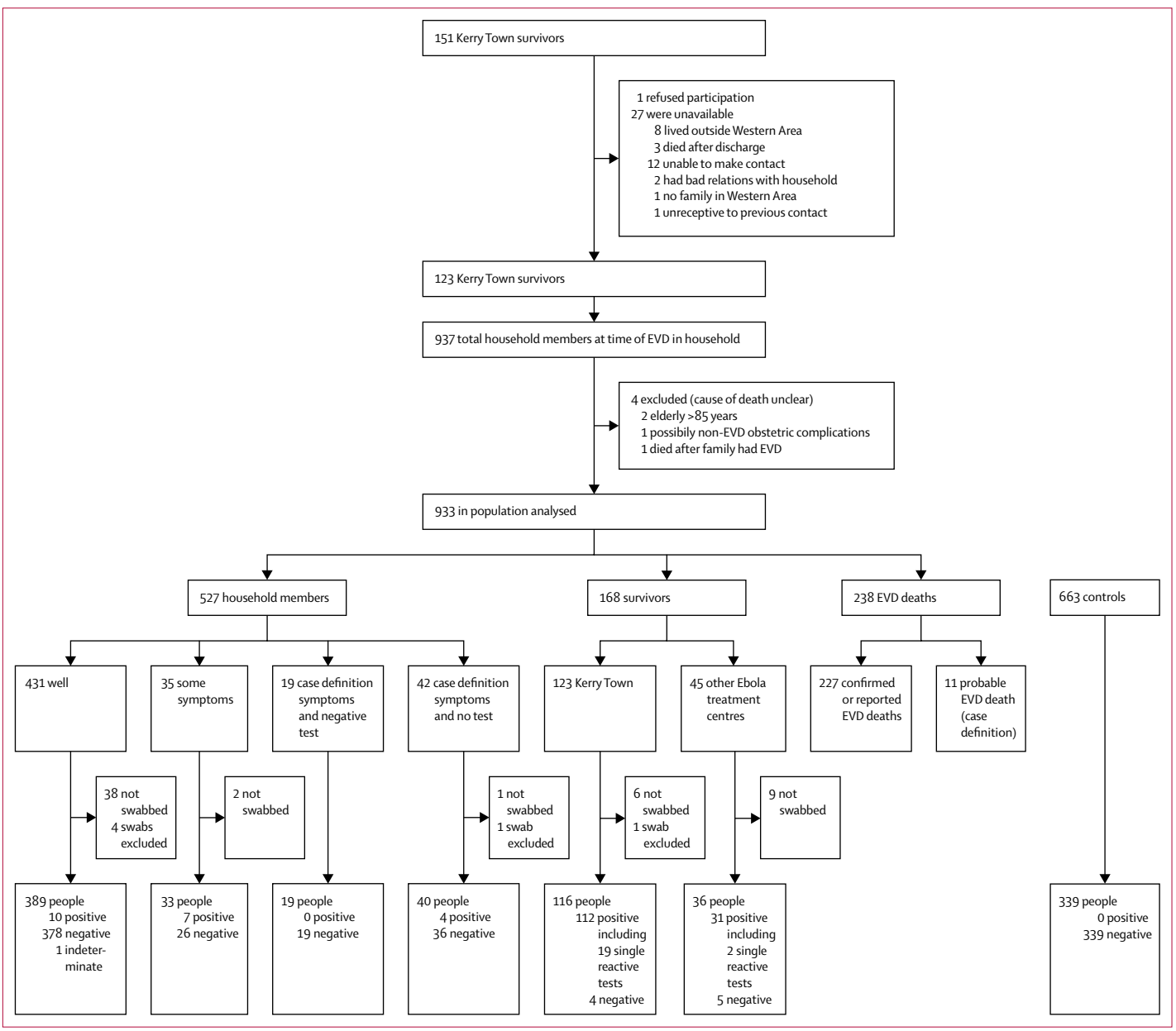

Figure 1: **Flow chart of study participants**
Households were defined as those who ate from the same pot. They included everyone who stayed there at the time Ebola was in the household, including those who were not normally resident. Of those not swabbed, most were absent; eight refused (all had been asymptomatic) and four had died since Ebola. Of the six excluded swabs, three were miscoded and three were not found. EVD=Ebola virus disease.

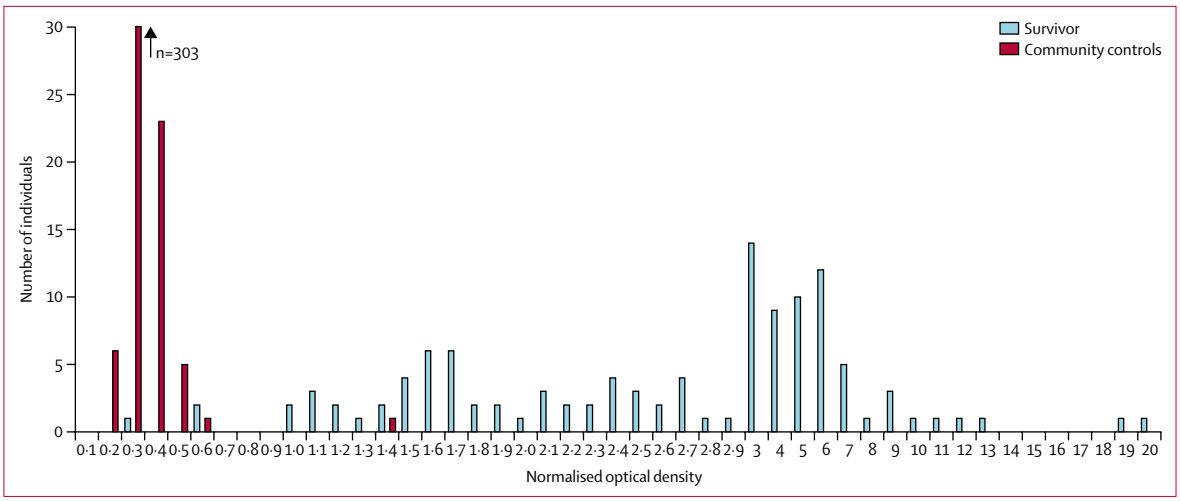

Figure 2: **Normalised optical densities of the first test in samples from 116 Kerry Town survivors and 339 Sierra Leone controls**

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 χ^2 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

For the **raw data** see http://doi.org/10.17037/DATA.41

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[15] but the household included another survivor so was visited. Of the remaining survivors, eight lived outside Western Area, three had died,[15] 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

	Total	Positive			Negative				Indeterminate	IgG positive/total*	IgG positive (95% CI)
		RR	R	RUR	RUU	UUU	UU	U	RRUU		
Community controls	339	0	0	0	1	0	25	313	0	0/339	0·0% (0–1·08)
Kerry Town survivor	116	92	19	1	1	2	1	0	0	93/97	95·9% (89·8–98·9)
Household member: survivor from other Ebola treatment centre	36	29	2	0	0	2	3	0	0	29/34	85·3% (68·9–95·0)
Household member: asymptomatic	389	10	0	0	17	8	76	277	1†	10/388	2·6% (1·2–4·7)
Household member: symptomatic	92	10	0	1	1	2	8	70	0	11/92	12·0% (6·1–20·4)
Symptoms fitting case definition/no PCR test	40	3	0	1	1	1	3	31	0	4/40	10·0% (2·8–23·7)
Symptoms fitting case definition/PCR negative	19	0	0	0	0	1	0	18	0	0/19	0·0% (0–17·6)
Symptomatic not fitting case definition	33	7	0	0	0	0	5	21	0	7/33	21·2% (9·0–38·9)

Because of limited availability of kits, not all samples could be retested. We retested all positives (except some from known survivors of Ebola virus disease but including all those nearer the cutoff), all negatives from EVD survivors, and a sample of other negatives, prioritising those nearer the cutoff. We did third tests on any samples with discrepant results after two tests. For those samples with only one previous result, which were retested on the last available plate, we retested in duplicate in case any discrepancies arose. R=reactive. U=unreactive. *Total individuals; those with only a single reactive test available or indeterminate results excluded. †Retested because of borderline results; mean of all normalised optical densities 1·0 (SD 0·4; appendix p 5).

Table 1: **Prevalence of Ebola IgG positivity in samples from Ebola virus disease survivors, household contacts, and community controls, Sierra Leone, 2015**

repeatedly reactive and considered positive (table 1). Defining positive as two reactive tests gives a sensitivity of 95·9% (95% CI 89·8–98·9; 93 of 97 samples; table 2).

Among the community controls, all but one sample were unreactive on the initial test (338 [99·7%] of 339 samples). This sample was unreactive on second and third tests (NODs 1·41, 0·33, and 0·32). There were no further reactive results among 25 samples that were retested. Since no control sample was considered positive, specificity was 100% (95% CI 98·9–100).

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 asymptomatically. 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 asymptomatically. 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

	Sensitivity (proportion of Kerry Town survivors reactive on test)		Specificity (proportion of community controls unreactive on test)	
	n/N	% (95% CI)	n/N	% (95% CI)
Single test	113/116	97·4% (92·6–99·5)	338/339	99·7% (98·4–99·99)
Confirmed	93/97	95·9% (89·8–98·9)	339/339	100% (98·9–100)

Results are presented on the basis of a single test, and using the rule that all reactive results should be confirmed by a second test.

Table 2: **Sensitivity and specificity of the oral fluid Ebola virus antibody test**

See Online for appendix

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	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% (6·1–20·4)	
Sex								
Male	161	3	1·9% (0·0–5·3)	0·53	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)	0·11	3	1	33·3% (0·8–90·6)	0·001
2–4	43	0	0·0% (0·0–8·2)	0·06 (trend)	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% (0·0–23·2)	
15–19	52	1	1·9% (0·0–10·3)		7	0	0·0% (0·0–41·0)	
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	2	100·0% (15·8–100)	0·06
Handled fluids	39	4	10·3% (2·9–24·2)	0·06 (trend)	17	1	5·9% (0·1–28·7)	
Direct wet contact	120	1	0·83% (0·0–4·6)		41	6	14·6% (5·6–29·2)	
Direct dry contact	68	0	0·0% (0·0–5·3)		13	1	7·7% (0·2–36·0)	
Indirect wet contact	11	0	0·0% (0·0–2·9)		2	0	0·0% (0·0–84·2)	
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)	0·10	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	4·5% (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	5	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	1	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% (3·8–5·3)		24	2	8·3% (1·0–27·0)	
>16	76	2	2·6% (3·2–9·2)		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% (0·0–41·0)	0·89
Most days	125	3	2·4% (0·5–6·9)		27	3	11·1% (2·4–29·2)	
Every day	183	6	3·3% (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	11·1% (1·4–34·7)	1·0
Most days	72	2	2·8% (0·3–9·7)		23	3	13·0% (2·8–33·6)	
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	71	9	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	4	16·0% (4·5–36·1)	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)

	Asymptomatic				Any symptoms			
	Total (n)	IgG positive			Total (n)	IgG positive		
		n	% (95% CI)	p		n	% (95% CI)	p
(Continued from previous page)								
Spouse Ebola survivor								
No	372	10	2·7% (1·3–4·9)	1·0	86	9	10·5% (4·9–18·9)	0·15
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)	0·18	9	1	11·1% (0·3–48·2)	0·45
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**

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 (r^2=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 (95·9%).

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;[16] 4·0% (four of 101) in Kikwit;[17] 21·4% (12 of 56) in Gabon;[18] 45·9% (11 of 24) among highly exposed contacts in Gabon;[4] 1·0% (two of 210) in Uganda,[3] and 6·5% (12 of 185) in Kono, Sierra Leone,[19] 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.[20]

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 asymptomatically infected individuals, and between adults and children, have been noted previously.[17,21,22] The slight excess of missed symptomatic infections in children younger than 5 years is consistent with underdiagnosis in this group.[11] There was no evidence that any of the seropositive results were due to late transmission via semen:[23] only two spouses of EVD survivors were seropositive and both were male.

WHO guidelines for EVD survivor care[24] 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

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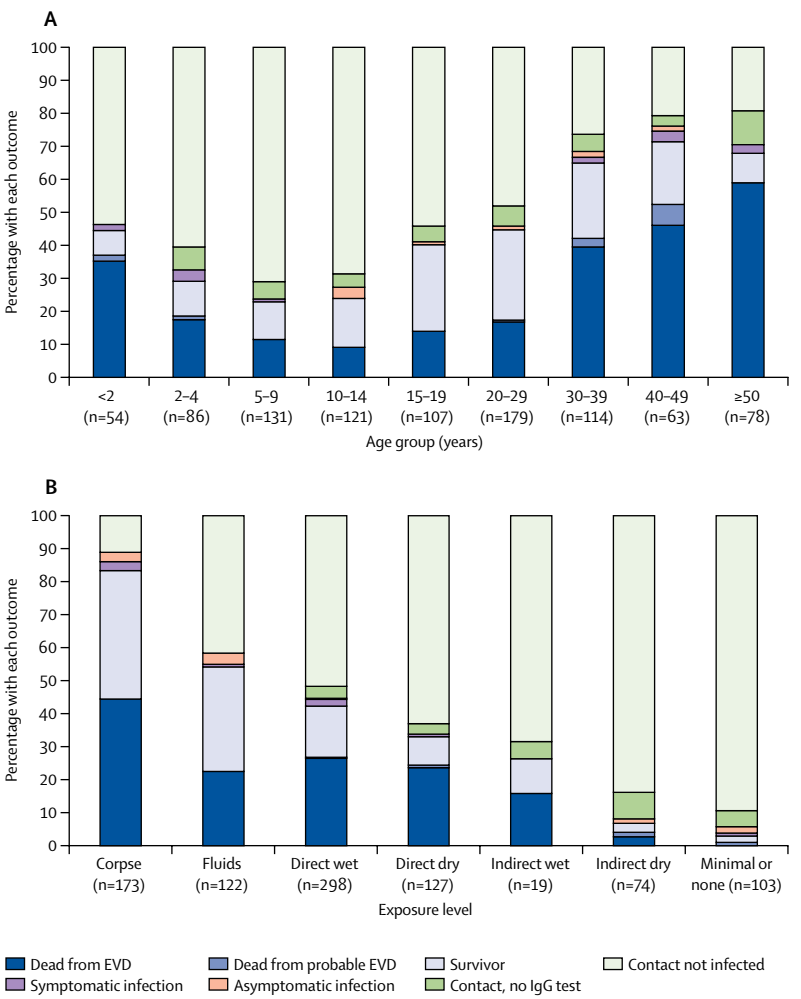

Figure 3: **Ebola manifestation and risk in households of survivors of Ebola virus disease (A) by age group in all members and (B) by exposure level (excluding the primary cases in each household)**
The primary cases were excluded for (B) so that the outcomes for each type of contact in Ebola-affected households could be seen. Information on deceased household members was provided at interview by the surviving household members. Exposure levels were determined from the interviews with all household members. Exposure levels are defined as follows: corpse, touched body of someone who died of EVD; fluids, direct contact with body fluids of a wet case (ie, an EVD case with diarrhoea, vomiting, or bleeding); direct wet, direct contact with a wet case (including nursing and personal care, sharing a bed, breastfeeding an EVD-positive child); direct dry, direct contact with a dry case (ie, an EVD case without wet symptoms); indirect wet, indirect contact with a wet case (eg, washing clothes or bed linen); indirect dry, indirect contact with a dry case; minimal or none, minimal contact (eg, shared meals) or no known contact. See Bower and colleagues[12] for details. EVD=Ebola virus disease.

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,[17,25,26] 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,[12,27–29] 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-affected 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 asymptomatically 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 JTS. 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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Supplementary appendix

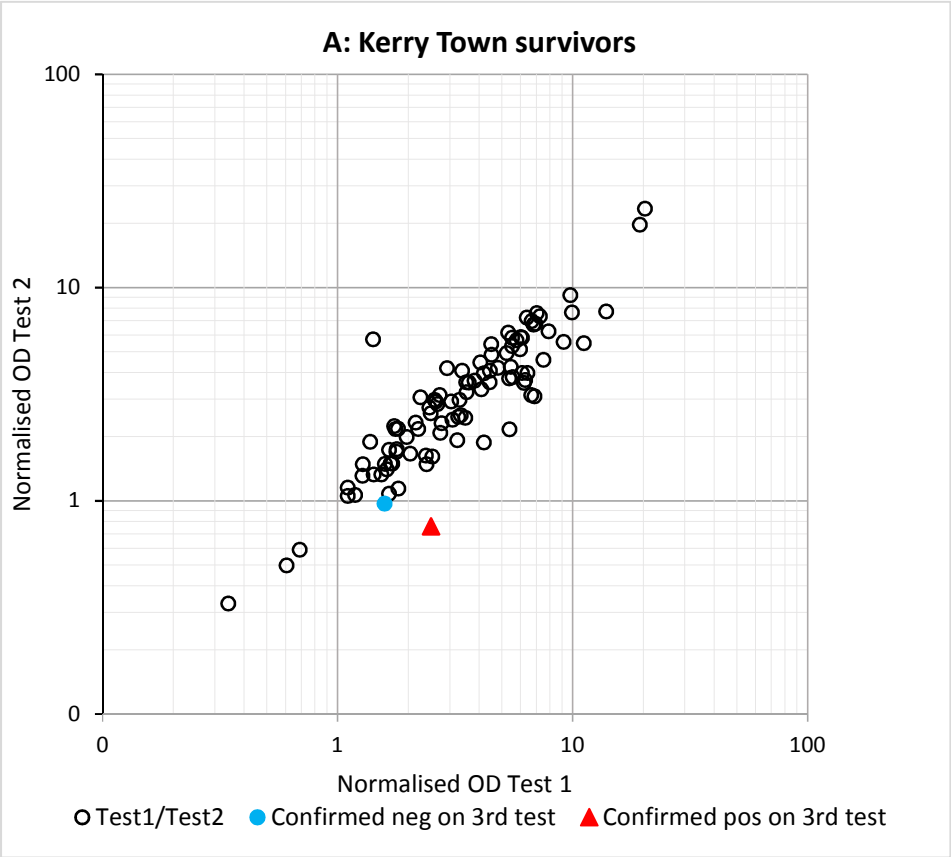
This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

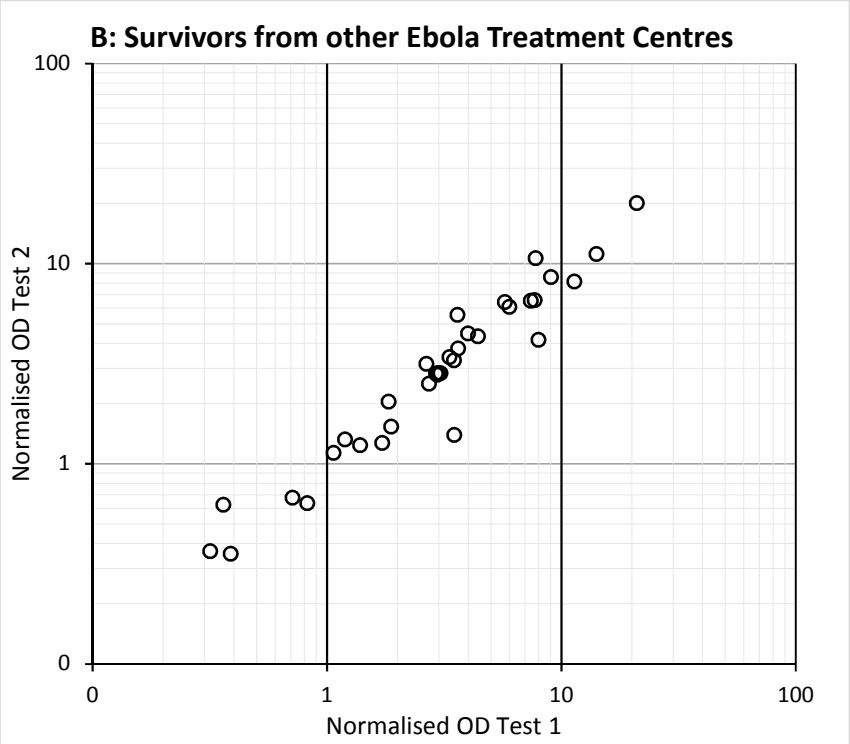
Supplement to: Glynn JR, Bower H, Johnson S, et al. Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus. *Lancet Infect Dis* 2017; published online Feb 27. http://dx.doi.org/10.1016/S1473-3099(17)30111-1.

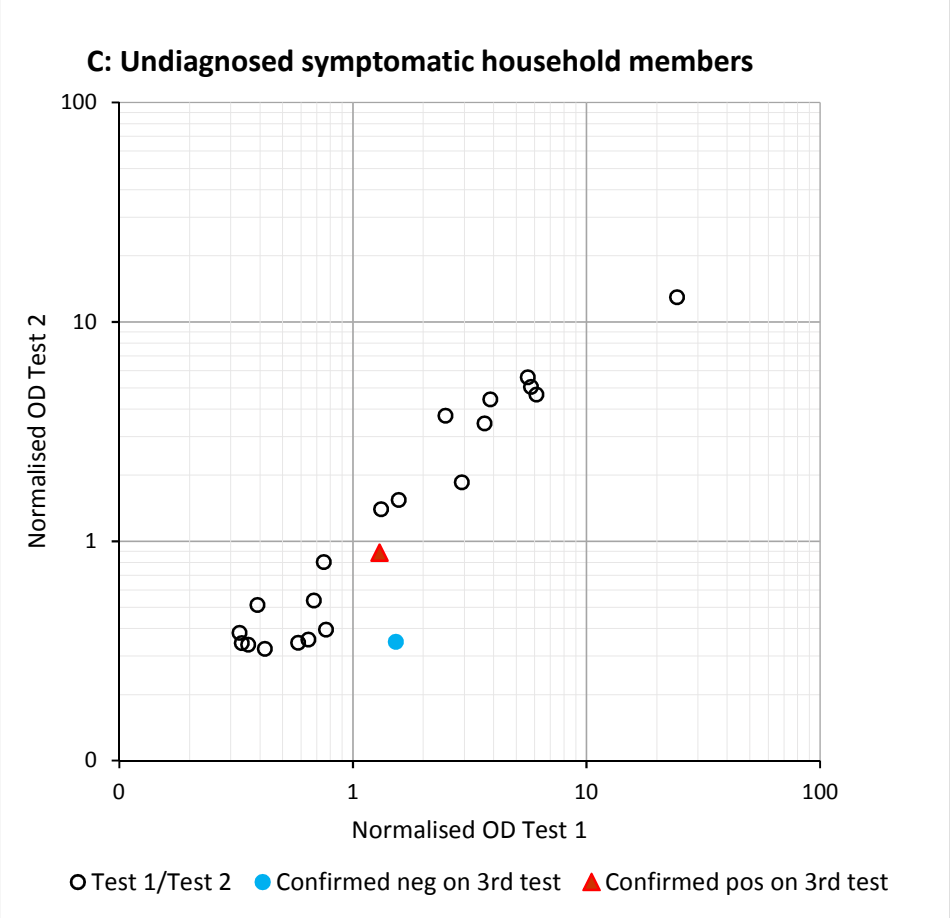
Appendix: Asymptomatic infection and unrecognised Ebola Virus Disease in Ebola-affected households in Sierra Leone: a cross sectional study using a new non-invasive assay for antibodies to Ebola virus

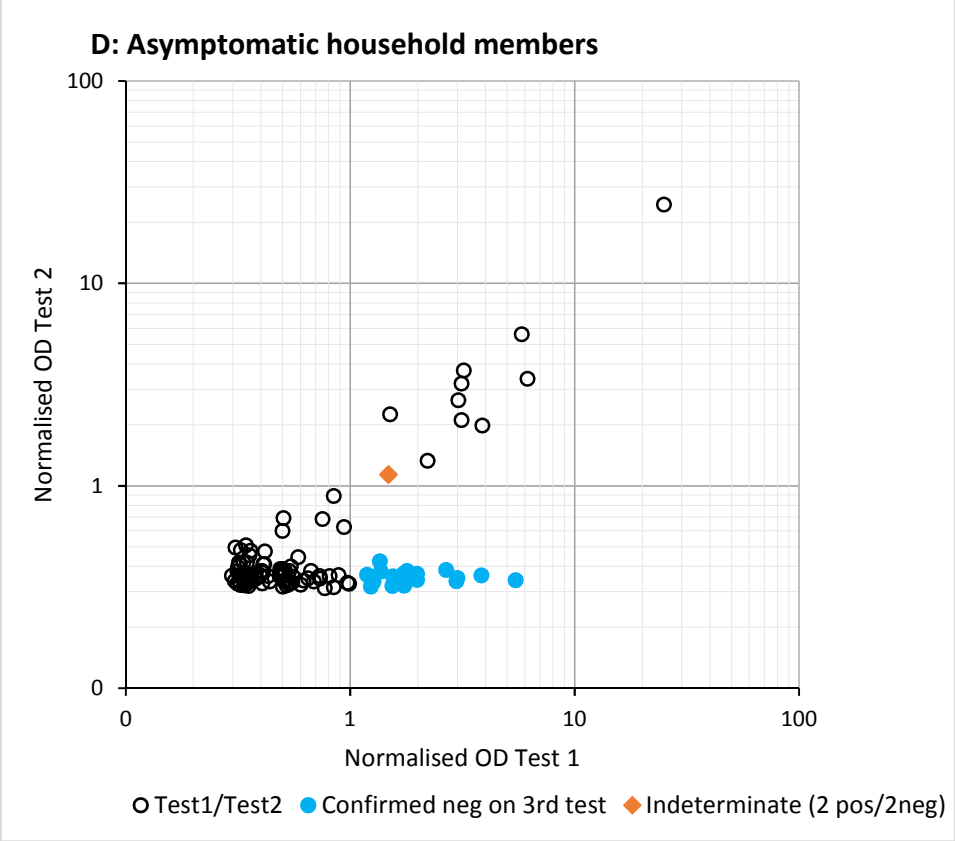
Judith R Glynn J, PhD, Hilary Bower, MSc, Sembia Johnson, BSc, Catherine F Houlihan, PhD, Carla Montesano, PhD, Janet T Scott, PhD, Malcolm G Semple, PhD, Mohammed S Bangura, Alie Joshua Kamara, Osman Kamara, Saidu H. Mansaray, Daniel Sesay, Cecilia Turay, Steven Dicks, MSc, Raoul E Guetiya Wadoum RE, Msc, Vittorio Colizzi, PhD, Francesco Checchi, PhD, Dhan Samuel, PhD, Richard S Tedder, FRCPath

Figure 1: Comparison of normalised optical density (OD) in samples tested in duplicate. (A) Among 97 Kerry Town Survivors; (B) among 34 Ebola survivors from other Ebola Treatment Centres; (C) among 22 symptomatic household contacts; (D) among 112 asymptomatic household contacts; (E) among 26 community controls. Samples that gave discrepant results (reactive/unreactive) are highlighted and the results on subsequent testing are indicated. Samples tested in duplicate were: all those from contacts and controls that were reactive on the first test; all those from survivors that were unreactive on the first test; and a selection of other samples including those closer to the cut-off (see Table 1 in the main text).




B: Survivors from other Ebola Treatment Centres
100
10
1
0
Normalised OD Test 2
0
1
10
100
Normalised OD Test 1


C: Undiagnosed symptomatic household members
100
10
1
0
Normalised OD Test 2
0
1
10
100
Normalised OD Test 1
Test 1/Test 2
Confirmed neg on 3rd test
Confirmed pos on 3rd test


D: Asymptomatic household members
100
10
1
0
Normalised OD Test 2
0
1
10
100
Normalised OD Test 1
Test1/Test2
Confirmed neg on 3rd test
Indeterminate (2 pos/2neg)

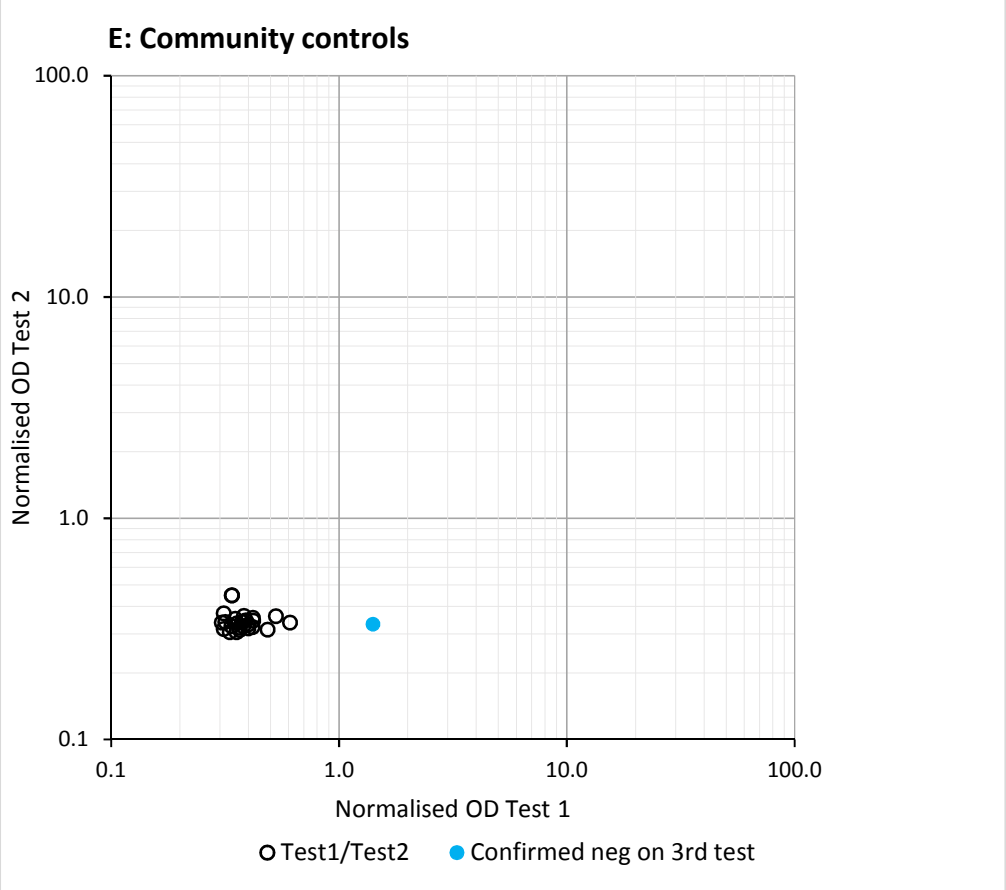

E: Community controls
100.0
10.0
1.0
0.1
Normalised OD Test 2
0.1
1.0
10.0
100.0
Normalised OD Test 1
Test1/Test2
Confirmed neg on 3rd test

Figure 2: Normalised optical density (OD) results from samples with initially discrepant results, by participant group; and from Kerry Town (KT) survivors with negative results. The different results from each sample are presented vertically.

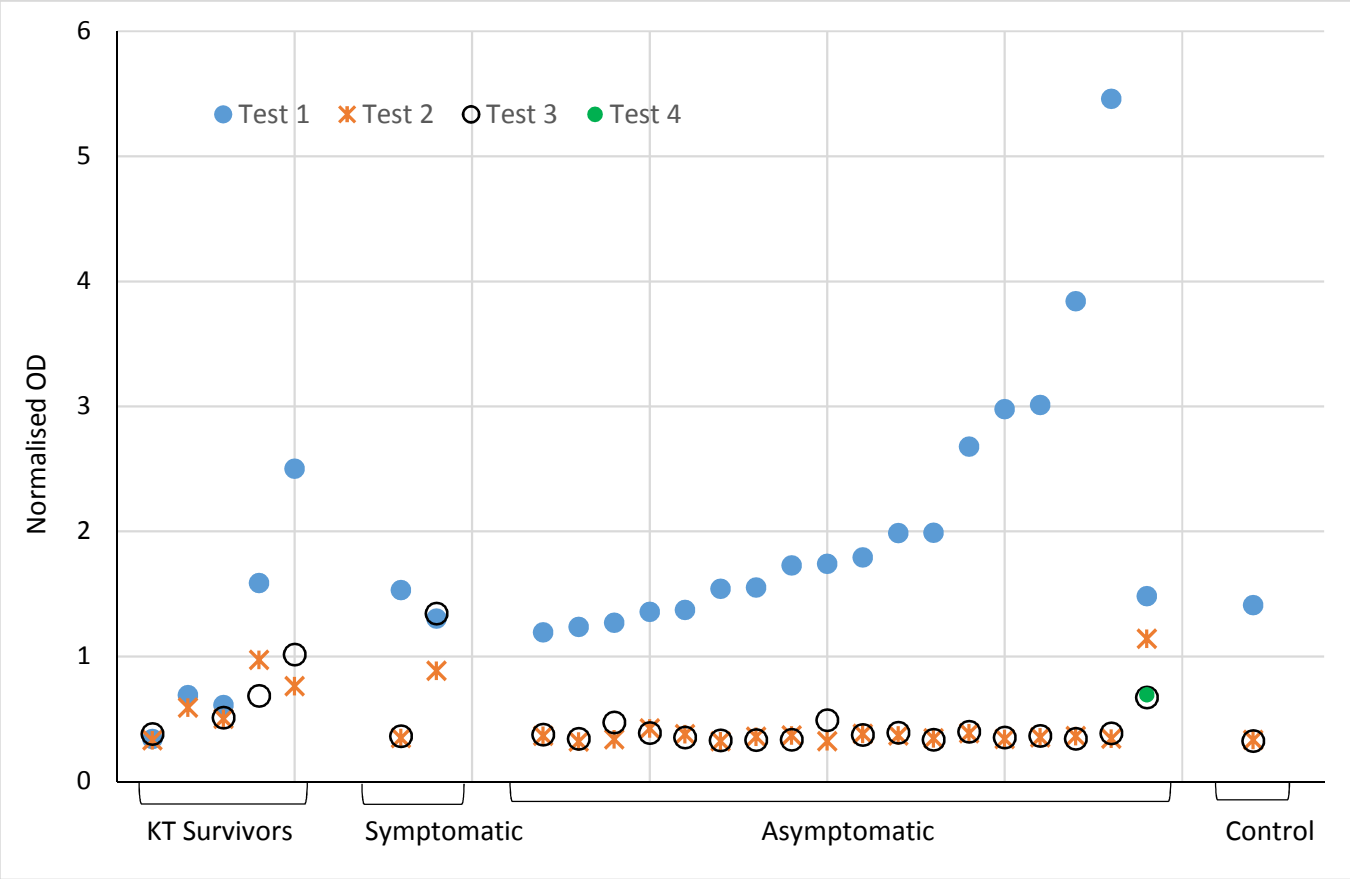


Figure 3. Box plot of normalised optical densities (OD) in samples confirmed seropositive, by participant group. The mean of all tests per sample was used.

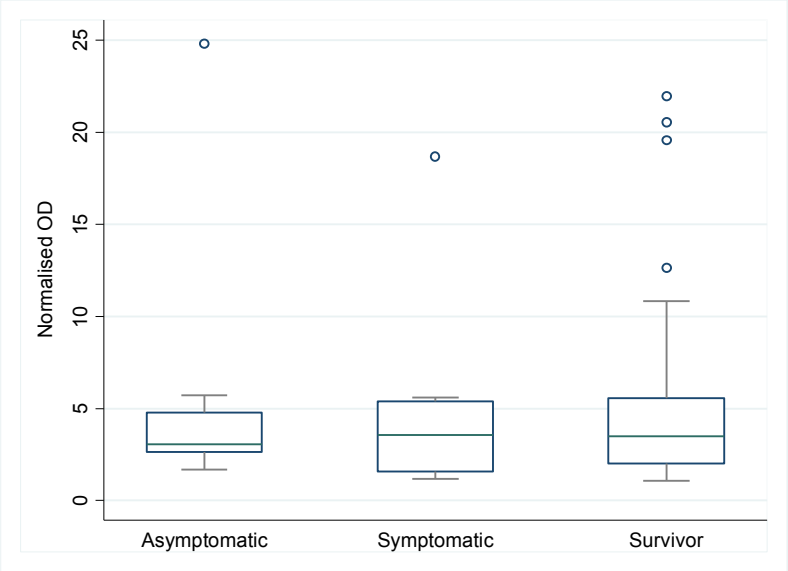


Figure 4. Distribution of normalised optical density (OD) in samples from survivors (Kerry Town and other Treatment Centres) by time since admission. The mean of all tests per sample was used.

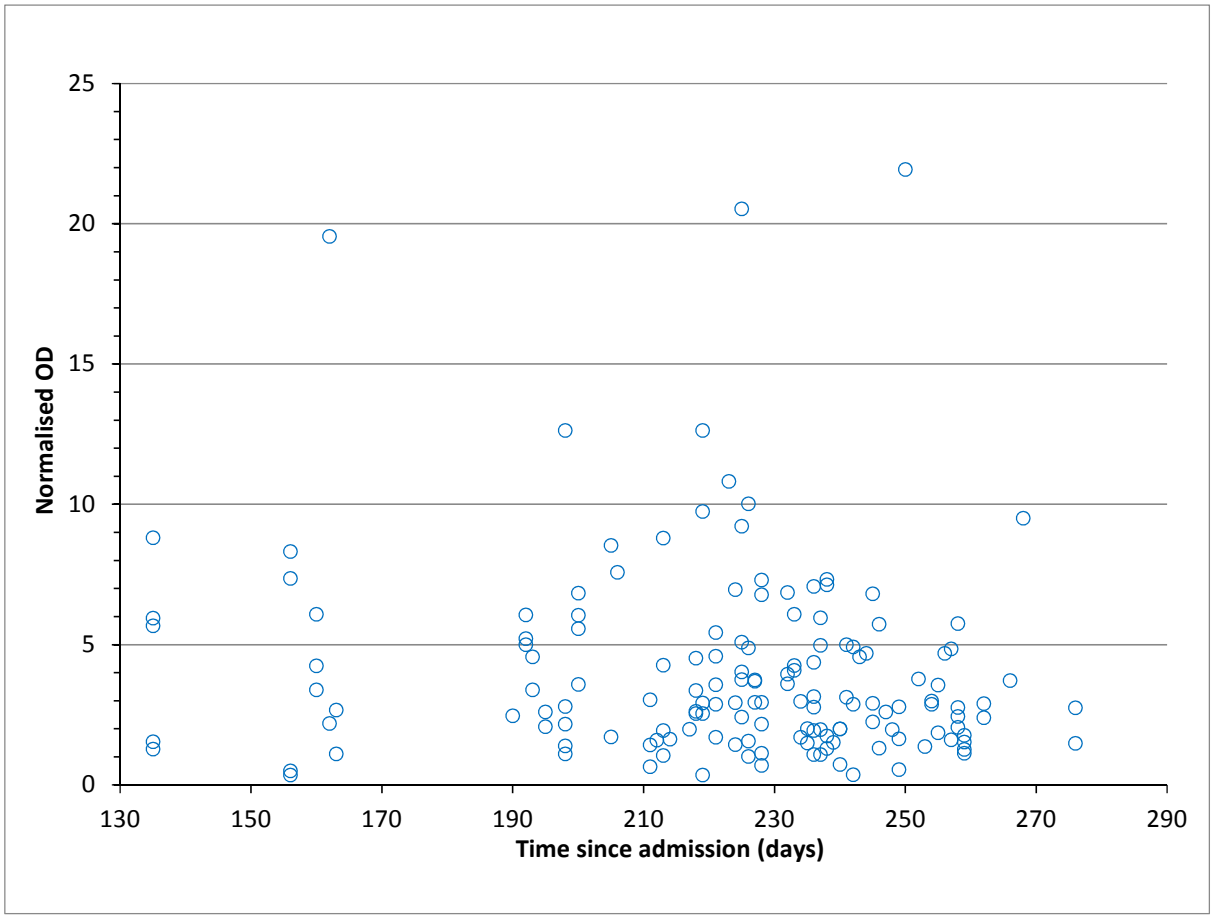


Figure 5. Distribution of normalised optical density (OD) in samples from survivors (Kerry Town and other Treatment Centres) by age. The mean of all tests per sample was used.

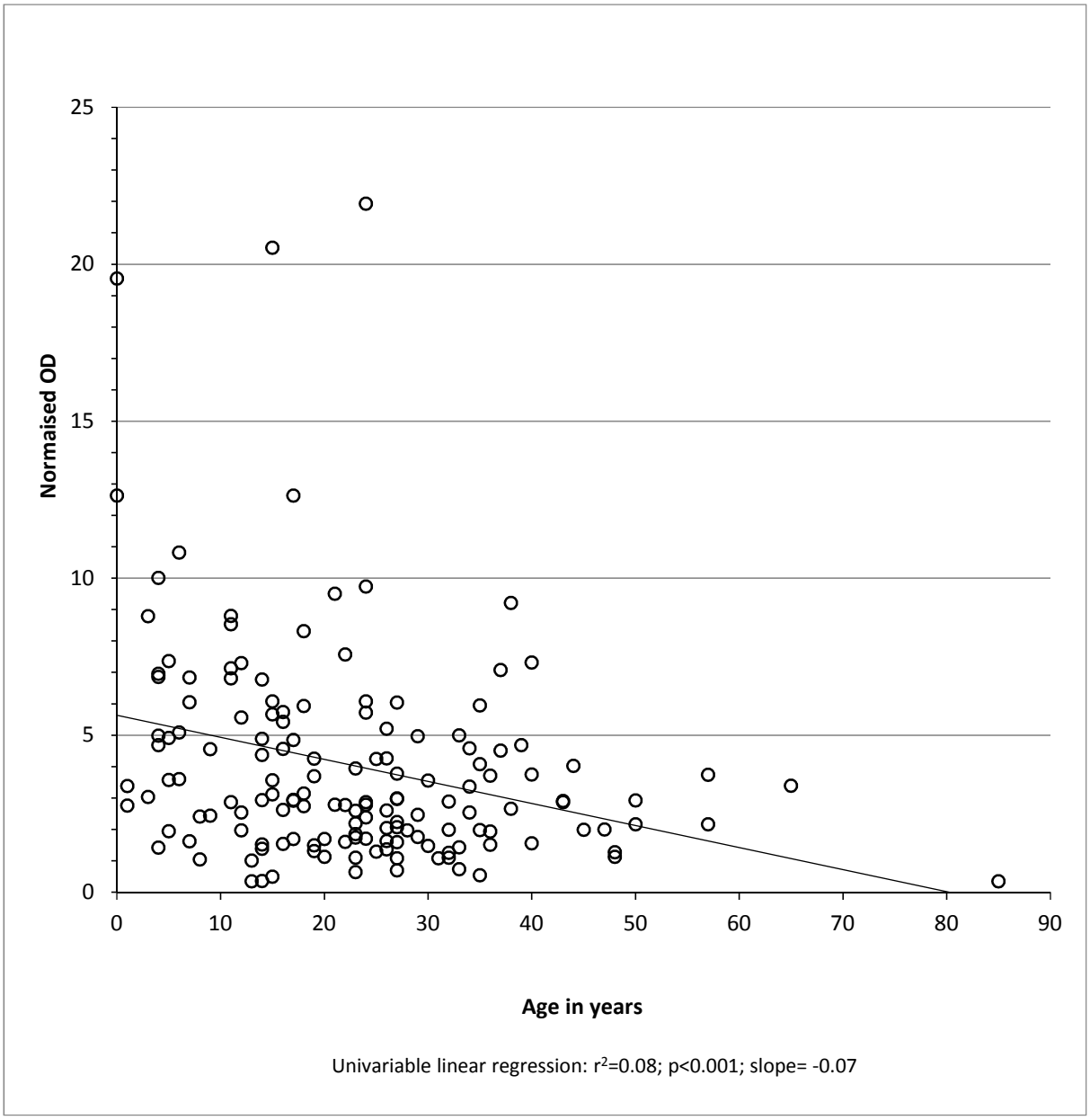


Table 1: Asymptomatic and symptomatic undiagnosed infections as a proportion of all EBOV infections in survivor households

	Died EVD	Survivor	Symptomatic undiagnosed	Asymptomatic infection	All EBOV infections
	n (%)	n (%)	n (%)	n (%)	N
Overall	238 (55.7)	168 (39.3)	11 (2.6)	10 (2.3)	427
Age group*					
<5	36 (67.9)	13 (24.5)	4 (7.5)	0	53
5-14	26 (40.6)	33 (51.6)	1 (1.6)	4 (6.3)	64
≥15	173 (56.4)	122 (39.7)	6 (2.0)	6 (2.0)	307
Sex					
Female	135 (53.3)	106 (41.9)	5 (2.0)	7 (2.8)	253
Male	103 (59.2)	62 (35.6)	6 (3.4)	3 (1.7)	174

* Age missing for 3

Paper 6: Variability in intrahousehold transmission of Ebola Virus, and estimation of the household secondary attack rate

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The Journal of Infectious Diseases

BRIEF REPORT

Variability in Intrahousehold Transmission of Ebola Virus, and Estimation of the Household Secondary Attack Rate

Judith R. Glynn,[1] Hilary Bower,[1] Sembia Johnson,[2,a] Cecilia Turay,[2] Daniel Sesay,[2] Saidu H. Mansaray,[2] Osman Kamara,[2] Alie Joshua Kamara,[2] Mohammed S. Bangura,[2] and Francesco Checchi[3,b]

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Transmission between family members accounts for most Ebola virus transmission, but little is known about determinants of intrahousehold spread. From detailed exposure histories, intrahousehold transmission chains were created for 94 households of Ebola survivors in Sierra Leone: 109 (co-)primary cases gave rise to 317 subsequent cases (0–100% of those exposed). Larger households were more likely to have subsequent cases, and the proportion of household members affected depended on individual and household-level factors. More transmissions occurred from older than from younger cases, and from those with more severe disease. The estimated household secondary attack rate was 18%.

Keywords. Ebola; transmission chains; intrahousehold; risk factors; secondary attack rate.

Although funerals and healthcare settings play an important role in the spread of Ebola virus, community transmission, mostly between family members, accounts for the majority of transmissions [1, 2]. Yet few studies have assessed transmission patterns within households, and what determines whether the infection is contained or spreads.

Studies of risk factors for Ebola virus disease (EVD) have focused on the exposure (infection is most likely following contact with dead bodies and bodily fluids [3–5]) and on the characteristics of the person exposed, with lower attack rates in children than adults [4, 6]. Less emphasis has been given to the characteristics of the source cases (other than the severity of disease that they had [7]) or of the households that may be associated with onward transmission, although behavioral and environmental factors are likely to influence exposure patterns [8]. A study of 27 households in Kikwit, Democratic Republic of Congo (DRC), in 1995 found no association between household characteristics and secondary attack rates [5].

Little is known about who transmits (except for a small number of reconstructed transmission chains; see, eg, [1, 2, 7]). In Yambuku, DRC, in 1976, the secondary attack rate was higher in closer relatives and from female source cases [9]. Two studies in Liberia found no difference in transmission by sex; in one [10], but not the other [8], there was less transmission from children than from adults.

In a study of 94 households of survivors, we have previously estimated exposure-specific and age-specific attack rates [4], risk factors for the acquisition of Ebola in young children [11], and the extent of asymptomatic infection [12]. In this analysis we reconstruct the likely within-household transmission chains to assess factors influencing transmission and who probably transmitted to whom; and estimate the household secondary attack rate, a key parameter for transmission modeling studies [13].

METHODS

In July–September 2015, all survivors from Kerry Town Ebola Treatment Centre living in Western Area, Sierra Leone, and their household members, were invited for interview, as described elsewhere [4, 11]. Transmission chains were created for each household, based on the contact patterns described by the household members. We did not attempt to ascertain onset dates, given the time that had elapsed before interview, but all households only experienced 1 period with EVD cases. See Supplementary Figure 1 for definitions and Supplementary Figure 2 for an illustration of how the generations of transmission were derived.

Individual written informed consent was obtained from all participants (or their parents/guardians for those aged <18 years) before interview and sample collection. Permission for the study was granted by the Sierra Leone Ethics and Scientific Review Committee, and the Ethics Committee of the London School of Hygiene and Tropical Medicine.

Statistical Analysis

We investigated whether any household transmission occurred (using logistic regression), and the proportion of household members infected (using generalized linear models), by characteristics of the primary case(s) and the household.

At the individual level, we assessed the characteristics of cases that were associated with transmission, including severity of illness, classified by symptoms while at home (wet symptoms [ie,

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diarrhea, vomiting, or bleeding] or only dry symptoms) and survival. We used negative binomial regression, because the number of subsequent cases was overdispersed, adjusted all analyses for household size (by including the number of people exposed as an exposure parameter), and allowed for household clustering using robust standard errors. All analyses used Stata version 14 software.

RESULTS

Household members of 123 of the 151 Kerry Town Ebola survivors were living in Western Area and available for interview: 1 survivor refused and the others lived outside the area or were unavailable [4]. They lived in 94 households, which altogether contained 937 individuals, of whom 427 were infected with Ebola virus (including 10 asymptomatic infections and 11 undiagnosed symptomatic infections identified by serology [12], and 238 deaths). Four individuals with unclear causes of death are included as noncases. Household size varied from 1 to 27 people, with up to 21 EVD cases in a single household (see Supplementary Figure 3*A*).

Most households had a single primary case; 8 households had 2 cases, 1 had 3 cases, and 1 had 4 co-primaries (Supplementary Figure 3*B*). We excluded the 1 single-person household and 1 individual who already had EVD before joining a household with EVD cases. Two adults with unclear age were included in the largest adult age category (15–44 years).

Household-Level Analyses

In the univariable analysis, household size and crowding, but no other available household-level measures, were associated with the risk of any subsequent cases occurring (Table 1). All 25 households in which a primary case died at home had subsequent cases. Onward transmission was more common from primaries with wet symptoms than dry symptoms, but 4 households with primary cases with dry symptoms had onward transmission. Subsequent cases were also more common in households with older primary cases.

In multivariable analysis the associations with crowding and older primaries were lost, and the only factors influencing the risk of any secondary transmission were the number exposed and the severity of illness of the primary case(s). The crude odds ratio (OR) for the association with number exposed (1.3; 95% confidence interval [CI], 1.1–1.5, for each additional person exposed) was only slightly reduced by adjusting for the severity of illness of the primary case (OR, 1.2; 95% CI, 1.0–1.5).

Several factors were associated with the proportion of household members infected (Table 1). After adjustment in the generalized linear model, the proportion was higher in more crowded households, households with older people, and if the primary was ≥45 years old, head of household, female, or had more severe illness (Supplementary Table 1). Households that included a healthcare worker or were infected later in the epidemic had a lower proportion infected.

Individual-Level Analyses

A third of those infected with Ebola virus transmitted to someone else in the household (139/425 [33%]; Table 2). More than half of those who transmitted (55%) transmitted to 2 or more people (Supplementary Figure 4). Of those who transmitted, 108 died, 29 became survivors from treatment centers, 1 was an unrecognized symptomatic case, and 1 was an asymptomatic household member with positive serology (who was the most likely source for her 1-week-old baby).

Factors associated with onward transmission are shown in Table 2. After adjusting just for household size and household clustering, the likelihood of onward transmission was similar for males and females and was higher from older cases, from primary cases, from those with more severe disease, and from healthcare workers and household heads.

In the multivariable analysis, the associations with household head and healthcare workers were lost. The associations with age and severity of illness were reduced but were still strong. To see whether the excess risk of transmission from those who died was entirely due to contact with the corpse, further analysis excluded those dying at home: The adjusted incidence rate ratio for transmission was 3.1 (95% CI, 1.9–5.2) comparing wet cases who died away from the home to wet cases who survived.

Supplementary Table 2 shows the characteristics of the likely sources for all nonprimary cases in the households. There was little evidence for assortative or disassortative transmission between the sexes, but there was disassortative transmission by age: Children were infected more by those aged 15–44 and less by those aged ≥45 than would be expected by chance. The households had up to 5 generations of transmission (Supplementary Figure 3*B*). The proportion of cases infected as secondary cases, rather than in subsequent generations, was lower among young children (Supplementary Table 2).

Household Secondary Attack Rate

The 109 primary/co-primary cases gave rise to 201 secondary cases (Supplementary Table 2, including 7 with asymptomatic infections) among 827 exposed household members, giving a secondary attack rate of 24% and reproduction number in the first generation of intrahousehold transmission of 1.8. The overall proportion of household members infected (household attack rate), excluding the primary cases, was 38% (317/827). As survivor households tend to have more cases (increasing the chance that some survived), these attack rates are likely to be overestimates. The case fatality rate for this epidemic was around two-thirds so we can adjust for this bias by assuming that for each household with only surviving cases, 2 households with the same number of only fatal cases were missed, and that these households were the same size as the households with only surviving cases. This adjustment gives an estimated household secondary attack rate of 18%, reproduction number of 1.2, and household attack rate of 28%. With this adjustment,

Table 1. Characteristics of Households and of the Primary Case(s) in Relation to Spread of Ebola Virus in the Household

Household Characteristic	No. of Households	Any Secondary Cases			Mean Proportion Infected (95% CI)	
		No.	(%)	*P* Value[a]		*P* Value[b]
No. of people (excluding primaries)						
≤5	27	13	(48)	.003	0.24 (.11–.37)	<.0001
6–10	36	28	(78)		0.35 (.25–.44)	
≥11	30	26	(87)		0.42 (.31–.53)	
Mean age of exposed (excluding primaries)						
<17	31	19	(61.3)	.2	0.22 (.13–.31)	<.0001
17–20	31	23	(74.2)		0.36 (.25–.47)	
≥21	31	25	(80.1)		0.43 (.31–.56)	
Level of crowding						
>3/room	30	22	(73)	.04	0.40 (.29–.52)	<.0001
2–3/room	32	27	(84)		0.33 (.23–.43)	
<2/room	29	16	(55)		0.28 (.15–.41)	
Access to water						
Sometimes	18	11	(61)	.2	0.28 (.11–.44)	.2
Most days	29	19	(66)		0.33 (.22–.45)	
Every day	45	36	(80)		0.36 (.27–.45)	
Access to soap						
Sometimes	26	19	(73)	.8	0.29 (.19–.39)	.4
Most days	18	14	(78)		0.46 (.29–.62)	
Every day	48	33	(69)		0.32 (.22–.41)	
Setting						
Rural	23	19	(83)	.2	0.30 (.21–.40)	.05
Urban	70	48	(69)		0.35 (.27–.43)	
Healthcare worker in household						
No	78	56	(72)	.9	0.33 (.27–.40)	.5
Yes	15	11	(73)		0.38 (.17–.59)	
Persons moved out of household						
No	78	54	(69)	.2	0.34 (.27–.41)	.6
Yes	15	13	(87)		0.33 (.19–.47)	
Period						
November–mid-December	47	34	(72)	.9	0.36 (.27–.45)	.001
Mid-December to March	46	33	(72)		0.32 (.23–.41)	
Primary[c]						
Illness while at home						
Dry symptoms	13	4	(31)	<.001	0.09 (0–.21)	<.0001
Symptoms unknown	2	2	(100)		0.25 (.0–1.0)	
Wet symptoms	39	24	(62)		0.31 (.21–.41)	
Died, location unknown	14	12	(86)		0.36 (.19–.52)	
Died at home	25	25	(100)		0.51 (.40–.62)	
No. of primary cases						
1	81	59	(73)	.7	0.36 (.29–.43)	.001
>1	12	8	(67)		0.21 (.072–.35)	
Child (<15 y)						
No	86	62	(73)	.97	0.34 (.28–.41)	.02
Yes	7	5	(71)		0.28 (.0–.60)	
Aged ≥45 y						
No	61	38	(62)	.004	0.27 (.19–.34)	<.0001
Yes	32	29	(91)		0.47 (.38–.57)	
Household head						
No	55	41	(75)	.5	0.36 (.28–.44)	.8
Yes	38	26	(68)		0.31 (.21–.41)	
Male						
No	43	33	(77)	.3	0.39 (.29–.49)	.01
Yes	50	34	(68)		0.30 (.21–.38)	

Mean proportion infected defined as the number of nonprimary cases divided by the number exposed (ie household members excluding the primary cases). Missing data: crowding for 2, water and soap for 1.

[a] χ^2 test.

[b] From generalized linear model. Given as *P* value for trend across categories if >2 categories.

[c] Where there was >1 primary, the variables are coded as present if at least 1 primary had that characteristic, and the most severe manifestation of disease was selected.

Table 2. Factors Influencing Onward Transmission of Ebola Virus Within the Household by Characteristics of the Source Cases, Sierra Leone 2014–2015

Characteristics of Source	Total No. Infected	No. (%) Transmitting	No. of Persons Infected by Each Case									Crude IRR[a]	(95% CI)	*P* Value	Adjusted IRR[b]	(95% CI)	*P* Value
			0	(%)	1	(%)	2	(%)	≥3	(%)	Mean						
Total	425	139 (32.7)	286	(67.3)	63	(14.8)	36	(8.5)	40	(9.4)	0.7						
Sex of source																	
Female	252	88 (34.9)	164	(65.1)	38	(15.1)	27	(10.7)	23	(9.1)	0.8	1					
Male	173	51 (29.5)	122	(70.5)	25	(14.5)	9	(5.2)	17	(9.8)	0.7	0.94	(.65–1.4)	.74			
Age of source																	
<5 y	51	6 (11.8)	45	(88.2)	5	(9.8)	0	(0.0)	1	(2.0)	0.2	1			1		
5–14 y	64	12 (18.8)	52	(81.3)	10	(15.6)	2	(3.1)	0	(0.0)	0.2	1.7	(.05–5.6)		2.6	(1.1–5.8)	
15–44 y	229	75 (32.8)	154	(67.2)	35	(15.3)	24	(10.5)	16	(7.0)	0.7	5.2	(1.8–15.0)		4.2	(2.1–8.4)	
≥45 y	81	46 (56.8)	35	(43.2)	13	(16.0)	10	(12.3)	23	(28.4)	1.7	12.6	(4.2–37.8)	<.001	4.8	(2.3–10.1)	<.001
Source was primary case																	
No	317	67 (21.1)	250	(78.9)	38	(12.0)	19	(6.0)	10	(3.2)	0.4	1			1		
Yes	108	72 (66.7)	36	(33.3)	25	(23.1)	17	(15.7)	30	(27.8)	1.8	6.7	(4.8–9.4)	<.001	3.0	(2.0–4.5)	<.001
Severity of illness of source at home																	
Dry: survived	65	8 (12.3)	57	(87.7)	7	(10.8)	1	(1.5)	0	(0.0)	0.1	1			1		
Dry: died away from home	5	1 (20.0)	4	(80.0)	0	(0.0)	0	(0.0)	1	(20.0)	0.8	7.4	(.74–74.8)		3.7	(.61–22.7)	
Unknown symptoms: survived	38	2 (5.3)	36	(94.7)	2	(5.3)	0	(0.0)	0	(0.0)	0.1	0.40	(.09–1.7)		0.46	(.10–2.2)	
Unknown symptoms: died away	15	3 (20.0)	12	(80.0)	2	(13.3)	0	(0.0)	1	(6.7)	0.3	1.7	(.35–8.3)		1.8	(.59–5.5)	
Wet: survived	85	21 (24.7)	64	(75.3)	12	(14.1)	6	(7.1)	3	(3.5)	0.4	3.3	(1.6–6.9)		2.8	(1.3–5.7)	
Wet: died away from home	37	30 (81.1)	7	(18.9)	10	(27.0)	6	(16.2)	14	(37.8)	2.1	16.6	(8.5–32.3)		8.6	(4.4–17.1)	
Died location unknown	138	36 (26.1)	102	(73.9)	18	(13.0)	14	(10.1)	4	(2.9)	0.5	2.3	(1.04–5.2)		2.2	(1.1–4.7)	
Died at home	42	38 (90.5)	4	(9.5)	12	(28.6)	9	(21.4)	17	(40.5)	2.7	18.4	(9.9–34.2)	<.001	9.8	(4.8–20.1)	<.001
Occupation of source																	
Not healthcare worker	414	131 (31.6)	283	(68.4)	61	(14.7)	35	(8.5)	35	(8.5)	0.7	1					
Health worker	11	8 (72.7)	3	(27.3)	2	(18.2)	1	(9.1)	5	(45.5)	3.0	4.6	(2.6–8.1)	<.001			
Source's position in household																	
Household member	360	105 (29.2)	255	(70.8)	50	(13.9)	30	(8.3)	25	(6.9)	0.6	1					
Household head	65	34 (52.3)	31	(47.7)	13	(20.0)	6	(9.2)	15	(23.1)	1.5	3.5	(2.3–5.3)	<.001			

Abbreviations: CI, confidence interval; IRR, incidence rate ratio.

[a]IRR adjusted for cluster and household size.

[b] IRR adjusted for cluster, household size and all variables in the model. *P* values from likelihood ratio test.

the median household size is reduced from 12 to 8, and the case fatality rate increased from 56% to 62%.

DISCUSSION

The households in this study had a variety of experiences, from those with a single EVD case and no subsequent spread to those with all members affected. Key drivers of household transmission included severity of illness and increasing age of the source. Household size was an important determinant of initial spread, but did not influence the total proportion infected once adjusted for other factors. Those with only dry symptoms were less likely to transmit, but one-third of households with primary cases with dry symptoms had subsequent cases.

The association with age of the source was not fully explained by severity of illness, or the fact that primary cases (who have more susceptibles to transmit to) tend to be older [4], or by the tendency for young children to be infected in later generations (Supplementary Table 2). Possible explanations include young children being cared for by the parent who was also the source of their infection, and the respect given to older people, leading to people ministering to them. Our study contradicts inferences of a modeling study, which predicted more transmission from children [14]. Lower transmission from children was also found in transmission chains in Liberia [10], with no difference by age found in a study based on contact tracing data [8].

The association with crowding was expected, but the lack of association with sanitation is surprising. Few households moved people out, and where this did happen it may have been too late to avert exposure. We found less transmission later in the epidemic, suggesting improved knowledge of what to do, and helped by a greater availability of Ebola care beds; having a healthcare worker in the household also reduced transmission. Associations with more transmission if the primary case was head of the household or female were not supported in the individual-level analysis, after adjustment for other factors.

The household secondary attack rate was high. At 18%, it is closer to reports from Kikwit, DRC (16%) [5] and Nzara/Yambio, Sudan (13%) [15] than to Yambuku (8%) [9] or the 6% estimated for Sierra Leone (which relied on matching names and addresses from case report forms) [13], or 4% in Liberia (based on shared surnames and communities in contact tracing data) [8].

This analysis relied on histories collected in interviews 4–9 months after the events. By interviewing the household members as a group, we hoped to maximize recall. We did not attempt to record dates of onset, so have not used the serial interval but have based the transmission chains on the reported order of events, and types of contact, favoring higher levels of exposure where multiple sources were possible. Some misclassification is likely and our method may have contributed to the association with severity of illness in the individual-level analysis, but not to the associations with severity of illness in the household analysis or to the higher transmission from those who died away from the home than from those who survived. This last finding, which is in contrast to findings in Liberia and Guinea [7], may be explained by higher viral loads while at home in those who subsequently died. The association of transmission with severity of illness underscores the importance of early identification and isolation of cases.

CONCLUSIONS

Initial spread in the household was more likely in larger households, and cases with more severe disease, particularly deaths, and older cases had more onward transmissions. Our estimate for reproduction number in the first generation of household transmission of only a little above 1, and the reduced proportion of household members affected later in the epidemic, suggest that it should be feasible to curtail intrahousehold transmission more rapidly.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Appendix

Variability in intra-household transmission of Ebola virus, and estimation of the household secondary attack rate

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Table S1. Risk factors influencing proportion infected with Ebola virus in survivor households, Sierra Leone 2014-2015. Results from generalised linear model.

	Crude			Adjusted[a]		
Household	OR	CI	p	OR	CI	P
No. of people						
	1.1	1.0-1.1	<0.0001	1.0	0.97-1.04	0.9
Mean age of exposed						
<17	ref		<0.0001	ref		<0.0001
17-20	1.9	1.3-2.8		2.4	1.5-3.8	
≥21	3.0	2.1-4.4		5.3	3.1-0.2	
Level of crowding						
Decreasing crowding[b]	0.70	0.58-0.85	<0.0001	0.67	0.53-0.86	0.002
Healthcare worker in household						
vs none	1.1	0.80-1.6	0.5	0.51	0.33-0.79	0.002
Period						
Late vs early	0.62	0.47-0.82	0.001	0.43	0.29-0.64	<0.0001
Primary						
Illness while at home						
Dry symptoms	ref		<0.0001	ref		<0.0001
Symptoms unknown	2.9	0.75-11.1		0.85	0.20-3.5	
Wet symptoms	4.8	2.2-10.3		2.8	1.2-6.5	
Died, location unknown	3.8	1.7-8.7		4.8	1.9-12.0	
Died at home	9.6	4.4-20.6		5.0	2.2-11.8	
Aged >=45						
vs <45	1.8	1.4-2.4	<0.0001	2.0	1.4-2.0	<0.0001
Head of household						
vs not	0.95	0.71-1.3	0.8	3.3	2.0-5.5	<0.0001
Male primary						
vs only female	0.70	0.53-0.92	0.01	0.43	0.29-0.64	<0.0001

[a] Adjusted for all the factors shown in the table . Factors were included if they had an association with the outcome in the adjusted model at the 5% level. The number of people in the household was included because it had a large effect on the risk of any secondary spread, although it was not associated with the proportion infected, as seen here.

[b]Linear trend across categories

Table S2: Characteristics of likely source cases for individuals infected with Ebola virus in their households, Sierra Leone, 2014-15

						Characteristics of non-primary cases										
Likely sources of transmission	**Female**	***%***	**Male**	***%***	***P*[a]**	**<5y**	***%***	**5-14y**	***%***	**15-44y**	***%***	**≥45y**	***%***	***P*[a]**	**Total**	***%***
	189	*100.0*	118	*100.0*		48	*100.0*	58	*100.0*	154	*100.0*	47	*100.0*		307	
Sex of source																
Female	121	*64.0*	71	*60.2*		34	*70.8*	42	*72.4*	85	*55.2*	31	*66.0*		192	*62.5*
Male	68	*36.0*	47	*39.8*	0.50	14	*29.2*	16	*27.6*	69	*44.8*	16	*34.0*	0.06	115	*37.5*
Age of source																
<5 years	7	*3.7*	1	*0.90*		0	*0.0*	2	*3.4*	3	*1.9*	3	*6.4*		8	*2.6*
5-14 years	7	*3.7*	7	*5.9*		4	*8.3*	3	*5.2*	6	*3.9*	1	*2.1*		14	*4.6*
15-44 years	89	*47.1*	60	*50.8*		27	*56.3*	36	*62.1*	68	*44.2*	18	*38.3*		149	*48.5*
≥45 years	86	*45.5*	50	*42.4*	0.34	17	*35.4*	17	*29.3*	77	*50.0*	25	*53.2*	0.04	136	*44.3*
Relationship of source to infected person																
Spouse	32	*16.9*	17	*14.4*		0	*0.0*	0	*0.0*	32	*20.8*	17	*36.2*		49	*16.0*
Child	13	*6.9*	2	*1.7*		0	*0.0*	0	*0.0*	8	*5.2*	7	*14.9*		15	*4.9*
Mother	55	*29.1*	26	*22.0*		22	*45.8*	25	*43.1*	32	*20.8*	2	*4.3*		81	*26.4*
Father	15	*7.9*	19	*16.1*		10	*20.8*	6	*10.3*	17	*11.0*	1	*2.1*		34	*11.1*
Sibling	24	*12.7*	24	*20.3*		3	*6.3*	7	*12.1*	31	*20.1*	7	*14.9*		48	*15.6*
Grandchild	4	*2.1*	1	*0.0*		0	*0.0*	0	*0.0*	0	*0.0*	5	*10.6*		5	*1.6*
Grandparent	8	*4.2*	8	*7.6*		8	*16.7*	3	*5.2*	5	*3.2*	0	*0.0*		16	*5.2*
In-laws	9	*4.8*	1	*0.85*		0	*0.0*	1	*1.7*	5	*3.2*	4	*8.5*		10	*3.3*
Niece/nephew	3	*1.6*	3	*2.5*		0	*0.0*	1	*1.7*	4	*2.6*	1	*2.1*		6	*2.0*
Cousin	4	*2.1*	2	*1.7*		0	*0.0*	3	*5.2*	3	*1.9*	0	*0.0*		6	*2.0*
Aunt/Uncle	9	*4.8*	7	*5.9*		2	*4.2*	10	*17.2*	4	*2.6*	0	*0.0*		16	*5.2*
Other	13	*6.9*	8	*6.8*	n/a	3	*6.3*	2	*3.4*	13	*8.4*	3	*6.4*	n/a	21	*6.8*
Position of source in household																
Household member	123	*65.1*	86	*72.9*		38	*79.2*	43	*74.1*	91	*59.1*	37	*78.7*		209	*68.1*
Household head	66	*34.9*	32	*27.1*	0.15	10	*20.8*	15	*25.9*	63	*40.9*	10	*21.3*	0.01	98	*31.9*
Source was health care worker																
No	166	*87.8*	108	*91.5*		46	*95.8*	57	*98.3*	133	*86.4*	38	*80.9*		274	*89.3*
Yes	23	*12.2*	10	*8.5*	0.31	2	*4.2*	1	*1.7*	21	*13.6*	9	*19.1*	0.004	33	*10.7*
Severity of illness of source at home																
Dry symptoms: survived	4	*2.1*	5	*4.2*		2	*4.2*	3	*5.2*	4	*2.6*	0	*0.0*		9	*2.9*
Dry symptoms: died away from home	3	*1.6*	1	*0.85*		1	*2.1*	2	*3.4*	0	*0.0*	1	*2.1*		4	*1.3*
Unknown symptoms: survived	1	*0.53*	1	*0.85*		1	*2.1*	1	*1.7*	0	*0.0*	0	*0.0*		2	*0.65*
Unknown symptoms: died away from home	3	*1.6*	2	*1.7*		2	*4.2*	0	*0.0*	2	*1.3*	1	*2.1*		5	*1.6*
Wet symptoms: survived	23	*12.2*	12	*10.2*		11	*22.9*	13	*22.4*	11	*7.1*	0	*0.0*		35	*11.4*
Wet symptoms: died away from home	49	*25.9*	31	*26.3*		9	*18.8*	11	*19.0*	44	*28.6*	16	*34.0*		80	*26.1*
Died symptoms: location unknown	32	*16.9*	27	*22.9*		10	*20.8*	16	*27.6*	24	*15.6*	9	*19.1*		59	*19.2*
Died at home	74	*39.2*	39	*33.1*	0.78	12	*25.0*	12	*20.7*	69	*44.8*	20	*42.6*		113	*36.8*
Position of source in transmission chain																
Primary case	133	*68.5*	68	*56.2*		21	*43.8*	31	*53.4*	113	*69.8*	36	*76.6*		201	*63.8*
Secondary case	45	*23.2*	42	*34.7*		17	*35.4*	24	*41.4*	37	*22.8*	9	*19.1*		87	*27.6*
Tertiary case	12	*6.2*	9	*7.4*		6	*12.5*	3	*5.2*	11	*6.8*	1	*2.1*		21	*6.7*
Quaternary or more	4	*2.1*	2	*1.7*	0.12	4	*8.3*	0	*0.0*	1	*0.62*	1	*2.1*	<0.001[b]	6	*1.9*

Notes: Missing data in subsequent cases: 8 subsequent cases with multiple possible sources of infection included only in analysis of position of source in transmission chain; 2 no information on source of infection.

Of the 54 who died away from home whose place of death was known, 49 died in Ebola Treatment or Holding Centres, one died with a traditional healer, one was asked to leave the home and died on a bus, and three moved to other households (two to the same household).

[a] Chi^2 or Fisher's exact tests. [b] Chi^2 using linear regression.

n/a = not applicable (numbers in subgroups too small)

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Figure S1: Definitions used

Household: People eating from the same pot who were resident while members of the household had EVD. Includes people who had not been resident in the household before Ebola occurred (e.g. external family members who joined the household to care for sufferers).

Case: EVD survivors from treatment centres; those reported by the family to have died of Ebola or who died and had symptoms fitting the case definition of EVD; and people with positive IgG to Ebola (symptomatic or asymptomatic).

Primary case: The first person with symptoms in the household. For some households more than one person was described as having symptoms at the same time, usually following a common external exposure. They have been treated as co-primaries.

Subsequent case: Cases in the household who were not primary cases.

Wet case: Case of EVD with diarrhoea, vomiting or bleeding.

Dry case: Case of EVD without diarrhoea, vomiting or bleeding.

Levels of exposure: This 8-level scale was created a priori based on literature and discussion with Ebola treatment centre staff. The highest level was contact with the body of those who died of Ebola; then direct contact with body fluids of those with Ebola, including breast feeding, then direct contact with "wet" cases; then direct contact with dry cases; indirect contact with a wet case (eg with washed clothes); indirect contact with a dry case; minimal contact (eg shared utensils); no contact known.

Transmission/source of infection: The most likely routes of transmission were estimated from the contact histories. For example, if someone was reported to have had a direct physical contact with a case while they were ill, this case was taken as the likely source for that transmission. If more than one contact was reported we selected the one more likely to transmit, i.e. the one with the greatest degree of exposure, based on the levels of exposure and contact patterns described.

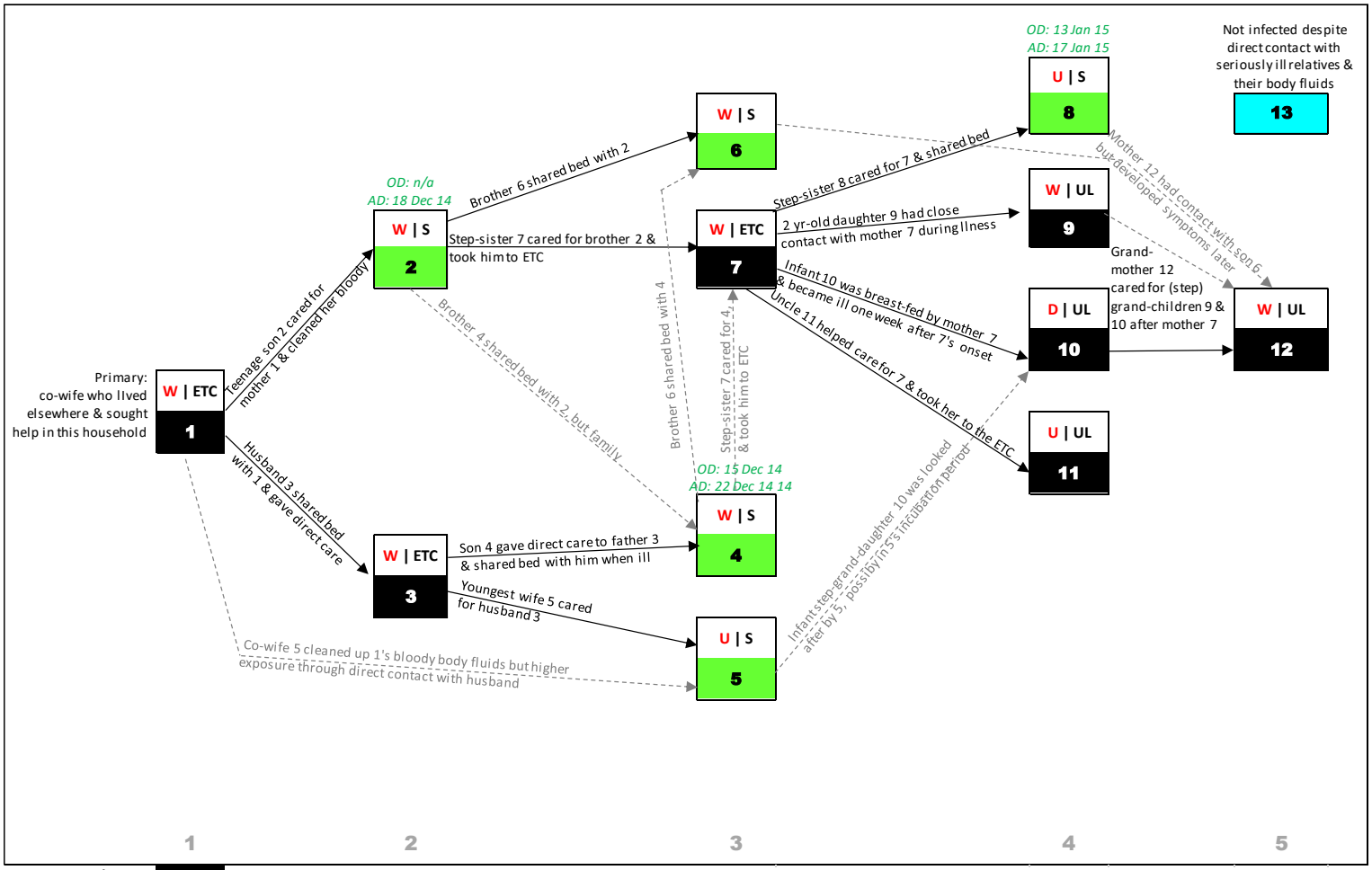
Generation of transmission: Primary cases are the first generation. Secondary cases are those thought to have been infected by the primary cases; tertiary cases those infected by the secondary cases, and so on.

Household secondary attack rate: Secondary cases/(household members - primary cases)

Household attack rate: Subsequent cases/(household members - primary cases)

Reproduction number (R) in the first generation of transmission: secondary cases/primary cases in the household

Figure S2: Illustration of reconstruction of transmission chains in a large and complex household



Legend

- Dead hh member
- Surviving hh member
- Not infected
- **W** Wet symptoms at home
- **D** Dry symptoms at home
- **U** Symptoms at home unknown
- **S** Survivor from any ETC
- **ETC** Died in Ebola Treatment Centre
- **UL** Death location unknown
- *OD/AD* Onset date/Admission date to ETC (dates have been changed to protect anonymity but retain perspective)
- Most likely transmission route
- Alternative transmission routes

***Notes**: The household shown had the highest number of likely generations of transmission in the study and a high attack rate (92%). It was chosen to illustrate the complexity of possible transmission routes and the factors considered. Where more than one transmission route was possible, we took the highest risk exposure as the most likely transmission route. For example, 2is considered the source of 7 because he had symptoms earlier than the alternative source 4.*

Figure S3. Experience of Ebola in 94 households of Ebola survivors, Sierra Leone 2014-15 (a) Showing deaths, cases of EVD and asymptomatic infections (b) Showing generations of transmission. Each column represents a different household.

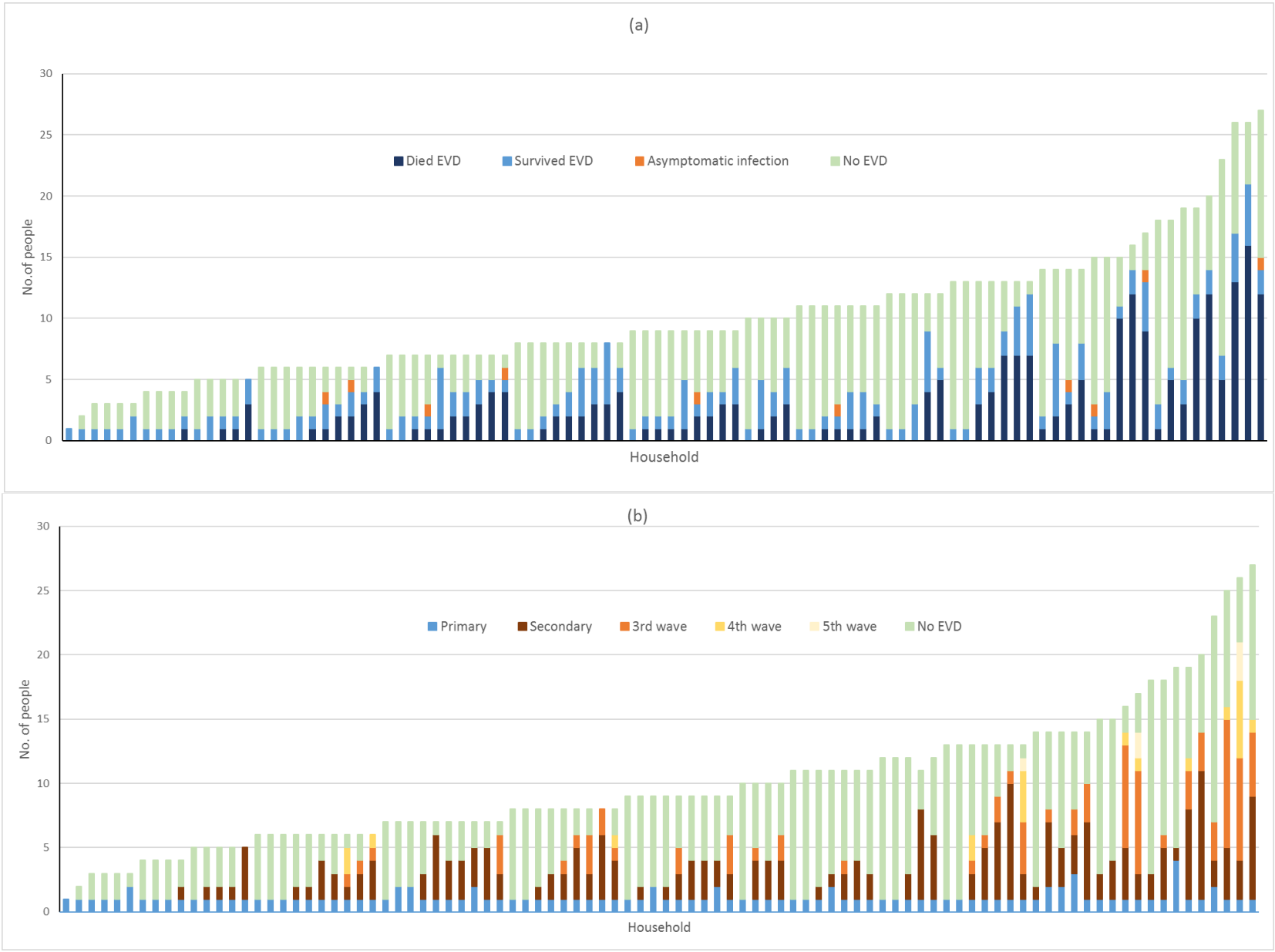
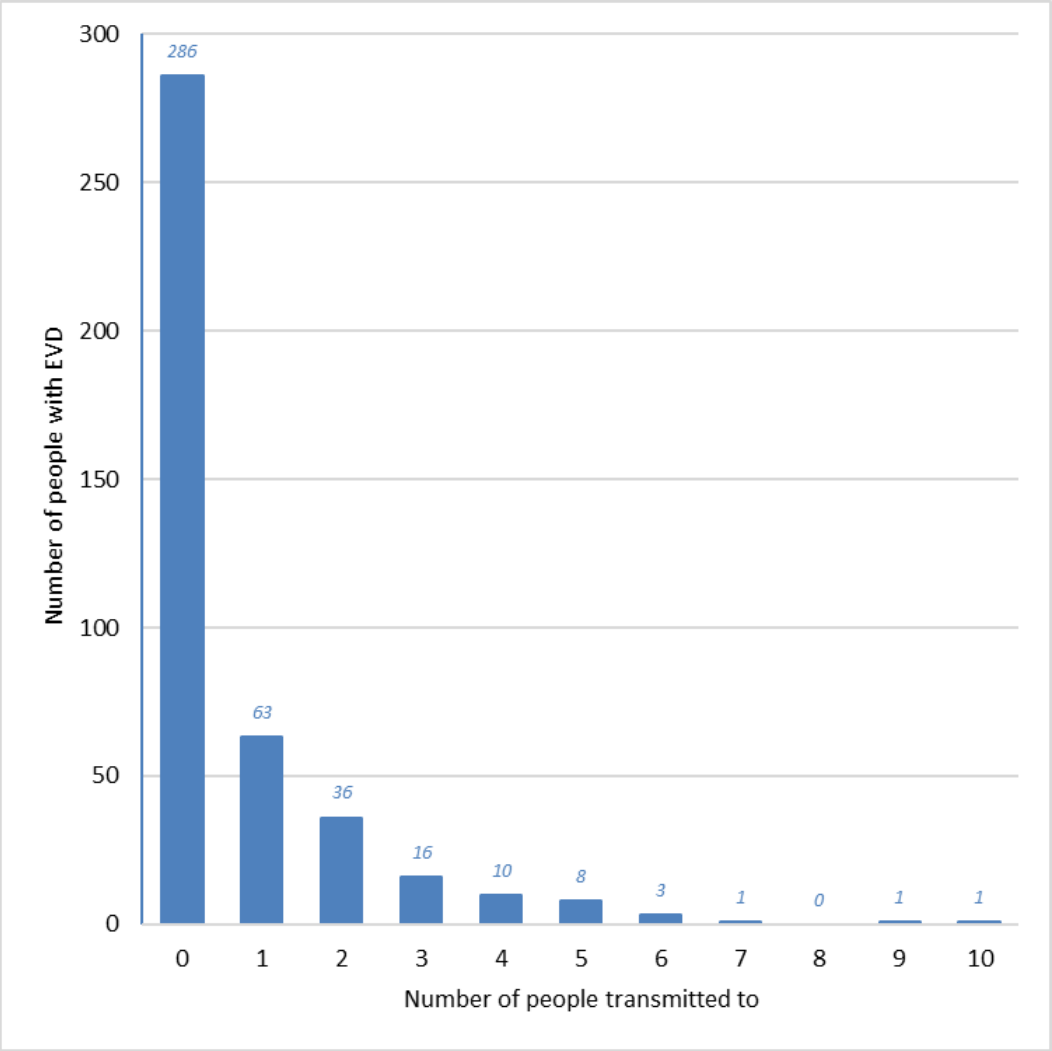


Figure S4. Number of intra-household transmissions per EVD case in households of Ebola survivors, Sierra Leone 2014-15



Appendix

Paper 7: Delivery of an Ebola virus-positive stillborn infant in a rural community health centre, Sierra Leone, 2015

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Thesis Title	Ebola virus transmission and disease severity in Sierra Leone 2013-16		
Primary Supervisor	Professor Jimmy Whitworth		

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Where was the work published?	American Journal of Tropical Medicine and Hygiene		
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Delivery of an Ebola Virus-Positive Stillborn Infant in a Rural Community Health Center, Sierra Leone, 2015

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Abstract. We report the case of an Ebola virus (EBOV) RNA–negative pregnant woman who delivered an EBOV RNA–positive stillborn infant at a community health center in rural Sierra Leone, 1 month after the mother's last possible exposure. The mother was later found to be immunoglobulins M and G positive indicating previous infection. The apparent absence of Ebola symptoms and not recognizing that the woman had previous contact with an Ebola patient led health workers performing the delivery to wear only minimal personal protection, potentially exposing them to a high risk of EBOV infection. This case emphasizes the importance of screening for epidemiological risk factors as well as classic and atypical symptoms of Ebola when caring for pregnant women, even once they have passed the typical time frame for exposure and incubation expected in nonpregnant adults. It also illustrates the need for health-care workers to use appropriate personal protection equipment when caring for pregnant women in an Ebola setting.

On January 13, 2015, the U.S. Centers for Disease Control and Prevention (CDC) field laboratory in Bo, Sierra Leone, identified Ebola virus (EBOV) RNA in an oral swab from a stillborn infant by quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) for Ebola *NP* and *VP40* genes. The swab, taken in accordance with Sierra Leone Ministry of Health and Sanitation (MoHS) protocol to investigate all deaths regardless of symptoms or exposure during the outbreak, tested positive with cycle threshold values of 16 in both targets, indicating a high viral load. At the time of delivery, the infection status of the mother was unknown.

A team from Médecins Sans Frontières (MSF) traveled to the village where the infant was born to find the mother and investigate the chain of transmission. The 20-year-old woman was found healthy and living with her husband who had been discharged from the MSF Ebola Management Center (EMC) in Bo on December 16, 2014. Despite her insistence that she had had no symptoms of Ebola virus disease (EVD), she agreed to be tested for EBOV.

On January 15, a whole blood sample was collected from the mother in the Bo Hospital Transit Center and analyzed at the CDC field laboratory. The sample was negative for both EBOV RNA targets. CDC laboratorians re-extracted RNA and reran assays on the maternal and infant samples with the same result. Later enzyme-linked immunosorbent assays performed by CDC Atlanta on the maternal sample to detect antibodies specific to Ebola found it positive for immunoglobulin M (IgM, titer ≥ 1:1,600) and immunoglobulin G (IgG, titer ≥ 1:400), indicating the mother had had a recent EBOV infection (Table 1).[1] Virus isolation attempts with the infant swab were positive. Isolation was not attempted with the maternal sample because of negative qRT-PCR and positive IgM results.

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To learn more about possible transmission routes for the woman's infection, an MSF team conducted separate qualitative interviews with the woman and her husband in their village (Figure 1). On December 3, 2014 the husband developed symptoms (vomiting, fatigue, loss of appetite, and headache) after carrying his sick uncle to the community health center 9 days earlier. The uncle, who spent only a few hours in the pregnant woman's house, died at the health center shortly after arriving and was diagnosed EBOV positive using RT-PCR testing of a postmortem oral swab.

The husband's brother and a friend, who helped carry the uncle to the health center but did not live with the couple or the uncle, developed symptoms on December 3 and 4, respectively. On December 6, all three men tested EBOV positive (Table 1), and their households were quarantined for 21 days. The brother died in the Transit Center; the husband and his friend were admitted to the MSF EMC and discharged cured on December 16 and 21, respectively. Both husband and wife separately reported that they did not have sexual intercourse on his return home, due to EMC health education, but also because of the woman's pregnancy.

The woman was approximately 7-month pregnant when her husband became symptomatic. Although she did not sleep in the same bed once he became ill, she did provide close contact care for him, including bathing and massage. On January 21, when CDC, MoHS, and World Health Organization officials interviewed the woman using a standardized questionnaire to collect information about potential symptoms of Ebola in the month before onset of labor, she reported experiencing intense fatigue and loss of appetite, abdominal pain, jaundice, eye pain, sensitivity to light, and confusion. During the qualitative interviews conducted by MSF on February 9, she reported experiencing severe back pain and a "gush of water" from her vagina on the day after her husband went to Bo (December 7), leakage of bloody fluid from her vagina, loss of appetite, and intense fatigue. But she did not describe the latter symptoms noted in the standardized questionnaire and in the absence of clinical opinion at the time, it is difficult to determine which report is more accurate (Table 2).

TABLE 1
Test results for samples collected from persons in the chain of transmission, Bo, Sierra Leone, 2014–2015

Patient	Specimen collection date	Specimen source	Ebola virus load result (CT value)*	Other results
Wife	January 15, 2015	Blood	Negative (> 40:> 40)	IgG positive (≥ 1:400) IgM positive (≥ 1:1,600)
	–	Repeat†	Negative (> 40:> 40)	–
Stillborn Infant	January 10, 2015	Oral swab	Positive (16:16)	–
	–	Repeat†	Positive (20:19)	–
Husband	December 7, 2014	Blood	Positive (28:28)	–
Husband's Brother	December 7, 2014	Blood	Positive (18:19)	–
Husband's Friend	December 7, 2014	Blood	Positive (26:26)	–
Uncle	November 24, 2014	Oral swab	Positive (26:26)	–

CT = cycle threshold; IgG = immunoglobulin M; IgM = immunoglobulin M.
*The first value corresponds with the *NP* gene and the second value corresponds with the *VP40* gene.
†The sample from the infant and wife were retested on January 17, 2015.

Since the woman associated her symptoms with pregnancy and anxiety regarding her husband's illness, she did not seek medical care nor reveal her condition to anyone, including the contact tracer. When her husband returned from the EMC on December 15, the couple still did not believe any of her symptoms were related to Ebola. They were also under the impression that they could not seek medical care while in quarantine. Thus, the woman only sought medical care on December 28 after completing the 21-day quarantine. She reported being treated for an infection and vaccinated against tetanus and was told to return home until she was closer to term. After walking 3 miles home, she told the village traditional birth attendant (TBA) that the baby had stopped moving. The TBA was unable to find a fetal heartbeat and advised her to seek medical care again. She sought care at a second health center where she was given medication, which the TBA believed was to induce labor.

On January 10, 2015, she went into labor, walked to the health center where she had first sought care and delivered a stillborn infant. The fetus was deformed and macerated. Since the woman had no symptoms and was no longer in quarantine, and because the fetus was already emerging when the mother arrived, the nurse assisting wore minimal personal protection equipment (PPE) consisting of gloves and an apron during the delivery. The village TBA, who had accompanied the woman, wore no PPE during the delivery. The nurse followed MoHS guidelines and requested a safe burial for the infant and an oral swab was collected. Once the stillborn infant was identified as EBOV RNA positive, the nurse and TBA were quarantined but did not develop any EVD symptoms during the 21-day period. No additional cases were reported in the couple's village or surrounding settlements served by the health center.

To our knowledge, this is the first report of a delivery of an EBOV-infected stillbirth in a rural community health center, and the case raises a number of important issues about providing care for pregnant women outside an EMC setting during an Ebola outbreak. Most notable is the increased risk of exposure that occurs for health workers when clinical symptoms are not reported, and they do not take into account epidemiological indicators, such as previous contact with a positive case, when assessing the risk of infection.

There is limited information about maternal and fetal outcomes related to EBOV infection. Previous reports have documented only pregnancies managed in an EMC setting,[2–4] noting that pregnant women tend to have more severe disease and worse outcomes than nonpregnant women. Mupapa and others reported a case fatality rate of 95.5% among pregnant women compared with 70% in nonpregnant women in an Ebola outbreak in Kikwit, Democratic Republic of the Congo, in 1995 and that the majority of the 15 EBOV-infected pregnant women presented with serious symptoms more often than EBOV-infected nonpregnant women.[2]

Recently, Baggi and others highlighted the potential for more positive outcomes, describing two symptomatic pregnant women admitted to an EMC in Guinea who survived vaginal delivery with supportive care. However, both delivered EBOV-infected stillborn infants, and the authors warned of the possibility of pregnant EVD survivors being referred to local health-care centers for delivery, potentially exposing maternity staff to a high risk of infection.[3]

The case we describe did not become severely ill in the way people in West Africa have come to expect of those suffering from Ebola. The woman did not recognize her symptoms as being related to Ebola, nor did the health workers who assisted her, despite miscarriage being a defining

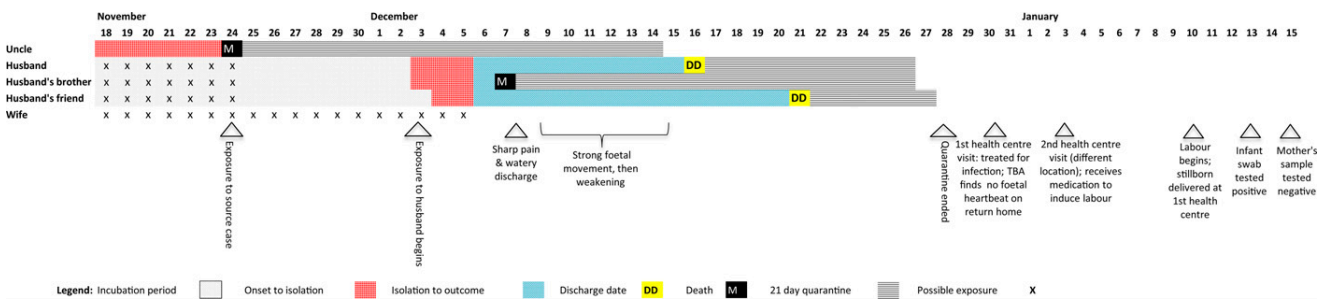

FIGURE 1. Chains of transmission associated with an Ebola-positive stillborn infant, Bo District, Sierra Leone, November 2014 to January 2015.

TABLE 2

Symptoms reported by the subject during the case investigation compared with Sierra Leone MoHS case definition[5]*

	Standardized questionnaire by CDC/MoHS/WHO on January 21	Qualitative interview by MSF investigation team on February 9
Acute fever (> 38°C)	No	No
Headache	No	No
Abdominal pain	Yes	No
Intense fatigue	Yes	Yes
Loss of appetite	Yes	Yes
Generalized or articular pain	No	No
Difficulty in swallowing	No	No
Difficulty in breathing	No	No
Nausea or vomiting	No	No
Hiccups	No	No
Miscarriage	No	No
Diarrhea	No	No
Unexplained bleeding	No	Yes

CDC = Centers for Disease Control and Prevention; MoHS = Ministry of Health and Sanitation; MSF = Médecins Sans Frontières; WHO = World Health Organization.

*A suspect case of Ebola is defined as contact with a clinical case and acute fever (> 38°C), or contact with a clinical case and three or more of the above symptoms, or acute fever and three or more of the concerning symptoms above, or any unexplained bleeding or miscarriage.[5]

characteristic of Ebola during pregnancy. In the absence of the more recognizable symptoms of Ebola (i.e., fever, vomiting, and diarrhea), the woman's symptoms were misinterpreted as being caused by anxiety and pregnancy complications. We must, however, also acknowledge the difficulty of knowing for sure the clinical history of this case, given that fear of isolation and stigmatization may have influenced what the woman was willing to reveal.

The relatively long time between events and the assumption that there was no risk after completing a 21-day quarantine may also have reduced the alertness of the health workers. We believe the mother was most likely infected through contact with the uncle or with her husband after he developed symptoms and not as a result of sexual contact post recovery since the woman's description of the start of her intrauterine fetal death supports an earlier infection date. The possibility of a long delay between fetal death and delivery in EBOV-affected pregnancy has been previously seen.[6] An MSF technical guidance paper cites a woman admitted to the EMC in her 5th month of pregnancy, who had an EBOV-infected stillbirth 32 days after she tested RT-PCR negative.

Although there is also scant information currently in the literature about mild or asymptomatic EBOV infections, Akerlund and others recently reported a case in Monrovia, Liberia, of a pregnant woman with suspected premature rupture of membranes who did not meet EVD case definition or report an EVD contact but tested EBOV positive with a high viral load 3 days before becoming symptomatic. Blood, urine, oral, and vaginal fluids were all positive before the onset of obvious EVD symptoms, leading the authors to suggest that pregnancy might alter EVD presentation and progression and warn of the challenges this may present for health-care workers.[7]

All these cases point to a critical requirement for health workers, especially in the community where laboratory results will rarely be available before care, to be informed that the risk of EBOV transmission cannot be excluded even if the pregnant woman does not appear to have symptoms and her last known exposure to an EBOV-positive contact was more than 21 days prior. It is also important that all pregnant women who are EVD survivors or have been in contact with a confirmed EVD case are followed until the end of their pregnancy and encouraged to deliver in an appropriately prepared health-care facility.

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Disclaimer: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

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