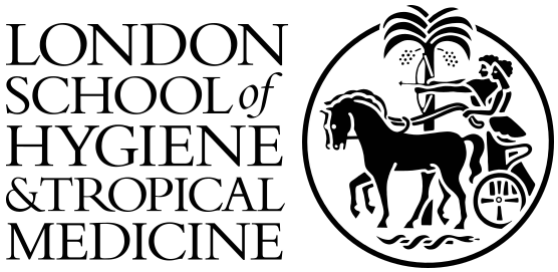

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Epidemiology and control of Ebola Virus Disease (EVD) in Sierra Leone: analysis of data from the Médecins Sans Frontières (MSF) response, 2014-15.

Grazia Marta Caleo

Thesis submitted in accordance with the requirements for

the degree of Doctor of Philosophy

of the University of London

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Department of Infectious Disease Epidemiology
Faculty of Epidemiology and Population Health

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

Funded by Médecins Sans Frontières Operational Centre Amsterdam (MSF-OCA)

Research group affiliation(s): MRC International Statistics and Epidemiology Group, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK.

Declaration of own work

I, Grazia Marta Caleo, 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.

Signed: [redacted] Date: 31st January 2021

Abstract

Aims and Objectives

This thesis stems from work conducted with Médecins Sans Frontières in Sierra Leone, during the 2014-2015 Ebola virus disease (EVD) outbreaks. The thesis addresses 3 main public health knowledge gaps and aims to improve the response to future EVD outbreaks by:

1) Understanding the factors that influenced EVD transmission and community compliance with control measures;
2) Estimating the performance of the WHO EVD case definition, and what components could improve it;
3) Estimating the design effect and mortality rates, and discussing how to improve future surveys for highly-clustered diseases.

Methods

The methods corresponding to each gap were:

1) A mixed-methods study in one village experiencing sustained transmission in Sierra Leone;
2) A review and meta-analysis exploring performance of WHO EVD case definitions against laboratory confirmed EVD;
3) Two population-based clustered surveys, in Sierra Leone.

Results

Study 1 identified that non-compliance with public health guidelines was a consequence of the failure of the response to orientate itself according to the needs and values of the community.

Study 2 estimated that the WHO EVD case definition performed sub-optimally to identify cases (sensitivity 81·5% (74·1-87·2%); specificity 35·7% (28·5-43·6%)). Inclusion of intense fatigue as a key symptom and contact history could improve performance, but these changes would require collaboration with, and trust of, affected communities.

Study 3 identified a high degree of clustering in community-based surveys of EVD, this contributed to imprecise mortality estimates, which have limited utility when assessing the impact of disease. Estimated the design effect along with methodological suggestions provided can inform future surveys for similar highly clustered diseases.

Conclusion

The thesis highlights that outbreak patterns are linked to social and cultural environments. Community influence key public health practices (i.e. case definitions). Recognition of transmission risks from responding organization requires a respectful and compassionate approach, understanding of social norms and adapted community-lead interventions.

Acknowledgements

The work presented in this thesis would not have been possible without the contribution, encouragement and guidance of several colleagues, friends, family, and patients.

Thanks to Professor Helen Weiss, my supervisor. Despite my long periods in the field, between outbreaks and family duty, she has always welcomed me back to continue this work. She has mentored and inspired me in this winding journey.

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Thanks to my sister Chiara. Despite my work taking me far from her and from where I come from, she understood that I also belong to what I do.

This thesis is for Suma, Jusu, Steven, Gebbeh, Charlse, Isha, Mohamed, Baby, Eritta, Fatimata, Sahar, children that I cared for and I that saw dying in the first weeks of my mission in Kailahun, and for Yatta and Isatta, the first children that I saw surviving. Each of them gave me insight and courage to look in remote villages for their family and community.

I often arrived too late. This thesis has helped to reconcile myself with them.

Preface

The aim of the PhD is to address evidence gaps that pose major clinical and public health challenges for agencies involved in outbreak management of Ebola Virus Disease (EVD). The thesis shows the need to re-frame how MSF and other response organizations operate and relate to the community in the context of an outbreak. Through work with patients and communities, outbreaks can become an opportunity to reinforce a non-existent health system and to create opportunities for access to safe and equitable care.

The work presented in this thesis reflects part of the work I have done as a medical doctor and Public Health advisor for Médecins Sans Frontières Operational Centre Amsterdam (MSF-OCA) during the 2014-2016 EVD outbreak in Sierra Leone.

As a staff member of MSF-OCA, I was deployed to Kailahun, Sierra Leone in June 2014, which was for several months the epicentre of the EVD outbreak. At the Ebola Management Centre (EMC), I supported patient triage, and set up the data management systems.

Outside the EMCs, I was responsible for EVD case investigations and supported the Ministry of Health (MoH) on surveillance and the use of EMC data to identify areas of transmission and to plan interventions.

In October 2014, I set up a community surveillance system in slum areas in Freetown. This supported the development of a novel MSF community strategy which ensured that the community were centrally involved in surveillance and were consulted to identify chains of transmission, and that households under quarantine were able to receive timely food, water, and mental health support[1].

In December 2014, I was appointed by MSF-OCA to coordinate and provide technical support to the field epidemiologists deployed by MSF-OCA in Sierra Leone. In 2015, I performed several field visits to support the design and implementation of population studies. In 2016, I supported to respond to the last EVD flare-up and supported Ministry of Health in Tonkolili District to reinforce health facility base surveillance[2].

In 2018 and 2019, I provided technical assistance and field work during two further EVD outbreaks in remote and conflict-affected areas in the Democratic Republic of Congo (DRC).

My PhD and field work led to improvement of MSF and MoH outbreak control practices in DRC, and informed the development of MSF-OCA epi-anthropological approach on EVD outbreak response. This approach has recently shaped the development of MSF-OCA Community guidelines for the novel

[1] Review of MSF-OCA surveillance and alert response in Freetown during the Ebola outbreak: lessons learned and challenges, MSF-OCA internal report. Available at https://www.researchgate.net

[2] Integrated Disease Surveillance and Response (IDSR), Tonkolili District, Sierra Leone, MSF-OCA internal report 2016.

coronavirus disease 2019 (COVID-19) response, and the design of a qualitative study exploring community perception toward COVID-19 in Sierra Leone.

My role in this PhD was the conceptualisation of the project, the design of the studies, and leading the development of study protocols, field implementation, data management and dissemination. I also led the review and metanalysis of the literature to explore performance of WHO EVD case definitions. I am first author on the 3 papers, reflecting my leadership role in the work.

Structure and methodological approach of the thesis

This thesis is structured in a research paper style and comprises of three papers, with an introduction, discussion and linking material. The first chapter provides an overview of Ebola Virus Disease (EVD), EVD transmission, history of EVD outbreaks, public heath control measures, diagnostic methods, EVD clinical presentation, current treatments and vaccines, and a review of the salient events of the EVD outbreak in Sierra Leone.

Chapter 2 provides the context to the work done by MSF-OCA in Sierra Leone and summarises the challenges faced while responding to the outbreak. The gaps and challenges experienced during field work informed the conceptualisation of the PhD and the development of the research objectives.

Chapter 3 (Objective 1; Research paper 1) aims to understand transmission dynamics and factors associated with non-adherence to control strategies in the context of sustained EVD transmission in a village in Kailahun district. Supplementary material included a link to the study protocol and mix-methods data collection tools designed for the study.

Chapter 4 (Objective 2; Research paper 2) addresses the performance of WHO EVD case definitions and risk scores, which are crucial for accurate surveillance tools to detect suspected cases for referral and as screening tools for clinicians to support admission and laboratory testing decisions at Ebola health facilities. Supplementary material included PRISMA-DTA checklist, search strategies and additional analysis.

In Chapter 5 (Objective 3; Research paper 3) I implemented and analysed data from two retrospective mortality studies implemented in 2015 to estimate overall and EVD-specific mortality rates in two areas where MSF suspended critical health interventions and refocussed on EVD care. This chapter highlights the challenges of using retrospective mortality studies for highly clustered disease outbreaks like EVD and suggests solutions to this problem. The supplementary material included a link to the study protocol and data collection tools designed for the study.

In Chapter 6, I summarise the key findings from the thesis, and discuss the contribution of this work to future and current EVD and non EVD outbreak responses, including identification of areas that warrant further research.

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List of acronyms and abbreviations

List of acronyms and abbreviations

AR	Attributable risk
AUC	Area under the curve
BDBV	Bundibugyo virus
BOMV	Bombali virus
CCCs	Community Care Centres
CDC	Centers for Disease Control and Prevention
CEVD	Chronic Ebola virus disease
CFDA	China Food and Drug Administration
CFR	Case fatality rate
CI	Confidence intervals
CLEA	Community-Led Ebola Action
CMR	Crude mortality rate
COVID-19	Novel coronavirus disease 2019
Ct	Cycle threshold
DEFF	Design effects
DERCs	District Ebola Response Centres
DRC	Democratic Republic of the Congo
EBOV	Ebola virus
EHUs	Ebola Holding Units
EMA	European Medicines Agency
EMC	Ebola Management Centre
EPI	Expanded Programme on Immunization
ETC	Ebola Treatment Centre
EUAL	Emergency Use Assessment and Listing
EVD	Ebola virus disease
FDA	Food and Drug Administration
GP	Glycoprotein
GPS	Geographic positioning system
HCWs	Health care workers
HRs	Hazard ratios
HSROC	hierarchical summary receiver operating characteristic

List of acronyms and abbreviations

IDP	Internally displaced people
IHREC	International Health Regulations Emergency Committee on Ebola Viral Disease
IPC	Infection Prevention Control
IQR	Interquartile range
IRR	Incidence rate ratio
LSHTM	London School of Hygiene & Tropical Medicine
MDA	Mass Drug Administration
MARV	Marburg virus
MEURI	Monitored Emergency use of Unregistered and Investigational interventions
MoH	Ministry of Health
MoHS	Ministry of Health and Sanitation
MSF	Médecins sans Frontières
MSF-OCA	Médecins Sans Frontières Operational Centre Amsterdam
NERC	National Ebola Response Centre
OCV	Oral Cholera Vaccination
PALM	Pamoja Tulinde Maisha trial
PAR	Population attributable risk
PCR	Polymerase chain reaction
PHAC	Public Health Agency of Canada
PHEIC	Public Health Emergency of International Concern
PHUs	Peripheral health units
PPE	Personal protective equipment
PPS	Proportionally to population size
PREVAIL	Partnership for Research on Ebola Virus in Liberia
PRISMA-DTA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses-diagnostic test accuracy studies
PTSD	Post-traumatic stress disorder
RCT	Randomized controlled trial
RDTs	Rapid diagnostic test
RESTV	Reston virus
ROC	Receiver Operating Characteristic
ROC	Republic of Congo.
RT-PCR	Real-time reverse transcription PCR assays
SAEs	Serious Adverse Events
SAGE	Scientific Advisory Group for Emergencies

List of acronyms and abbreviations

SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SGBV	Sexual and gender-based violence
STRIVE	Sierra Leone Trial to Introduce a Vaccine Against Ebola
SUDV	Sudan virus
TAFV	Taï Forest virus
WHO	World Health Organization

Chapter 1: Introduction

1.1 Ebola virus Diseases (EVD)

Ebola virus (EBOV) is an RNA virus belonging to genus *Ebolavirus* of the Filoviridae family (Mononegavirales order). This family includes other five genera: Cuevavirus, Dianlovirus, Marburgvirus, Striavirus, and Thamnovirus. (1, 2). The genus *Ebolavirus* consists of six biologically distinct species that differ in genomic structure, host variety and geographic distribution: Ebola virus (EBOV, previously Zaire ebolavirus), Sudan virus (SUDV), Bundibugyo virus (BDBV), Taï Forest virus (TAFV, formerly Côte d'Ivoire ebolavirus), Reston virus (RESTV) and Bombali virus (BOMV) (1). These were identified between 1976 and 2018.

EBOV, SUDV and BDBV have caused large and recurrent outbreaks, known as Ebola virus disease (EVD) (3-5). In contrast, there has only been one documented case of TAFV which was not fatal and RESTV has caused only asymptomatic infection (3). BOMV was discovered in 2018, and its pathogenic potential is still unknown (6). RESTV is the only species that appears to circulate in South-eastern Asia (7). The rest of the Ebolaviruses circulate in Africa, where an estimated population of 22 million people in 22 countries live in areas considered at risk of EVD outbreaks (8). Currently, EBOV is the most studied member of the Filoviridae family, but all filoviruses are deemed to follow similar biological function (4).

The World Health Organization (WHO) classify EVD as a zoonosis which can cause fatal illness in humans and non-human primates, and list Ebola and Marburg viruses (MARV) as Category 4 Pathogens, requiring advanced biocontainment facility (Biosafety Level 4 (BSL-4)), and immediate notification (9-11).

Table 1 summarizes the members of the genus Ebolavirus known to be pathogenic in humans (4).

Table 1. Members of the genus *Ebolavirus* known to be pathogenic in humans, adaptation form Heinz *et al.* (4)

Genus	Species	Virus	Country	Year of discovery
Ebolavirus	*Zaire ebolavirus*	Ebola virus (EBOV)	DRC, Gabon, RC, Guinea, Liberia, Sierra Leone	1976
	Sudan ebolavirus	Sudan virus (SUDV)	DRC, South Sudan	1976
	Taï Forest ebolavirus	Taï Forest virus (TAFV)	Ivory coast	1994
	Bundibugyo ebolavirus	Bundibugyo virus (BDBV)	Uganda, DRC	2007
DRC= Democratic Republic of the Congo, ROC= Republic of Congo.				

1.2 Transmission

Bats are the putative reservoir hosts and efforts to explore genetic diversity, habitat suitability, and clarify reservoir(s) for filoviruses are ongoing (4, 12-14). Fruit bats of the Pteropodidae family seems to play a major role in the transmission cycle of *Ebolavirus*, by infecting intermediate hosts (including monkeys, great apes, duikers and pigs) via saliva and/or faeces (4, 14). The intermediate host then plays an amplification role and can transmit infection to human (through consumption or handling of infected bush meat) with subsequent human-to-human transmission in the community and health facilities (13). The risk of "spillover events" (defined as transmission of the virus from wild animal populations to humans), depends on a range of interplay factors in host-viral dynamics, including human behaviour, animals' susceptibility and anthropogenic land-use (15). A recent modelling paper predicts a 1.75 to 3.2-fold increase of EVD spillover events in Africa by 2070 (15).

Human-to-human transmission occurs primarily via unprotected direct contact with body fluids of symptomatic persons or those who have died from EVD, or occasionally through contact with contaminated fomites (i.e., needles, clothes) (13, 16-19). Aerosol transmission has been documented in experimental studies, but its role in human transmission remains unknown (20).

The time interval from infection or exposure to infection to onset of symptoms (i.e., incubation period) is 2-21 days, and infectivity starts with onset of symptoms (9). However, the virus can be detected by real-time reverse transcription PCR assays (RT-PCR) (see section 1.5 Diagnosis and diagnostic methods) in biological fluids (faeces, tears, sweat, semen, breast milk and cerebrospinal fluid) and sites (conjunctiva, rectum, vagina, aqueous humour) months after infection, when symptoms are no longer present and RT-PCR blood tests for virus RNA are negative (19, 21, 22).

EBOV has been isolated and detected from semen of Ebola survivors respectively 82 days and 18 months after symptom onset (19, 23); and decline of EBOV in semen over time have been documented (23). Although sexual transmission has been described, the risk of transmission from EVD survivors via sexual intercourse is considered low (24, 25). A retrospective study conducted in West Africa after the most intense EBOV transmission period (February 2015), investigated a series of 13 possible viral persistence–derived transmission of EBOV events (VPDTe)[3] (26). In four events, authors found strong evidence[4] of sexual transmission from male survivors, and in one event transmission was documented 65 weeks after recovery (26). A cohort study in Liberia which followed 171 EVD male survivors found detectable viral semen in 7.0% of survivors at 12 months, but no secondary cases despite participants

[3] VPDTe was defined: as: person-to-person transmission of EBOV from an EVD survivor (source) to another person (recipient) that occurred >21 days after the source case recovered from acute infection.
[4] Strong evidence for sexual transmission of Ebola virus, was defined based on the following 3 criteria: Epidemiologic investigation identified sexual contact between recipient or index person and single proposed/probable source AND EBOV detected in single proposed/probable source's semen or vaginal secretions (by a vaginal swab) by RT-PCR AND Sequencing indicates high likelihood of transmission to recipient

reporting unsafe sexual intercourse during this period (27). Current WHO interim guidelines advise all male EVD survivors to practice safer sex (i.e., abstinence or correct and consistent condom use) for at least 12 months after symptom onset or until their semen has twice tested negative (28).

Among female EVD survivors, there is no evidence of EBOV transmission from contact with vaginal fluids (29), but mother-to-child transmission has been documented during pregnancy (30). One case report has documented virus persistence in immunologically-privileged sites (i.e. placenta, amniotic fluid, umbilical cord blood cord blood) and in foetus tissue after the disappearance of EBOV RNA from maternal blood (31). Another case report documented an EBOV-positive stillbirth from a woman with serological evidence of prior asymptomatic EVD, suggesting the possibility of ongoing infectivity during the convalescent period (32). A literature review found consistent evidence of EBOV RNA in pregnancy-related fluids and tissues both during acute EVD (positive blood RT-PCR for EBOV RNA) and after recovery, with a potential risk of secondary infection to healthcare workers and community members (33). A recent case report from Democratic Republic of the Congo (DRC) documented for the first time the birth of two healthy neonates with negative blood tests for EBOV RNA, born to positive mothers (positive blood RT-PCR for EBOV RNA) who received investigational monoclonal antibody before delivery (34). To mitigate possible secondary transmission during pregnancy and delivery, recent WHO guidelines recommend that childbirth and pregnancy complications should be managed at Ebola Treatment Centres (ETC) and that Ebola Infection Prevention and Control (IPC) measures should be used in pregnant women with acute EVD and in EVD convalescent pregnant (35).

EBOV has also been detected in breastmilk, although the evidence of transmission via breastfeeding is conflicting (36-38). This may be due to the timing of testing the breastmilk, as it is likely that during the convalescent phase of the disease, the virus is detectable in breast milk but not in maternal blood (37, 39). However, given the high case fatality rates (CFR) associated with exposure in infants and neonates, WHO recommended that mothers with EVD confirmed should stop breastfeeding and be separated from their children until they have received two consecutive negative EBOV breastmilk tests by RT-PCR, separated by 24 hours (35).

Transmission risk depends on viral load, which is associated with disease severity, culminating in death (5); CFR varies from 20% to 90% depending on the virus species (3, 40, 41). Funeral events may start a “super-spreading event”, in which contact with a case at their most infectious stage can amplify transmission within a community (13). As documented in 2014 in Guinea, 85 confirmed cases were traced back to a single funeral (42), and a specific protocol has been developed to guide safe and dignified burial in an EVD outbreak context (43).

Transmission can also occur through nosocomial contacts, and this is a major cause of amplification of EVD outbreaks which hampers control efforts (44-46). Infections among Health care workers (HCWs) have been documented since the first EVD outbreaks in 1976 in Zaire (now DRC), and southern Sudan

(now South Sudan), with catastrophic impact on the health system (47-49). In the 1976 South Sudan outbreak, the hospitalization of one positive case led to 41 HCW dying, and one-third of the 220 hospital staff acquiring infection (50). In the same year iatrogenic amplification due to use of infected needles was documented in a missionary hospital in Yambuku, DRC (47). In 1995, in Kikwit DRC, 80 HCW were infected in a regional hospital, and in 2000 in Uganda, 425 cases (including 17 HCW) were linked to nosocomial transmission (50). During the 2014-2016 EVD West African outbreak, 815 health staff were infected (for 635 the outcome was available), among those with known outcome 418 died (51). In Sierra Leone, 221 HCWs died from EVD and the ratio of skilled health staff to population size was dramatically reduced from 17.2/10,000 people before the outbreak to 3.4/10,000 at the end of the EVD outbreak (52). In the 2018-2020 outbreak in North Kivu/Ituri, DRC, 5% of all cases were among HCWs (171 cases) (53). Current WHO guidelines focus on the critical aspect of IPC, availability and correct use of personal protective equipment (PPE), patients' triage, and training for health staff (54).

1.3 EVD outbreaks

Since its discovery in 1976 in two simultaneous and unrelated outbreaks in Zaire (now DRC), and southern Sudan (now South Sudan), EVD has been considered a severe rare infection causing localised outbreaks (47, 55). Historically, EVD outbreaks have been detected when a symptomatic case reaches a health facility, initiating nosocomial infection and amplification of transmission amongst patients and health staff (47). Between 1976 and 2012, twenty-three EVD outbreaks and isolated cases were documented, with the largest reported in Uganda (n=425 cases), and the most lethal in DRC (CFR=90%) (3, 50, 56, 57). Outbreaks repeatedly affected DRC (n=6), Uganda (n=5), Gabon (n=4), Republic of the Congo (n=3) and Sudan (n=3) (57, 58) (Figure 1).

In 2014, two unrelated EBOV outbreaks occurred in DRC and West Africa. In DRC, the outbreak started in August 2014 and was controlled within 3 months. It resulted in 66 cases and 49 deaths (58). In contrast, the 2014-2016 West African outbreak caused 28, 639 cases and 11,316 deaths in 6 countries over 2 years (59). The outbreak began in December 2013 in Guinea (n=3,804)[5]. In March 2014 the first case was notified in Liberia (causing 10,675 cases), in May 2014 the first EVD case was notified in Sierra Leone (n=14,124), in July 2014 in Nigeria (n=20), in August 2014 in Senegal (n=1) and October 2014 in Mali (n=8). Between 2014 and 2015, imported EVD cases from West Africa arrived in the USA (n=4), UK (n=1), Italy (n=1) and Spain (n=1) (59, 60) (Figure 1-2).

In August 2014, WHO declared the West African EVD outbreak to be a Public Health Emergency of International Concern (PHEIC), which represent a public health 'extraordinary event' and a "public health risk to other States" (61). The outbreak was "extraordinary" in term of number of cases and deaths, geographical distribution (urban and rural areas) and the time required to control it. In September 2014, the US Centers for Disease Control and Prevention (CDC) published forecasts indicating a catastrophic scenario of 1.4 million EVD cases unless 70% of cases were safely isolated (62). This announcement, coupled with advocacy efforts, triggered a number of international commitments to support the response effort, such as UN Mission for Ebola Emergency Response (UNMEER), although the majority of these were not delivered until after the peak of the epidemic had been passed (63).

In 2015, after two incubation periods (42 days) since the last EVD patient had tested negative, WHO declared Liberia (9 May), Sierra Leone (7 November) and Guinea (29 December) free of EBOV transmission (64). Subsequently all three countries experienced small and isolated EVD outbreaks which were controlled with a combination of early detection and integrated interventions, resulting in a limited number of secondary cases (64). Finally, on 29th March 2016, WHO declared that the EVD

[5] Guinea: WHO officially declared EVD outbreak on March 2014 when the first EVD case was confirmed, but the index patient, was retrospectively identified in December.

outbreak no longer represented a PHEIC and the recommendations adopted in response to the outbreak were lifted (65).

Virology studies suggest that the West African EVD outbreak strain was closely related to the Zaire strain circulating in a previous outbreak in DRC (97% homology, but classified as a different clade) and most likely had circulated in zoonotically in West Africa since 2004 (66, 67). The rapid and widespread West African EBOV transmission was due to a number of factors including weak surveillance, scant IPC in hospitals, non-specific initial clinical presentation, traditional burial procedures, porous borders, distrust of authority, late response and lack of knowledge about the disease and control strategies (64, 68).

Between 2017 and 2020, DRC experienced four additional unrelated EBOV outbreaks including an area of protracted conflict (North Kivu and Ituri provinces) (69-72). The outbreak in North Kivu/Ituri is the second largest protracted EVD outbreak after the 2014-2016 West Africa outbreak, with 3481 cases, among those 2299 died, it lasted from August 2018 to June 2020 (73). In July 2019, following a case in Goma (the capital of North Kivu province, a city of 2 million people on the border with Rwanda) and imported transmission from DRC in Uganda, the International Health Regulations Emergency Committee on Ebola Viral Disease (IHREC) declared the North Kivu/Ituri outbreak to be a PHEIC event (74). During this outbreak, important advances occurred on use of investigational drugs, and widespread EVD immunization campaign with promising preliminary results (4, 75) (see section 1.7 Treatments and vaccines). However, this outbreak further challenged response organizations, and their modus operandi toward communities who have been largely neglected by humanitarian organizations (75-77). The outbreak unfolded in a war zone, with over one million internally displaced people (IDP), and was characterized by chronic tension between local civilian and the government (78, 79). The response was further exacerbated by the community withdrawing from the responding organization due to fear of being misdiagnosed with EVD and being forced to be isolate far from family and community in dedicated Ebola isolation centres, or due to the concern of being exposed to EVD in general health facilities (77) (see section 1.4, point 7 community engagement and barriers with EVD response). During the outbreak, several attacks to Ebola isolation centres and health facilities occurred, with causalities including HCWs and patients (80, 81). Use of military to support implementations of control strategies further contributed to distrust and reinforced the belief that the EVD response was part of a larger political agenda to further control North Kivu (77).

Figure 1. History of Ebolavirus outbreaks 1976-2020 from CDC (58)

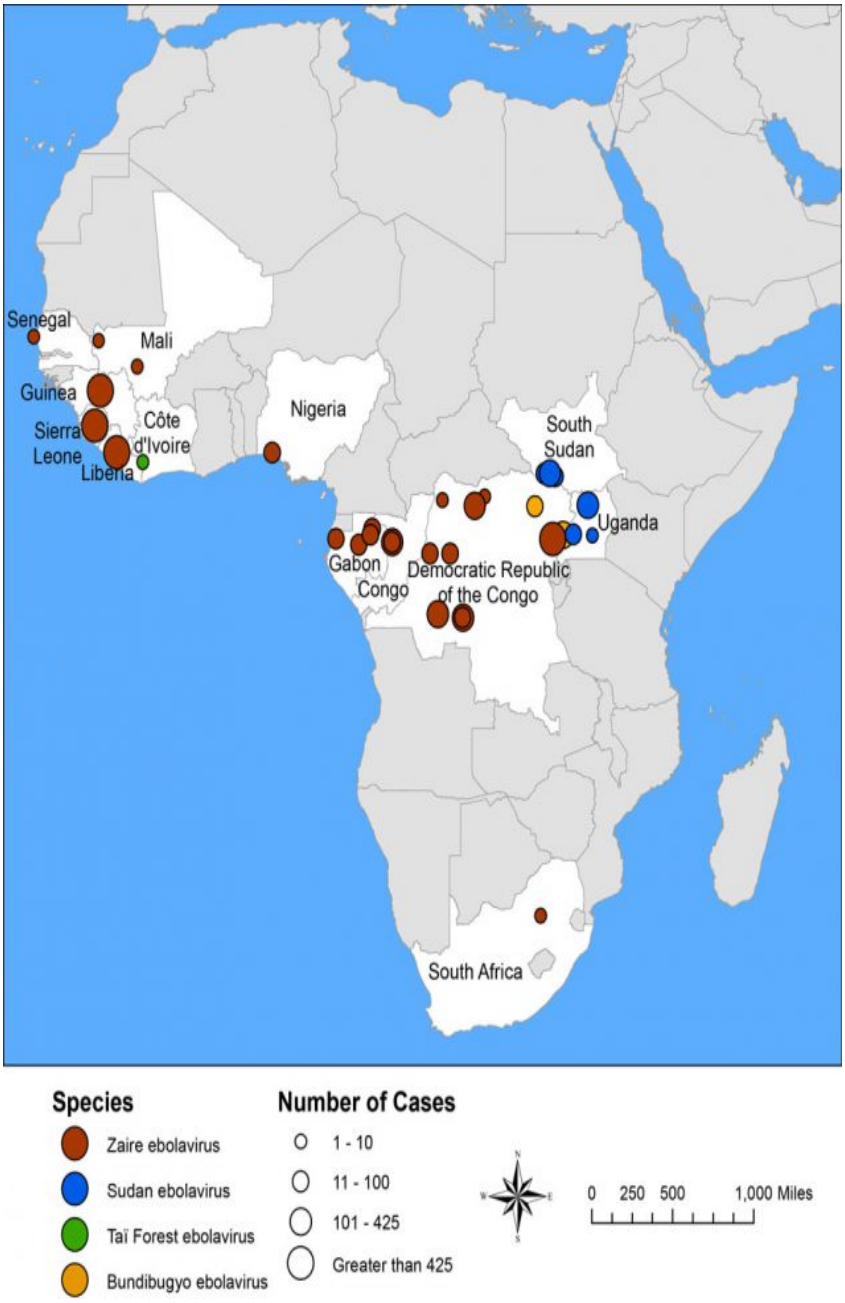


Figure 2. Countries affected by 2014-2016 EVD West Africa outbreak, (‘‘darker shades of blue indicate higher numbers of confirmed EVD cases’’), original map from Bart SM (60)

1.4 Public health interventions to control EVD outbreaks

Based on understanding of transmission routes, from previous outbreaks, seven public health pillars (plus access to laboratory facilities, see section 1.5 Diagnosis and diagnostic methods) have been critical to control EVD outbreaks occurring between 1976 to 2016 (82-85).
Toward the end of the 2014-2016 West Africa EVD outbreak, the availability of vaccines supported outbreak response (see section 1.7 Treatments and vaccines).

The public health pillars were surveillance, contact tracing, case isolation, safe and dignified burial, IPC, social mobilization and community engagement. The aims of each pillar are given below.

1. Surveillance: To detect the index case, monitor population transmission, and identify areas for intervention;

2. Contact tracing: To monitor contacts of cases for 21 days since their most recent EVD exposure and to timely isolate exposed contacts who become symptomatic;

3. Case isolation in specific EVD centres**:** To prevent secondary transmission in communities and health facilities through the use of Ebola isolation centre (i.e., Ebola Management or Treatment Centres (EMC/ETC)), with specific IPC measures including PPE;

4. Safe and dignified burial**:** To prevent secondary transmission during funeral ceremonies of those who have died or suspected to die from EVD; using trained burial teams and specific IPC procedures;

5. IPC ring approach to non-Ebola health facilities: To prevent secondary infection in health workers and health facilities using; enhanced IPC and triage approach in areas of intense EVD transmission;

6. Social mobilisation: To disseminate messages on infection, transmission, and control strategies,

7. Community engagement and documented barriers for communities to engagement with EVD response. To actively consult communities by create a participatory infrastructure; gathering epidemiological and behavioural information to improve effectiveness and relevance of public health interventions.

The ethical aim of community engagement is to enhanced protection, benefits, legitimacy, and shared responsibility between public health actors and affected populations (86). However, those ethical principles have been challenged during EVD outbreaks, with consequent practical and moral dilemmas which have also impacted the performance of the other pillars (87). For instance, affected communities were often consulted only after months of uncontrolled EVD transmission, or retrospectively at the end of outbreak, or when critical security incidents have occurred (88, 89).

In Sierra Leone, public health EVD responses have taken insufficient account the views and experience of affected population, with community left out of the decision-making process (89). This has fuelled misinformation and compromised the credibility of public health actions due to scepticism about the disease's existence, and speculation about the use of the virus to suppress specific population groups. (89-91). Barriers faced by the community included the use of public health messages that emphasised that EVD was a deadly disease, and lack of incorporation of local beliefs and practices into patient care, as well as distance to isolation centres, as identified in previous EVD outbreaks in Uganda, Angola, DRC and West Africa (92-95).

In the early months of the EVD outbreak in Sierra Leone, the community witnessed family members forcibly taken from their homes (88). Several families reported never hearing back from the relative taken to the Ebola centre (96). Other's patients were buried in mass graves, against local practice and beliefs. These factors all contributed to fears about dying alone in an Ebola isolation centre (94).
Poor community engagement has been in part ascribed to the architecture of the humanitarian system often rigid and driven by a biosafety perspective, where community engagement has not been prioritized as integral part the moral duties from responding actors (97). During the EVD West Africa outbreak, the fluid nature of social dynamics wasn't acknowledged, with the response system unable to understand, negotiate, and adjust interventions over time and across contexts (98). Use of militarised escorts to implement EVD interventions in volatile contexts (i.e., North Kivu/Ituri, provinces in DRC) also contributed to community withdrawal, the deterioration of security, and consolidation of population distrust in the response effort (76).

Public health stakeholders have often assumed that is responsibility of communities to meet the needs of responders, to hand over their relatives to an uncertain and often fatal outcome in Ebola facilities, to report their family, friends and neighbours to disease surveillance staff, and to then have limited freedom and livelihoods (99, 100). Use of vertical coercive EVD control measures have exacerbated pre-existing tension and contributed to compromise trust into public-health interventions (87). For instance, in Liberia, use of an incentive scheme to increase case reporting of suspected cases was refused by the community and perceived to further increase social disruption (101). Instead, consulted communities suggested improving EVD reporting by addressing specific health problems (e.g.

providing food for families in quarantine, increasing communication between Ebola facilities and families, increasing basic health services, providing psychosocial support for affected families, and including Ebola survivors in teams involved on active case-finders and contact-tracers) (101).

Stigma associated with EVD infection has also been described as an additional factor that has contributed to generate rumours, community disengagement, with consequent delay on access to care, and outbreak control (102).

Failure to recognise the social dimension and value of traditional burial rituals on management of EVD deaths during burial procedures have further discouraged community on reporting deaths and adhere to strict biomedical burial procedures (103, 104). In Liberia, cremation procedures used to manage EVD deaths have contributed to social breakdown, increasing inequalities among people who could afford private funerals and disadvantaged people who were obliged to accept cremation (105). Following the outbreak community have express the desire to have a form of formal commemoration to retrospectively honour EVD deaths (106).

Inconsistent distribution of aid to affected communities have further contributed to social disruption and poor community compliance (105, 107). EVD outbreaks have occurred in settings with fragile health systems, and use of significant resources dedicated to EVD has led to neglect other relevant local health burden. Thus, resources for EVD control have been perceived largely inconsistent with the rest of health and humanitarian needs (107, 108). In Sierra Leone, significant changes in EVD-related behaviour (i.e., prompt referrals to treatment and safe burials) were documented when community meetings were held to identify priority area of interventions and when identified needs were consistently delivered (109). This was consistent with other successful public health models that engaged more meaningfully with communities, strengthening proximity and encouraging mutual dialogue (110, 111).The discrepancies between different actors on how to set up participatory dialogue and practices with affected population has also contributed inconsistency on community engagement and on design and delivery of EVD interventions (89).

Finally, in retrospect, interventions which failed to respect, sympathize and recognise community in their multiple social and believe dimensions have ultimately become barriers for community to engage with responding organization, and for organization to meaningful engage and work with community (93).

1.5 Diagnosis and diagnostic methods

Clinical presentation of patients with EVD is non-specific, and can be confused with other endemic tropical diseases, including malaria (see section 1.6 Case definition and clinical presentation) (4, 5, 11).

Rapid and accurate diagnosis of EVD is therefore crucial for outbreak control to interrupt the chain of transmission, support patient management (i.e., isolation and discharge), follow EVD survivors during the convalescent phase, and retrospectively identify paucisymptomatic or asymptomatic cases (54, 112).

Current EVD diagnostics include: i) molecular techniques to detect viral RNA nucleic acids, ii) rapid antigen test to detect viral proteins, and iii) serological assays to detect host antibodies produced against the virus (113).

For EVD confirmation, WHO recommends the use of molecular technique such us RT-PCR which detect viral RNA in blood and non-blood specimens. RT-PCR results are expressed in cycle threshold (Ct), and are used as indicators of viral load (114). The lower the Ct value the higher the viral load, the minimum RT-PCR detection limit is 1000 virus RNA copies per mL of blood (5). However, RT-PCR can detect copies of viral RNA, but does not distinguish between viable, infectious, virus or residual RNA, therefore interpretation of Ct value needs to be always interpreted together with clinical and contact history of patients (25, 115, 116). For instance, RNA virus can be undetectable by RT-PCR in the first 1–3 days after symptom onset, and a second blood specimen is required for any negative test if taken within 72 hours of onset of symptoms. references (54). Viremia peak 3 to 7 days after disease onset, and decline under the detection threshold 2 to 3 weeks after the onset of symptoms (5).

WHO defines EVD survivors as ‘a person: with a confirmed positive result on RT-PCR testing for Ebola virus on any body fluid who subsequently recovered; AND/OR who is IgM and/or IgG positive on serological testing for EVD and has not been vaccinated against Ebola virus’ (117).

Current interim WHO guideline indicated that patients diagnosed with EVD are eligible for discharge from Ebola isolation facilities if they had ≥3 days without any symptoms and signs and negative blood RT-PCR; for patients with persisting symptoms discharge from isolation is considered after two RT-PCR negative blood tests, conducted 48 h apart (54). However, in other centres in Europe and USA, which cared for EVD patients during the 2014-2016 West Africa outbreak, discharge criteria varied according centres; with some centres using as discharge criteria negative RT-PCR tests on blood and other non-blood fluids (i.e., urine) and EBOV cell culture under biosafety level 4 containment (118).

Patients who survive EVD, can have a prolonged convalescence period, in those patients, RT-PCR is used to detect virus persistence in other non-blood body fluids (e.g. semen) and or immuno-privileged sites (e.g. eyes, testes, meninges) (see section 1.2 Transmission) (5, 119) (Figure 3).

There are currently no available and validated diagnostics tests which could detect Ebola virus prior to the onset of symptoms, and diagnostic tests may vary on the panel of species which they are able to detect (i.e., EBOV, SUDV, BDBV) (113).

During the 2014-2016 West Africa outbreak, WHO added the Xpert® Ebola Test (Cepheid AB - Solna, Sweden) to its list of RT-PCR Ebola diagnostics eligible procurement (120-122). (Table 2, section A)

Use of rapid antigen test as screening tools in patients meeting clinical and epidemiological criteria compatible with EVD, is recommended by WHO in remote settings where access to RT-PCR is not immediately available or in settings where number of cases has overtaken isolation and laboratory capacity; any rapid antigen test specimens (either positive or negative) must be retested by RT- PCR for confirmation (123). The performance (i.e., sensitivity and specificity) of molecular diagnostic (RT-PCR) and rapid antigen tests are currently benchmarked against the PCR Trombley assay, which is considered the gold standard (124). WHO define an acceptable performance for rapid antigen tests when sensitivity is > 95%, and specificity is > 99% (125).

Guidance on the use of rapid antigen test is based on a WHO Interim Guidance published in 2015. At that time Corgenix ReEBOV was the only rapid antigen test identified by WHO as appropriate for use, but it is no longer on the market (126). Currently the only rapid antigen test approved by WHO and the FDA (Food and Drug Administration) is the OraQuick Ebola Rapid Antigen, which can identify antigens/protein associated with EVD both in the blood from symptomatic patients and in oral fluid taken post-mortem (120, 127, 128) (Table 2, Section B).

An evaluation of the performance of other non-approved rapid antigen tests in a cohort of 205 samples from positive and negative EVD patients during the 2014-2016 West African outbreak found good performance (sensitivity 77.06%, specificity 91.67%) but still below the acceptable performance criteria set by WHO against the reference standard RT-PCR (129) (Table 2, Section C).

Usually, molecular diagnostic (RT-PCR) and rapid antigen tests are used to identify EVD acute infection when patients are symptomatic and virus levels or virus protein are high in patients' blood (3-10 days after the onset of symptoms) (Figure 3). Both methods are also used post-mortem to test cadaveric fluid (oral swab) to retrospectively identity EVD deaths (113, 120, 128). As mentioned previously, RT-PCR is also used in the convalescent period to detect virus persistence in survivors in other non-blood body fluids (5).

Serological tests are used to retrospectively identify paucisymptomatic or asymptomatic cases when RNA virus is not detectable in the blood and when Ebola-specific antibodies are present instead (130, 131). In 1955, in the Kikwit outbreak in Democratic Republic of the Congo (DRC), a serological study among 29 EVD survivors found that IgM antibodies appeared 2-9 days after symptom onset (persisting up to 168 days since the first test) while IgG appear 6-18 days after onset of symptoms (persisting up

to 749 days since the first test) (132). Recently, serological studies have been conducted in Republic of Congo, where recurrent EVD outbreaks had occurred, reporting seroprevalence of IgG Ebola-specific antibodies of 2.5%, reaching prevalence of 4% among rural populations (133). Another study, in DRC, found antibody seroprevalence of 18.7% among pygmies' communities (134). In DRC, other authors have documented a sero-reactivity of 28.1% for IgG among 565 healthcare workers without history of EVD but living in an area where in 2014 an EVD outbreaks have been previously documented (135).

More recently a cross-sectional seroprevalence study was implemented to rebuild chain of transmission in Guinea where the index case of the West Africa was first reported, the study involved 237 individual and identified additional eight previously undetected seropositive survivors, illustrating the use of serology in understanding the chain of transmission (136).

There are few performance studies of serological tests, however a cross-sectional seroprevalence study among contacts of 151 EVD survivors found high sensitivity (95.9% (95% CI 89.8–98.9) and high specificity (100% (95% CI 98.9–100) in one serological essay not yet validated by WHO (131) (Table 2, section D). Similar results were observed with another serological study among 94 EVD survivors using a different serological assay (137) (Table 2, section E).

In summary, each diagnostic tool should be used and interpreted according to phase of infection, clinical presentation and contact history of patients. Each can inform outbreak response, through the identification of infectious cases (when RT-PCR is positive in blood or RT-PCR is positive in oral swab in EVD death), supporting follow up of survivors (by identifying RNA persistence in non-blood bodily fluids, such as semen in EVD survivors) or rebuilding chains of transmission to identify paucisymptomatic or asymptomatic case (i.e., serological test to detect IgM or IgG).

Table 2 summarizes the main categories of diagnostic test for EVD and their performance.

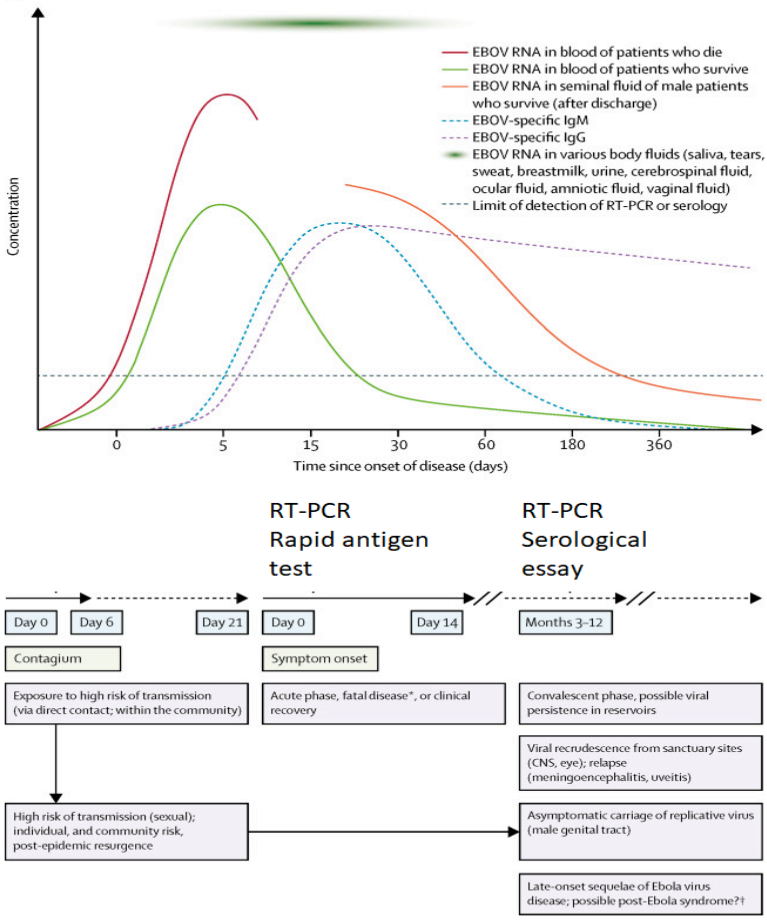
Table 2. Main categories of diagnostic test for EVD and performance

Section	Commercial name	Diagnostics category	Specimen used to assess performance	Acute infection Or survivor/convalescent phase	Reference standard	Performance		WHO approval for emergency procurement	Reference
						Sensitivity (95%CI)	Specificity (95%CI)		
A	Xpert® Ebola Test manufactured (Cepheid AB - Solna, Sweden)	RT-PCR	Whole blood	Acute	Trombley RT-PCR assay	100%, (84.6%–100%)	95.8% (91.8%–98.2%).	Yes	(122)
B	OraQuick® Ebola Rapid Antigen Test Kit (Cadaveric Oral fluid and Whole Blood) EUAL	rapid antigen test	Cadaveric oral fluid	Acute and post-mortem	Xpert Ebola Test (Cepheid)	94.12% (83.76-98.77)	100.00% (98.11-100)	Yes	(128)
C	DPP® [&] Ebola Antigen System (Chembio Diagnostic Systems, Inc.)	rapid antigen test	Serum	Acute	Trombley RT-PCR assay	77.06% (67.8–84.3)	91.67% (83.8–96.1)	No	(129)
D	GPδTM [catalogue 0501-016]; IBT Bioservices, Rockville, MD, USA	Serological assay	Oral swabs	Contact of survivors	PCR-confirmed EVD survivors and from the community controls	95.9% (89.8–98.9)	(100% (98.9–100)	No	(131)
E	Multiple Analyte Profiling technology (xMAP; Luminex Corp., Austin, TX)	Serological assay	Plasma	Survivor/convalescent	Commercial EBOV NP and GP enzyme-linked immunosorbent assays (ELISAs; Alpha Diagnostic, San Antonio, TX).	95.70%* (89.6-98.3)	99.10%.* (94.9-99.8)	No	(137)

[&]DPP® Ebola Antigen presented high accuracy when tested on samples with low Ct value (high viral load)

* Sensitivity and specificity varied according to antigen used, reported sensitivity and specificity for least two antigens

Figure 3. Time sequence of the evolution of the Ebola virus infection and disease and diagnostic method; adaptation form Ebola virus disease Prof Denis Malvy *et al.* (5)



1.6 Case definition and clinical presentation

The initial clinical symptoms and signs of EVD are non-specific and overlap with other common tropical diseases (including malaria, Typhoid fever, Lassa fever, Meningococcal disease, shigellosis, plague, leptospirosis, anthrax, relapsing fever) (4, 11). This non-specificity contributes to delays in detection of cases, the confirmation of an outbreak, and timely implementation of control measures (9, 11).

EVD WHO case definition used during outbreak encompass four main categories: suspect, probable confirmed cases, and non-case, according to clinical presentation, history of contact, and laboratory results (138) (Table 3).

The understandings of the clinical presentation and route of transmission of EVD have been established by retrospective review of exposure history, contact tracing and clinical observation (3, 5, 139, 140). These have indicated that people are not contagious until they develop symptoms (9). EVD incubation period (time interval from infection to onset of symptoms) range from 2-21 days depending on the species and route of transmission[6] (5, 9, 140). Typically, the disease evolution is rapid (within 10 days of symptom onset), and following three main clinical phases (5):

1. First phase: 1-3 days: flu-like symptoms (i.e., fatigue, myalgia);
2. Second phase: 4-7 days: presentation of both "wet" symptoms (i.e., vomiting, diarrhoea (up to 10 litres per day)), and "dry" symptoms (headache);
3. Third phase: 7-10 recovery or death (bleeding that occur in less than half of affected patients);

Additionally, chest pain, hiccups, miscarriage, cough, conjunctival injection, neurological and ocular symptoms have also been reported (5).

The virus induces direct tissue damage, through a paradoxical cascade of inflammatory responses that can ultimately result in multiple organ failure and onset of a septic shock-like syndrome (141) (see Annex 1.1 Underlying EVD pathophysiology). A systematic review and metanalysis reported difference in clinical features between fatal cases and EVD survivors with bleeding, vomiting, and diarrhoea observed in more than 60% of patients who died compared to EVD survivors (142).

During the convalescent period, longitudinal studies and systematic reviews have described several clinical and immunological alterations among EVD survivors, including arthralgia, encephalitis, post-

[6] Route of exposure and variation in mean incubation period (6·3 days, versus in infection known to be due to injection versus 9·5 days exposures by contact). 140. Breman JG PP, Johnson KM, White MK, Mbuyi M, Sureau P, Heymann DL, Van Nieuwenhove S, Mccormick JB, Ruppol JP, KIntokI V, Isaacson M, Van der Groen G, Webb PA, Ngvete K. The epidemiology of Ebola haemorrhagic fever in Zaire, 1976. Amsterdam: Elsevier Science. 1978:Pages 103-24.

traumatic stress disorder (PTSD), hearing loss, uveitis, inflammation of one or both testicles, changes in menstruation, impotence, persistence of immune activation and inflammatory pathways (143-152).

These are described collectively as post Ebola syndrome or chronic Ebola virus disease (CEVD) (144, 153).

Among a cohort of 277 survivors, high viremia levels during the acute phase of EVD infection were associated with an increased risk of uveitis (adjusted odds ratio [aOR] 3.33, 95% CI 1.87–5.91) compared to those without uveitis (150).

However, it is unclear whether the EVD-associated syndrome is caused by direct effect of virus or to immune complex deposition or dysregulated immune response (154).

Annex 1 provides an overview of the likely underlying pathophysiology of EVD, the groups at most risk of negative outcomes, and malaria co-infection.

Table 3. World Health Organization (WHO) EVD case definitions used during EVD outbreaks, to define suspect, probable, confirmed cases and non- case (138)

Suspect	**Probable**	**Laboratory confirmed**	**Non-Case**
a. Any person, alive or dead, suffering or having suffered from a sudden onset of high fever and having had contact with: - a suspected, probable or confirmed Ebola or Marburg case; - a dead or sick animal; **OR** **b**. Any person with sudden onset of high fever and at least three of the following symptoms: - headaches - lethargy - anorexia / loss of appetite - aching muscles or joints - stomach pain - difficulty swallowing - vomiting - difficulty breathing - diarrhea - hiccups; **OR** **c.** Any person with inexplicable bleeding; **OR** **d.** Any sudden, inexplicable death	**a.** Any suspected case evaluated by a clinician; **OR** **b.** Any deceased suspected case (where it has not been possible to collect specimens for laboratory confirmation) having an epidemiological link with a confirmed case	**a.** Any suspected or probably cases with a positive laboratory result. Laboratory confirmed cases must test positive for the virus antigen, either by detection of virus RNA by reverse transcriptase-polymerase chain reaction (RT- PCR), **OR** by detection of IgM antibodies directed against Marburg or Ebola.	**a.** Any suspected or probable case with a negative laboratory result. "Noncase" showed no specific antibodies, RNA or specific detectable antigens.
N.B. During an outbreak, case definitions are likely to be adapted to new clinical presentation(s) or different modes of transmission related to the local event			

1.7 Treatments and vaccines

1.7.1 Patient management and investigational treatments

Before and during the 2014-2016 West Africa EVD outbreak, standard care of EVD cases was focused on addressing the main clinical symptoms and signs including: i) supportive care to balance volume deficits and hypoperfusion (using oral rehydration or intravenous crystalloid infusion); ii) treatment of presumptive co-infection (i.e., malaria treatment, antibacterial therapies); ii) mitigation of symptoms like nausea, vomiting, pain (using of antiemetic agents, and opiates); iv) mental health support for patients and families, and (v) nutritional support (4, 5). Correction of electrolyte levels and hypoglycemia were introduced in patient care following the introduction of point-of-care biochemistry testing during the West Africa outbreak (155). Patient care is provided in dedicated Ebola isolation centre where trained HCWs and specific IPC protocols must be in place (54).

In August and September 2014, WHO, with a committee of experts, listed investigational therapy which showed antiviral efficacy and safety in vitro or in animal models, and postulated that investigational therapeutics could be offered to EVD patients in clinical trials, or under emergency or compassionate use basis (although this last option would not allow sufficient evidence about safety and efficacy) (156, 157). Since 2014, studies using a range of investigational therapies have been implemented (158), in isolation centres, with a common aim of reducing EVD fatality by lowering viral replication, curbing the inflammatory response and facilitating the innate and adaptive immune responses to clear the virus (5).

In 2014 in Guinea, a multicentre non-randomised comparative study assessed the survival benefit of Favipiravir (T-705) and standard of care (a synthetic drug with antiviral activity) versus historical controls who received only standard care (159). The study enrolled 126 patients and 540 historical controls (patients treated at the same EMC centre in the previous months). In the final analysis 99 patients received Favipiravir and standard of care. Interim analysis showed that in the intervention group viremia was a strong predictor of mortality; mortality was 91% (95% CI 78.8%–91.1%) in the group of patients with high viral load (Ct value < 20) compared to a mortality of 20% (95% CI 11.6%–32.4%) in patients with Ct value ≥ 20 (159), $p < 0.001$. Both 95% CIs for the mortality estimates included the predefined target values (baseline value in the centres before the trial) of 30% (for patents with Ct value ≥ 20) and 85% (for patients Ct value < 20). Thus, the study could not prove efficacy, in particular for patients with high viremia, but provided strong evidence for the rationale to stratify patients by Ct value when designing similar trials (159). Results from this study were judged by the Ministry of Health and Public Hygiene of Guinea sufficient to add Favipiravir, under a compassionate use, to standard of EVD care in this Country (160).

In 2014 in Liberia, a retrospective cohort study of EVD patients compared mortality between those who received two different antimalarial treatments in two different periods when the centre had a shortage of the first-line malaria drugs (artemether-lumefantrine) (161). The mortality risk was significantly lower among the 194 patients receiving artesunate-amodiaquine than among the 71 patients receiving artemether-lumefantrine (50% vs 64%; risk ratio=0.69, 95%CI 0.54-0.89 adjusted for confounding variables (age, sex, Ct value, time from symptom onset to admission, malaria test result, type of standard care and bed occupancy on the day of patient admission) (161). However, the study had several limitations including the observational design, that could not prove a definite survival effect, and authors recommended future study for stronger evidence.

Between 2014 and 2015, in Sierra Leone, a non-randomised two-centre comparative study assessed the survival benefit of convalescent blood transfusions versus standard care (162). Among the 69 participants (43 in the intervention group (one dropped out in a second stage), 25 in the control group), the odds ratio for survival was 2.3 (95% CI 0.8–6.5) in the blood convalescent group (162). However, the small sample size and the non-randomised design limited the conclusions about efficacy to reduce mortality.

In 2015 in Guinea, a larger non-randomised comparative study with historical controls also assessed the survival benefit of convalescent plasma versus standard care (163). This study enrolled 84 patients given convalescent plasma and compared with and 418 historical controls (EVD patients isolated at the same centre in the previous months). The risk of mortality was 31% in the intervention group and 38% in the control group (risk ratio=0.88 (95% CI 0.51–1.51), adjusted for age and viral load) (163). Again, this study did not provide evidence of survival benefit of convalescent plasma.

In March 2015, the PREVAIL II (The Partnership for Research on Ebola Virus in Liberia) study started in Guinea, Sierra Leone, Liberia and United States (164). This was first and only multicentre randomised controlled trial (RCT) during the West Africa outbreak. Patients were randomly assigned in a 1:1 ratio to receive ZMapp (monoclonal antibody cocktail) plus standard of care versus vs standard of care alone. In Guinea, standard of care also included Favipiravir following the earlier study (159). In the study a probability ≥ 97.5% was required to establish efficacy. Overall, 72 patients were enrolled, stratified by baseline Ct value (≤22 vs. >22) and country of enrolment. Overall, the risk of mortality was 22% in the ZMapp group vs 37% in the control group. In the study the Bayesian estimate of the absolute difference in mortality, between the intervention group and the control group, was −14 percentage points, and the relative difference was −38%, giving a 91.2% posterior probability that the intervention was superior to the non-intervention; this value was below the established probability threshold (≥ 97.5%) for declaring superiority of the investigational treatment. The frequentist results estimated a mortality difference of −15 percentage points (95% confidence interval [CI], −36 to 7) and a relative risk of 0.60 (95% CI 0.25 to 1.27). However, the study did not reach the threshold (≥97.5%)

required to establish superiority to standard care; the results were suggestive of effectiveness but not statistically conclusive.

During the 2014-2015 West Africa outbreak, an additional four studies were conducted: i) two non-randomised single-arm intervention studies with concurrent controls using TKM-130803 (interfering RNA product) and Interferon β-1a (immunomodulators) respectively in Sierra Leone and Guinea; ii) a non-randomised single-arm intervention study with historical controls using Favipiravir in Sierra Leone; and iii) a non-randomised single-arm intervention study without controls using Brincidofovir (antiviral drug) in Liberia, however none of them could prove survival benefit (165).

Case reports on patients infected in West Africa but treated in USA and other European country were also published (166). Overall, 27 EVD patients received care outside West Africa. Among these, 23 (85%) received investigational therapies (with 19 (70%) receiving more than one investigational therapy) (166). Overall, 7 patients (26%) received invasive mechanical ventilation, among those 5 received also continuous renal replacement therapy. Five of the patients died, giving a CFR of 18.5% (95%CI 6.3%-38.1%) for the group treated outside West Africa compared to an estimated CFR of 62.9% (95% CI 61.9–64.0%) among patients in West Africa (167). Ethical concerns were raised about access to investigational therapy, as fewer than 5% of patients in West Africa had access to investigational therapy versus 85% of those treated outside the region (168). Similar ethical concerns were raised in Guinea for EVD pregnant women, where access to investigational drugs outside clinical trials was extremely challenging (169).

Lessons learned during the 2014-2016 West Africa outbreak on study design, implementation and outcome predictors (e.g. the importance of stratifying by Ct value in RCTs) were utilised during the 2018-2020 second largest EVD outbreak in DRC (North Kivu and Ituri provinces). During this outbreak four investigational drugs were given to patients under a WHO protocol of Monitored Emergency use of Unregistered and Investigational interventions (MEURI) within an RCT (Pamoja Tulinde Maisha (PALM) trial) (170). The PALM trial was a multicentre RCT, in which patients were stratified by Ct value (≤ 22 or > 22) and ETC of enrolment. The primary end point was mortality by 28 days. All participants received standard care and were randomised with an allocation ratio 1:1:1:1 to receive either ZMapp™ (control arm), remdesivir (a broad-spectrum antiviral), mAb114 (single monoclonal antibody, obtained from memory B cells from a survivor of the 1995 Kikwit DRC outbreak) or REG-EB3 (monoclonal antibody cocktail, obtained by mice that encode human antibody). ZMapp was chosen as the control condition, based on the PREVAIL II trial results (164). The REGN-EB3 group was added in a second version of the study protocol, therefore data from this group was compared with patients ZMapp group (called in the analysis the ZMapp subgroup), who were enrolled the time or after the REGN-EB3 group was added. From November 2018 to August 2019, the PALM trial enrolled 681 EVD patients in four centres. Interim results of the study showed strong evidence of improved survival

for patients receiving MAb114 or REGN-EB3, compared with those receiving remdesivir or ZMapp, leading the independent study committee to recommend suspension of the remdesivir or ZMapp trial arms. The final results showed evidence that patients receiving Mab114 or RWGN-EB3 had better survival outcomes than patients receiving ZMapp. The mortality risk was 35.1% (61/174 patients) in the MAb114 group, compared with 49.7% (84/169 patients) in the ZMapp group (the difference was −14.6 percentage points (95% confidence interval [CI],−25.2 to −1.7; P=0.007); and 33.5% (52/155 patients) in the REGN-EB3 group, compared with 51.3% (79/154 patients) in the ZMapp subgroup (the difference was a−17.8 percentage points (95% CI,−28.9 to −2.9; P=0.002). In addition the study observed that overall the proportion of patients who died was lower (27.1% (42 out 155)) among patients who reported history of vaccination (rVSVΔG-ZEBOV-GP vaccine, see section 1.7.2 Vaccines) than among those who reported no previous vaccination (48.4% (225 out 465)). However, patients who reported vaccination were also more likely to have lower viremia (higher Ct values), better hepatic markers and early access to care since onset of symptoms (e.g. mean of 3.8 days among the vaccinated group versus a mean of 5.9 days in the non-vaccinated); further studies will better elucidate the relationship between previous vaccination and EVD outcome. The RCT included also pregnant women and children aged under five years respectively, thus providing the first preliminary data on safety and efficacy for these populations. The PALM trial results informed the recent WHO guidelines that now recommend "access to and use of investigational therapeutics under MEURI be carefully considered for each individual patient, including for vulnerable populations such as pregnant women and paediatric patients, as appropriate given the available data", and that "In general, the expert panel recommends consideration of factors such as disease severity and risks/benefits of investigational therapy (including adverse effects in pregnant or paediatric populations)" (35).

Between October and December 2020, the FDA approved the Inmazeb (also known as REGN-EB3) and the Ebanga (also known as mAb114), for both paediatric and adult populations (171, 172).

However, despite the enormous progress, there remains a lack of evidence on efficacy of drugs that could penetrate immune privileged tissue (i.e. eyes, brain, testes) thus mitigating late patient sequelae and the virological reservoir in EVD survivors, along with combination of therapy to target multiple EVD strains (5).

The availability of specific EVD therapeutics has contributed to a shift in the language, design, and functions around Ebola isolation centres as places where patients were supported to places where patients can access treatment - thus we now refer to Ebola Treatment Centres (ETC) rather than Ebola Management Centres (EMC). These centres have a dual function – prevention (interrupting the transmission chain by isolating infectious cases), and treatment (providing specific individual treatment to improve survival).

However, despite the availability of new effective therapeutics, the main challenge remains to improve health seeking behaviour, so people attend ETCs and to build community trust toward responding organisations. This is demonstrated by the low number of cases treated (n=681) versus the overall number of cases (n=3481) detected during the 2018-2020 North Kivu/Ituri outbreak. Findings from a community survey conducted in North Kivu DRC at the time of the outbreak, shortly before the PALM trials started, found that, among the 931 participants, 32% and 25% of survey respondents respectively had low trust in local authorities and believed that EVD was not real. In the study, both factors were associated with a reduced likelihood of seeking health care and with poor compliance with preventive behaviours such as acceptance of Ebola vaccines (173).

1.7.2 Vaccines

In August 2014, WHO called for fast-track development of Ebola vaccines as part of the public heath road map for the Ebola response in West Africa (85). On November 2014, a special Strategic Advisory Group of Experts (SAGE), working Group on Ebola Vaccines and Vaccination was established (174). WHO set up an Emergency Use Assessment and Listing (EUAL) to accelerate the procedure and availability of Ebola candidates vaccines during public health emergency (175).

Since the 2014-2016 West Africa outbreak, 15 Ebola vaccines candidate underwent clinical development (176); vaccines encompassed three main categories: i) non-replicating vector vaccines, ii) replicative vector vaccines, and iii) other types of vaccines (177).

Between 2015 and 2017, two different vaccines have been domestically approved these included respectively the prime/boost candidate vaccine based on rVSV- and Ad5-vectored components (GamEvac-Combi) licensed by the Ministry of Health of the Russian Federation based; and the monovalent vaccine based on recombinant adenovirus type-5 vector (Ad5-EBOV) licenced by China Food and Drug Administration (CFDA) (177), Table 4.

In 2019, the vesicular stomatitis virus–Zaire Ebola virus (rVSV-ΔG-ZEBOV-GP) was approved by the European Medicines Agency (EMA) and was licensed for marketing as Ervebo® in the European Union (178). This vaccine has also been granted approval for clinical use by United States and has been pre-qualified by WHO (179). This vaccine is a live attenuated replication competent vaccine based on the vesicular stomatitis virus which was genetically modified to express a surface glycoprotein (GP) of EBOV Kikwit 1995 strain (180) (Table 4); originally developed by Public Health Agency of Canada (PHAC) it is currently licenced by Merck. The clinical approval for rVSV-ΔG-ZEBOV-GP, was supported by data on safety, immunogenicity and efficacy gathered from clinical trials conducted in Africa, Europe and North America (181-190).

Clinical trials started in 2014, when a multicentre Phase 1 trial was designed to assess the safety, side-effect profiles, and immunogenicity of single dose rVSV-ΔG-ZEBOV-GP (182). The study enrolled 158 healthy participants and was implemented in 4 sites across Europe and Africa. In 3 sites the trial was design as an open-label, uncontrolled study assessing ascending vaccine doses (ranging from 300,000 to 20 million PFU). In the fourth site the trial was a double-blind, randomized, placebo-controlled, assessing doses of 10 million and 50 million PFU. The study was initially stopped since 22% of participants had reactive arthritis, the study resumed using a lower dose of vaccine and showed immunogenicity and no serious Adverse Events (SAEs) related to the vaccine were reported.

Between February to March 2015, the Partnership for Research on Ebola Vaccines in Liberia I (PREVAIL I) implemented a randomised, double-blind, placebo-controlled trial evaluating safety and immunogenicity of the rVSV-ΔG-ZEBOV-GP vaccine and the chimpanzee adenovirus type 3-vectored Ebola virus vaccine (ChAd3-EBO-Z) enrolling overall 1500 adults (186); in this cohort antibody responses was present one months after vaccination (in 70.8% of people who received the ChAd3-EBO-Z vaccine and in 83.7% of those who received the rVSV-ΔG-ZEBOV-GP), response persisted at 12 months (in 63.5% of participants receiving ChAd3-EBO-Z and in 79.5% of those who received the rVSV-ΔG-ZEBOV-GP).

Between March 2015 and July 2016, in Guinea, a phase 2 clinical trial targeting frontline health workers was implemented (189). The study was an open-label, non-randomized trial aiming to assess safety of a single dose of rVSV-ΔG-ZEBOV-GP which enrolled 2016 participants and 99 controls. Minor adverse events (headache, fatigue, arthralgia) were reported by 70% of participants 3 days after vaccination, and 2 of the 8 SAEs were identified during follow-up and occurred in pregnant women.
Between April and August 2015, in Sierra Leone the STRIVE (Sierra Leone Trial to Introduce a Vaccine Against Ebola) clinical trials started; it was a randomized controlled phase II/III trial to assess efficacy, immunogenicity, and safety of vaccine of rVSV-ΔG-ZEBOV-GP among 8,673 frontline and workers health care workers; participants were randomized to receive either rVSV-ΔG-ZEBOV-GP at enrolment or 18–24 weeks after enrolment (190); the study could not prove vaccine efficacy since it was implemented when the number of case in Sierra Leone was low but confirmed, data on safety and immunogenicity.

A follow up analysis of STRIVE data reported pregnancy outcome among 84 women health workers accidentally vaccinated during the STRIVE trial; women were either in early pregnancy or became pregnant less 60 days after vaccination (191). The study reported no congenital anomalies among the 44 out of 51 live births who were examined; the frequency of pregnancy loss was 45% (14/31) among immediate vaccinated women compared to 33% (11/33) in the group assigned to deferred vaccination (unadjusted RR 1.35 (95% CI 0.73–2.52); p = 0.34). In this study the difference in pregnancy loss between the 2 groups was not statistically significant, solid conclusion could not be drawn due to the small size was and the retrospective study design (191).

Between March 2015 to January 2016, in Guinea a Phase 3 open-label, cluster-randomised ring trial was undertaken in 2015-2016 in Guinea and Sierra Leone to assess the efficacy of a single dose of rVSV-ΔG-ZEBOV-GP (184). The study used a ring design in which a list of contacts and contacts of contacts was created for each EVD positive case. The list constituted a “ring” or cluster. Each cluster was then randomly selected to either received immediate vaccination or delayed vaccination (21 days

later). The primary outcome was laboratory-confirmed EVD with onset 10 days or more from randomisation. The trial included 4539 contacts and contacts of contacts, in 51 clusters randomly assigned to immediate vaccination, and 4557 contacts and contacts of contacts in 47 clusters randomly assigned to delayed vaccination. The trial showed an efficacy of 100% (95% CI 68.9–100.0) p=0.005), with mild adverse events. The study also showed the feasibility of implementing a ring-vaccination approach during an EVD outbreak.

Use of ring vaccination was previously successfully implemented to eradicate smallpox in the 1970s (192). The aim of this approach is to create a ring of people immune around each confirmed case, thereby interrupting secondary transmission. Contacts are people who lived in the same household or had direct contact (unprotected care or prepared the body for a funeral ceremony) with a confirmed case. Contacts of contacts are neighbours or people living in the same geographical area.

On June 2017, the SAGE recommend that in the occurrence of additional EVD outbreaks to use the ring approach with rVSV-ΔG-ZEBOV-GP vaccine under the Expanded Access framework (also called compassionate use, while safeguarding ethical and good clinical practice precautions), which allows to use of investigational vaccine outside of clinical trials (193, 194). SAGE also recommended consideration of other vaccines if a new outbreak was caused by species other than Zaire, and to include not just the social network of EVD cases (the "ring") but to expand immunization to HCWs and front-line workers in area of EVD transmission or area at risk of further expansion (193, 194).

In April 2019, WHO reviewed data generated by Ebola vaccine manufacturers on two candidate vaccines: the adenovirus 26 vectored glycoprotein/MVA-BN (Ad26.ZEBOV/MVA-BN) vaccine developed by Johnson & Johnson (J&J), and the CanSino-Beijing Institute of Biotechnology (Ad5-EBOV) vaccine (195). The Ad26.ZEBOV/MVA-BN is monovalent non-replicative adenovirus type vectored vaccine encoding Ebola virus glycoprotein (Ad26.ZEBOV), followed by a booster by a multivalent modified vaccinia Ankara-vectored vaccine encoding glycoproteins from Ebola, Sudan, and Marburg viruses as well as the nucleoprotein of Tai Forest virus (MVA-BN-Filo) (196). It is administrated as a two-dose vaccine, designed to induce long-lasting protection against to the EBOV, SUDV, TAFV and MARV (196, 197). Data on safety and immunogenicity came from a Phase 1 study of healthy volunteers (n=87) in the UK that showed minor adverse events and seroconversion at 1 year, with persistence of vaccine-induced T-cell response (198). These data were consistent with another Phase 1 clinical trials conducted in Uganda and Tanzania which showed that Ad26.EBOV prime/MVA-BN-Filo boost elicited humoral responses at 12 months following immunization with minor adverse events (199). Another Phase 2 clinical trial assessed three different two-dose of Ad26.ZEBOV/MVA-BN among 423 participants, confirming safety and immunogenicity (200). All evidence gathered from

previous clinical trials indicated that the vaccine could be used as a pre-emptive tool in population at risk of transmission.

Lessons learned from the implementation of ring vaccination in West Africa were capitalized in the 2018 Équateur province EVD outbreaks in DRC and, further consolidated during the North Kivu/Ituri epidemic (2018-2020) (201). As per SAGE recommendations, during the 2018-2020 North Kivu/Ituri EVD outbreak ring vaccination activities using with rVSV-ΔG-ZEBOV-GP started under the Expanded Access framework principle covering 303 905 people (53). Apart from the ring approach a second approach was also used in North-Kivu/Ituri called the 'targeted geographic vaccination' were vaccination was offered to everyone in the neighbourhood or village where a confirmed EVD case was reported (202, 203) (Figure 4). Interim analysis on 679 rings (including 91,492 contact and contacts of contacts, and 28,888 HCWs) vaccinated using rVSV-ΔG-ZEBOV-GP during the 2018-2020 EVD outbreak in North Kivu/Ituri Ebola outbreak; estimated a vaccine efficacy of 97.5% (95%CI 95.8 – 98.5%) for people vaccinated with an onset of illness more than 10 days after vaccination and 88.1% (95%CI 79.9-92.9%) for all those with EVD, regardless of the timing of illness onset (204).

Over the course of the 2018-2020 North Kivu/Ituri outbreak, SAGE made specific outbreak recommendations on EVD immunization strategies and target population; SAGE consideration were based on epidemiological risks, review of evidence on new promising vaccines, and deterioration of security (205, 206). Over time SAGE recommendations were implemented by the MOHS in the 2018-2020 North Kivu/Ituri these included (202, 205, 206):

1. Implementation and evaluation of Ad26.ZEBOV/MVA-BN vaccine in area where there was no active transmission;
2. Continuation to offer rVSV-ΔG-ZEBOV-GP to –''all people at high risk of Ebola infection including those who have been in contact with a person confirmed to have Ebola, all contacts of contacts, and others determined to be at high risk of contracting Ebola'';
3. Granted access to rVSV-ΔG-ZEBOV-GP vaccine to pregnant women beyond their first trimester of pregnancy and to lactating women, if identified as case contacts;
4. When security or stigmatization make difficult to reach communities, complement immunization strategy with a temporary pop up strategy (i.e., vaccination sites set up at health posts, rather than near the homes of patients infected with Ebola);
5. Adjusting rVSV-ΔG-ZEBOV-GP vaccine dosage according to risk of exposure (i.e., highest risk (contacts and contacts of contacts) receiving a dose of 0.5ml of vaccine instead of 1ml (dosage equal to that used in ring vaccination trial in Guinea in West Africa); lower-risk / people potentially involved in tertiary transmission receiving a dose of 0.2 ml.

More recently, a risk and benefit analysis of rVSV-ΔG-ZEBOV-GP has been published in a systematic review, which summarised data from clinical trials conducted since 2014 (151). This review included 17,600 adults and 234 children who over time were vaccinated in different trials; and confirmed the safety profile with fewer than 2% of participants experiencing SAEs. The analysis concluded that the vaccine was able to elicit an immune response, with neutralizing antibodies persisting up to 365 days postvaccination, and was deemed to be effective to prevent EVD when vaccinated within 10 days.

On January 2021, WHO and other partners announced the setup of a global Ebola vaccine stockpile of rVSV-ΔG-ZEBOV-GP to facilitate rapid access to vaccines during outbreaks (207). Currently the Ad26.ZEBOV/MVA-BN and the ChAd3-EBO-Z vaccines are progressing to be approved for clinical use (4, 186).

Despite these remarkable achievements, questions remain about the optimal strategy to achieve rapid immunity versus long term immunity (i.e., the use of single-shot rVSV-ΔG-ZEBOV-GP vs use of prime–boost approach that could provide more durable protection). It also remains important to consider that current evidence of safety and efficacy of experimental vaccines are a tangible result of the trust that local populations and health staff have put into the research community and their contribution to generate evidence and practices to control EVD (208, 209).

Annex 1.2 summarise key SAGE positions and recommendations on EVD immunization, 2014-2020 (210, 211). The summary of statement and recommendations are direct quotes from SAGE guidance and the list is not exhaustive but focuses on the main considerations and recommended changes over time.

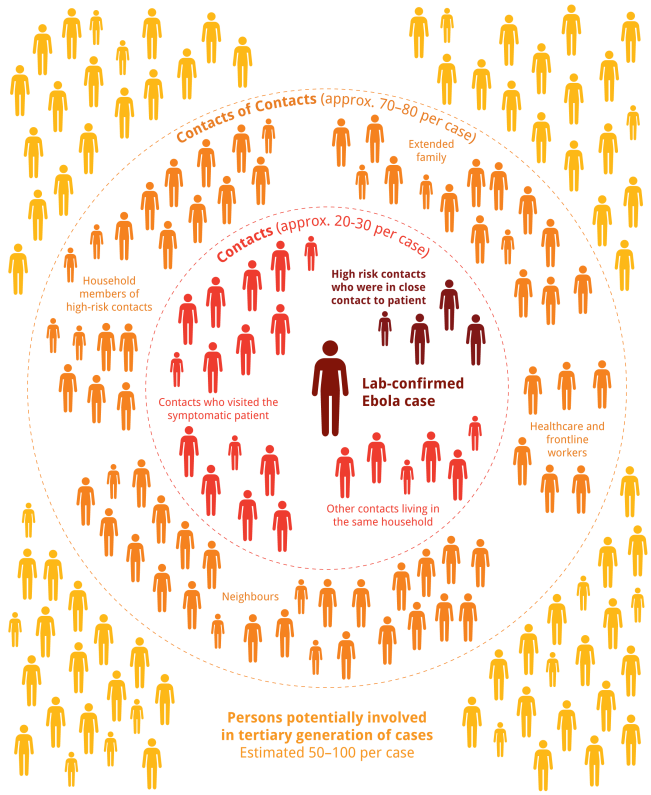
Table 4. Overview of the main vaccine categories, adapted from Baptiste Martin *et al*.(212)

Categories	Vaccine	Phase evaluated*
Non-replicative vector-based Ebola vaccines General advantage: Better tolerability profile General constrains High dosage of viral particles is needed to elicit a significant response Might require the injection of several doses	Ad26-ZEBOV/MVABN-Filo Janssen Vaccines (Johnson & Johnson), USA	1-3
	ChAd3-EBOV-Z Sabin Vaccines Institute & GSK, USA/Russia EBOV	1-2
	Ad5-EBOV CanSino Biologics Inc. & Beijing Institute of Biotechnology, Chine (RPC) Approved by CFDA	1-2
Replicative vector-based Ebola vaccines General advantage: Highly efficient with relatively low dosage	rVSVΔG-ZEBOV-GP Merck, Germany Approved by EMA, FDA and Pre-qualified by WHO	1-3
	Evac-Combi Gamaleya Research Institute of Epidemiology and Microbiology, Russia Approved by MOH of Russian Federation registered vaccine on 28/12/2016 (no. LP-003390).	1-4

General constrain Some safety concerns associated with the replicative vector-based vaccines		
Others	Delta VP30 University of Tokyo & Waisman Biomanufacturing, Japon et USA	1
	INO-4201, Inovio Pharmaceuticals, USA	1
	EpivacEbola Rospotrebnadzor, Russie	1
	Nanoparticle recombinant Ebola GP vaccine Novavax, USA	1

***Phase-1**: small trials in healthy individuals with the aim to evaluate the safety and immunogenicity
Phase-2: recruits hundreds to thousands of subjects to identify safety, immunogenicity, and efficacy.
Phase-3: recruits thousands of subjects, is essential for registration and approval to market of a vaccine, assess the effect of the final formulation and dose. (Ref. The clinical development process for a novel preventive vaccine: An overview)
Phase-4: monitor stages on adverse effects, and long-term immunity after the vaccine is licensed and marketed.

Figure 4. Ring immunization strategy used in North Kivu/Ituri, 2018-2020 EVD outbreak, from WHO,- Ending an Ebola outbreak in a conflict zone (203)



'first ring'= direct contact;

'second ring' = contact of contacts (those in direct contact with the first ring (i.e., neighbours and HCWs);

'third ring'= contacts of the second ring (3 degrees of separation from the suspected case).

1.8 EVD outbreak in Sierra Leone, 2014-2016

Sierra Leone is located in West Africa, the country is bordered by Guinea in the north and east, and by Liberia in south and east. During the 2014-2016 EVD epidemic, Sierra Leone reported 14,124 cases and 3,956 deaths, in an estimated population 7.1 million in 2015 (40% residing in urban context) (64, 213, 214). Similar to Guinea and Liberia, this was the first time that Sierra Leone was affected by an EVD outbreak.

The outbreak unfolded in a context of an extremely fragile health system, with the highest maternal and under-5 mortality indicators, with 45% of the population in a state of food insecurity, recovering from the effects of a protracted civil war (1998–2002) (213, 215, 216).

The first cases in Sierra leone were reported in May 2014, among people returning from the funeral of a well-known traditional healer, who was reported to have treated infected patients in Guinea. Following this funeral, human-to-human transmission started in the District (217). The outbreak spread to the neighbouring Kenema District in June 2014. In Kenema Government Hospital (KGH), one of the largest nosocomial outbreaks started, 66 healthcare workers were infected (69% case fatality) representing the largest cluster of health workers in the West Africa outbreak (218). By mid-October 2014, all the 14 Districts reported at least one case, by the end of December more than 9,000 cases were recorded (217). By the end of January, EVD incidence declined and the country moved to a phase of response where the focus was on the transition from low transmission to the end of the outbreak, by increasing capacity for case finding and contact tracing (219).

In November 2015, WHO declared the end of the epidemic in Sierra Leone (64). However, in January 2016 Sierra Leone had two new cases, but transmission was rapidly controlled. In March 2016, WHO declared Sierra Leone to be Ebola-free (65); since then no new cases have been reported.

Table 5 summarises the key salient events in the EVD outbreak in Sierra Leone.

To control the outbreak, the following public health measures were implemented in addition to the previous mentioned control pillars (se section 1.4 Public health interventions to control EVD outbreaks):

1. Ebola Holding Units (EHUs; set up on May 2014): screening centres in existing health facility where suspect patients, received initial general care and waited for testing before been reefed to EMC if confirmed; aim to timely isolate case, prevent nosocomial transmission in non-Ebola facilities and refer to EMC only positive patients (220);

2. Quarantine (set up in August 2014): Individuals and households exposed to EVD undergo observation and restriction of movements for the duration of the incubation period since their last

possible exposure. Households were supposed to receive water and food supply for the duration of quarantine (221);

3. Bylaw (set up in August 2014): Law making criminal offence to shelter patients suspect of EVD, imposing a prison sentence of up to two years (222);

4. 1-1-7 hotline call system (set up in August 2014): To enhance Ebola surveillance the Government of Sierra Leone repurposed an existing national, toll-free telephone line (1-1-7 system) requiring communities to report all deaths and suspected Ebola cases (223);

5. Operation Western Area Surge (September and December 2014): A door-to-door Ebola awareness campaign searching for suspect cases mainly implemented in Freetown (217);

6. District Ebola Response Centres (DERCs), set up in October 2014, to provide at District level coordination and response (224);

7. National Ebola Response Centre (NERC), set up in October 2014 set up in Freetown to provide national-level coordination and response (see Annex 3) (224);

8. National IPC training program (set up between October and December 2014); the aim was to prevent nosocomial transmission, it enrolled 1,200 peripheral health units (PHUs) across the Country (225);

9. Community Care Centre (CCCs); set up in November 2014: Small facilities inside the community were used to isolate suspected and probable cases of EVD while they waited for lab results, prior to transfer to an EMC. The aim was to reduce the delay to isolation, thus preventing onward transmission, to increase acceptance of isolation from patients and the community, and to increase community engagement towards the response. This was mainly implemented in districts without EMC facilities and in rural area (226, 227);

10. Community-Led Ebola Action (CLEA); set up in November 2014: to support communities to conduct their own risk analysis, understand transmission routes and identify key actions to prevent EVD transmission; one of the main outcomes of this initiative was to connected communities with the DERCs (111);

11. Mass Drug Administration (MDA) (December 2014–January 2015): A two-round, door-to-door, campaign, with artesunate–amodiaquine (ASAQ) covering over 2.7 million people. The campaign was conducted in 8 Districts with high burden of malaria and affected also by EVD. The aim was to reduce malaria morbidity at community level to decrease health services burden and isolation of febrile non-EVD cases to EMC with consequent risks of nosocomial transmission (228);

12. National IPC guidelines (May 2015): to set standards during routine healthcare for safe and high-quality patient care (229);

13. Ring vaccination (second flare up in January 2016): The rVSVΔG-ZEBOV-GP vaccine was used to vaccinate EVD contacts and contacts of contacts using a ring approach (overall 325 contacts and 255 contacts of contacts were vaccinated (184) (see section 1.7.2 Vaccines).

Annexe 1.3 provides a visualization of the response frame in Freetown or in similar DERCs, and Annex 1.4 provides the bed capacity and number of cases in November 2014 in Sierra Leone.

1.8.1 EVD outbreak impact and lessons learned: a few practical examples

The 2014-2016 EVD outbreak was devastating for Sierra Leone. The World Bank estimated an impact of $1.9 billion on the local economy (230). The Ministry of Health and Sanitation (MoHS) reported that at least 295 health care workers had EVD, among those 221 died, with a consequent reduction of skilled health workers (from 17.2/10,000 people before the outbreak to 3.4/10,000 at the end) and a 23% reduction on health delivery services (52). Overall, 8,345 children were left orphans, and 3,034 people were EVD survivors with consequent social and health needs (52).

Sierra Leone gained important experience and lessons learned from this catastrophic outbreak. For instance, prior to EVD outbreak Sierra Leone had no IPC programme or staff trained on IPC (229).

MoHS also revitalised the Integrated Disease Surveillance and Response (IDSR) as an essential public health tool to timely recognise and respond to epidemic-prone diseases (231).

Practical examples of application of lessons learned and improved public health practices were: i) timely response to the 2017 floods and landslides in Freetown, with the first implementation of a pre-emptively Oral Cholera Vaccination (OCV) campaign (232); ii) set up of ethical frame to Ebola data to support research, and to supporting families to locate the graves of family members died during the EVD outbreak (96); iii) recognition of crucial role of communities in outbreak preparedness and response and recovery (233); iv) standardization of data and decentralization of response during the current novel coronavirus disease 2019 (COVID-19) (Personal communication with Médecins Sans Frontières Operational Centre Amsterdam (MSF-OCA)team in Sierra Leone).

At the end of the EVD outbreak, MSF-OCA committed to Sierra Leone and set up medical intervention to improve maternal and child indicators in a Tonkolili District (234). I personally observed and contributed those important public health shift in Sierra Leone; during the support I provided to the MOHS to strength IDSR in Tonkolili District, implementing and evaluating the Cholera the vaccination

in Freetown[7,8], supporting the reconciliation project to support family to locate family grave, and the recent support to design and analysis of a community qualitative assessment conducted in May 2020 at the start at the start of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in the Country (see Chapter 6).

However, despite several improvements and lesson learned there are still extensive gaps to provide adequate services, with shortages of personnel, challenges in supply of drugs, and laboratory services. This has led recently to an increasing commitment of MSF-OCA in Sierra Leone also on TB care in another rural district.

7 Integrated Disease Surveillance and Response (IDSR), Tonkolili District, Sierra Leone, MSF-OCA internal report 2016.
8 Field visit report support to Oral Cholera Vaccination (OCV), Freetown, 2017 G.Caleo, MSF-OCA internal report 2017

Table 5. Key events in the Ebola outbreak in Sierra Leone, 2014-2016 (46, 217-219, 235, 236)		
Date		Description
2014	May	• Outbreak began in Kailahun, on the border with Guinea.
	June	• A state of emergency was declared and Kailahun and Kenema Districts soon became the epicentres of the outbreak; • In late June, MSF opened an EMC, which continued to operate until January 2015, when the District was declared free; • In Kenema two wards of the government hospital, previously dedicated to Lassa fever, were converted for isolation of EVD cases. It became an important place of disease amplification, 66 healthcare workers were infected representing the largest cluster of health workers in the West Africa outbreak; • The first cases of EVD were reported in Freetown, the Sierra Leone capital, and Port Loko, eventually spreading to all the 14 Districts.
	July	• Ebola Operations Centre (EOC) set up to support coordination of response; • School closed and reopened in April 2015.
	August	• The President declared a national state of emergency and quarantine was imposed to affected households; • The government also designed a law to inflict a severe penalty, up to two years of prison, on any people hiding suspect cases; • Between August and December the country entered the first phase of response during which efforts focused on increasing the number of beds, hiring and training teams in safe and dignified burial, and strengthening surveillance systems and social mobilization capacities.
	September	• A campaign known as the surge was implemented (repeated in December 2014) in which the country was locked down for 3 days, and active door to door case-finding was mainly implemented in the capital and Western Area District; • During this period hospitals lacked PPE, training and an adequate triage system.
	October	• The National Ebola Response Centre (NERC) was set up in Freetown to provide national-level coordination and response; (Annex1.3 example in Freetown). • At District-level, DERC's were set up. In each affected district a central command was established.
	November	• The country reported 4,828 cumulative confirmed cases, with the highest burden in Freetown where 150 cases per week were reported; • Only 7 EMCs were operating in the country, bed capacity was insufficient, resulting in patients being transported to distant EMCs and CCCs (Annex 4Bed capacity and number of cases); • CLEA model developed.
	December	• Gradually, bed capacity increased with an estimate 3.6 beds for every confirmed and probable case in December 2014. Distribution of EMC and CCCs were uneven leaving districts with active transmission still uncovered; • Doctors in Sierra Leone went on strike
2015	January	• An extensive antimalarial campaign, covering 2.5 million of people in 8 districts was delivered; • By the end of January, EVD incidence declined.
	March	• National Lockdown (3 days) .
	April-August	• STRIVE stated (Vaccine trials for frontline health workers)
	June	• Curfew imposed in Porto Loko and Kambia.
	November	• On 7 November WHO declared that the Sierra Leone EVD epidemic was over; • Sierra Leone then moved to a phase aimed at enhancing surveillance, managing residual risks and rapid identification and response to flare ups, including the use of vaccine; • Systematic oral swabbing of all deaths was implemented.
2016	January	• A new case of EVD was detected in Tonkolili District; the patient died in the community and generated a secondary case.
	March	• On 17 March WHO declared Sierra Leone to be Ebola-free for the second time

Annex 1.1 Underlying EVD pathophysiology, groups at risk of negative outcomes, and malaria-coinfection

Underlying EVD pathophysiology

EVD symptoms reflect the likely underlying pathophysiology (4). The virus seems at first to "sabotage" the immune system (innate and acquired) which enables uncontrolled virus replication, followed by the vascular system thereby inducing blood leaking and hypotension (4, 237). The cascade and dissemination start with infection of dendritic cells and macrophages, inactivating Interferon and releasing cytokines that amplify an inflammatory response with initial damage of blood vessels and initiate coagulation. Immune responses impair adaptive immune response by causing T-cell exhaustion and apoptosis (238). Susceptibility to secondary infections might be induced by lymphocyte depletion (139).

The virus than infects reticuloendothelial and microvascular endothelial cells, further compromising vascular integrity and causing blood leakage. Infection of hepatocytes induces cell damage, disseminated intravascular coagulopathy (DIC), with risk of thrombosis and bleeding (139). Further dysregulation of blood pressure is due to damage of adrenal glands that are unable to produce steroids (crucial to regulate the immune response, and blood pressure) (139). Damage to renal tubular cells contribute to renal dysfunction. Damage to the gastro-intestinal tract with consequent diarrhoea additionally contributes to risk of hypotension, shock and death (139, 237). Weakness and muscle aches are caused by myositis and elevation of markers of muscle cell damage (4).

Virus kinetics study of among EVD patients found that viral load tends to peak early in patients who survive compared with patients who do not (239). This could be explained by a previous study that observed that early and rapid increase in antibody level supported activation of cell mediated response, and virus clearance among survivors (240). Immune response seems to play a crucial role in possible relapses, due to resumption of viral replication in survivors (241). Severity of the disease was found to be associated associate with high viral load, cytokines, and chemokines and likely the outcome of direct cytopathic effects of viral replication and paradoxical host responses to infection (242, 243).

Groups at risk of negative outcomes

Previous studies have reported an association between age and EVD CFR; high fatality rates among children less than five and adults older than 40 have been consistently reported (244-249) as has an association between viral load at admission and CFR (247, 250-253). During the West Africa outbreak, a study including 6,191 EVD confirmed cases across West Africa estimated that CFR was higher in people aged over 45 years compared to patients aged 16- 44 years old (254) (manuscript supplementary materials page SA19). In this study, CFR, among people aged over 45 years, varied from 71.4% (95%

CI 67.1-75.4) in Guinea, 76.5 % (95% CI 71.6-80.8) in Liberia, to 82.3% (95% CI 78.5-85.5) in Sierra Leone (254). In contrast among patients aged 16- 44 years old CFR varied from 56.3% (95% CI 53.5-59.0) in Guinea, 66.5% (95% CI 63.7-69.1) in Liberia, to 70.3% (95% CI 67.9- 72.7) in Sierra Leone (254).

Over the last EVD outbreaks the reported proportion of children infected, has varied from 9% in the Kikwit DRC outbreak in 1995, nearly 20% during the West African in 2014-2016, 29% during the 2018-2020 North Kivu/Ituri, in DRC, and 41% in Gulu, Uganda (Sudan EVD) in 2000–2001 (53, 64, 255, 256). Although children have accounted for a lower proportion of those infected, the risk of deaths in this group is significant.

Children can be exposed to body fluids during baptism, close contact with mother, care giver or neighbours or acquire infection while attending health facilities (46, 257). In a cohort study conducted among 282 EVD positive children (less than 13 years old) admitted in 11 Ebola holding centres in Sierra Leone during 2014-2015 EVD outbreak, the overall CFR in this age group was 57% (95% CI 51%–63%) but reaching a CFR of 70% in infants (258). In this cohort age was the strongest predictor of death (adjusted OR 0.92 [95% CI 0.86–0.98] per 1-year increment in age) (258). Another large retrospective study included 1,147 children under 16 years of age infected across the 3 affected countries in the West Africa outbreak (254). The study reported, for EVD confirmed cases, a CFR of 85.5% (95% CI 74.7-92.2) for children under one years old versus 50.3% (95% CI 45.7-55.0) for children aged between 10-15 years old (254). In this cohort the mean incubation period was shortest in children younger than 1 year of age (6.9 days (95% confidence interval [CI], 5.1 to 9.5) compared to children 10 to 15 years of age (9.8 days (95% CI, 8.7 to 11.1) (254). Likely indicating a more rapid progress on infection among younger children, this is further exacerbated by the difficulties of recognising EVD symptoms in children thus hampering, early detection, and timely case management (253, 259).

Historically EVD in pregnancy has been associated with high rates of maternal mortality, stillbirths and abortions (30, 260, 261). Before the 2014-2016 West Africa outbreak, the largest cohort of 82 pregnant women with suspected EVD came from the 1976 epidemic in DRC; in this cohort CFR was 89% (47); in this cohort the high CFR was attributed to iatrogenic and in-hospital EVD-transmission (47). In 1995, during the Kikwit DRC outbreak, only one out of 15 suspected EVD pregnant women survived, in all of them haemorrhagic signs were observed (261). However, a more recent retrospective cohort study including 175 EVD confirmed women of reproductive age (15– 49 years) in 5 Ebola treatment units (ETU) in Liberia and Sierra leone, reported no significant EVD specific mortality (46% vs 54%, P = .60) between EVD pregnant (n=13) and nonpregnant women (n=162) in the same age group (262). Although survivor pregnancy testing bias cannot be ruled out (pregnancy test only became standard from December 2014) difference in survival estimates between historical and more recent data might be due to difference on how EVD was ascertained over time (e.g., in the historical data EVD diagnosis

was mainly based on clinical data vs PCR-RT in more recent study) and exposure to infection (e.g., iatrogenic exposure in historical data versus non iatrogenic). Further studies are needed to investigate outcomes in pregnant women compared to non-pregnant in the same reproductive age group. Recently specific guidelines were developed for women acquiring EVD during pregnancy and or breastfeeding, indicating that pregnant women should have access to the same supportive and investigational treatments used for non-pregnant women (35).

While for EVD pregnant women, recent evidence are more positive, foetal outcomes of children born to mothers infected with EVD remain poor (262); before the West Africa outbreak, fifteen neonates have been reported be born alive, but all died within few weeks after birth (263). During the West Africa outbreak only one neonate born from an EVD women (who did not received investigational therapy), is known to have survived to EVD, the neonate received investigational treatments (ZMapp, leukocyte transfusion, and remdesivir); and today is still alive and healthy (169, 264). A case series from the North Kivu/Ituri 2018-2020 EVD outbreak, reported a neonate born EVD negative to a EVD survivor mother (265). During the same outbreak another case report, documented the survival of two neonates enrolled in the PALM trials (34). Mothers and infants received investigational therapy, both neonates were born blood PCR-RT negative, and were discharged alive from the ETC. Unfortunately, despite receiving investigational therapy, both mothers died short after delivery (34).

Malaria-coinfection

Although malaria is a prevalent in countries where EVD outbreaks had previously occurred, before the 2014-2016 EVD West Africa outbreak, there had been limited evaluation of the possible negative effect of malaria co-infection among EVD patients (266). A retrospective cohort study conducted in Liberia, found that risk of death was 36% lower (risk ratio, 0.64; 95% CI 0.49-0.85) among the 272 EVD positive patients who received artesunate–amodiaquine. However, this effect was lost among the 65 patients co-infected with malaria (risk ratio, 1.00; 95% CI 0.54-1.85) (161). Another retrospective cohort study conducted in Guinea between 2014 to 2015, found that among the 97 patients EVD positive, mortality for EVD cases was 58%, rising to 86% if patients had both EVD and malaria co-infection (267). Another retrospective cohort study of patients admitted to 3 Ebola centres in Sierra Leone; found that among the 254 EVD positive patients, 21% had co-infection malaria; mortality for EVD cases alone was 52%%, rising to 66% if patients had both EVD and malaria infection (268), in this cohort mortality risk was increased both in patients positive to EVD and malaria (adjusted hazard ratio 9.36, 95% CI 6.18–14.18, $p<0{\cdot}0001$), and among patients EVD positive but malaria negative (5.97, 4.44–8.02, $p<0{\cdot}0001$), compared to the group negative to both EVD and malaria (268).

A modelling study found that administration of preventive antimalaria treatment to all EVD contacts, is cost saving, and would reduce the probability of EVD contacts to be misclassified and wrongly admitted to an Ebola isolation centre. In this study, in the wet season, preventive antimalaria would

reduce the probability of admission by 29% in Liberia, by 33% in Sierra Leone, by 36% in Guinea for contacts aged <5 years old; and by 10% (in Sierra Leone and Guinea) and 11% in Libera for contacts aged 15 years or older (269).

Current WHO recommendations include empirical treatment of malaria for patients isolated in Ebola treatment centre (54).

Annex 1.2 SAGE key positions and recommendations on EVD immunization 2014-2020

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Date		Key positions/considerations	Keys recommendations
2014	October	SAGE confirmed that it would provide expert advice on the deployment of Ebola vaccines on an emergency basis, as needed in response to requests from WHO. A consultation on potential Ebola vaccines and therapies followed to review plans for safety studies of 2 candidate vaccines – one utilizing a vesicular stomatitis virus (rVSVZEBOV) and another utilizing a chimpanzee adenovirus (ChAD3-ZEBOV), both expressing Ebola Zaire surface glycoproteins. Randomized controlled trials (RCTs) are the best study design, but when not feasible, alternatives may be considered, including cluster-randomized and step-wedge designs. The consultation noted that investigation of any candidate intervention must not detract attention from implementation of effective clinical care, rigorous infection prevention and control, careful contact tracing and follow-up, effective risk communication, and social mobilization	SAGE confirmed that it would provide expert advice on the deployment of Ebola vaccines on an emergency basis, as needed in response to requests from WHO. Subsequently SAGE was asked to immediately establish a SAGE working group on Ebola vaccines and vaccination.
2015	April	SAGE was asked for feedback on framework to develop guidelines to support planning, implementing and monitoring vaccination once a vaccine becomes available. SAGE stressed the importance of transparent and prompt sharing of information on the trial protocols and data from the phase 3 clinical trials, and the need for a greater role for WHO in facilitating the sharing of information so that results between studies will generate the greatest benefit for policy decision-making.	SAGE supported the proposed framework for making recommendations, but asked that it be made explicit that the identification and prioritization of target populations for vaccination will be based on a thorough assessment of risks (from disease as well as from vaccination) and benefits. Further development of the Emergency Use Assessment and Listing procedure being developed by WHO, which would allow use of a vaccine in the context of a Public Health Emergency of International Concern, be done in close consultation with relevant regulatory authorities, including those of the affected countries. Future use of unproven Ebola vaccines should be in the context of studies that would generate safety and effectiveness data.
		Lessons learnt from several EVD vaccines candidate and outbreak response was unprecedented; this experience has informed the development of a blueprint for research preparedness and rapid response for future epidemics.	Vaccination during outbreaks should be part of an integrated strategy and complement other public health measures to interrupt transmission. It does not substitute for full-time personal protective equipment

Annex 1.2 SAGE key positions and recommendations on EVD immunization 2014-2020			
Date		Key positions/considerations	Keys recommendations
	October	Interim results from a Phase 3 trial suggest that rVSV-ΔG-ZEBOV is efficacious, safe, and likely to be effective at the population level when delivered during an EVD outbreak, using a ring vaccination strategy Data on safety in children, pregnant women, and those with underlying medical conditions are insufficient to draw conclusions.	use, contact tracing and other infection control measures. Regular reviews of the epidemiological data should inform adjustments to the delivery strategies throughout the outbreak. Potential strategies include ring vaccination, geographic targeting of an area (mass vaccination) and vaccination of front-line workers. Pregnant women and infants have very high case fatality rates and may benefit from the indirect effects of their close contacts being vaccinated. Researchers should share data from pregnant women who were inadvertently vaccinated, and from HIV-positive subjects if included in the ongoing trials. Future trials should consider collecting data from children, adolescents, pregnant and lactating women, and immunocompromised individual. Efforts to develop vaccines against filoviruses other than ZEBOV, such as Sudan, Bundibugyo and Marburg should be pursued. Community-based participatory approaches to engage participants in all stages of clinical trials, including design, monitoring and evaluation, should be implemented Pre-approved and pre-positioned protocols and local research capacity strengthening in countries at risk for outbreaks should be put in place to facilitate rapid implementation of relevant studies
2016	April	Promising data are emerging from the Ebola vaccine trials. Pending regulatory approval, WHO is developing a country-based "Expanded Access Brigade" to facilitate use until an Ebola vaccine is licensed.	No additional recommendations made.
2017	April	Should an Ebola disease outbreak occur before the candidate vaccine is licensed, SAGE recommended that the rVSVΔG-ZEBOV-GP vaccine be promptly deployed under the Expanded Access framework, with informed consent and in compliance with Good Clinical Practice.	Ring vaccination, as used in the Phase 3 study in Guinea, is the recommended delivery strategy. This should be adapted to the social and geographic conditions of the outbreak areas and include people at risk including but not limited to: (i) contacts and

Annex 1.2 SAGE key positions and recommendations on EVD immunization 2014-2020			
Date		Key positions/considerations	Keys recommendations
		If the outbreak is caused by an Ebola virus species other than Zaire, consideration should be given to the use of other candidate vaccines that target the putative viral species. Available evidence on candidate Ebola vaccines, especially duration of protection, is insufficient to formulate conclusive recommendations regarding mass vaccination of the general population or vaccination of health-care workers in the absence of an outbreak.	contacts of contacts; (ii) local and international health-care and front-line workers in the affected areas and (iii) health-care and front-line workers in areas at risk of expansion of the outbreak. Further research is required to establish the acceptability of vaccines for use in health-care workers, duration of protection conferred by various candidate vaccines, cross protection between virus species and number of doses required, including need for boosting doses.
2018	October	If an outbreak is caused by an Ebola virus strain other than Zaire, consideration should be given to using candidate vaccines that target the respective viral strain. Currently, 1 multivalent vaccine (Ad26.ZEBOV/MVA-BNFilo) is in phase 2 of clinical development. SAGE noted that opportunities should be sought to assess the efficacy of other candidate EVD vaccines, such as in health care and front-line workers in areas that are not at high risk for EVD and are thus not eligible to receive the rVSV-ZEBOV vaccine in current study protocols and SAGE recommendations. Particular consideration should be given to the inclusion of pregnant and lactating women into vaccine research. Data on use of the vaccine in paediatric populations in such trials should be recorded. SAGE reviewed the data on the risks and safety of vaccinating pregnant women with the replicating live virus vaccine rVSVZEBOV. The preliminary results of a risk–benefit analysis to compare the safety of rVSV-ZEBOV vaccination in pregnancy with the risk of acquiring EVD in a setting of ring vaccination were examined. The risk for acquiring EVD of unvaccinated people, including pregnant women, in vaccination rings is very low (0.12%, 95% CI 0.02; 0.28) at a vaccination coverage of eligible people of ≥50%, probably as a result of herd immunity. It was noted that the data were insufficient to establish the risk for EVD of vaccinated rings at lower coverage. Data on the safety of rVSV-ZEBOV vaccination in pregnancy are relatively limited.	Should an EVD outbreak due to the Zaire strain occur before a candidate vaccine is licensed, rVSV-ZEBOV vaccine should be promptly deployed within the expanded access framework, with informed consent and in compliance with good clinical practice. Ring vaccination, as used in the phase-3 study in Guinea, is the recommended strategy for delivery, to be adapted to the social and geographical conditions of the outbreak areas and include people at risk:(i) contacts and contacts of contacts, (ii) local and international health care and front-line workers in affectedareas and (iii) health care and front-line workers in areasat risk due to extension of the outbreak. A geographically targeted vaccination strategy may be considered in when it is impossible to identify the individuals who make up ring vaccination cohorts because of serious security, social or epidemiological issues. In this case, the geographical area immediately around a case of EVD, such as a village or a neighbourhood, is most likely to include those individuals who were contacts or contacts of contacts of the index case SAGE recognized that a decision on whether to offer rVSV-ZEBOV, a systemically replicating vaccine virus, to pregnant women is complex, with ethical, clinical, epidemiological and social considerations.

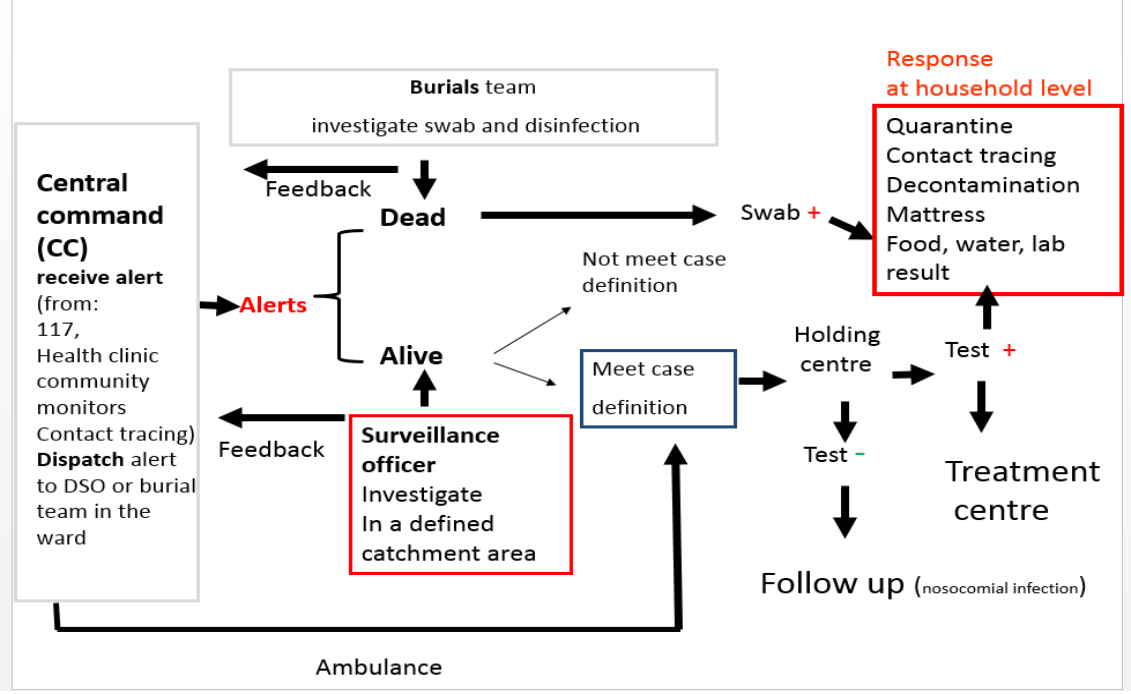
Annex 1.2 SAGE key positions and recommendations on EVD immunization 2014-2020			
Date		Key positions/considerations	Keys recommendations
			Inclusion of pregnant women in an EVD vaccine research protocol depends on local national regulatory authorities and local ethics review committees. SAGE encourages these bodies to assess the benefits and risk of offering rVSV-ZEBOV to pregnant and lactating women during an outbreak. As front-line and health workers are at increased risk of exposure in an outbreak, SAGE recommends that national authorities consider offering the EVD vaccine to those who are pregnant or lactating, with an informed consent procedure.
2019	February	In exceptional circumstances, i.e. settings where because of serious security, social or epidemiological issues, ring vaccination (i.e. identification of contacts and contacts of contacts) cannot be adequately implemented, geographic targeted vaccination is recommended as an alternative strategy.	Interim recommendations Consideration is given to the use of any of new vaccines to vaccinate HCWs and FLWs in the neighbouring areas where there is a possibility of spread. Use of rVSV-ZEBOV-GP vaccine in pregnant women currently remains limited to the EVD outbreak affected areas in DRC and should be continuously evaluated based on the emerging data on the safety and efficacy of the vaccine in this target population.
	April	In modelling studies, geographically targeted mass vaccination and ring plus were less efficient in terms of cases averted, doses of vaccine required and cases prevented per 100 vaccine doses than ring vaccination. Ring vaccination is currently the most effective strategy in this outbreak of Ebola virus disease in the DRC. Targeting broader geographical areas should remain a fallback strategy if contact tracing is not feasible and vaccine supplies are sufficient.	Known attack rates and case fatality rates (CFRs) among women and young infants outweigh these potential risks in favor of the use of the vaccine in these groups. SAGE considered that the high rates of attack and fatality in these groups and the accumulating data on vaccine safety and efficacy for other groups justify inclusion in the ongoing ring vaccination in North Kivu of infants aged 6–12 months and lactating women. As data on the safety of the vaccine in infants aged 6–12 months accumulate, inclusion of infants from6 weeks of age should be considered. SAGE previously recommended that consideration be given to urgent evaluation of new candidate vaccines. This recommendation remains to be implemented.

Annex 1.2 SAGE key positions and recommendations on EVD immunization 2014-2020			
Date		Key positions/considerations	Keys recommendations
			Manufacturers of candidate vaccines against Ebola virus disease prioritize strategies to generate data on safety, immunogenicity and possibly efficacy in pregnant women, lactating women and infants.
	May (Interim)	the Democratic Republic of Congo (DRC) has deteriorated with a large increase in the number of cases . A major factor in this rise is an increase in critical security incidents that have dramatically affected the ability to identify, follow up and vaccinate contacts successfully. This context challenges the implementation of ring vaccination based on the identification of contacts and contacts of contacts, as recommended by SAGE in April 2017 and confirmed by SAGE during its April 2019 meeting. Further, a potential vaccine shortage may manifest in case the outbreak expands further and/or is prolonged.	SAGE deliberated on the following recommendations on implementation of novel strategies and adjusted dose regimes: **Implementation of innovative operational strategies:** **Pop-up vaccination –** In this approach, already successfully implemented to address security issues and tensions with the community vaccination is implemented at an agreed and temporary, protected vaccination site, at a distance from the residence of the contacts, often a health facility) and; **Targeted geographic vaccination** – In this approach, already successfully implemented to address security issues, all the contacts and contacts of contacts of all cases reported in a given village or neighbourhood are enumerated and invited for vaccination simultaneously. **Revised vaccination strategy to adjust the target population for ring vaccination to include a second and third barrier of immunized individuals around each incident case.** (I) Continue to offer as a priority rVSV-ZEBOV-GP vaccine and vaccinate those at higher risk of Ebola including contacts and contacts of contacts and health care workers (HCWs) and front line workers (FLWs) in affected Aires de Santé. (ii) Offer rVSV-ZEBOV-GP and vaccinate to those who can potentially be involved in the tertiary generation of cases (e.g. 3rd level of contacts) to create a barrier around the contacts of contacts in affected Aires the Santé. (iii) Offer a vaccine other than rVSV-ZEBOV-GP to those at some risk of Ebola in Aires de Santé with

Annex 1.2 SAGE key positions and recommendations on EVD immunization 2014-2020			
Date		Key positions/considerations	Keys recommendations
			cases, although at a lower risk than those described in (i) and (ii) above. SAGE recommends that these lower risk populations would be vaccinated with the J&J vaccine with informed consent. The latter ideally implemented as per the SAGE recommendation from April 2019 which outlines that studies using other candidate vaccines should be done in this context: **3)Alternative dosing for the rVSV-ZEBOV-GP vaccine.** To ensure vaccine continues to be available and offered to individuals at greatest risk of Ebola during this outbreak and in order to secure the availability of the rVSV ZEBOV-GP in the mid-term, SAGE revised the following proposal, based on an analysis undertaken by the U.S. Food and Drug Administration to exceptionally adjust the vaccine dose for the currently available lots of rVSV-ZEBOV-GP being used in DRC: For those at higher risk of Ebola including contacts and contacts of contacts including HCWs and FLWs in the affected areas: offer a vaccine dose with a similar potency to that used in the Guinea Ebola ça suffit trial (i.e. 2 x 107 pfu). (ii) For those who can potentially be involved in the tertiary generation of case (e.g. 3rd level of contacts), a 5-fold dose adjustment compared with the current dosing of the vaccine is recommended (in relation to the potency of the vaccine lots being used in DRC). The 5-fold dose reduction in the broader population was motivated on immunological considerations related to dose-response analysis using a 4.8-fold dose reduction in various subpopulations and seroconversion rates in those groups at 28 days after vaccination and later, noting this dosing regimen provides a reasonable risk-benefit trade-off for protection.

Annex 1.2 SAGE key positions and recommendations on EVD immunization 2014-2020			
Date		Key positions/considerations	Keys recommendations
			SAGE supports the adjusted dosing administration as proposed above. SAGE acknowledges, that since the vaccine is available in 10-dose vials at 1 ml/dose, that a 2-fold and a 5-fold reduction in dose could be readily implemented by injection of 0.5 mL and 0.2 mL, respectively.
	October	Strategies and a mechanism will be built for creation of a stockpile and allocation for outbreak response and for preventive use of EVD vaccine outside of outbreaks, which will be reviewed by SAGE for endorsement	SAGE will develop a priori defined criteria for implementation of the dose reduction if future vaccine supply is significantly constrained and the outbreak is not contained.
2020	March/ April	At a future SAGE meeting, it is expected that possible recommendations for off-label use for specific populations such as young infants, children and pregnant and lactating women will be discussed to allow best use of the recently licensed rVSV-ZEBOV vaccine in future outbreaks.	SAGE recommended that a comprehensive review be conducted of the recent experience of Ebola virus vaccine implementation and policy development during an outbreak response in order to inform future processes for the development of recommendations, the use, and the monitoring of un-licensed vaccines in emergency and outbreak response situations.
N.B. All statement and recommendations are direct citations from SAGE reports			

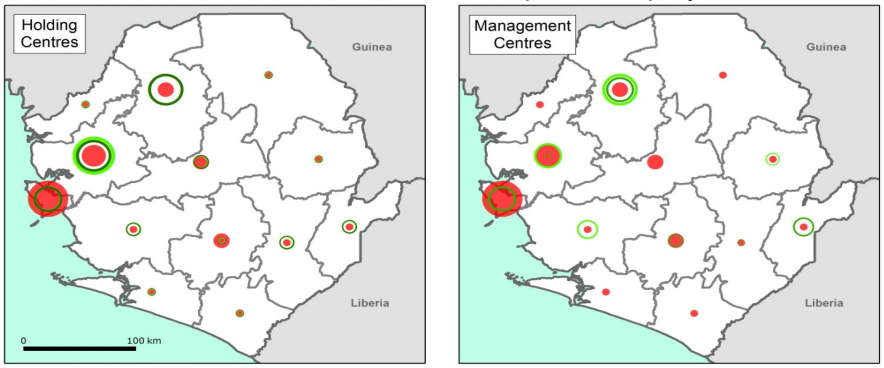
Annex 1.3 Visualization of the National Ebola Response Centre (NERC)



- District Surveillance Officer (DSO) were responsible for working with local chiefs, community monitors and local health facilities for the timely detection of new cases (both deaths and living). The local population could report alerts by calling the 177 or reporting to DSO, local chief. The DSOs were in charge of verifying the alerts and calling the central command to request an ambulance or a burial team depending on the outcome of investigation.
- The central command dispatched ambulances and allocated patients according to bed availability.

Annex 1.4 Confirmed cases by District, available and existing bed capacity in November 2014

Sierra Leone: new Ebola cases 10-16 Nov 2014, with current and planned bed capacity



Ebola Holding Centres (EHC)

District	New cases	Cumul. cases	Current EHC beds	Planned EHC beds
Bo	31	220	20	20
Bombali	40	697	210	210
Bonthe	1	3	19	7
Kailahun	0	558	49	49
Kambia	4	56	7	7
Kenema	0	493	29	29
Koinadugu	25	73	10	10
Kono	12	57	20	20
Moy amba	7	144	26	26
Port Loko	86	711	209	352
Pujehun	0	28	24	24
Tonkolili	27	300	40	40
Western Areas	211	1627	138	138

Map symbol colours

- Current bed capacity (where facilities are open)
- Planned bed capacity (facilities open, planned or under construction)
- Number of confirmed new ebola cases 10-16 November

Map symbol sizes

1 25 50 100 200 300+

Data sources

Ebola cases: Ministry of Health health.gov.sl
Bed availability: National Ebola Response Centre 18 Nov 2014 data.hdx.rwlabs.org

Ebola Management Centres (EHC)

District	New cases	Cumul. cases	Current EMC beds	Planned EMC beds
Bo	31	220	50	50
Bombali	40	697	110	210
Bonthe	1	3	0	0
Kailahun	0	558	72	72
Kambia	4	56	0	0
Kenema	0	493	25	25
Koinadugu	25	73	0	0
Kono	12	57	0	40
Moy amba	7	144	0	100
Port Loko	86	711	22	190
Pujehun	0	28	0	0
Tonkolili	27	300	0	0
Western Areas	211	1627	200	810

MEDECINS SANS FRONTIERES ARZTE OHNE GRENZEN

Date: 19/11/2014 **Contact:** MSF-CH Field GIS Officer msfch-sierraleone-egis@geneva.msf.org **Filename:** SLE_Epi_SL_CumAnd21DayAndBeds_District
This map is for information purposes only and has no political significance. The boundaries and names shown and the designations used on this map do not imply official endorsement or acceptance by MSF.

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Chapter 2: MSF-OCA in Sierra Leone 2014-2016 EVD outbreak

2.1 MSF-OCA response activities, challenges, and definition of research objectives

This chapter provides the context for this thesis, by summarizing the main work undertaken by MSF-OCA during the EVD outbreak (both within the EMC and in the community) and the subsequent definition of the PhD research objectives.

Since the Kikwit DRC EVD outbreak in 1995, MSF has gained important experience supporting the response to several Filovirus outbreaks (e.g. EBOV, BDBV SUDV, Marburg) in multiple countries (e.g. DRC, Gabon, Uganda, South Sudan, Angola, ROC) (1-5). Early MSF interventions mainly focused on water, sanitation and hygiene, creation of isolation wards, health workers protection. Subsequently, there was increasing emphasis on patient care and surveillance. Documentation of adaptation of protocols on burials, home disinfection and psychosocial support to affected families during Marburg outbreaks have been previously reported by MSF as key interventions to respectfully and successfully engage with community (5).

However, despite MSF updating its guidelines regularly, lessons learned from the response to Filovirus outbreaks were mainly gained by two sections of MSF with limited systematic transfer of knowledge and practices to other MSF operational centres.

Thus, MSF-OCA gained his first experience EVD response only during the 2014-16 EVD outbreak in Sierra Leone. Over the 2 years of the outbreak response, the MSF-OCA strategy shifted from patient management to interventions to prevent infection and mortality at community level. At the end of the outbreak mobile clinics for EVD survivors and referral for eyes complications surgery were set up.

Overall, 1,948 patients were referred to three EMCs run by MSF-OCA in Kailahun, Bo and Tonkolili Districts in Sierra Leone (Table 6). In October 2014, at the height of the outbreak in Freetown, MSF-OCA set up a community surveillance project in a slum area where general transmission was reported[9]. This was the first time that surveillance was the primary component of an MSF-OCA intervention for Ebola and also, given there was no MSF-OCA EMC in Freetown, the first time that their role was not directly linked to an EMC.

Multidisciplinary teams were set up to strengthen community surveillance while addressing humanitarian gaps for quarantined households in slum areas. In January 2015, in Tonkolili and Bombali

[9] Review of MSF-OCA surveillance and alert response in Freetown during the Ebola outbreak: lessons learned and challenges, MSF-OCA internal report. Available at https://www.researchgate.net

Districts, to identify area of transmission a new rapid mapping method was implemented in collaboration with local community and MoHS (6).

The work in Kailahun informed the conceptualization of Objective 1 (Chapter 3). Much of what is known about the EVD epidemic in Kailahun District, including the large number of cases, inpatient case fatality rate and routes of transmission, came from the MSF EMCs (7-11). Ebola was previously unknown to the local population. As such, there was no knowledge either amongst health-care workers or in the community about routes of transmission and strategies to control it such as safe burial, isolation of patients, quarantine, and contract tracing (12). The reactions of the population were characterised by fear and distress (13).

Understanding the factors which determined population willingness and the ability to comply with protective measures is essential but was largely undocumented. To address this evidence gap, I designed a mixed-method study to explore the factors which influenced EVD transmission dynamics and community compliance with control measures in a rural village that experienced sustained EVD transmission and high mortality burden, despite frequent community engagement visits in the village by MSF teams. The study also offered insights into the humanitarian and operational aspects that MSF should enhance to adapt EVD health interventions to local realities to reflect community perspectives and priorities.

Objective 2 (Chapter 4) was conceptualised in response to the work done at triage while admitting patients in the three EMCs, and during surveillance to identify suspect cases at community level in Freetown. Identifying suspected cases at EMCs and at community level was challenging due to the limited effectiveness of the WHO EVD case definitions. There had been no systematic and rigorous evaluation of the performance of WHO EVD case definitions. Such an evaluation was needed to guide communities and public health practitioners to improve the effectiveness and efficiency of identification and management of suspected cases during an EVD outbreak.

The third research question (Objective 3, Chapter 5) was conceptualised in the aftermath of the EVD outbreak in Bo District, where MSF-OCB was running a 200-bed secondary level referral hospital. In October 2014, MSF suspended health services due to the high risk of EVD nosocomial transmission and concerns about staff safety (14). The hospital had been operating since 2003, providing 8,000 paediatric, and 2,500 emergency obstetrical and gynaecological admissions per year. The closure of the MSF hospital in Bo District was perceived to contribute to an increase in mortality in the local area, however in the absence of a functional national civil registration and strong surveillance system in place there was limited capacity to measure the extent of the crisis. Thus, I designed two retrospective mortality surveys were to benchmark the crude and EVD specific mortality. The studies offered empirical estimates mortality rates and design effects. This was the first reported estimation of design

effect for EVD, and prompted reflection on the utility of using of retrospective mortality studies for highly-clustered diseases.

The research was led by the candidate and conducted as part of a collaborative approach with other MSF-OCs, Ministry of Health (MOH) as well as other relevant academic institutions.

Table 6. Characteristics of patients admitted to MSF EMCs, 2014-2015

(Excluding dead on arrival (n=61, positive 44), transferred and defaulters (n=52), Total patients referred to EMCs 1,948)

Patients characteristic	**Confirmed patients N=1,338**	**Non cases N=534**	**Total N=1,872**
Age, in years			
Median (IQR)	27.85 (16-39)	30.08 (20-40)	28.49 (17-40)
< 5 (%)	77 (5.75)	46 (8.61)	123 (6.57)
5-14 (%)	224 (16.74)	46 (8.61)	270 (14.42)
15-54(%)	941 (70.33)	381 (71.35)	1,322 (70.62)
≥ 55(%)	96 (7.17)	61 (11.42)	157 (8.39)
Sex (%)			
Male	661 (49.40)	328 (61.42)	989 (52.83)
Female	677 (50.60)	206 (38.58)	883 (47.17)
Outcome. N (%)			
Died	581 (43.4)	37 (6.92)	618 (33.66)
Cured	757 (56.6)		

2.2 Rationale for the PhD

The 2014-2016 EVD outbreaks in West Africa posed serious clinical and epidemiological challenges, in part due to limited investment in EVD research in previous EVD outbreaks. It was also the first time that MSF-OCA had been part of an EVD outbreak response, and intensive efforts were made to collect extensive data to improve patient care, and to conduct community studies to inform more humane and effective future interventions.

The research objectives were developed targeting specific questions that continue to pose major obstacles for agencies involved in outbreak response and local MoHS. The PhD was part of a consolidation process on lessons learned for MSF-OCA, and documentation of use of operational research conducted under outbreak conditions.

2.3 Aim of the PhD

The aim is to address evidence gaps on three main areas around community EVD experience and transmission, the accurate identification of EVD cases, methodological constraints on estimating EVD burden for clustered diseases at population level. These issues continue to pose major clinical and public health challenges for agencies involved in EVD outbreak management.

Research objectives

The objectives of this thesis are to:

1. Understand of population-level transmission dynamics and factors associated with compliance to EVD control in a rural village that experienced sustained EVD transmission in Kailahun District, Sierra Leone;
2. Assess the performance of the WHO EVD case definition and other EVD screening scores, to support surveillance and admission testing decisions at EMCs;
3. Estimate and discuss the utility of empirical mortality rates and design effects for highly-clustered disease outbreaks like EVD.

Table 7 summarises evidence gaps, objectives, and clinical or policy implication identified by this thesis.

The study protocols for Objectives 1 and 3, were approved by the Independent Ethics Review Board of Médecins Sans Frontières (MSF), and the Internal Review Board of the Sierra Leone MoHS.

Table 7. Synopsis of the evidence gaps addressed by the thesis, its objectives, clinical or policy implication, corresponding chapter and research paper

Evidence gap	PhD Objective	Clinical or policy implication	Chapter and research paper
Lack of knowledge of the factors that influence EVD transmission dynamics and community compliance with control measures over time.	Investigate drivers of EVD transmission and community perspective toward EVD control in Kailahun District, Sierra Leone.	To inform responses to future EVD outbreaks.	Chapter 3; Paper 1
Investigation of concerns about the performance of WHO EVD case definitions and lack of evaluation of its performance.	Assess performance of the WHO EVD case definitions and other screening scores, to support surveillance and admission testing decisions at EMCs.	To guide communities and public health practitioners to improve the effectiveness and efficiency of identification and management of suspected cases during EVD response.	Chapter 4; Paper 2
Lack of a benchmark of EVD mortality, and guidance on survey methods for highly clustered diseases.	Measure overall and EVD mortality rates and the design effects in two areas where MSF suspended paediatric interventions, Bo District, Sierra Leone.	Prompt reflection on the utility of using retrospective mortality studies for highly clustered diseases.	Chapter 5; Paper 3

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Chapter 3: (Research paper 1) The factors affecting household transmission dynamics and community compliance with Ebola control measures: a mixed-methods study in a rural village in Sierra Leone

3.1 Preamble

This chapter addresses the lack of in-depth understanding of transmission dynamics and factors associated with non-adherence to control strategies in the context of sustained EVD transmission in an EVD-affected village in Kailahun district, Sierra Leone conducted in April 2015.

This research paper was published in the BMC Public Health in 2018, and it is reproduced as follows with no revisions or adaptation from the published manuscript. The original publication is included at the end of this thesis.

3.2 Citation

Caleo G, Duncombe J, Jephcott F, Lokuge K, Mills C, Looijen E, Theoharaki F, Kremer R, Kleijer K, Squire J, Lamin M, Stringer B, Weiss HA, Culli D, Di Tanna GL, Greig J. The factors affecting household transmission dynamics and community compliance with Ebola control measures: a mixed-methods study in a rural village in Sierra Leone. BMC Public Health. 2018 Feb 13;18(1):248.

3.3 Cover sheet

The Research Paper Cover Sheet is enclosed on the following pages.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	210968	**Title**	Dr
First Name(s)	Grazia Marta		
Surname/Family Name	Caleo		
Thesis Title	Epidemiology and control of Ebola Virus Disease (EVD) in Sierra Leone: analysis of data from the Médecins Sans Frontières (MSF) response, 2014-15.		
Primary Supervisor	Professor Helen Weiss		

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

SECTION B – Paper already published

Where was the work published?	BMC Public Health		
When was the work published?	13 February 2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	n/a		
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was first author on this paper. I conceived and drafted the study protocol. I analysed the data with GDT support. I drafted the manuscript, and then incorporated feedback from the co-authors. I oversaw the manuscript submission process, and revised the manuscript, as necessary, to respond to input from peer review

SECTION E

Student Signature	Grazia Marta Caleo
Date	December 4th 2020

Supervisor Signature	Helen Weiss
Date	December 4th 2020

3.4 Abstract

Background

Little is understood of Ebola virus disease (EVD) transmission dynamics and community compliance with control measures over time. Understanding these interactions is essential if interventions are to be effective in future outbreaks. We conducted a mixed-methods study to explore these factors in a rural village that experienced sustained EVD transmission in Kailahun District, Sierra Leone.

Methods

We reconstructed transmission dynamics using a cross-sectional survey conducted in April 2015, and cross-referenced our results with surveillance, burial, and Ebola Management Centre (EMC) data. Factors associated with EVD transmission were assessed with Cox proportional hazards regression. Following the survey, qualitative semi-structured interviews explored views of community informants and households.

Results

All households (n=240; 1161 individuals) participated in the survey. 29 of 31 EVD probable/confirmed cases died (93·5% case fatality rate); six deaths (20·6%) had been missed by other surveillance systems. Transmission over five generations lasted 16 weeks. Although most households had ≤5 members there was a significant increase in risk of Ebola in households with >5 members. Risk of EVD was also, associated with older age. Cases were spatially clustered; all occurred in 15 households.

EVD transmission was better understood when the community experience started to concord with public health messages being given. Perceptions of contact tracing changed from invading privacy and selling people to ensuring community safety. Burials in plastic bags, without female attendants or prayer, were perceived as dishonourable. Further reasons for low compliance were low EMC survival rates, family perceptions of a moral duty to provide care to relatives, poor communication with EMC, and loss of livelihoods due to quarantine. Compliance with response measures increased only after the second generation, coinciding with the implementation of restrictive by-laws, return of the first survivor, reduced contact with dead bodies, and admission of patients to the EMC.

Conclusions

Transmission occurred primarily in a few large households, with prolonged transmission and a high death toll. Return of a survivor to the village and more effective implementation of control strategies coincided with increased compliance to control measures, with few subsequent cases. We propose key recommendations for management of EVD outbreaks based on this experience.

Keywords: Ebola virus disease, transmission dynamics, community perception.

3.5 Manuscript

3.5.1 Background

The first case of Ebola virus disease (EVD) in Sierra Leone is believed to have occurred in mid-May 2014, in a remote village of Kailahun District (estimated population 465,048) (1, 2). On 12th June 2014, the President of Sierra Leone declared a state of emergency in the district (3). The last case was recorded in Kailahun in mid-December 2014 and the Ministry of Health and Sanitation (MoHS) declared Kailahun District free from human-to-human transmission on 22nd January 2015, following 42 continuous days without a confirmed case (1). Médecins sans Frontières (MSF) opened an Ebola Management Centre (EMC) in Kailahun on 26th June 2014 to support the district MoHS (4). The MSF EMC was the only functioning Ebola management centre in the district, responsible for isolating 63·0% of confirmed cases. In total, the district MoHS reported 565 confirmed EVD cases in the population of Kailahun (attack rate 0·12%), including 287 deaths (case fatality rate [CFR] 51·0%) (5).

Evidence-based interventions for EVD control include early detection of cases through effective surveillance and contact tracing, admission of symptomatic cases to EMCs where staff adhere to high standards of infection control procedures, and safe burials by trained teams (6, 7). Quarantine measures were also widely implemented (8), and by-laws imposed that included travel restrictions and penalties for hiding suspected cases (9).

The transmission dynamics of the West Africa EVD epidemic have, so far, been reconstructed from EMC and surveillance data, and mathematical modelling (4, 10-12). However, poor surveillance systems and limited EMC capacities are likely to have resulted in underestimation of the true extent of the outbreak, limiting the ability to understand the dynamics and experience of the epidemic at community level, in particular in Sierra Leone, the country most affected by the West Africa EVD outbreak (13, 14).

Little is known of the factors that influence EVD transmission dynamics and community compliance with control measures over time. Such understanding is essential if interventions are to be effective, particularly in areas like Sierra Leone with no previous local EVD experience. In order to address this knowledge gap and inform future responses, we conducted an in-depth mixed-methods study in a rural village in Kailahun District that experienced prolonged EVD transmission during the outbreak.

3.5.2 Methods

To enable assessment of behaviour adaptation over time, we used data from MSF EMC patient registers to select a village in the District that had experienced a very protracted EVD outbreak. We then conducted a mixed-methods study combining data gathered via a cross-sectional survey and semi-structured interviews in this selected village. The cross-sectional survey data were used to reconstruct

the dynamics of transmission. Semi-structured interviews were used to document community perception, resistance, and adaptation to response strategies. Survey and interview data were triangulated with data from the safe burial and MoHS surveillance databases to verify the reconstruction of the EVD transmission, and explain changes in transmission and behaviour over time.

Cross-sectional survey

All consenting households in the village were included in the cross-sectional household survey. A trained MSF team, using a validated instrument for household mortality studies and EVD case investigation forms, collected demographic data from household heads on household members, births, arrivals, departures, deaths, illnesses (including signs and symptoms compatible with the EVD case definition), and history of contact with individuals symptomatic for EVD (15, 16). Verbal consent for participation was obtained from the head of each household after a briefing about the aim of the survey, the questions and duration of the questionnaire, and the option to end the interview at any time if wished.

The household survey was conducted in April 2015, with a recall period for responses between May 2014 (date of the first reported EVD case in the district) and the date of the survey. A local events calendar was developed to aid recall. MSF-EMC patient registers were used to verify the date of admission, symptoms, laboratory confirmation of EVD, and outcomes of patients admitted to the EMC. Each household in the village was enumerated and listed; from this list we randomly selected the households for the semi-structured interview.

Geographic positioning system (GPS) data were used to map the village layout and location of all households. Data were de-identified and entered into a password-protected electronic database.

Semi-structured interviews

At the end of the cross-sectional survey, semi-structured interviews were conducted with key community informants and selected households. Households were divided into two groups based on whether they had experienced at least one EVD case or no EVD cases. Ten households were randomly selected for interview from each group (total of 20 interviews).

A purposive approach was used to select key community informants: traditional healers; biomedical health-care providers; and community leaders including tribal authorities, heads of community groups, and religious leaders. The heads of the selected households and key community informants were interviewed after verbal consent to participate was obtained. Participants were briefed about study objectives, questions and duration of interview, and the option to leave the study at any time. All interviews were semi-structured, took place in a private space, and were conducted by a trained MSF team.

Interviews were conducted in the local language using an interpreter to translate and back translate to English. The local events calendar developed for the household survey was also used in the semi-structured interviews. Topic guides directed interviewers to explore changes over time in perceptions of EVD and perspectives related to EVD response activities including contact tracing activities, the MSF EMC, the safe burial team, and quarantine. Interviews explored how these EVD control strategies were implemented and how these accorded with cultural beliefs. The topic guide was the same for household and key informant groups except for an additional section in the key informant guide, regarding how the outbreak started in the village. After initial data analysis had been completed, a summary narrative was compiled and shared with the village in the format of a story. Participant validation was achieved in this way in order to refine findings (17).

Case definitions

World Health Organization (WHO) EVD case definitions were used to define suspect, probable, and confirmed cases (16). A suspect case was defined as: any person, alive or dead, suffering or having suffered from sudden onset of high fever and having had contact with a suspect, probable, or confirmed EVD case or with a dead or sick animal; any person with sudden onset of high fever and at least three relevant symptoms (headaches, vomiting, anorexia/loss of appetite, diarrhoea, lethargy, stomach pain, aching muscles or joints, difficulty swallowing, breathing difficulties, hiccup); any person with inexplicable bleeding; or any sudden, inexplicable death. A confirmed case was defined as anyone with a positive quantitative reverse transcription polymerase chain reaction (PCR) result. PCR cycle threshold (Ct) results were used as indicators of viral load. The lower the Ct value the higher the viral load (18). A probable EVD case was defined as anyone who met the clinical case definition and had a history of contact with a person with confirmed EVD, but who did not have a confirmed laboratory test result (16).

Data analysis

Cox proportional hazards regression models were fitted to estimate hazard ratios (HRs) and 95% confidence intervals (95% CI) for the association between EVD (probable and confirmed cases) and covariates previously documented to be associated with EVD, including household size, sex, and age (19, 20). Events were dated by epidemiological week and used as the time parameter in the Cox model. Cox shared frailty models were used to allow for within-household correlation.

The crude mortality rate (CMR) and EVD-specific mortality rate were estimated as deaths during study period/(mid-period population at risk x duration of period), where mid-period population at risk accounted for births, deaths, arrivals, and departures during the recall period (21). Mortality rates were expressed as deaths per 10,000 per day. The attributable risk percent (AR%) and population attributable risk percent (PAR%) were used to estimate excess mortality risk due to EVD in the exposed households and village level, respectively.

The proportion of EVD cases isolated by admission to the EMC and the proportion of people who died from EVD and received safe burial were assessed by comparing cases reported in MoHS surveillance, EMC, and burial team data with cases (confirmed and probable) identified through the household survey.

Transmission dynamics were constructed using contact history, and described using transmission chains. Relationships between individuals were categorised as nuclear (immediate family), extra nuclear (extended family), and social (neighbours and friends).

Statistical analyses were carried out using Stata 14.0 (Stata Corporation, Texas-USA); maps were generated using QGIS™ software (version 2.14, http://qgis.org). Participant responses from all semi-structured interviews were translated and transcribed at the time of the interview. Key community informants and household interview data were analysed separately using an inductive framework approach via an iterative process of coding and categorization (using ©NVivo 10) leading to the identification of emerging themes. The former contributed to the description of initial phase of the outbreak along with documenting the village experience over time, and the latter to exploring affected and unaffected household experience.

3.5.3 Results

Study population

The village consisted of 240 households (1161 individuals); all heads of households gave consent to participate. The median age of villagers was 18 years (interquartile range [IQR] 7-34 years), with 44·4% (n=515) younger than 15 years old. Approximately half the villagers were female (52·7%). Household size ranged from 1-17 people, with a median size of 5 (IQR 3-6).

Transmission dynamics

Overall, 31 EVD cases (15 confirmed, 16 probable) were identified, giving an overall attack rate in the village of 2·7%. The index case was an adult male who was resident in a city that was a known EVD hotspot in June-July 2014. In late July 2014, while symptomatic, he travelled back to his village of origin and died 1 week after his return. Table 8 details the possible routes of EVD transmission that were reported by his household and key informants. There was no record of the index case being tested for EVD, although he was reportedly taken to a holding centre for testing.

Following death, the index case was buried in an unsafe manner by community members, many of whom had unprotected contact with the body. It is believed that this may have started the chain of person-to-person transmission in the village. Transmission lasted for 16 weeks, with 30 cases arising

over five transmission generations: 11 cases in the 1st generation, seven in the 2nd, five in the 3rd, four in the 4th, and two in the 5th. For the one remaining case, a traditional birth attendant, a clear source of infection and transmission generation was not established (Figure 5). The time from exposure to symptom onset was ≤2 weeks for all cases with known exposure. The first survivor came back to the village in week 35 (late August), after 7 weeks of transmission, when most of the cases in the village had already occurred.

Amongst the secondary cases with known exposure: 38·0% (11/29) had, as sole exposure, contact with a symptomatic person who was a probable/confirmed case; 10·3% (3/29) had a history of attending a funeral; and almost half (14/29; 48·2%) had history of both contact with a symptomatic person and a funeral exposure. The proportion of cases exposed via a funeral decreased over time from 90·9% (10/11) in the 1st generation to 71·4% (5/7) in the 2nd, 40·0% (2/5) in the 3rd, 25·0% (1/4) in the 4th, and none in the last. Contact with a symptomatic person increased from 72·7% (8/11) in the first to 100·0% in the following generations. Among the 30 secondary cases, 28 died (93·3%) and two survived (6·7%).

There was strong evidence of clustering of EVD (p <0·0001), with all cases occurring in 15 of the 240 households (Figure 6). Thirty-two percent of cases occurred in two households, in which cases occurred over three- and four-generation chains.

Most secondary cases were exposed via the nuclear (57·6%; 17/30) or extended family (30·0%; 9/30). Affected households had a median of seven members (IQR 6-8), and non-affected households a median of three (IQR 2-4) (p <0·0001).

Factors associated with EVD

EVD was associated with older age and household size in unadjusted analysis; these associations became stronger after adjustment for both variables and sex (Table 9). The rate of EVD was similar by sex (aHR 1·03; 95% CI 0·49-2·17 for females vs males), but was greater among those aged 15-54 years (aHR 23·04; 95% CI 3·06-173·12) and ≥55 years (aHR 57·28; 95% CI 7·03-466·33) compared with those aged 5-14 years, and among those living in larger (>5 members) (aHR 56·53; 95% CI 19·64-162·73) compared with smaller households (Table 9).

Mortality

Of the 31 cases (index case plus 30 secondary cases), 29 died (CFR 93·5%; 95% CI 78·6-99·2%). Thirteen of 15 confirmed cases and all 16 probable cases died. About half (55·2%) of EVD deaths were among females; three were pregnant and miscarried at home.

The community reported five non-EVD deaths during the recall period. The CMR for all causes of death (EVD and other) was 0·97 per 10,000 per day. EVD-specific CMR was 0·83 per 10,000 per day and the non-EVD CMR was 0·14 per 10,000 per day.

The AR% for death associated with EVD was 99·5% (95% CI 98·6-99·8) among the exposed households, while the PAR% for death associated with EVD in the whole village was 84·5%.

Admission to the MSF EMC

In mid-August 2014, cases started to be admitted to the EMC. Of the 31 cases, 15 were admitted to the EMC and had Ebola infection confirmed by PCR testing. Twelve cases had an exact date of symptom onset recorded, with a median time from first onset of symptoms to admission of 4·0 days (IQR 3-5). The median time to admission was 5·0 days in the first generation (IQR 4-7), falling to 1·0 day in the last generation (IQR 0-1). The mean Ct value at admission was 21·8 (SD 4·5). Among the confirmed cases at EMC, 12 (80·0%) presented at admission with at least one wet symptom (diarrhoea, vomiting, or bleeding).

Burial, quarantine, and contact tracing

Of the 29 EVD deaths, 13 (44·8%) occurred within the EMC; five deaths in the community then had a safe burial by the burial team. Six deaths (20·6%) were captured during the survey but were not listed in the EMC, MoHS surveillance system and/or safe burial database. A further five people who died were reported by families to have been transported to an MSF or local Government hospital, however, there was no record of those patients in the EMC database. Contact tracing was reported to have occurred starting in late July; one in five village households reported they had been under contact tracing and quarantine measures. However, in August 2014, when 18 secondary cases had already occurred, the entire village was put under restriction of movements.

Community perception, resistance, and adaptation to response activities

Semi-structured interviews were conducted with 38 participants: 10 households reporting EVD cases (affected households (AH)), 10 households with no cases (UH), and 18 key community informants (CI).

Introduction of EVD in the village

When discussing how EVD had been introduced to the village, all participants referred to a single member or index case in the family or community, ranging from a family visitor to a health worker.

> "The man [index case] brought Ebola here. He used to treat people in [city] that was a hotspot at the time. When he got sick, he came here to see traditional healers and a herbalist came from Guinea to treat him using traditional herbs." – (CI09_m)

"An ambulance came to collect him and take him to [XX] holding centre. It was anecdotally reported that he tested negative, so some relatives went to pick him up. People were very happy, so they came to greet him/celebrate." - (CI04_m)

Misgivings toward Ebola

Initially, it was difficult for villagers to believe that infection could spread through everyday person-to-person contact. This perception was compounded by a climate of mistrust of authorities, fear of death, and lack of understanding of complex health messages such as the importance of isolation of those infected.

"We had never seen a sickness like this before, where you touch someone and you die." - (CI12_m)

"It seemed like someone had poisoned our village; many, many, many people died. It was similar to other diseases [e.g. malaria]." – (CI16_m)

"We thought it was a curse; some people thought that it was some kind of traditional medicine that was being thrown on them." - (CI13_f)

"People thought it was a conspiracy between the President and the westerners, who needed blood. They thought that if you go to the EMC, you will die." - (CI03_m)

"People didn't believe it: like war, we didn't believe it could come here. There was lots of arguing - some people thought Ebola wasn't real. They thought it was something sent by God." - (CI04_m)

"People were hiding symptoms and deaths because they were scared of the camp [EMC]; by the time they were found and the ambulance called, they were already dead." - (CI11_m)

"Early on, people were hiding if they were sick. By the time we knew they were sick, they weren't alive long enough to send them to the EMC (1-2 days)." - (UH05_m)

"We beat the contact tracers - we thought they were responsible for our relatives' deaths because they went for training at the same time [end of July] XX [index case] got sick." - (CI16_m)

"At the start, people hated the contact tracers - they beat them. One man in particular was beaten almost to death." - (CI17_f)

"The man [index case] came with a letter that said he should be isolated for 21 days. But we didn't understand what 'isolation' meant." - (CI16_m)

Change in perception

The perception of EVD held by the villagers changed when information received from contact tracers and the MSF health promotion team was consistent with what villagers observed in their lives at the community level. Implementation of the by-laws on travel and penalties for non-reporting cases supported the understanding of the severity of the outbreak by villagers, and helped them accept that control measures were intended to protect and help the community.

> "When we saw that people touched sick people and got sick, we could see the communication of it and realised that it is real." - (CI13_f)

> "Sensitisation from different sources [MSF/MoHS/radio] started to make sense; symptoms in our loved ones were exactly the same as they were telling us." - (CI11_m)

> "We realised that no contact was good, after a while, we saw the benefit." - (CI15_m)

> "But we had to follow the law we had to pay 500,000 Leones if there was a sick person found in the house." - (CI12_m)

> "It was for our own safety - to avoid touching bodies. To help them to stop the spread of Ebola. The word 'safe' equals 'help'." - (CI16_m)

Behaviour adaptation

Understanding of the route of transmission, and observing survival of cases admitted to the EMC supported changes in behaviour and adaptation by the community. This mainly occurred in late August coinciding with the return of a survivor, reduced contact with dead bodies, restriction of movements and isolation of patients.

> "When we heard about people surviving people's attitude changed." - (CI03_m)

> "We would go far away from the person and inform contact tracers who will call an ambulance to remove them to the camp [EMC]." - (UH04_f)

> "Initially, it [burial team] was not good but when we saw that the deaths increased, we knew it was for our own safety." - (AH02_m)

The village implemented a number of local measures to prevent spread between households.

> "During the outbreak, some people even devised their own preventive measures, like stopping children from playing football so they don't have contact with each other, and stop visit other households." - (CI09_m)

"Traditional birth attendants stop doing deliveries." - (CI17_f)

Understanding control strategies and constraints

All strategies such us MSF/EMC, MSF health promotion, contact tracing, burial practices, quarantine/restriction of movements were understood by the community as helping to control EVD. However, resistance to specific practices that were perceived as offensive to socio-cultural norms was reported; this resistance continued until the value of such practices was understood.

MSF/EMC

The EMC was understood to help people survive:

"Without the camp [EMC] - we would have no survivors." - (CI04_m)

However, communication regarding the status of admitted patients was perceived as poor:

"We received no information while they were still alive. When they died, a nurse who worked at the camp [EMC] told us." - (CI14_f)

"When the ambulance went with XX to the camp [EMC], some family members went to visit and they learned that he had died." - (CI07_m)

The MSF health promotion team were perceived as empowering the community:

"It gave the Community Health Workers a zeal to call ambulances; they empowered us. They sensitised us about preventive methods and no touch." - (CI06_m)

"Helped to decrease cases." - (CI12_m)

"We learned not to touch other people, and use water and soap." - (CI15_m)

Contact tracing

Contact tracing was perceived as a mechanism to remove people from the community who were thought to be a risk, which initially created mistrust. This gave contact tracers a reputation for invading privacy and disrupting family and community life and sending people to their deaths.

"There was no sensitisation about why contact tracers were here. They would just call the ambulance and collect people to the EMC." - (CI01_m)

"We didn't like the contact tracers; called them murderers." - (CI02_f)

> "Invasion of privacy - it was not their business to investigate our household." - (CI04_m)

> "We didn't like the fact that they were involving themselves in our affairs, we thought contact tracers were selling us to other people and that they were too inquisitive." - (CI17_f)

However, contact tracers were valued once people understood that they were trying to protect people and prevent the spread of Ebola:

> "It is our culture to touch people when they are sick, so if you don't take people out of the village, people will touch them." - (CI11_m)

> "Without contact tracers we would have continued touching people. Instead, sick people were collected to the camp [EMC]." - (CI16_m)

> "Otherwise we would have far more deaths." - (CI14_f)

> "Contact tracers should be empowered with training to stop the spread." - (CI13_f)

Burial practices

The value of safe burials was understood:

> "Without the burial team, the disease would have spread because touching dead bodies is bad." - (CI02_f)

However, burials were initially seen as lacking honour in terms of how they were performed, specifically the use of plastic bags, and the lack of burial clothes and prayers. Respondents also commented on the lack of women in the burial team and on the arrival of the teams in the village already dressed in personal protective equipment (PPE).

> "Plastic bags are not traditional - there is no honour when you bury people this way." - (CI03_m)

> "Praying was not allowed." - (CI09_m)

> "Sometimes, in dreams, my husband appears and says 'I have no clothes'." - (AH06_f)

> "Men burying women is not good; women should be part of the burial team." - (CI17_f)

> "We weren't happy about it. Before the outbreak, if a chief dies or a special person dies, they are buried by other special people. Now, we can't do that. There is no clothes, no dressing - and men are burying women, which is a problem for us." - (CI11_m)

> "People were afraid of the burial team when they came dressed in full protective clothes. They thought they were ghosts." - (CI03_m)

In October, the burial procedures were improved to incorporate greater respect for local tradition:

> "We couldn't pray before, either, but now we can." - (CI03_m)

> "Now they [burial team] dress in protective clothes in the village." - (CI11_m)

Quarantine/restriction of movements

The community understood the value of quarantine:

> "Because of quarantine, we couldn't spread Ebola to other households." - (AH07_m)

However, people were also angry about quarantine:

> "It destroyed many things, especially farming, our crops were destroyed and there is no food available now." – (CI15_m)

In September, quarantine measures were improved by incorporation of food supply to quarantined households:

> "We had no food at the start. They should have given us food like they did in other households at the end." - (AH06_f)

Affected versus unaffected households

Both affected and unaffected households were sensitive to law enforcement and were in favour of stricter methods to control Ebola in the future. The consequences of quarantine, in terms of financial and emotional impact and stigma, were harsher in affected households compared with non-affected households, since non-affected households were only directly impacted when the entire village was quarantined.

> "Seven members of my family were taken to EMC. They all died there. Everyone would yell at us, 'you brought Ebola here!' I didn't - my brother did. But I still felt guilty." - (AH03_m)

Affected households provide some insight into factors that led to continued transmission in some homes but not in others, and why within-household transmission continued even when between-household transmission was reducing:

> "We could not abandon sick people – we must care for [them]." - (AH05_f)

> "People didn't come around - it was like the devil was here." - (AH04_m)

3.5.4 Discussion

Our study provides a comprehensive description of EVD in one village in Kailahun District, Sierra Leone that experienced sustained EVD transmission during 2014. We attempt to capture the complexities of the social context influencing outbreak control in this specific epidemic. We documented that immediate family members of large households were at greater risk of being infected, and because of the larger number of inhabitants, these households were more likely to maintain transmission. This finding corroborates insights from other studies. This may imply that future responses to an EVD outbreak could justify prioritization of affected large households and their immediate family members, in particular when human resources are insufficient to address the scale of the outbreak (19, 22).

Within affected households, transmission was maintained by the need to provide care for sick relatives, with cases continuing to occur over several generations. Compliance with response measures increased only after the second generation, coinciding with the return of a survivor, and strict implementation of other components of the EVD response, such as restriction of movements, reduced contact with dead bodies, and isolation of cases. However, this changing context only occurred after 7 weeks of transmission, when most of the cases in this outbreak had already died.

In particular, return of survivors to the village after treatment prompted a shift toward belief in Ebola and increasing acceptance of control measures. Late return of survivors prevented teams from building trust within the community. At the time that survivors returned, the village was experiencing a peak in case numbers, the MSF EMC was reaching the limit of its capacity (100 beds), and communication with households was primarily to inform of deceased loved ones, thus contributing to community fear and despair. People reported avoiding the MSF EMC because of poor survival rates, which reinforced the community perception of the EMC as a place where people die. One approach to improving community understanding and uptake of EMC services in future could include developing the role of an EMC-village liaison, whose role would be to support timely communication with communities about the status of relatives throughout admission. Use of EMC-village liaisons could acknowledge the gap in understanding of health system workers as to why patients may undermine control measures when faced with the need to look after their loved ones. Contact tracers, could potentially play this liaison role; and

therefore have the potential to be seen as providing something positive to the community rather than just reporting and tracing cases.

Reduced misgivings and doubt about Ebola were crucial to influencing attitudes toward control measures. This change likely occurred once the health messages given to the community mirrored their reality. Once Ebola transmission was understood, the perceptions of contract tracing changed from invading privacy, selling people to working collectively toward community safety. The community then participated in control measures by setting up a number of local strategies such as stopping babies being delivered in the community, preventing children from playing contact games together, and not visiting other households. These strategies contributed to outbreak control, as observed by other authors (23). Our findings emphasised the importance of the community having a role in tailoring outbreak responses. Following a localised governance approach may permit incorporation of accepted local social norms from the outset of intervention efforts, making them more acceptable and therefore effective.

Clear communication of complex health messages was challenging, but played a role in the acceptance of EVD control measures. It was essential that the community understood there was a 21-day incubation period, the importance of EMC isolation (both self-imposed and institutional), and that a single negative test result could not rule out disease during the incubation period. Other authors described similar issues for messaging in Sierra Leone and in previous outbreaks (24, 25).

Similarly to the rest of the country, the age structure of the village was young, with those under age 15 accounting 44% of the population. The limited life experience of youth, and particularly collective experience with death from exposure to body fluids (e.g. ”touch someone and you die”) or with infection prevention and control concepts (e.g. “we didn't understand what 'isolation' meant”) may have contributed to delays in understanding and adoption of the necessary responses, rather than villagers being deliberately uncooperative. However, we documented that regardless of age, the population in general suffered an overwhelming level of inexperience toward this disease and its impact. Response agencies must acknowledge community demographic structure and perspectives on the presence of EVD in parallel with launching control measures cognisant of their baseline understanding.

Our study findings show nuanced perceptions toward quarantine as both a way to control the spread of Ebola, and a cause of social and livelihood disruption, which challenged compliance, as reported by other researchers (26). This argues for such social disruption to be taken into account when planning how best to protect affected people and control transmission.

Safe burial using plastic bags, lack of burial clothes, and the absence of women in the burial team were described as showing a lack of honour for the deceased. Burials were described as being more compliant to control measures when practices such as community prayer were permitted. In addition, the burial team started to dress in PPE after arrival in the village as now recommended by WHO Guidelines (27). Additional measures that can be implemented without compromising safe burial, such as including

female members in the burial team, and safe alternatives to plastic burial bags, would further enhance community acceptance compliance, and should be included in EVD control guidelines.

The comprehensive design of this study enabled every household in the village to be surveyed, and therefore a number of deaths were captured by our survey, that were not identified by MOHS surveillance, EMC, or burial data. All cases and deaths detected were spatially clustered; this is a key finding since traditional methods to estimate mortality rely on cluster sampling approaches, which in this case could have generated either an under- or over-estimation of EVD, depending on whether the limited number of affected households was randomly selected. This is an important element to take into account while trying to benchmark the burden of highly clustered diseases like EVD. Even in a highly affected community, clustering of disease means that household sampling is likely to miss many households unless an appropriate estimate of intra-cluster correlation is available. It is noted that it would not have been feasible to carry out exhaustive studies on the wider population in the middle of the EVD outbreak. In future, we recommend developing alternative methods of sampling to estimate disease and mortality that account for the highly clustered nature of diseases such as EVD.

Strengths and limitations

A major strength of this study is its mixed methods design, which provides a deeper understanding and explanation of the social reactions to dealing with EVD at community level. Half of the EVD cases in this study were not confirmed by PCR. However, they met the suspected case definition, died, had clear epidemiological links with a confirmed case, and some generated secondary cases, some of which were confirmed EVD. The number of deaths may have been under-reported, as villagers may have feared a penalty for not adhering to the mandatory notification by-law. However, it should also be noted that the study was well perceived by villagers, as demonstrated by the participation of the entire village, their help in documenting the transmission chains, and their willingness to tell the story of the village outbreak. We cannot exclude underestimation of the burden of EVD infection in the village by missing mild or asymptomatic cases. We also collected data on morbidity at the time of the outbreak, and three living people reported history of symptoms compatible with EVD, and a history of exposure, but they were never tested or isolated and thus not included in the analysis. If they were true cases, our EVD mortality may be overestimated, however, when we did include these cases in the analysis it did not change our findings significantly. The true EVD infection rate could be known only via a serological study (28).

Incorrect recall of the timing of deaths may have occurred, but the impact of Ebola makes this less likely, and the use of a local community calendar of events aided recollection of timing. In addition, we validated dates and symptoms for cases admitted to the Kailahun EMC, MOHS surveillance, and buried by the burial team. We were able to rebuild accurate dates for the events of each case we identified, validated across multiple data sources.

For the qualitative part of the study, we acknowledge it was more difficult to definitively link community behaviour change with specific measures or events. Furthermore, we recognise that those are reported perceptions recollected at the time of the outbreak, however, these were consistent among the different people interviewed and suggested a shift in the way the community expressed their ideas of EVD. We acknowledge that perception of changes in the village may have been influenced by the differing roles played by community informants, and in relation to the differing experiences of affected vs. unaffected households.

It is also important to note that our observations were based on a single, high-burden village. Our findings are therefore likely to be generalizable to similar rural settings with high levels of transmission. However, it is possible that the outbreak and response would be different in villages with lower levels of transmission, as experience of the disease was an important driver of behavioural change.

Finally, the main limitation of our qualitative work was that questions regarding burial practices seemed to provoke a limited depth of response in particular among affected households. This may have been because respondents were still affected by their loss.

3.5.5 Conclusion

In this high-burden village, transmission was maintained by a small number of large households; the outbreak was controlled in this community only after prolonged transmission and a high death toll. A key recommendation emerging from these findings is to ensure that large households and immediate family members are prioritized in control and prevention activities. There is also a need to develop novel sampling methods appropriate for estimating mortality for highly clustered diseases like EVD.

Our findings provide practical information on how future interventions could be implemented more humanely and effectively. We emphasise the following factors: recognising the role of communities for their contribution in controlling outbreaks; identifying community liaison roles which can keep families informed of their relatives' progress in the EMC; ensuring survivors are engaged to increase community trust to delegate care to EMCs; conveying complex health messages around incubation periods and infectivity clearly to the community; using appropriate alternatives to burial in plastic bags; including women in burial teams; and compensating quarantined households and communities to ensure they can maintain and re-establish livelihoods.

Factors underlying delays in implementing control measures included community belief or otherwise in the presence of EVD, lack of trust, and the toll imposed by interventions such as safe burial procedures and the social disruption of quarantine. Early understanding of social norms and experiences and the ability to link this to localised strategies and adapted health interventions would be essential.

Including these findings in future recommendations for outbreak control policy could help to improve the accuracy of mortality estimates and avoid unnecessary deaths and protracted suffering in future outbreaks.

Abbreviations

AR%: Attributable risk percent; CFR: Case fatality rate; CI: Confidence intervals; CMR: Crude mortality rate; Ct: Cycle threshold; EMC: Ebola Management Centre; EVD: Ebola virus disease; GPS: Geographic positioning system; HRs: Hazard ratios; IQR: Interquartile range; MoHS: Ministry of Health and Sanitation; MSF: Médecins sans Frontières; PAR%: Population attributable risk percent; PCR: Polymerase chain reaction; PPE: Personal protective equipment; WHO: World Health Organization.

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Availability of data and material

Data are available under the MSF data sharing agreement. Requests to access data can be made to data.sharing@msf.org.

Authors' contributions

GC, JG, CM, and FJ conceived the idea; JD and EL implemented the study; and GC wrote the first and final drafts. GC, BS, FT, DC, and GDT contributed to the analysis and interpretation. JG, KL, RK, KK, JS, ML and HW reviewed early drafts. KL, JG, BS, and HW reviewed the late draft. All authors have given signed or electronic approval to be authors on the manuscript. All authors read and approved the final manuscript.

Authors' Information

GC, JG, FT, DC, KL are associated with the Manson Unit, Médecins Sans Frontières (MSF)

KL is associated with the National Centre for Epidemiology and Population Health, Research School of Population Health, Australian National University

JD, CM, EL, RK, KK are associated with Médecins Sans Frontières (MSF) Amsterdam

FJ is associated with the Department of Veterinary Medicine, University of Cambridge

JS, ML are associated with the District Health Management Team, Ministry of Health and Sanitation, Kailahun, Sierra Leone

BS is associated with Médecins Sans Frontières (MSF) London

HW is associated with MRC Tropical Epidemiology Group, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine

GDT is associated with the Centre for Primary Care and Public Health, Queen Mary University of London

Ethics approval and consent to participate

The study protocol was approved by the Ethics Review Board of MSF, the Internal Review Board of the Sierra Leone MoHS, and The London School of Hygiene & Tropical Medicine (LSHTM). Verbal consent for participation was obtained from the head of each household after a briefing about the aim of the survey, the questions and duration of the questionnaire, and the option to end the interview at any time if wished.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Table 8. Possible sources of infection for the index case

Contact with EVD patient(s) in the course of his work as a pharmacist
Contact with EVD patient(s) when he had a tooth extracted at a local Government Hospital, which was, at that time, a major hotspot of EVD
Contact with a traditional healer, who reportedly came from Guinea to treat the index case, who may have been infected
Contact with EVD patient(s) at a holding centre he was taken to for testing because he was symptomatic following the tooth extraction
Unknown source of infection (e.g. community)

Table 9. Demographic characteristics of the study participants and risk factors for EVD

	Entire village N	EVD infected N (% of village)	Crude hazard ratio	95% CI	p	Adjusted hazard ratio	95% CI	p	Adjusted hazard ratio from shared frailty Cox	95% CI	p
Sex											
Male	549	13 (2·4%)	ref			ref			ref		
Female	612	18 (2·9%)	1·24	0·61–2·54	0·55	1·03	0·49–2·17	0·92	1·19	0·52–2·73	0·68
Age group (years)											
< 5	174	4 (2·3%)	7·87	0·88–70·48	0·0001	6·02	0·66–54·39	< 0·0001	6·10	0·63–58·63	0·12
5–14	341	1 (0·3%)	ref			ref			ref		
15–54	552	18 (3·3%)	11·27	1·50–84·45		23·04	3·06–173·12		20·26	2·48–165·09	0·005
≥ 55	94	8 (8·5%)	31·57	3·94–252·48		57·28	7·03–466·33		53·06	5·89–477·66	< 0·0001
Household size											
≤ 5 members	973	4 (0·4%)	ref			ref			ref		
>5 members	188	27 (14·4%)	37·15	12·99–106·19	< 0·0001	56·53	19·64–162·73	< 0·0001	56·08	16·38–191·92	< 0·0001

Figure 5. EVD transmission generation, according to week of onset

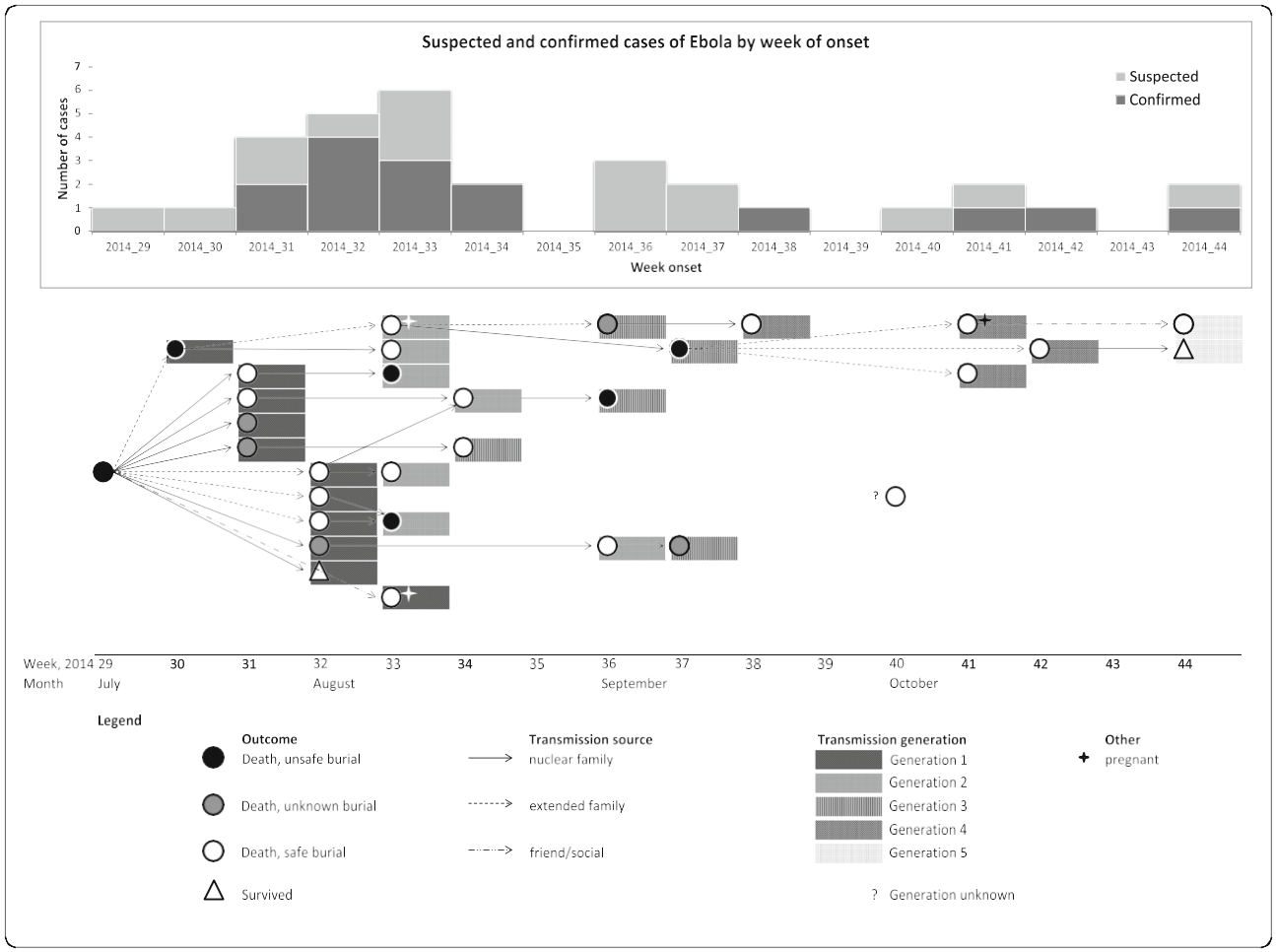
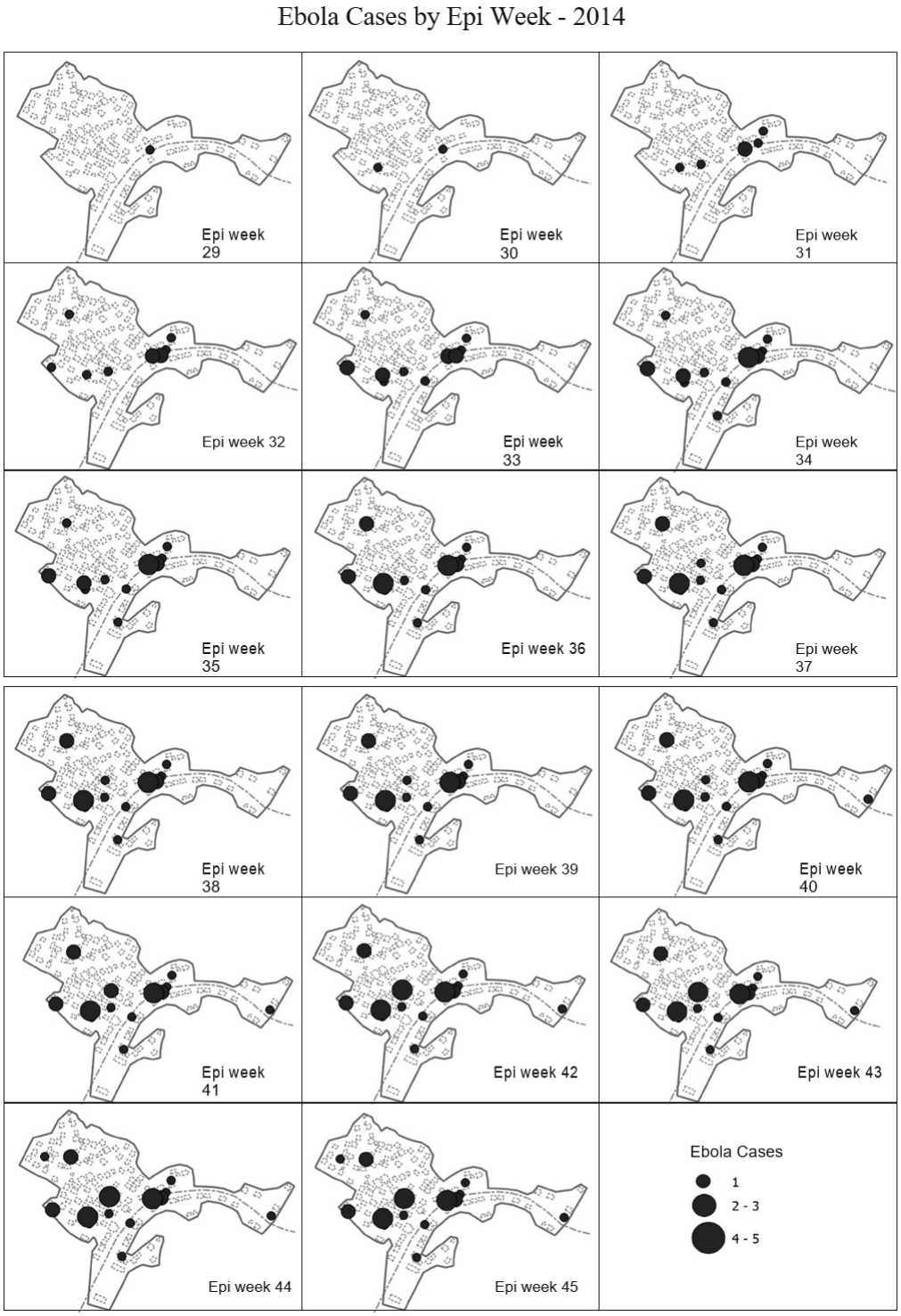


Figure 6. Geographical distribution of cases over time, weeks 29 –week 45



3.5.6 Reference

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3.6 Supplementary material

The detailed study protocol is publicly available on the MSF research platform at this link: https://remit.oca.msf.org/studies/173

Chapter 4:(Research paper 2) Clinical and epidemiological performance of WHO Ebola case definitions: a systematic review and meta-analysis

4.1 Preamble

In this chapter I present the work undertaken to address the lack of systematic and rigorous evaluation of the performance of WHO EVD case definitions and other clinical and epidemiological characteristics such as symptoms and signs at admission and contact history, against the reference standard (laboratory confirmation of Ebola virus infection).

This was investigated through a systematic review and meta-analysis of studies published in English between June 13, 1978, and Jan 14, 2020.

The paper was published in the Lancet Infectious Diseases in November 2020, and it is reproduced as follows with no revisions or adaptation from the published manuscript. The original publication is included at the end of this thesis.

4.2 Citation

Caleo G, Theocharaki F, Lokuge K, Weiss HA, Inamdar L, Grandesso F, Danis K, Pedalino B, Kobinger G, Sprecher A, Greig J, Di Tanna GL. Clinical and epidemiological performance of WHO Ebola case definitions: a systematic review and meta-analysis. Lancet Infect Dis. 2020 Nov;20(11):1324-1338.

4.3 Cover sheet

The Research Paper Cover Sheet is enclosed on the following pages.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	210968	**Title**	Dr
First Name(s)	Grazia Marta		
Surname/Family Name	Caleo		
Thesis Title	Epidemiology and control of Ebola Virus Disease (EVD) in Sierra Leone: analysis of data from the Médecins Sans Frontières (MSF) response, 2014-15.		
Primary Supervisor	Professor Helen Weiss		

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

SECTION B – Paper already published

Where was the work published?	Lancet Infect Dis		
When was the work published?	November 2020		
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SECTION C – Prepared for publication, but not yet published

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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was first author on this paper. I conceived the he review. I extract data from original manuscripts. I analysed the data with GDT support. I drafted the manuscript, and then incorporated feedback from the co-authors. I oversaw the manuscript submission process, and revised the manuscript, as necessary, to respond to input from peer review

SECTION E

Student Signature	Grazia Marta Caleo
Date	December 4th 2020

Supervisor Signature	Helen Weiss
Date	December 4th 2020

4.4 Summary

Background

Ebola virus disease case definition is a crucial surveillance tool to detect suspected cases for referral and as a screening tool for clinicians to support admission and laboratory testing decisions at Ebola health facilities. We aimed to assess the performance of the WHO Ebola virus disease case definitions and other screening scores.

Methods

In this systematic review and meta-analysis, we searched PubMed, Scopus, Embase, and Web of Science for studies published in English between June 13, 1978, and Jan 14, 2020. We included studies that estimated the sensitivity and specificity of WHO Ebola virus disease case definitions, clinical and epidemiological characteristics (symptoms at admission and contact history), and predictive risk scores against the reference standard (laboratory-confirmed Ebola virus disease). Summary estimates of sensitivity and specificity were calculated using bivariate and hierarchical summary receiver operating characteristic (when four or more studies provided data) or random-effects meta-analysis (fewer than four studies provided data).

Findings

We identified 2493 publications, of which 14 studies from four countries (Sierra Leone, Guinea, Liberia, and Angola) were included in the analysis. 12021 people with suspected disease were included, of whom 4874 were confirmed as positive for Ebola virus infection. Six studies explored the performance of WHO case definitions in non-paediatric populations, and in all of these studies, suspected and probable cases were combined and could not be disaggregated for analysis. The pooled sensitivity of the WHO Ebola virus disease case definitions from these studies was 81·5% (95% CI 74·1–87·2) and pooled specificity was 35·7% (28·5–43·6). History of contact or epidemiological link was a key predictor for the WHO case definitions (seven studies) and for risk scores (six studies). The most sensitive symptom was intense fatigue (79·0% [95% CI 74·4–83·0]), assessed in seven studies, and the least sensitive symptom was pain behind the eyes (1·0% [0·0–7·0]), assessed in three studies. The performance of fever as a symptom varied depending on the cut-off used to define fever.

Interpretation

WHO Ebola virus disease case definitions perform sub-optimally to identify cases at both community level and during triage at Ebola health facilities. Inclusion of intense fatigue as a key symptom and contact history could improve the performance of case definitions, but implementation of these changes will require effective collaboration with, and trust of, affected communities.

Funding

Médecins Sans Frontières (MSF).

4.5 Manuscript

4.5.1 Introduction

Ebola virus disease case definition is a crucial surveillance tool to detect suspected cases for referral and as a screening tool for clinicians to support admission and laboratory testing decisions at Ebola health facilities. However, there have been long-standing concerns about the poor performance of the WHO Ebola virus disease case definitions, including the inability to distinguish Ebola virus disease from common diseases such as malaria and typhoid fever (1-3).

The scale of the 2014–16 west African Ebola epidemic further challenged the operational use and validity of the WHO case definitions in detecting suspected cases at the community level and allocating patients appropriately to high-risk or low-risk wards for testing at specialised isolation centres (4).Consequently, during and since this epidemic, organisations involved in the Ebola virus disease response have estimated the sensitivity and specificity of the WHO case definitions and its constituent symptoms and signs, and developed alternative definitions and risk scores to identify clinical and epidemiological factors that could predict infection under outbreak conditions(5, 6). Discordance on the use of WHO Ebola virus disease case definitions with consequent delay on outbreak control and community disengagement have been reported in west Africa and, in the current outbreak, in the Democratic Republic of the Congo along with its bordering countries (7-9).

However, the operational use and performance of those definitions and risk scores has not been rigorously evaluated. Such an evaluation is needed to guide communities and public health practitioners to improve the effectiveness and efficiency of identification and management of suspected cases during Ebola virus disease responses.

We aimed to assess the performance of the WHO Ebola virus disease case definitions and other clinical and epidemiological characteristics, such as symptoms and signs at admission and contact history, as the index test or test under assessment, against the reference standard of laboratory-confirmed Ebola virus infection.

4.5.2 Methods

Search strategy and selection criteria

For this systematic review and meta-analysis, we searched PubMed, Scopus, Embase, and Web of Science, without regional restrictions, for studies in English published between June 13, 1978 (when the first Ebola virus disease outbreaks were reported on), and Jan 14, 2020 (10,11) We also endeavoured

to capture data on the current outbreak of Ebola virus disease in the Democratic Republic of the Congo by contacting relevant people involved in the response.

The search terms included “Ebola”, “EVD infection”, “case definition”, “admission symptoms”, “sensitivity”, “specificity”, “likelihood”, “score”, “classification”, “validity” and “performance” (Appendix, pp 5-6).

We included observational retrospective studies that estimated the sensitivity and specificity of WHO Ebola virus disease case definitions and other clinical and epidemiological characteristics (symptoms and signs at admission and contact history) against the reference standard (laboratory confirmation of Ebola virus infection), and studies that developed, or externally validated, predictive risk scores (based on a combination of symptoms and signs, and epidemiological information) to predict the risk of being positive for Ebola virus.

We also included studies looking at sensitivity and specificity of WHO case definitions for Ebola or Marburg virus infections because they belong to the same family of viruses (Filoviridae) and share the same case definitions, and the reference standard is laboratory confirmation of infection (12).

We excluded studies on the sensitivity and specificity of diagnostic tests, animal and vaccine studies, studies of survivors of Ebola virus disease, and studies on predictors of outcomes or severity of Ebola virus disease, community surveillance, and outbreak and clinical management. Studies specifically on frequency of symptoms at admission were also excluded as a previous review exists (13).

Two reviewers (GC and FT) independently screened all titles and abstracts to identify those meeting the selection criteria, and a third author (LI) arbitrated for studies without consensus. A full-text review was then done for these articles, and their bibliographies were assessed for other eligible studies. We extracted data on author, year of publication, country, virus, period of data collection, study design, study objective, outcomes measured, setting in which data were collected (eg, Ebola treatment centres), age of population included in the study, study size including number of patients who were negative and positive for Ebola virus, diagnostic method, limitation of individual studies, and performance of the WHO Ebola virus disease case definitions, and individual symptoms and signs, and epidemiological links or contact history with known patients with Ebola virus disease.

Performance data extracted included sensitivity, specificity, predictive values and risk score, and area under the receiver operating characteristic (ROC) curve (AUC). We developed a spreadsheet to compile extracted data based on the Cochrane data tool (14). The primary data extracted from each article were checked by a second researcher (FT). No protocol was developed for this study.

WHO Ebola virus disease case definitions were used to define suspected, probable, and confirmed cases, which varied by context and period of outbreak. In 2014 in Sierra Leone, WHO included miscarriage as an additional symptom (eg, abdominal pain) or sign (eg, vaginal bleeding) to the existing

definitions (12,15) For paediatric populations, the modified WHO case definition used in Sierra Leone was evaluated (figure 7) (15).

Data analysis

We derived the numbers of true positive, false negative, true negative, and false positive cases in each study using data provided in each article for each symptom and sign, and WHO Ebola virus disease case definition. Sensitivity and specificity are correlated, and univariate measures of heterogeneity, such as I^2, are not suitable to report heterogeneity in diagnostic test accuracy reviews (16). We used bivariate and hierarchical summary ROC (HSROC) models for meta-analysis (17,18).

The bivariate model provides estimation of a summary of sensitivity and specificity, whereas the HSROC model provides the estimation of a summary curve from studies that have used different thresholds, the 95% confidence region for the summary point, and the 95% prediction region. The prediction region graphically illustrates between-study heterogeneity as well as the bivariate relationship between sensitivity and specificity (19). Only studies that used comparable thresholds, symptoms and signs, or definitions were combined using these methods.

Given that HSROC models cannot be fitted when there are data from fewer than four studies, for some symptoms and signs we did a random-effects meta-analysis to calculate pooled estimates for sensitivity and specificity (20).Compared with bivariate and hierarchical models, pooled estimation from random-effects meta-analysis could slightly overestimate point estimation, so estimates from the random-effects model are provided for completeness.

We summarised, without any further re-analysis, studies that developed or externally validated risk scores for predicting Ebola virus infection. Scores were used to identify individuals with a higher or lower risk of Ebola virus infection during screening at Ebola health facilities. To obtain the risk scores, these studies used the regression coefficients of independent risks obtained by multivariable logistic regression against Ebola virus infection and then converted regression coefficients into an integer-based point-scoring system. Reviewed studies assigned positive and negative risk scores with calculated AUC to epidemiological, demographic, and clinical characteristics. Positive values indicated higher risk of Ebola virus infection and negative values indicated higher risk of another infection such as malaria or typhoid.

Values assigned to the risk score varied by study; therefore, a meta-analysis of risk scores was not done, but instead evidence was systematically reviewed. For comparability, we reclassified the risk scores reported in the included studies into categories, from very low risk to very high risk (appendix p 7). STATA 15 was used for statistical analysis.

PRISMA guidelines for Diagnostic Test Accuracy Studies (PRISMA-DTA) were followed (appendix pp 2–4) (21).

Role of the funding source

GC, KL, AS, and JG were employed by the funder, and participated in planning the study, carrying out the research, and writing the report. The funder of the study had no further 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.

4.5.3 Results

Of the 2493 studies initially screened using the article title, 143 were deemed to be potentially eligible on the basis of the abstract, and their full-text articles were assessed. Of these studies, 18 met the inclusion criteria, but three were excluded because data on sensitivity and specificity could not be extrapolated (appendix p 8). One was excluded because it is yet unpublished (FG). Of the 14 included studies, 11 were full manuscripts (5,6,22,24,25,27,29–33), one a letter (28), one an oral plenary abstract (26), and one a conference poster (23) (the author of this poster was also contacted and they provided an abstract with additional data [Kuehne A, Epicentre, Paris, France, personal communication]; table 10). 13 studies were published between 2015 and 2019 and assessed Ebola virus disease in the west Africa outbreak (seven in Sierra Leone (5,6,25,26,30,32,33), four in Guinea (24,27,28,31), and two in Liberia (23,29)). The remaining article was published in May, 2010, assessing Marburg virus in Angola (22).

Overall, 12 021 people with suspected disease were included, of whom 4874 were confirmed as positive for Ebola virus infection. Study populations varied from 75 to about 2847 (table 10). All studies, apart from the national surveillance study, included patients who presented alive to health facilities for assessment. The national surveillance study included all cases (suspected, probable, and confirmed), including patients both alive and deceased, identified in both the community and health facilities. Eight studies' data were from single Ebola treatment centres (23,27–33), with the remaining using a national surveillance list (24), three from Ebola holding units (5,25,26), and two from hospitals screening patients for Ebola virus disease while still functioning as general health facilities (6,22). All studies covered distinct patient groups from different periods and geographical areas, except for two studies from Guinea (24,27). Although these two studies covered overlapping patient groups, they reported on different clinical and epidemiological characteristics (WHO case definition performance vs symptom performance) (24,27).

All selected manuscripts analysed all ages combined, except one author who assessed, in two different studies, the sensitivity and specificity of 2014 WHO Ebola case definitions and also developed a risk score specifically for the paediatric population (younger than 13 years) (25,26).

Six studies explored the performance of a WHO case definition in non-paediatric populations (5,22,24,29–31). In all of these studies, suspected and probable cases were combined and could not be

disaggregated for analysis. The following results therefore apply to this combined group of suspected and probable cases. The pooled sensitivity was 81·5% (95% CI 74·1–87·2) and pooled specificity was 35·7% (28·5–43·6; figure 8). One study assessed WHO 2014 case definitions for a paediatric population (younger than 13 years old); the sensitivity was 98·0% (95% CI 95·0–99·0) and specificity was 5·0% (3·0–7·0) (25).

When WHO subdefinitions were assessed, history of contact and symptoms had high specificity compared with clinical symptoms alone, ranging from 62·3% (95% CI 49·8–73·5) to 94·4% (95% CI not provided in original paper; table 11). The highest sensitivity (100·0%) was documented for the WHO subdefinitions in which fever was not mandatory. Among studies using clinical symptoms and signs alone, the definition including three or more symptoms (intense fatigue, confusion, conjunctivitis, hiccups, diarrhoea, and vomiting) had the highest specificity (79·1% [95% CI not provided in original paper]). Unexplained death had high specificity (92·8% [95% CI not provided in original paper]) but the lowest sensitivity (14·2% [95% CI not provided in original paper]; table 11).

For children, the highest specificity (97·0% [95% CI not provided in original paper]) was with a case definition of contact, fever, and conjunctivitis, or contact, fever, anorexia, and two of abdominal pain, diarrhoea, or male sex (older than 2 years; table 11) (26).

Seven articles developed a risk score (22,23,25,29,31–33) and among those five (25,29,31–33) did an internal validation (using bootstrap or test and training methods) and one assessed a risk score according to outbreak prevalence in a paediatric population (25). An eighth study (28) externally validated the score developed by Oza and colleagues (33) without developing an alternative score. Of the 44 potential predictors of Ebola virus infection included across the seven studies that developed risk scores, 20 were found to be positive or negative predictors (figure 9). The score system ranged from very low to very high risk, with intermediate categories varying across studies (appendix p 7).

One study created a malaria sensitive score aiming to discriminate between Ebola virus infection and malaria infection, which indicated a predictor power of 89·6% (95% CI 86–93) to discriminate Ebola virus positive versus negative, reaching a discrimination power of 98·5% (95% CI not provided in original paper) during the malaria season (32). The same study obtained similar results (AUC 76·8% [95% CI not provided in original paper] vs 75·0% [70·0–80·0]), when externally validating the scores developed by Levine and colleagues (29,32).

The study validating Oza and colleagues' algorithm found poorer performance in their cohort (AUC 58% [95% CI 56–61] vs 83·0% [79–86]) (28,33).

The highest performing score was developed by Hartley and colleagues (32), a key difference being referral time (figure 9). For the adult population (six studies 22,23,29,31–33), a positive risk score for infection was associated in more than one study with each of the following five characteristics:

epidemiological link (eg, history of contact), diarrhoea, conjunctivitis, unexplained bleeding, difficulty swallowing (also called dysphagia; figure 9).

Fever was assessed at different thresholds (>38·0°C or ≥38·5°C), and inclusion of fever in the final predictive score was only reported by two studies (31,32) (figure 9). Discordant values were assigned across studies (either positive or negative) for anorexia or loss of appetite, muscle pain (also called myalgia), and abdominal pain.

For the paediatric population (one study 25), positive predictors were age (2 years or older), sex (male), epid-emiological link, diarrhoea, conjunctivitis, fever (>38·0°C), anorexia or loss of appetite, and abdominal pain. Negative predictors were difficulty swallowing, rash, headache, and difficulty breathing (also called dyspnoea; figure 9). The same study compared two different time periods over the Ebola virus disease 2014–16 outbreak in Sierra Leone (high prevalence in October, 2014 [77% of suspected cases testing positive], and low prevalence in March, 2015 [4% of suspect cases testing positive]): a low cutoff for the risk score (with high sensitivity) performed better at periods of high prevalence transmission, and a high cutoff with high specificity performed better during low prevalence (25). Similarly, the positive predictive value decreased from 93% to 31%, and the negative predictive value increased from 23% to 90% when comparing high (early) to low (late) transmission periods in the Ebola virus disease outbreak in another study in Liberia in an all ages population (23).

Eight studies measured sensitivity and specificity of individual symptoms at admission, assessing a total of 35 symptoms (5,22–24,27,29–31). The pooled sensitivity per symptom ranged from 79·0% (95% CI 74·4–83·0) for intense fatigue (seven studies) to 1·0% (0·0–7·0) for pain behind the eyes (three studies). By contrast, the pooled specificity ranged from 98·0% (95% CI 91·0–100·0) for pain behind the eyes to 32·3% (95% CI 25·8–39·4) for intense fatigue (appendix p 9).

Haemorrhagic symptoms and signs were the most specific indicator of infection. Other symptoms and signs with high specificity included confusion, coma, hiccups, rash, and sore throat with specificity ranging from 92·0% (95% CI 91·0–94·0) for hiccups to 97·8% (95% CI 95·2–99·0) for rash (appendix p 9). Performance of fever was assessed by seven studies, but each one used a different definition of fever (5,22,23,27,29–31). The optimal performance (definition that achieved best balance between maximising sensitivity vs maximising specificity) for fever was a threshold at ≥38·5°C (sensitivity 80·2% [95% CI 69·2–88·2]; specificity 82·6% [71·2–90·3]; table 12).[31] In the random- effects analysis, a threshold at greater than 38·0°C (three studies 22,27,29) gave a pooled sensitivity of 80·0% (95% CI 69·0–90·0) and specificity of 25·0% (17·0–33·0; table 12).

Seven studies assessed sensitivity and specificity of an epidemiological link (5,22–24,29–31). Across these studies, the sensitivity of an epidemiological link ranged from 21·6% (95% 17·9–25·6) to 100·0% and specificity ranged from 29·0% (95% CI 19·0–41·3) to 86·0% (95% CI 74·0–94·0). The most sensitive definition was history of contact with a person with confirmed Ebola virus infection (100·0%; table 12).

The most specific definition was direct contact with an individual potentially infected with Marburg virus or his or her body fluids, or direct contact during funeral practices (22).

4.5.4 Discussion

Our results indicate that, for all ages combined, the WHO case definitions have a sensitivity of 81·5% and a specificity of 35·7%. The sensitivity is not high enough to achieve acceptable false negative rates, particularly in low-prevalence settings, the primary requirement for community-based screening. The low specificity results in high numbers of false positives and thus potentially unnecessary admissions to Ebola treatment centres, with associated risk of nosocomial transmission and costs of managing suspected cases (1). As a consequence, a large number of people who do not have Ebola virus disease will experience unnecessary invasive procedures, risk of being infected with Ebola virus, isolation from family, fear of being stigmatised, and delay to appropriate care, and community mistrust in response activities will increase.

In our meta-analysis, fever had low specificity (25·0%), except for when defined as a threshold at 38·5°C or more (82·6%), and the WHO case subdefinition had 100% sensitivity only when fever was not a mandatory criterion. In the risk score systematic review, the association of fever with Ebola virus infection was not consistent across studies, with only two studies including it in the final predictive score. Presence of fever is likely to be related to the stage of infection at admission, with previous studies reporting absence of fever in a large proportion of suspected cases at admission (34). This finding is consistent with a recent Ebola seminar reporting that fever was absent in at least 10% of the cases in the west Africa outbreak (35).

Therefore, exclusion of fever from the case definition at the community level is likely to increase the sensitivity of the case definition. Intense fatigue was the most sensitive symptom (79·0%) that could be used at the community level to facilitate early referral of suspected cases and prevent community transmission.

The meta-analysis did not identify any individual symptom or sign having an optimal trade-off between sensitivity and specificity. Conjunctivitis, unexplained bleeding, difficulty swallowing, and diarrhoea were individual symptoms and signs with the best discriminatory accuracy in the studies that explored risk score for the all-age population and with the exception of diarrhoea all had high specificity (>80%) in the studies that explored their performance. However, these symptoms and signs could also be a proxy for late-stage disease when the virus infects endothelial cells, compromising vascular integrity, with massive tissue injury resulting in disseminated intravascular coagulopathy with risk of thrombosis, bleeding, and damage to the adrenal glands and gastrointestinal system (36–38). These symptoms and

signs could enable health practitioners to prioritise patients for admission to an Ebola treatment centre when resources are scarce but are less useful at the community level because they appear at a late stage of the disease when transmission risk is the highest.

None of the studies assessed miscarriage, despite it being included in the December, 2014, WHO case definition (15). History of miscarriage and other associated pregnancy complications (eg, stillbirth) could help to identify cases that can be a major source of nosocomial transmission in general health facilities (39).

Although only one study focused on a paediatric population, this study used data from 11 Ebola holding units and included a large population of children (1006), providing useful guidance for this age group (26).

The WHO paediatric definition had very high sensitivity (98·0%) but very poor specificity (5·0%). When the same authors assessed a WHO subdefinition (including contact, fever, and conjunctivitis, or contact, fever, anorexia, and two of abdominal pain, diarrhoea, or male sex [older than 2 years]), the sensitivity dropped markedly to 23·0% but the specificity improved to 97·0%. The optimal fever temperature cutoff for the paediatric population was not explored. However, in another study of a paediatric population of patients with confirmed Ebola virus disease admitted to one Ebola treatment centre in Sierra Leone, 25% of children aged 5 years and younger were afebrile (40). This difference might be due to several factors: how fever was assessed (either reported in their history or measured at admission), age groups included (younger than 13 years vs younger than 5 years), period of data collection (August–March, 2015, vs June–Dec, 2014) when seasonality of other febrile illnesses could have influenced fever prevalence, background Ebola virus transmission rates, and viraemia at admission and time since onset of symptoms.

The paediatric analysis did not explore sensitivity and specificity of individual symptoms and signs at admission for children. Alongside the fact that they might have different clinical presentations compared with adults, children are more likely to experience adverse outcomes from Ebola virus disease and are less able to report symptoms and history of contact.

Similarly, pregnant women with non-Ebola virus disease-related complications usually present with symptoms (such as bleeding and abdominal pain) that mimic Ebola virus infection (39). As suggested elsewhere, the paediatric and pregnant women populations might require adaptation of case definitions that take into account their specific characteristics (41–43). None of the selected manuscripts explored the performance of WHO Ebola case definitions among pregnant women. Therefore, further evidence specifically applicable to children and pregnant women is required to develop appropriate tools for screening for Ebola virus disease in these populations.

Reported history of contact was a strong predictor for paediatric and adult populations, often performing better than many of the clinical symptoms included in accepted case definitions, as also reported by other studies (44). However, it is likely that this is an underestimate of the potential performance of actual contact history in screening for Ebola virus disease.

Levels of disclosure of self-reported clinical information and contact history depend on community engagement with intervention strategies, including trust in the health-care provider. Therefore, to improve WHO case definition performance, effective and trusted collaboration with communities is essential to ensure reliable understanding and reporting of such crucial epidemiological information. Equally, it is the responsibility of response agencies to understand the underlying pattern of Ebola virus transmission, local traditions, coping mechanisms, and family dynamics in order to identify people at risk of infection. Genetic sequencing has also been put forward as a tool for identifying chains of transmission when contact history is unknown(45).

One of the limitations in interpreting the results of this meta-analysis is that all the evidence reviewed, apart from the national surveillance study, came from patients triaged at health facilities or Ebola isolation centres. Thus, this meta-analysis might represent only cases with severe symptoms, limiting generalisability to the performance of these screening criteria at the community level and in early stages of disease. Second, there was significant heterogeneity between selected studies, and considerable variation in the quality of data on clinical symptoms and recollection of patients' history, with different variables and thresholds used in each study, and limited data on co-infection. For example, fever is a key symptom in the WHO case definitions, but different temperatures were used to define fever, which could explain the between-study heterogeneity. Inconsistency on thresholds for fever and the decision to include fever or not have been reported in the Democratic Republic of the Congo and in four neighbouring countries (9).

For the two studies with overlapping patient populations, performance of WHO case definitions was assessed only using national surveillance data, with Ebola treatment centre data for these patients being assessed for only individual symptoms or WHO subdefinitions. These two studies were therefore not included together in pooled estimations, so the cohort overlap would not have affected results. Individual studies mentioned small sample size and poor quality of data as part of their limitations.

A range of contextual factors related to study setting will affect the performance of Ebola virus disease case definitions, including seasonally occurring diseases such as malaria and Lassa fever, which have a similar clinical presentation to Ebola virus disease. Such factors will affect the generalisability of our findings to other settings. In addition, only two of the recommended risk scores were externally validated (28,32), limiting the generalisability of those scores because performance appears to vary across outbreak periods and populations.

Finally, there is potential for publication language bias because we considered only studies in English. However, for Guinea, a French-speaking country, we included data from national surveillance and two major Ebola treatment centres; therefore, we consider that bias due to language restrictions was minimised in our results. We included peer-reviewed abstract and poster data to capture data on paediatric populations and additional evidence for all age cohorts, and we sought unpublished evidence from French- speaking countries.

This systematic review is relevant to inform public health practitioners in the current Ebola virus disease outbreak in the Democratic Republic of the Congo, in which only 8% of suspected cases isolated are confirmed, possibly because of inconsistent use of WHO case definition at community and health facility levels (46).

In conclusion, this first systematic review and meta-analysis of the strengths and limitations of the WHO Ebola virus disease case definitions highlights the need for further studies to assess consistent thresholds for fever, to explore viraemia and symptoms and signs at admission, and to externally validate risk scores for Ebola virus infection. The sensitivity and specificity of WHO Ebola case definitions could be improved by excluding fever and instead including both intense fatigue and history of contact. However, reliable disclosure of reported symptoms and history of contact requires effective collaboration with, and the trust of, affected communities. To achieve this trust and collaboration, responding organisations must recognise the paramount role of communities in controlling transmission and ending outbreaks. We also identified important gaps related to the paediatric and pregnant population, which must be addressed through future research.

Contributors

GC, KL, and HAW conceived the idea of this study. GC and FT undertook the literature review and extracted the data with help from LI. GC wrote the first and final drafts of the manuscript. GC, KL, HAW, and GLDT contributed to the analysis and interpretation of the data. KL, HAW, FG, KD, BP, GK, AS, JG, and GLDT reviewed early and late drafts of the manuscript, and all authors have given signed or electronic approval to be authors on the manuscript.

Declaration of interests

We declare no competing interests.

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4.5.5 Research in context

Evidence before this study

There have been long-standing concerns about the poor performance of WHO case definitions for Ebola virus disease, including their inability to distinguish Ebola virus infection from common tropical diseases. We did a systematic search of the scientific literature using PubMed, Scopus, Embase, and Web of Science, without regional restrictions, for research articles published in English between June 13, 1978, and Jan 14, 2020. We used the search terms "Ebola", "EVD infection", "case definition", "admission symptoms", "sensitivity", "specificity", "likelihood", "score", "classification", "validity" and "performance". We also contacted relevant experts. We found that different organisations have attempted to assess the performance of WHO Ebola case definitions and developed alternative definitions and risk scores. However, there has been no systematic and rigorous evaluation of those studies. Such an evaluation is needed to guide communities and public health practitioners to improve the effectiveness and efficiency of identification and management of suspected cases during an Ebola virus disease outbreak.

Added value of this study

To our knowledge, this study is the first systematic review and meta-analysis that assesses the performance of the WHO Ebola virus disease case definitions, and other clinical and epidemiological characteristics such as symptoms and signs at admission and contact history, against the reference standard (laboratory confirmation of Ebola virus infection). Our analysis provides the most comprehensive evidence on the limitations of WHO case definitions and its constituent symptoms and signs, and predictive risk scores. We show that the WHO case definitions perform sub optimally to identify cases at both the community level and during triage at general and specialist health facilities. The performance of fever as a symptom varied depending on the cut off used to define fever. The most sensitive symptom was intense fatigue. History of contact was a key predictor for the WHO case definitions and for risk scores. This study identifies important gaps related to the paediatric and pregnant population and highlights the need to use consistent thresholds (e.g., for fever) to explore viraemia and symptoms at admission, and to externally validate risk scores for Ebola virus infection.

Implications of all the available evidence

Inclusion of intense fatigue as a key symptom could improve the sensitivity, the primary requirement for community-based screening, of WHO and alternative case definitions. Inclusion of contact history will improve specificity, resulting in a lower number of false positives and thus a lower number of unnecessary admissions to Ebola health facilities. These improvements will contribute to reduced isolation from family, fear of being stigmatised, delay to appropriate care, and community mistrust in response activities.

Figure 7. WHO Ebola virus disease case definitions for all ages and the paediatric population

	WHO case definitions (August, 2014) all ages[12]	**WHO case definition (December, 2014) all ages in Sierra Leone[15]**	**Late 2014 WHO case definition for paediatric population in Sierra Leone[15]**
Suspected	Any person, alive or dead, suffering or having suffered from sudden onset of high fever and having had contact: • a suspect, probable, or confirmed Ebola virus disease case • with a dead or sick animal (for Ebola) • a mine (for Marburg); OR any person with sudden onset of high fever and at least three of the following symptoms: • headaches • lethargy • anorexia or loss of appetite • aching muscles or joints • stomach pain • difficulty swallowing • vomiting • difficulty breathing • diarrhoea • hiccups; OR any person with inexplicable bleeding; OR any sudden, inexplicable death	Any person having had contact with a clinical case and presenting with acute fever (>38°C); OR having had contact with a clinical case (suspected, probable, or confirmed) and presenting with three or more of the symptoms below; OR presenting with acute fever and presenting with three or more of the symptoms below: • headache • nausea or vomiting • loss of appetite • diarrhoea • intense fatigue • abdominal pain • generalised or articular pain • difficulty in swallowing • difficulty in breathing • hiccups • miscarriage; OR any person with unexplained bleeding or miscarriage; OR any unexplained death	Any child with fever and either one symptom (in children younger than 5 years), two symptoms (in children aged 5–12 years), or more than three symptoms (in children older than 12 years); for children younger than 1 years old, maternal history is very important
Confirmed	Any suspected or probable cases with a positive laboratory result; laboratory-confirmed cases must test positive for the virus antigen, either by detection of virus RNA by RT-PCR, or by detection of IgM antibodies directed against Marburg or Ebola	Any person with a positive PCR test for Ebola or Marburg virus	Any person with a positive PCR test for Ebola or Marburg virus
Probable	Any suspected case evaluated by a clinician; OR any deceased suspected case (where it has not been possible to collect specimens for laboratory confirmation) having an epidemiological link with a confirmed case	A suspect case that is known to have had contact with a known case (suspected, probable, or confirmed); OR any person who is, on clinical or epidemiological grounds, very likely to have Ebola or Marburg	Not further specified

Table 10. Overview of articles included in the systematic review and meta-analysis

	Country	Virus	Period of data collection	Design	Objective	Outcomes	Setting of data collection	Age of study population	Patients positive for Ebola virus/ total patients	Method (reference standard) and timing of Ebola virus confirmatory testing	Limitations
Roddy et al (2010)[22]	Angola	Marburg	March–July, 2005	Observational retrospective study of data at admission	Evaluate the diagnostic validity of individual patient clinical and epidemiological characteristics and WHO-recommended case definitions for Marburg haemorrhagic fever, and develop a data-derived diagnostic algorithm for Marburg haemorrhagic fever that improves the WHO-recommended definitions	Sensitivity and specificity of WHO case definition, WHO case subdefinitions, symptoms at admission, and epidemiological link; and risk score	Screening at one hospital	All ages	41/102	Quantitative PCR on admission	Small sample; only saw patients at admission; data only captured Marburg haemorrhagic fever; hospital-based data collection; detailed data not available for all Marburg haemorrhagic fever cases; only presenting symptoms were recorded; highlights the necessity of collecting high-quality clinical and epidemiological data during outbreaks; over-representation of individuals with more serious symptoms that required hospital admission; no reported validation (external or internal)
Kuehne et al (2015)[23]	Liberia	Ebola	August, 2014–March, 2015	Observational retrospective study of data at admission and clinical results	Study the discriminative accuracy (sensitivity, attributable frequency, diagnostic test odds ratio, area under the receiver operating characteristic curve) of clinical signs, contact history, and combinations thereof	Sensitivity and specificity of WHO case subdefinitions, symptoms at admission, and epidemiological link; and risk score	One Ebola treatment centre	All ages	1235/1832	Quantitative PCR on admission	Reporting bias; poor data quality; conference poster and abstract data (Kuehne A, Epicentre, Paris, France, personal communication); no reported validation (external or internal)
Levine et al (2015)[29]	Liberia	Ebola	September, 2014–January, 2015	Observational retrospective study of data at admission	Develop a clinical prediction model that can help to guide care for patients with suspected Ebola virus disease,	Sensitivity and specificity of WHO case definition, symptoms at	One Ebola treatment centre	All ages	160/382	Quantitative PCR on admission	Data collected only at admission, different stages of disease process; data might not be representative of all patients

					provide specific parameters for isolation and admission to treatment centres, and maximise resource use	admission, and epidemiological link; and risk score					with Ebola virus disease; poor data quality; small sample; patients pre-screened by Ebola treatment units (ambulance travel); only assessed 14 variables; no reported external validation, only internal validation
	Country	**Virus**	**Period of data collection**	**Design**	**Objective**	**Outcomes**	**Setting of data collection**	**Age of study population**	**Patients positive for Ebola virus/total patients**	**Method (reference standard) and timing of Ebola virus confirmatory testing**	**Limitations**
Lado et al (2015)[s]	Sierra Leone	Ebola	May, 2014–December, 2014	Observational retrospective study of data at admission	Identify clinical characteristics that were predictive of Ebola virus disease diagnosis and assess the accuracy of suspected Ebola virus disease case definitions	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link	One Ebola holding unit	All ages	464/724	Quantitative PCR on admission	Small sample; poor accuracy on reporting of symptoms and history; no access to patients who chose not to present to hospital or did not have access; no reported validation (external or internal)
Arranz et al (2016)[30]	Sierra Leone	Ebola	December, 2014–March, 2015	Observational retrospective study of data at admission	Compare the clinical characteristics of confirmed cases (patients with Ebola virus disease) and non-confirmed cases (patients without Ebola virus disease), assess the diagnostic validity of initial symptoms used in WHO case definition to diagnose Ebola virus disease in a low-incidence situation	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link	One Ebola treatment centre	All ages	31/75	Quantitative PCR on admission	Only data at admission; poor data quality; retrospective design; small sample; no reported validation (external or internal)
Loubet et al (2016)[31]	Guinea	Ebola	December, 2014–February, 2015	Observational retrospective study of data at admission	Identify epidemiological, sociodemographic, and clinical variables associated with Ebola virus disease diagnosis and to create, based on these variables, a predictive score for identification of confirmed Ebola virus disease	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link; and risk score	One Ebola treatment centre	All ages	76/145	Quantitative PCR on admission	Data collected only at admission; poor data quality; retrospective design; patients included might have been reluctant to come to the Ebola treatment centre, and thus were more likely to present severe clinical presentation with late symptoms; temperature taking might be affected by several factors; small sample size; anorexia and temperature (the factors that in that study

											were associated with an increased likelihood of Ebola virus disease) are not easy to measure and interpret; no reported external validation, only internal validation
	Country	**Virus**	**Period of data collection**	**Design**	**Objective**	**Outcomes**	**Setting of data collection**	**Age of study population**	**Patients positive for Ebola virus/total patients**	**Method (reference standard) and timing of Ebola virus confirmatory testing**	**Limitations**
Hartley et al (2017)[32]	Sierra Leone	Ebola	December, 2014–November, 2015	Observational retrospective study of data at admission	Construct an easy-to-use and highly accurate triage scoring system that discriminates Ebola virus infection risk in a malaria-sensitive manner and improve the predictive accuracy for Ebola virus disease and malaria	Risk score	One Ebola virus treatment centre	All ages	158/566	Quantitative PCR on admission; rapid diagnostic malaria test (histidine-rich protein-II antigen rapid diagnostic kits were used)	Only the most prevalent symptoms at admission were included in the score; poor data quality; did not fully cover all the malaria season because the Ebola treatment centre was opened from December to June; recall bias
Fitzgerald et al (2017)[26]	Sierra Leone	Ebola	August, 2014–March, 2015	Observational retrospective study of data at admission	Refine the case definition and describe outcomes of admitted children	Sensitivity and specificity of WHO case subdefinitions	11 Ebola holding units	Paediatric population (younger than 13 years)	309/1006	Quantitative PCR on admission	Only included children younger than 13 years; oral plenary abstract; no reported external validation, only internal validation
Ingelbeen et al (2017)[27]	Guinea	Ebola	March, 2014– September, 2015	Observational retrospective study of data at admission	Describe the burden of non-cases in relation to the phase of the outbreak; determine the duration of their stay at the Ebola treatment centre and (potential) subsequent nosocomial infections; compare characteristics, outcome, and risk factors for death in confirmed cases and non-cases to improve the selection, diagnosis, and care of people with suspected Ebola virus disease	Sensitivity and specificity of WHO case subdefinitions and symptoms on admission	One Ebola treatment centre	All ages	822/2362	Quantitative PCR on admission; Xpert Ebola Assay (Cepheid GeneXpert, Sunnyvale, CA USA) on admission	The Ebola treatment centre for part of the outbreak was located within one hospital but then moved to another area in July; could not assess possible drivers for the large proportion of non- cases; no reported validation (internal or external)
Oza et al (2017)[33]	Sierra Leone	Ebola	November, 2014–March, 2015	Observational retrospective study of data at admission	Develop two Ebola risk scores to supplement the broad WHO case definition by further separating triaged patients based on their likelihood of being positive for Ebola virus	Risk score	One Ebola treatment centre	All ages	252/424	Quantitative PCR on admission; biochemistry laboratory tests with the Piccolo Xpress (Abaxis, Union City, CA, USA) and i-STAT (Abbott Point of Care, Princeton, NJ, USA) device	Only one treatment centre; investigated 14 commonly recorded symptoms; small amount and poor quality of patient data; excluded exposure as a potential predictor because of large amount of missing data or poor data quality; patients might not be representative of the overall population of suspect Ebola cases; no reported external validation, only internal validation

	Country	Virus	Period of data collection	Design	Objective	Outcomes	Setting of data collection	Age of study population	Patients positive for Ebola virus/total patients	Method (reference standard) and timing of Ebola virus confirmatory testing	Limitations
Hsu et al (2018)[24]	Guinea	Ebola	March– October, 2014	Observational retrospective study of surveillance data	Assess the diagnostic performance of the WHO suspected case definition by using epidemiological surveillance and diagnostic test	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link	National surveillance line list	All ages	1304/2847	Quantitative PCR (on admission and for deceased patients at the community level)	Unknown how representative the database was for all patients with Ebola virus disease; only 1412 patients had complete data to assess and analyse the WHO case definition; possible overestimation of performance of WHO definition because only common symptoms were recorded in the early stage of the outbreak; poor data quality; no reported validation (internal or external)
Fitzgerald et al (2018)[25]	Sierra Leone	Ebola	August, 2014– March, 2015	Observational retrospective study of data at admission	Develop a predictive score that could be used to tailor the paediatric case definition for suspected Ebola virus disease according to the clinical and epidemiological setting	Sensitivity and specificity of WHO case definition and risk score	11 Ebola holding units	Paediatric population (younger than 13 years)	309/1006	Quantitative PCR on admission	Only included children younger than 13 years; poor data quality; no data on the true Ebola status of people who did not meet the WHO case definition and were not admitted; no reported validation, only internal validation
Ingelbeen et al (2018)[28]	Guinea	Ebola	March, 2014– September, 2015	Observational retrospective study of data at admission	Validate risk score by Oza and colleagues[13]	Risk score	One Ebola treatment centre	All ages	805/2311	Quantitative PCR on admission; Xpert Ebola Assay (Cepheid GeneXpert) on admission	Did not propose another algorithm; letter; no reported external validation, only internal validation
Huizenga et al (2019)[6]	Sierra Leone	Ebola	September, 2014– November, 2015	Observational retrospective study of data at admission	Evaluate the pre-existing health-care infrastructure during the Ebola virus disease outbreak, and assess the provided health care and safeguard functionality of a health-care system for all patients not suspected to have or diagnosed with Ebola virus disease	Sensitivity and specificity of WHO case subdefinitions	Screening at one hospital	All ages	22/1556	Quantitative PCR on admission	Scant description of data; poor data quality; no reported validation (external or internal)

Figure 8. HSROC summary of sensitivity and specificity

HSROC=hierarchical summary receiver operating characteristic.

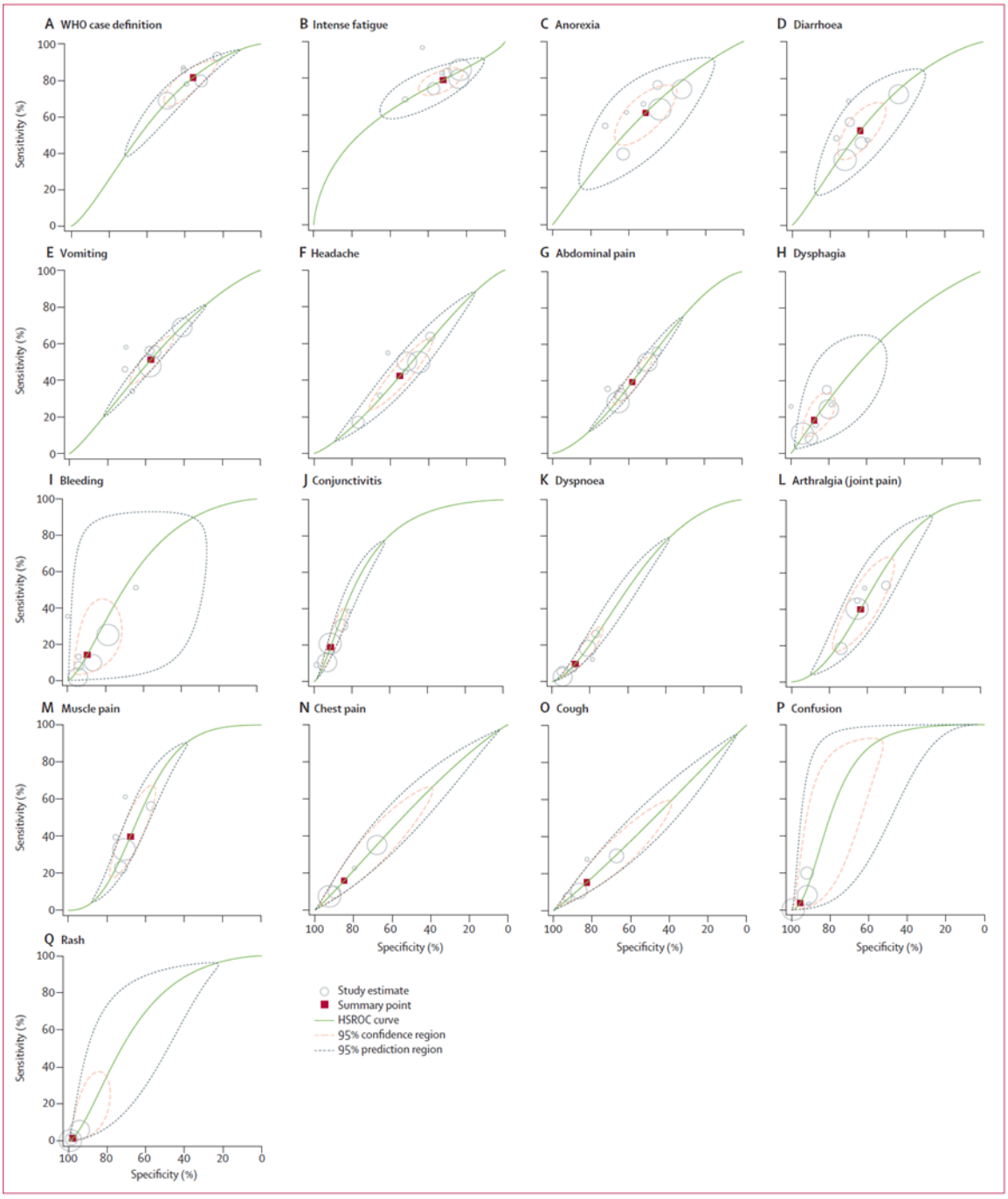

Table 11. Sensitivity and specificity of WHO Ebola virus disease subdefinitions against reference standard of laboratory-confirmed Ebola virus infection, in decreasing order of sensitivity

	WHO subdefinition	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Huizenga et al (2019)[6]	WHO definition, with the difference that fever with sudden onset is not a mandatory criterion	100·0%	42·5%*	2·4%*	100·0%
Fitzgerald et al (2017)[26]	Contact alone, fever (in children older than 2 years) OR fever and conjunctivitis (in children younger than 2 years)	94·0%*	35·0%*	Not provided	Not provided
Roddy et al (2010)[22]	Epidemiological link or a combination of myalgia or arthralgiaand any haemorrhage	79·0% (64·0–91·0)	73·0% (60·0–84·0)	Not provided	Not provided
Loubet et al (2016)[31]	WHO subdefinition 2 (temperature ≥37·5°C plus risk factor†)	75·0% (63·5–83·9)	62·3% (49·8–73·5)	Not provided	Not provided
Roddy et al (2010)[22]	WHO case definition (clinical criteria only‡)	73·0% (57·0–86·0)	43·0% (30·0–56·0)	Not provided	Not provided
Roddy et al (2010)[22]	Fever plus three or more symptoms§	68·0% (52·0–82·0)	46·0% (33·0–59·0)	Not provided	Not provided
Loubet et al (2016)[31]	Temperature ≥38·5°C plus risk factor†	68·4% (56·6–78·3)	82·6% (71·2–90·3)	Not provided	Not provided
Arranz et al (2016)[30]	Contact and three symptoms§	67·7% (51·3–84·2)	81·8% (70·4–93·2)	72·4% (56·1–88·7)	78·3% (66·3–90·2)
Loubet et al (2016)[31]	WHO subdefinition 3 (temperature ≥37·5°C plus clinical symptoms§)	67·1% (55·2–77·2)	76·8% (64·8–85·8)	Not provided	Not provided
Loubet et al (2016)[31]	WHO subdefinition 1 (risk factor plus clinical symptoms§)	63·2% (51·3–73·7)	66·7% (54·2–77·3)	Not provided	Not provided
Lado et al (2015)[5]	Three or more major symptomsfj	57·8% (52·1–61·4)	70·8% (64·7–76·4)	77·9% (73·1–82·3)	47·5% (42·3–52·7)
Arranz et al (2016)[30]	Fever and three symptoms§	58·1% (40·7–75·4)	50·0% (35·2–64·8)	45·0% (29·6–60·4)	62·9% (46·8–78·9)
Hsu et al (2018)[24]	Clinical criteria§	57·2%*	62·0%*	66·4%*	52·5%*
Ingelbeen et al (2017)[27]	WHO case definition (clinical criteria only‖)	56·9%*	46·4%*	36·3%*	66·8%*
Roddy et al (2010)[22]	Epidemiological link and two or more general symptoms§	54·0% (37·0–70·0)	91·0% (80·0–97·0)	Not provided	Not provided
Roddy et al (2010)[22]	Epidemiological link and three or more general symptoms§	54·0% (37·0–70·0)	93·0% (83·0–98·0)	Not provided	Not provided
Arranz et al (2016)[30]	Contact plus fever	48·4% (30·8–66·0)	77·3% (64·9–89·7)	60·0% (40·8–79·2)	68·0% (55·1–80·9)
Roddy et al (2010)[22]	Fever plus haemorrhage	44·0% (28·0–60·0)	72·0% (59·0–83·0)	Not provided	Not provided
Ingelbeen et al (2017)[27]	Three major signs**	27·7%*	79·1%*	41·5%*	67·2%*
Fitzgerald et al (2017)[26]	Contact, fever, and conjunctivitis OR contact, fever, anorexia, and two of abdominal pain, diarrhoea, or male sex (older than 2 years)	23·0%*	97·0%*	Not provided	Not provided
Kuehne et al (2015)[23]	History of contact, gastrointestinal symptoms†† and illness duration of >3 days	20·0%*	94·4%*	Not provided	Not provided
Hsu et al (2018)[24]	Unexplained death	14·2%*	92·8%*	72·0%*	45·2%*

*95% CI not provided in the original paper. †For example, being a health worker, have attended a funeral, and having contact with a relative suspect of having Ebola virus.

‡Fever plus three other symptoms or fever and haemorrhage. §Symptoms or criteria not specifed in original paper. fjThree or more symptoms among the following: intense fatigue, confusion, conjunctivitis, hiccups, diarrhoea, or vomiting. ||Acute fever and presenting three or more of the following: headache, anorexia or lack of appetite, lethargy, muscle or joint pain, breathing difficulties, vomiting, diarrhoea, stomach ache, difficulty swallowing, and hiccups; or any person with unexplained bleeding.

**As proposed by Lado and colleagues.[5] ††Diarrhoea, vomiting, and anorexia or loss of appetite.

Figure 9. Overview of risk score by symptoms and epidemiological characteristics

	AUC (95% CI) on own study database	AUC (95% CI) of Levine et al algorithm[29] on Hartley et al[32] database	AUC (95%CI) of Oza et al algorithm[33] on Ingelbeen et al[26] database	Epidemiological link	Referral (4–9 days)	Days since first symptom	Duration of illness >3 days	Gastrointestinal symptoms*	Male sex	Age (≥2 years)	Age (<2 years)	Diarrhoea	Conjunctivitis	Fever (>38·0°C)	Unexplained bleeding	Nausea or vomiting	Fever (≥38·5°C)
Hartley et al (2017)[32]	89% (86–93)	NA	NA	6	3	Y	NA	NA	NA	NA	NA	3	4	1	2	Y	NA
Oza et al (2017)[33]	83% (79–86)†	NA	58% (56–61)	NA	NA	NA	NA	NA	NA	NA	NA	2	2	Y	Y	1	NA
Loubet et al (2016)[31]	82% (77–87)	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	Y	NA	Y	NA	3
Fitzgerald et al (2018; paediatricpopulation)[25]	80%‡	NA	NA	2	NA	Y	NA	NA	1	2	Y	1	2	1	Y	Y	NA
Levine et al (2015)[29]	75% (70–80)	76%‡	NA	2	NA	NA	NA	NA	NA	NA	NA	1·5	NA	Y	Y	Y	NA
Kuehne et al (2015)[23]	53–59‡	NA	NA	+	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA
Roddy et al (2010)[22]	‡	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	Y	Y	Y	+	Y	NA

(Figure 9 continues on next page)

	Joint pain	Anorexia or loss of appetite	Muscle pain	Difficulty swallowing	Abdominal pain	Rash	Headache	Difficulty breathing	Fatigue, weakness, or asthenia	Hiccups	Cough	Diarrhoea or vomiting	Epigastralgia	Anuria	Haematuria	Disorientation	Hepatomegaly	Haemoptysis	Malaria infection	ORL haemorrhage	Dehydration	Haematochezia	Joint or muscle pain	Bleeding at injection site	Bloody gingivitis	Jaundice	Non-menstrual vaginal bleeding	Bloody diarrhoea	Haematemesis	Epistaxis
Hartley et al (2017)[32]	NA	Y	−2	2	Y	Y	Y	Y	Y	Y	NA	NA	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA
Oza et al(2017)[33]	NA	−1	NA	Y	Y	Y	−1	−1	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	NA	NA	NA	NA	NA	NA	NA
Loubet et al (2016)[31]	Y	2	Y	Y	Y	NA	Y	Y	Y	NA	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fitzgerald et al (2018; paediatric population)[25]	Y	1	Y	−1	1	−2	−1	−1	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Levine et al (2015)[29]	Y	1	1	1	−1	NA	Y	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kuehne et al (2015)[23]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Roddy et al (2010)[22]	+	Y	+	Y	Y	NA	Y	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	Y	Y	Y	Y	Y	Y

Predictive scores (numeric or + symbol) are shown in shaded cells (blue indicates positive scores and light pink indicates negative scores). Y indicates that the characteristic was assessed, but not used. AUC=area under the receiver operating characteristic curve. NA=not assessed. ORL=otorhinolaryngology. *Diarrhoea, vomiting, or anorexia or loss of appetite. †95% CI is taken from Ingelbeen et al (2018)[28] because, although Oza and colleages do not report 95% CIs in their manuscript, Ingelbeen and colleagues have externally validated Oza and colleagues' score and they do report the 95% CI. ‡95% CI, AUC, or both AUC and 95% CI not given in original paper.

Table 12. Sensitivity and specificity of fever, epidemiological link, or contact history, ordered by optimal performance

	Variable	Sensitivity (95% CI)	Specificity (95% CI)
Fever cutoff			
Loubet et al (2016)[31]	≥38·5°C	80·2% (69·2–88·2)	82·6% (71·2–90·3)
Loubet et al (2016)[31]	≥38·0°C	88·2% (78·2–94·1)	72·5% (60·2–82·2)
Loubet et al (2016)[31]	≥37·5°C	93·4% (84·7–97·5)	50·7% (38·5–62·9)
Kuehne et al (2015)[23]	History of fever	85·3%*	26·4%*
Lado et al (2015)[5]	≥37·5°C or referred	85·9% (82·4–89·0)	16·4% (12·0–21·6)
Arranz et al (2016)[30]	≥38·0°C or referred	61·3% (44·1–78·4)	29·5% (16·1–43·0)
Roddy et al (2010)[22]	>38·0°C	85·0% (71·0–94·0)	20·0% (11·0–32·0)
Levine et al (2015)[29]	>38·0°C	85·0% (79·0–91·0)	21·0% (16·0–27·0)
Ingelbeen et al (2017)[27]	>38·0°C	71·5%*	30·5%*
Pooled analysis†	>38·0°C	80·0% (69·0–90·0)	25·0% (17·0–33·0)
Epidemiological link			
Hsu et al (2018)[24]	Contact with infected persons or body fluid, handling of bushmeat, attendingthe funeral of a patient with Ebola virus disease	74·7%*	67·1%*
Roddy et al (2010)[22]	Epidemiological link‡	67·0% (50·0–81·0)	86·0% (74·0–94·0)
Arranz et al (2016)[30]	History of contact with a person with confirmed Ebola virus disease	100·0%	59·0% (43·5–74·4)
Levine et al (2015)[29]	Sick contact§	65·0% (58·0–73·0)	61·0% (54·0–67·0)
Loubet et al (2016)[31]	Health worker or having had contact with a person with suspected Ebola virusdisease or having attended funerals	81·5% (44·0–60·7)	29·0% (19·0–41·3)
Kuehne et al (2015)[23]	Contact to case	47·3%*	71·2%*
Lado et al (2015)[5]	Travel to an Ebola virus disease hotspot area, health-care work, funeral attendance, or contact with an ill family member or friendfj	21·6% (17·9–25·6)	84·6% (79·6–88·8)

Optimal performance is the definition that achieved best balance between maximising sensitivity versus maximising specificity. *95% CI not provided in original paper.

†The pooled analysis was used for the studies that had the same cut-off for fever (>38°C).[22,27,29] ‡Epidemiological link was defined as direct contact with an individual potentially infected with Marburg haemorrhagic fever or his or her body fluids or direct contact during funeral practices. §Direct or indirect contact with a patient with suspected or confirmed Ebola virus disease in the previous 21 days, including living in the same household or providing direct care for the patient. fjA contact is any personwho comes into contact with a case or suspected case by sleeping in the same household within the past month; direct physical contact with the case (dead or alive); touching his or her linens or body fluid; or attendance at a funeral of a person with confirmed or suspected Ebola virus disease.

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4.6 Supplementary material

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Infectious Diseases

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: Caleo G, Theocharaki F, Lokuge K, et al. Clinical and epidemiological performance of WHO Ebola case definitions: a systematic review and meta-analysis. *Lancet Infect Dis* 2020; published online June 25. https://doi.org/10.1016/S1473-3099(20)30193-6.

Contents

A. Methods Appendix

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	2-3
Definitions for data extraction	11	Provide definitions used in data extraction and classifications of target condition(s), index test(s), reference standard(s) and other characteristics (e.g. study design, clinical setting).	3, Table 1
Risk of bias and applicability	12	Describe methods used for assessing risk of bias in individual studies and concerns regarding the applicability to the review question.	Table 1
Diagnostic accuracy measures	13	State the principal diagnostic accuracy measure(s) reported (e.g. sensitivity, specificity) and state the unit of assessment (e.g. per-patient, per-lesion).	2-3
Synthesis of results	14	Describe methods of handling data, combining results of studies and describing variability between studies. This could include, but is not limited to: a) handling of multiple definitions of target condition. b) handling of multiple thresholds of test positivity, c) handling multiple index test readers, d) handling of indeterminate test results, e) grouping and comparing tests, f) handling of different reference standards	3

Page 1 of 2

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
Meta-analysis	D2	Report the statistical methods used for meta-analyses, if performed.	3;7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	3;7
RESULTS			
Study selection	17	Provide numbers of studies screened, assessed for eligibility, included in the review (and included in meta-analysis, if applicable) with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each included study provide citations and present key characteristics including: a) participant characteristics (presentation, prior testing), b) clinical setting, c) study design, d) target condition definition, e) index test, f) reference standard, g) sample size, h) funding sources	7;9-10, Table 1
Risk of bias and applicability	19	Present evaluation of risk of bias and concerns regarding applicability for each study.	Table 1

Results of individual studies	20	For each analysis in each study (e.g. unique combination of index test, reference standard, and positivity threshold) report 2x2 data (TP, FP, FN, TN) with estimates of diagnostic accuracy and confidence intervals, ideally with a forest or receiver operator characteristic (ROC) plot.	10-12, Figure 2 Figure 3 Table 2-3
Synthesis of results	21	Describe test accuracy, including variability; if meta-analysis was done, include results and confidence intervals.	10-12, Figure 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression; analysis of index test: failure rates, proportion of inconclusive results, adverse events).	Appendix page 7 and 9
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence.	12-14
Limitations	25	Discuss limitations from included studies (e.g. risk of bias and concerns regarding applicability) and from the review process (e.g. incomplete retrieval of identified research).	13-14, Table 1
Conclusions	26	Provide a general interpretation of the results in the context of other evidence. Discuss implications for future research and clinical practice (e.g. the intended use and clinical role of the index test).	12-14
FUNDING			
Funding	27	For the systematic review, describe the sources of funding and other support and the role of the funders.	7

Adapted From: McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, The PRISMA-DTA Group (2018). Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. JAMA. 2018 Jan 23;319(4):388-396. doi: 10.1001/jama.2017.19163.

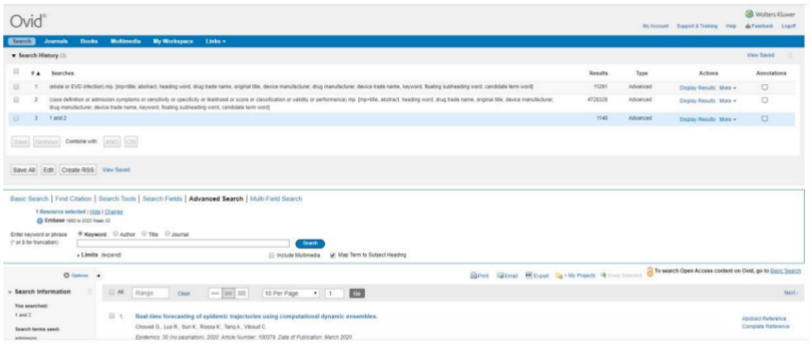
For more information, visit: www.prisma-statement.org.

A2. Systematic review search terms

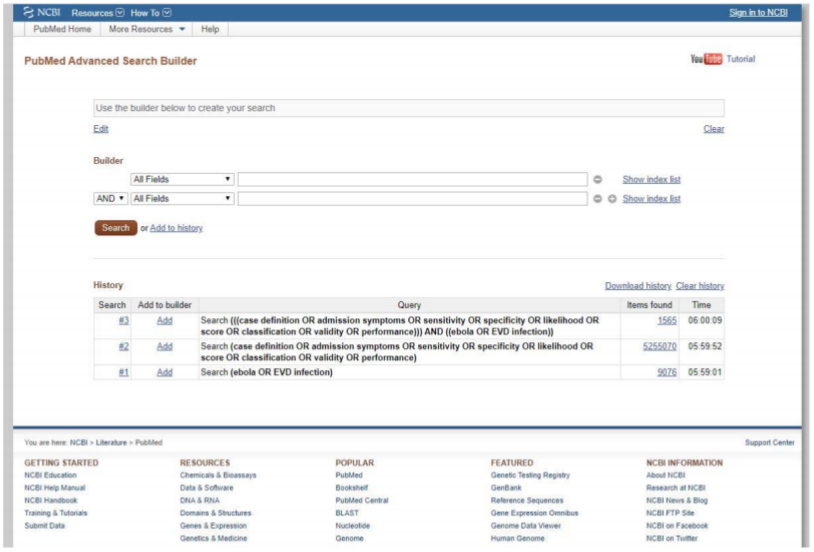
To identify eligible studies, we searched PubMed, Scopus, EMBASE, Web of Science, without regional restrictions. We included articles in English published from 13 June 1978 (when the first EVD outbreaks were reported on) to 14 January 2020.

The search terms used in our systematic review in order to identify the studies needed are: (ebola OR EVD infection) AND (case definition OR admission symptoms OR sensitivity OR specificity OR likelihood OR score OR classification OR validity OR performance)

Embase Search Criteria



PubMed Search Criteria



A3. Risk score

We summarised, without any further re-analysis, studies that developed or externally validated risk scores for predicting EV infection. Scores were used to identify individuals with a higher or lower risk of EV infection during screening at ETCs.

For comparability, we re-classified the risk scores reported in the included studies into categories, from very low risk to very high risk (Table S1)

Supplementary Table 1. Classification of the risk score for Ebola virus disease (EVD) infection across selected studies

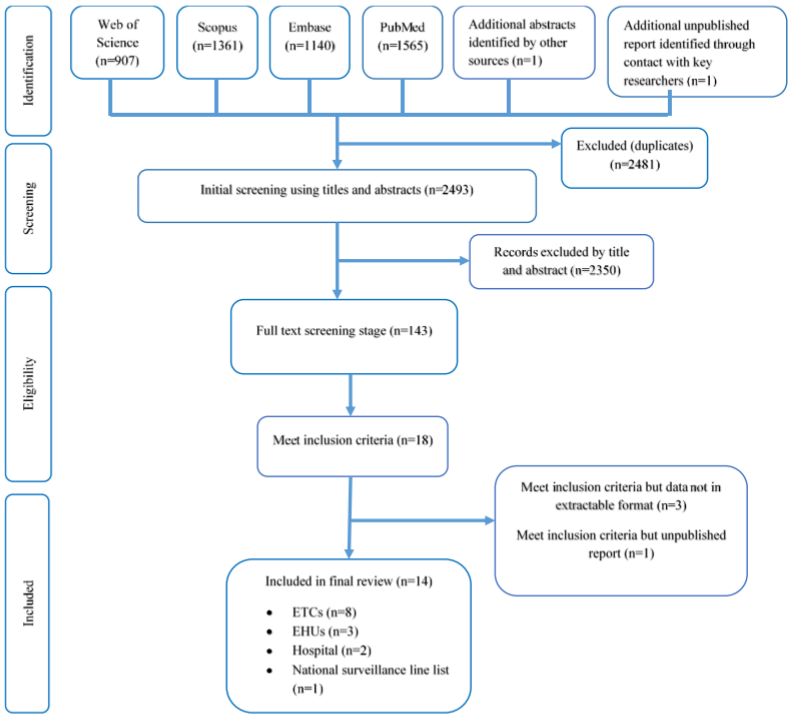
Classification	***Levine et al., 2015[29]***	***Loubet et al., 2016[31]***	***Hartley et al., 2017[32]***	***Oza et al., 2017 and Ingelbeen et al., 2018 [33, 28]***	***Fitzgerald et al., 2018 a[25]***
Very low risk	*≤0*	*-**	*≤1*	*-**	
Low	*1*	*0-2*	*2-4*	*- (lowest score: -3)*	*0-3*
Moderate	*2*	*2-4*	*5-7*	*0*	*-**
High risk	*3*	*≥4 (4-6)*	*8-11*	*+ (highest score: +5)*	
Very high risk	*≥4*	*-**	*≥12*	*-**	*7-10*

**In high prevalence, score ≥7 indicates high risk for a child being a case (0-13 years old).*

B. Results Appendix

B1. Systematic review flowchart

Supplementary Figure 2: PRISMA flow diagram depicting the number of articles at each of the identification, screening and eligibility stages



B2. Pooled sensitivity and specificity of individual symptoms

Supplementary Table 2. Performance of symptoms at admission ordered by number of studies

Symptom	Number of studies	Model	Sensitivity (95% CI)	Specificity (95% CI)
intense fatigue	7	Bivariate and HSROC	79·0 (74·4-83·0)	32·3 (25·8-39·4)
anorexia	7		61·0 (50·8-70·2)	51·3 (41·7-60·8)
diarrhoea	7		51·4 (42·2-60·6)	64·4 (56·7-71·3)
vomiting	7		51·2 (43·4-58·9)	57·4 (50·6-63·8)
headache	7		42·2 (30·9-54·2)	55·0 (45·0-64·5)
abdominal pain	7		39·3 (31·7-47·5)	58·0 (51·9-63·8)
dysphagia/difficulties to swallow	7		18·2 (12·3-26·2)	87·6 (82·0-91·7)
bleeding	7		14·3 (6·2-29·4)	89·5 (80·7-94·6)
conjunctivitis	6		19·0 (11·8-29·2)	91·1 (87·4-93·9)
hiccups	6	Random effects*	10·0 (9·0-12·0), I2=0· 00%	92·0 (91·0-94·0), I2=9·96%
dyspnoea	6	Bivariate and HSROC	9·8 (4·9-18·3)	87·8 (81·2-92·3)
arthralgia/joint pain	5		39·9 (28·0-53·1)	63·5 (55·6-70·7)
muscle pain	5		39·8 (28·3-52·5)	67·8 (62·1-72·9)
chest pain	4		15·8 (8·1–28·4)	84·7 (73·6-91·7)
cough	4		15·2 (8·4–25·8)	82·6 (71·5-90·0)
confusion	4		3·7 (0·6-19·6)	95·4 (89·1-98·1)
rash	4		1·5 (0·4-4·6)	97·8 (95·2-99·0)
'muscle or joint' pain	3	Random effects*	58·0 (47·0-69·0), I2=62·7%	53·0 (42·0-65·0), I2=73·1%
sore throat	3		8·0 (0·0-26·0), I2=99·3% 3·0	93·0 (81·0-99·0), I2=98·4%
jaundice	3		(0·0–9·0), I2=93·6%	97·0 (93·0-99·0), I2=82·7%
pain behind the eyes	3		1·0 (0·0–7·0), I2=98·1%	98·0 (91·0-100·0), I2=98·1%
haematemesis	2		20·0 (17·0–24·0), I2= 0·0%	97·0 (95·0-99·0), I2=0·0%
melena	2		4·0 (3·0-6·0), I2=0·0% 2·0	96·0 (94·0-98·0), I2=0·0%
coma	2		(2·0-3·0), I2=0·0% 83·9	95·0 (94·0-96·0), I2=0·0%
digestive symptoms	1		(70·9-96·8)	45·5 (30.7-60·2)
non-menstrual vaginal bleed	1		20·0 (8·0-39·0)	91·0 (75·0-98·0)
bloody gingivitis	1		17·0 (7·0-32·0)	93·0 (84·0-98·0)
bleed from injection site	1		12·0 (4·0-26·0)	97·0 (89·0-100·0)
back pain	1		6·5 (0·0-15·1)	93·2 (85·7-100·0)
epistaxis	1		5·0 (1·0-17·0)	98·0 (91·0-100·0)
epigastralgia	1		4·0 (1·0-12·0)	98·5 (91·0-99·9)
Neurological symptoms	1		3·2 (0·0-9·4)	90·9 (82·4-99·4)
loss of consciousness	1		3·1 (1·7-5·1)	97·6 (94·8-99·1)
haemoptysis	1		0·0 (0·0-9·0)	98·0 (91·0-100·0)

* I2 are reported where a random-effects models were performed (no. of studies <4). For hiccups the HSROC did not achieve convergence

Chapter 5: (Research paper 3) Methodological issues of retrospective surveys for measuring mortality of highly clustered diseases: case study of the 2014-16 Ebola outbreak in Bo District, Sierra Leone.

5.1 Preamble

In this chapter I present the analysis of two retrospective mortality surveys implemented to estimate mortality (due to EVD and non-EVD) and morbidity in areas where, due to the EVD outbreak, MSF suspended critical health interventions. The studies were carried out in rural and urban areas of Bo District, Sierra Leone during the 2014-2016 EVD outbreak.

The studies offered empirical estimates mortality rates and design effects. Study findings prompted reflection on the utility of using of retrospective mortality studies for highly-clustered diseases such as EVD. The manuscript of these studies is ready to be submitted

5.2 Cover sheet

The Research Paper Cover Sheet is enclosed on the following pages.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

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SECTION A – Student Details

Student ID Number	210968	**Title**	Dr
First Name(s)	Grazia Marta		
Surname/Family Name	Caleo		
Thesis Title	Epidemiology and control of Ebola Virus Disease (EVD) in Sierra Leone: analysis of data from the Médecins Sans Frontières (MSF) response, 2014-15.		
Primary Supervisor	Professor Helen Weiss		

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

SECTION B – Paper already published

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Plos One
Please list the paper's authors in the intended authorship order:	Grazia Caleo, Kamalini Lokuge, Katina Kardamanidis, Jane Greig, Jaroslava Belava, Emer Kilbride, Alhaji Sayui Turay, Gbessay Saffa, Ronald Kremer, Francesco Grandesso, Kostas Danis, Gianluca Ditanna, Helen A Weiss

Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am first author on this paper. I conceived and drafted the study protocol. I implemented the study. I analysed data with the support FG and HAW. I drafted the manuscript, and then incorporated feedback from the co-authors. I will oversee the manuscript submission process, and I will revise the manuscript, as necessary, to respond to input from peer review.

SECTION E

Student Signature	Grazia Marta Caleo
Date	December 4th 2020

Supervisor Signature	Helen Weiss
Date	December 4th 2020

5.3 Summary

Background

Cluster surveys are an accepted method for estimating mortality in humanitarian settings, but are subject potential limitations for highly-clustered outcomes such as Ebola Virus Disease (EVD). We used data from population-based surveys conducted during the 2014-2016 EVD outbreak in Sierra Leone to: i) estimate empirical mortality rates, ii) provide the first reported design effects (DEFF) for EVD and non-EVD mortality; iii) discuss the methodological limitations and operational utility of mortality estimated from cluster-sampled studies when DEFF is high.

Methods

We designed two population-based surveys conducted at the end of the EVD outbreak in Bo District, Sierra Leone, in urban and rural areas. In each area, 35 clusters of 14 households where selected with probability proportional to population size. We collected information on morbidity, mortality and changes in household composition during the recall period (May 2014 to April 2015). Rates were calculated for all-cause, all-age, under-5 and EVD-specific mortality. Crude and adjusted mortality rates were estimated using Poisson regression, accounting for the survey sample weights and the clustered design.

Results

The surveys were conducted in 70 clusters (total 980 households; 6,522 individuals). In total, 64 deaths were reported, of which 20 were attributed to EVD. The crude and EVD-specific mortality rates were 0.35/10,000 person-days (95%CI:0.23–0.52) and 0.12/10,000 person-days (95%CI:0.05–0.32), respectively. EVD deaths were reported in 15 households in 9 clusters, and non-EVD deaths were reported in 40 households in 31 clusters. Empirical DEFF for EVD mortality were higher than for non-EVD mortality (5.53 vs 1.53).

Conclusion

There was a high degree of clustering in these community-based surveys of EVD. This can contribute to imprecise mortality estimates with large confidence intervals, which have limited utility when assessing the impact of disease. Alternative design options would improve the utility of future surveys, and the empirical DEFF estimates we provide can inform more robust study designs in future retrospective surveys of highly clustered diseases such as EVD.

Conflict of interest statement

The authors have declared that no competing interests exist.

Funding

Médecins sans Frontières (MSF) provided funding for this study. HAW was funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement which is part of the EDCTP2 programme supported by the European Union. Grant Ref: MR/R010161/1

5.4 Manuscript

5.4.1 Background

In humanitarian contexts, surveillance is the accepted "gold standard" to measure public health outcomes and to estimate the impact of a crisis (including mortality) (1). During the 2014-2016 Ebola Virus Diseases (EVD) outbreak in Sierra Leone, vital statistics and surveillance systems were weak (2), necessitating reliance on estimates of the direct and indirect impact of the outbreak based on mathematical modelling and retrospective analysis of burial and health facility data (3-6).

Population-based cluster surveys can supplement surveillance data to estimate the severity of a crisis for advocacy or operational purposes (e.g. to prioritise areas for intervention) (7, 8). A previous study have explored the validity of cluster surveys versus systematic sampling methods for measuring crude mortality, reporting that both designs yielded similar estimations (9). Key methodological limitations of cluster surveys in humanitarian contexts include failure to calculate the optimal sample size, to sample proportionate to population size (PPS), to weight the sample during analysis, or to consider the design effect when calculating precision.(10) Recognised limitations of this methodology to measure clustered disease include the decreased precision due to high intra-cluster homogeneity, and therefore the recommendation to publish empirical estimates of design effects (DEFFs) to inform future studies (11).

This paper assesses the utility, strengths and limitations of clustered surveys for highly-clustered data, using data from two cluster surveys carried out in rural and urban areas of Bo District, Sierra Leone during the 2014-2016 EVD outbreak. The aims of the surveys were to estimate mortality (due to EVD and non-EVD) and morbidity in areas where, due to the EVD outbreak, Médecins Sans Frontières (MSF) suspended critical health interventions and refocussed on EVD care.

We carried out the surveys in full acknowledgement that EVD case distribution was clustered and that surveys estimates might be affected by that; thus, at design and implementation stages we attempt to mitigate for highly-cluster distribution.

The aims of this manuscript are to: i) report estimate crude and adjusted EVD mortality rates , ii) provide the first reported estimated DEFFs for EVD and non-EVD mortality during an EVD outbreak; iii)

discuss the methodological limitations and the operational utility of estimated mortality rates from cluster-sampled studies when DEFF is high.

5.4.2 Methods

Study setting, design and population

In September 2014, to reduce EVD mortality and transmission in the area MSF opened a 100-bed Ebola Management Center (EMC) in Bo township. Along with activities in the EMC, MSF conducted EVD outreach activities in Bo district, focusing on social mobilization, support of survivors, case finding, and case investigation efforts conducted in collaboration with the District Ebola Response Committee (DERC).

Prior to the EVD outbreak, MSF was running a 200-bed secondary level referral hospital in Bo town. The hospital was considered a lifeline for children and pregnant women coming from Bo and other districts; providing more than 8,000 paediatric and 2,500 emergency obstetrical and gynaecological admissions per year. However, in October 2014, MSF was forced to suspend health services due to increasing risk of EVD nosocomial transmission and concerns about staff safety (12).

The closure of the hospital was perceived to have contributed to an increase in mortality in the area. Thus, in the absence of strong routine surveillance and vital statistics, we conducted two surveys in MSF catchment areas in order to estimate EVD and non-EVD mortality and morbidity in Bo district during the Ebola outbreak. One survey was carried out in Bo rural areas which consist of 15 chiefdoms and 969 villages, with an estimated population of 538,751. A second survey was carried in Bo town (urban area of Bo District) consisting of 20 sections, with an estimated population of 178,446.

Study design

We used a two-stage population-based cluster survey design, an established methodology to estimate mortality rates in humanitarian and crisis settings (13-15). Surveys were implemented separately in two localities within Bo District: rural Bo chiefdom (rural area) and Bo town (urban area) to reflect differences in access to EVD care.

Recall period

In Sierra Leone the first EVD cases were reported on 24th May 2014 (16). The last confirmed case from Bo District was discharged on 26th January 2015(while the last confirmed case for the entire country was reported in March 2016). The recall period for the survey covered the period from May 2014 to April 2015 when the surveys were conducted (313 and 321 days for the rural and urban studies respectively). Group discussions with the Sierra Leone study team were used to design a local events calendar for the chosen recall period to improve the accuracy of the reported time of deaths using locally known events and household memories. The calendar incorporates relevant national awareness days,

religious observance events, and community events as well as salient events linked to the EVD outbreak (e.g. the declaration of a state of emergency) (8, 17).

Sample size

There is substantial inter-cluster variability of EVD transmission, with people living in an Ebola-affected households or village having a high household and village-level risk of EVD infection and mortality (e.g. due to attending the same funerals, and/or caring for a sick relative) (18). The between cluster-variability decreases the precision of a mortality estimates compared with a non-clustered sample of the same size. Prior to the EVD outbreak, the all-cause mortality rate in Sierra Leone was estimated between 0.5 and 0.7 deaths per 10000 people per day (19). In the absence of published DEFF estimation from prior EVD cluster surveys, we considered a range of sample size scenarios when designing our surveys, using different estimates of expected crude mortality rate (CMR), required precisions and assumed design effects (19, 20).

Based on simulations, the most likely set of estimates was considered to be a CMR of 1.0 deaths/10,000 person-days with a precision of ±0.5 deaths and a design effect of 4. Using ENA (2011) software, the required sample size for each area was calculated as 2390 individuals in 483 households. Since the value of DEFF increases with cluster size and reduced with cluster number, to further reduce the DEFF and increase the geographical variability we organized the survey in 35 clusters of 14 households (35x14) in each area instead of the classical (30x30) (21).

Sampling

Population estimations in Bo district (by village) and Bo town (by section) were obtained from the Local Ministry of Health and Sanitation (MOHS). These lists constituted the sampling frame from which the clusters were selected. In the first stage, villages/sections were selected with PPS. In the second stage, the starting household was chosen within a village (rural area) or within a section (urban area) using the standard World Health Organization (WHO) Expanded Programme on Immunization (EPI) methodology (22). A pen was spun and dropped on the ground in the central point of the village or section and a line drawn in its direction towards the edge of the village or section. Households along this line were counted, and one of these was selected using a random number table as the first to be interviewed in the villages/sections. To further reduce the DEFF that might occur through including geographically close households, subsequent households, were selected systematically as every n^{th} household (where n was determined as the total estimated households in the village/section divided by the number of households to be included); this was different from the WHO EPI approach, that does not introduce a step to select subsequent households. The next (n^{th}) household was selected by counting households to the left. If a household was empty, 2 further attempts were made later the same day before replacement. Replacement, including due to refusal, was with the next closest household on the left.

Definitions

The WHO EVD case definitions were used to define suspect cases based on history of contacts and signs and symptoms compatible with the EVD infection (Box 1) (23).

A household was defined as a person or a group of persons, related or unrelated, who lived together and who shared a common source of food. The head of household was defined as person aged 18 years and older who could give accurate information on demographics, illness, and mortality in his/her household and was present in the household during recall period.

Quarantine was defined as a household reporting separation (i.e., the household was cordoned off) and restriction of movement by the local authority following a positive EVD result in the household. Contact tracing was defined as a process of identifying, listing, and monitoring persons who had direct or indirect exposure to any confirmed, EVD case within the past 21 days.

Data management

Interviews were conducted with the head of each selected household. A trained MSF study team elicited information on household members, births, arrivals, departures, illnesses, deaths, place and circumstance of death. Medical records at the household level were used to re-build the possible cause and time of illness and death, if available. If all members of a selected household were deceased, the household composition and outcomes were reconstructed with help from family members and/or neighbors. Data on whether and when the household had been placed under quarantine and if any of the household members was put under contact tracing were also collected. The questionnaire and consent forms were verbally translated into the dominant local language Mende, which does not have a written tradition, and back-translated into English to ensure consistency. Study team were bi-lingual (speaking English and Mende). Group consensus on translations was sought during the training. Questionnaires were piloted prior to beginning the study. The detailed study protocol is publicly available on the MSF research platform (https://remit.oca.msf.org/studies/159).

Data analysis

We present descriptive analysis as means or medians (range) of numerical variables and proportions with 95% confidence intervals (CI) for categorical variables. Mortality rates per 10,000 per day were calculated using the mid-point population estimates as the denominator. Mid-point populations accounted for changes in household composition (births, deaths, and in- and out-migration) during the recall period. Rates were calculated for all-cause, all-age, under-5 mortality and EVD-specific mortality. Stratified linear and logistic regression models for continuous and binary outcome variables, respectively, have been fitted adjusting for age and sex. We calculated the crude and adjusted mortality rates, and incidence rate ratios (IRR) using Poisson regression. All analyses accounted for the survey sample weights and the effect of clustering induced by the two-stage sampling design. Data cleaning and statistical analysis were conducted using STATA v15 (Stata Corporation, TX, USA).

Ethical approval

The study protocol was approved by the Ethics Review Board of MSF, the Internal Review Board of the Sierra Leone MoHS, and the London School of Hygiene & Tropical Medicine (LSHTM). Approval to conduct the study was obtained from traditional authorities in all study sites prior to data collection. Participation was voluntary. Verbal informed consent for participation was obtained from the head of each household after a briefing about the aim of the study, the questions, survey and how their answers would be recorded, stored and used, duration of the questionnaire, and the option to end the interview or withdraw from the research at any time if wished. Confidentiality was protected during data collection and analysis. No personal identifying information was collected, and data were aggregated for analysis.

5.4.3 Results

Overview

The surveys were conducted in 70 clusters of 14 households (total 980 households; 6,522 individuals). Four households refused to participate (one in the rural and three in the urban area) and were replaced by the next consenting household. The rural area had a higher proportion of children under five (14.4%, 95%CI:13.3-15.5%) than the urban area (9.4%, 95%CI:8.4-10.4%) ($p<0.001$). The proportion of women was lower in the rural area (51.4%, 95%CI:49.6-53.2%) than in the urban area (54.6%, 95%CI:52.9-56.3%) ($p=0.01$) (Table 13).

Morbidity

Overall, 9.0% (n=586, 95%CI:7.2-10.1%) of the population surveyed reported that someone had been sick in their household at least once during the recall period. Prevalence of any morbidity was reported more frequently among household members aged over five compared to children under 5 (55.2% vs 47.8%) ($p<0.001$). The most frequently reported illness (all ages combined) was malaria/fever (n=358, 61.1%). Prevalence of suspected/probable EVD was 4.9% (n=29).

EVD survivors

In total, 9 people reported being EVD survivors, all of whom reported been admitted to MSF Bo EMC. Of these, 7 reported that their household was put under quarantine and contact tracing following their positive test. Only 6 of the 9 reported signs and symptoms and a contact history compatible with the suspect/probable EVD case definition.

Mortality

Overall, 36 out of 70 clusters (51.4%) reported deaths (16 clusters in the rural and 20 clusters in the urban area). In total, 64 deaths were reported, of which 18 were among children aged under 5 years old, giving crude and under-five mortality rates of 0.35/10,000 person-days (95%CI:0.23–0.52) and 0.91/10,000 person-days (95%CI:0.54-1.51) respectively (Table 14). All-cause mortality in the rural area was 25% higher (adjusted IRR 1.25; 95%CI: 0.67-2.33) than in the urban area (Table 14).

The most frequently reported causes of death were EVD (31.2%, n=20) and malaria/fever (18.7%, n=12). EVD was the main reported cause of death for those aged over 5 years (39.1%, n=18), while malaria/fever was the main cause for the under-5s (50.0%, n=9) (Table 15). For two children aged under-5, the case of death was attributed by family members to EVD, both in the rural area and in households experiencing more than one EVD case.

All households where EVD deaths were reported, experienced quarantine, and all except one, contact tracing. Nine EVD deaths (45.0%) met the suspect/probable EVD case definitions. Of the 20 EVD deaths, nine (45.0%) died at home, six (30.0%) in Bo MSF EMC, four (20.0%) in a non-Ebola health facility, and one (5.0%) in the ambulance.

The overall EVD-specific mortality rate was 0.12/10,000 person-days (95% CI:0.05–0.32). EVD-related mortality was 2.6 (adjusted IRR 2.6; 95%CI: 0.65–10) higher in rural area compared with that in the urban area (Table 16).

Design effects and cluster

The 29 EVD cases were reported in 20 households in 12 clusters, while the 358 malaria/fever cases were reported in 272 households in 62 clusters. Overall, 51.7% (15/29) of reported EVD cases occurred in six households. The DEFF for EVD infection was 28% higher compared to malaria/fever infection (7.01 vs 5.47) (Table 17).

The 20 EVD deaths were reported in 15 households in 9 clusters, with the 44 non-EVD deaths reported in 40 households in 31 clusters. The DEFF for EVD-specific mortality rate was 3.6 times higher compared to non EVD mortality rate (5.53 vs 1.53) (Table 16).

Five out of six clusters reporting more than one EVD cases where in the rural area versus one cluster in the urban area (83.3% vs 16.7%, p=0.063). Death at home was more frequent among clusters reporting more than one EVD case compared to clusters reporting one EVD case, however the difference was not statistically significant (26.7% vs 7.1%, p=0.385).

DEFF for EVD-specific mortality (overall 5.53) was 11 times higher in the rural area compared with the urban area (6.18 vs 0.58) (Table 16).

Overall, most of the DEFF was linked to one individual cluster reporting 6 out of 29 EVD cases identified overall in 3 households from the same village cluster.

5.4.4 Discussion

To our knowledge this is the first and largest population study conducted during the EVD outbreak in Sierra Leone, and the first study to estimate mortality rates and DEFF separately for urban and rural areas and for EVD and non-EVD. Mortality rate estimates were lower than expected based on the closure of the MSF hospital and the public health impact of the EVD outbreak. Only a small proportion of households and clusters reported EVD deaths and cases. This may in part be due to the high degree of clustering as indicated by the high DEFF, which contributes to imprecise mortality estimates with large confidence intervals, which therefore have limited utility when assessing the impact of disease.

Surveys in Freetown, Sierra Leone, and Monrovia, Liberia, estimated CMR and EVD-specific mortality rates covering a recall period which overlapped with our study, both studies were conducted with the assumption of an increase of mortality due to the outbreak. The Freetown study used our methodology (e.g. a two-stage population-based cluster but with a lower DEFF of 1.5) (24). In Monrovia a simple random sample of telephone numbers with remote interview was implemented, since a two-stage population-based cluster was deemed risky for the study team due to EVD transmission (25).

In Freetown, CMR was 0.52/10,000 persons/day (95%CI:0.29–0.76) and Ebola-specific mortality rate 0.19/10,000 person-days (95% CI:0.01–0.38) (24); empirical DEFF were not reported.

In Monrovia, CMR was estimated at 0.33/10,000 person-days (95%CI:0.25–0.43) and Ebola-specific mortality rate at 0.06/10,000 person-days (95%CI:0.03–0.11) (25). As in our study, EVD-specific mortality rate estimates had wide confidence intervals and consequently imprecise estimates. Both authors attributed the low mortality rates to improved access to care, enhanced hygiene practices due to the outbreak, and low reporting of deaths in particular for under-5s. These factors may also have contributed to the lower than expected mortality seen in our study in particular for the urban area.

At the start of the EVD outbreak in Monrovia, another study used a capture-recapture approach to estimate underreporting of EVD, showing that this method captured at least three times more EVD cases than officially reported (26). Lack of access to EVD surveillance data hampered the ability to consider capture-recapture or assess the correlation of our estimation with surveillance data. We recommend that future studies use capture-recapture methods or surveillance line lists as a sampling frame instead of the list of villages for cluster selection. Others have suggested a purposeful selection of clusters guided by knowledge of the spatial distribution of the outcome, instead of using the village sampling frame (27). These methods have the potential to allow investigation of clusters with already reported transmission, thus potentially providing more robust estimates and better allocations of resources. However, this approach might have the potential limitation of give estimates only relevant for the purposively selected areas with limited application to the wider population or exclude area affected but not selected due to other factors (i.e., lack of access, or unwillingness to report).

As suggested by other authors, to improve the precision for outcomes with high between-cluster variability, we increased the number of clusters and reduced their size (35x14 vs 30x30) (21). In spite of this, our empirical estimate had wide confidence intervals and a large DEFF. To improve precision, future studies should consider increasing the number and decreasing the size of sampled clusters even further, or using the variance partition coefficient to calculate separate sample sizes according to groups that show significantly different heterogeneity (28). This latter approach has been used in veterinarian epidemiology to analyze herd-level predictors.

At the implementation stage, to further mitigate DEFF, we introduced a step between households. This, however, induced us to skip neighbours of EVD-affected households who informally reported EVD infection to the study team. Future studies could consider involving community chiefs and key stakeholders on identification of affected households (8).

Other authors have used a snowball approach to capture maternal death (29), and proven to be cost effective to capture Visceral Leishmaniasis deaths (30). More recently, this approach was suggested to estimate the impact of SARS-CoV-2 transmissibility starting from contact networks (31).

Households in the rural area experienced a higher proportion of EVD deaths at home, and more clusters with more than one EVD case, possibly due to limited access to care along with weaker surveillance system delaying reaching rural areas (32). During the outbreak, EVD home deaths could be used by public heath actors as a key indicator to prioritise those households where secondary transmission is more likely to occur and prioritize them for EVD vaccination and more rigorous contact tracing for timely access to care.

Death at home along with different distribution patterns of households and household interaction is likely to have increased the area-specific degree of cluster homogeneity for EVD transmission, as demonstrated by the high DEFF for the rural area compared to the urban area for EVD deaths (6.18 vs 0.58). It is likely that urban households have less distinct borders, and more homogenous mixing and distribution of risk factors, and thus less within-household clustering. Future studies would be advised to consider different sampling methodologies for urban and rural area to allow for this.

Malaria/fever was by far the most common morbidity reported in all ages, and was the main cause of death for under-5-year-olds, and similar to EVD had high clustered patterns. Surveillance, at the time, was focused on EVD detection with limited attention to provide specific care for other endemic diseases like malaria (2). As previously suggested, malaria interventions should be prioritized in EVD outbreak responses, in particular for children aged under-5 and in rural settings (33). This will mitigate the additional morbidity and mortality burden, and stimulate the community to report to surveillance if health interventions are consistent with population needs.

In Table 18, we summarise challenges, proposed methods or design improvements to mitigate the limitations of surveys for highly-clustered data along with requisites and additional considerations.

Study limitations

As with many EVD studies, under-reporting of deaths due to the fear of penalty, stigma from other households, and dissatisfaction of how deaths have been handled by safe burial team cannot be excluded (34). However, a mitigating factor is that MSF was not associated with the punitive measures introduced and they had an established presence in Bo preceding the Ebola outbreak as a provider of free healthcare. Further, we had a high response rate to the survey, and found that households were willing to share information on EVD even if not randomly selected.

Misclassification of the cause of deaths (e.g. with malaria may not be distinguishable from EVD due to low performance of WHO EVD case definition) (35), and timing of death may have occurred, but, when available, we used medical records, clinical/contact history, info on quarantine and contact tracing and calendar of salient events to mitigate misclassification.

Under estimation of prevalence of any morbidity for children under 5 years old compared to household members aged five and over might have occurred, since interviews were carried out with head of households who are not necessarily caregivers for children under 5.

Finally, in our study most of the DEFF was attributed to one cluster. DEFF estimation could be underestimate if clusters or households inside affected clusters are not selected through the sampling approach used, hampering the true estimation of DEFF. A previous study found a similar spatially clustered EVD pattern (18). This is a key limitation of the methodology used that could be mitigated by taking into account design improvements described above.

5.4.5 Conclusion

For humanitarian organizations it is imperative to document the methodological limitations of studies and discuss the utility of estimates generated by common epidemiological tools used to quantify burdens and needs, in order to ensure accountability to affected populations, and best use of resources.

Our findings demonstrate a high degree of clustering in current methodologies for community-based surveys of EVD. The empirical DEFF estimates we provide can inform more robust study designs in future retrospective surveys of highly clustered diseases such as EVD, and the alternative design strategies proposed can improve the utility of future surveys for estimating mortality of such diseases.

Contributors

GC, KL, JG, conceived the idea, KK, JB and GC implemented the study, GC wrote the first and late draft. GC, FG, GDT and HAW contributed to the analysis. HAW, KL, and KD reviewed early drafts. All authors contributed to later drafts and approved the final submission.

Declaration of interests

We declare no competing interests.

Acknowledgments

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Availability of data and materials

Data are available under the MSF data sharing policy. Requests to access data can be made to data.sharing@msf.org.

Box 1 World Health Organization (WHO) EVD case definitions were used to define suspect, probable, and confirmed cases		
Suspect	**Probable**	**Laboratory confirmed**
a. Any person, alive or dead, suffering or having suffered from a sudden onset of high fever and having had contact with: - a suspected, probable or confirmed Ebola or Marburg case; - a dead or sick animal; **OR** **b**. Any person with sudden onset of high fever and at least three of the following symptoms: - headaches - lethargy - anorexia / loss of appetite - aching muscles or joints - stomach pain - difficulty swallowing - vomiting - difficulty breathing - diarrhea - hiccups; **OR** **c.** Any person with inexplicable bleeding; **OR** **d.** Any sudden, inexplicable death	**a.** Any suspected case evaluated by a clinician; **OR** **b.** Any deceased suspected case (where it has not been possible to collect specimens for laboratory confirmation) having an epidemiological link with a confirmed case	**a.** Any suspected or probable cases with a positive laboratory result. Laboratory confirmed cases must test positive for the virus antigen, either by detection of virus RNA by reverse transcriptase-polymerase chain reaction (RT-PCR), or by detection of IgM antibodies directed against Marburg or Ebola.

Table 13. Households characteristics and movements according to area, mortality studies Bo District

Variable	Urban	Rural	p-value for difference
No. clusters	35	35	
No. households sampled	490	490	
No. individuals at baseline (start recall period)	3,266	3,048	
No. departure (deaths/out-migration)	122	140	
No. arrivals (births/in-migration)	108	100	
Total number of individuals in study	3,374	3,148	
Mean household size (95%CI)	8.6 (7.9-9.2)	7.9 (7.3-8.6)	0.15
Mean age (95%CI)	22.9 (22.3-23.5)	22.7 (21.9-23.5)	0.71
% Children <5 years (95%CI)	9.4 (8.4-10.4)	14.4 (13.3-15.5)	<0.001
% Female (95%CI)	54.6 (52.9-56.3)	51.4 (49.6-53.2)	0.01

Table 14. Reported deaths and crude and under 5 years mortality rates, crude and adjusted incidence rate ratio, mortality studies Bo District

	N of deaths		Mortality rate (all ages) mortality rate per 10,000-person-days			Under-five mortality rate mortality rate per 10,000-person-days		
Area	**Total**	**< 5 years**	**Rate**	**[95% CI]**	**Design Effect**	**Rate**	**[95% CI]**	**Design Effect**
Overall	64	18	0.35	[0.23 – 0.52]	2.86	0.91	[0.54 – 1.51]	1.43
Urban	28	4	0.27	[0.19 – 0.38]	0.45	0.41	[0.13 – 1.30]	0.60
Rural	36	14	0.38	[0.23 – 0.62]	3.44	1.03	[0.59 – 1.77]	1.52
Crude incidence rate ratio (IRR)			1.41	[0.77 – 2.60]		2.51	[0.69 – 9.14]	
Adjusted IRR [1]			1.25	[0.67 – 2.33]		2.56	[0.71 – 9.28]	

[1] Adjusted for age group and sex

Table 15. Reported causes of death by age group, mortality studies Bo District

Cause of death	Five years old and over (N=46)		Under five years old (N=18)	
	#	%	#	%
EVD	18	39.1	2	11.1
Chronic diseases	8	17.4	-	-
Other*	6	13.0	1	5.5
Old Age	5	10.7	-	-
Malaria/Fever	3	6.5	9	50.0
Unknown	3	6.5	-	-
Trauma/Accident	2	4.3	1	5.5
Convulsion	-	-	2	11.1
Death during pregnancy or childbirth	1	2.2	-	-
Neonatal death	-	-	3	16.7

*Sickle Cell Disease, Candidiasis, 'Witch Gun', Swollen Foot, Abdominal Pain, Herpes Zoster, Hernia

Table 16. Reported deaths, EVD specific and non-EVD specific mortality rates, crude and adjusted incidence rate ratio, mortality studies Bo District

	N of deaths		EVD specific mortality rate per 10,000-person-days			Non-EVD-specific mortality rate per 10,000-person-days		
Area	**EVD**	**Non-EVD**	**Rate**	**[95% CI]**	**Design Effect**	**Rate**	**[95% CI]**	**Design Effect**
Overall	20	44	0.12	[0.05 – 0.32]	5.53	0.22	[0.16 – 0.32]	1.53
Urban	6	22	0.06	[0.02 – 0.14]	0.58	0.21	[0.14 – 0.31]	0.44
Rural	14	22	0.15	[0.05 – 0.43]	6.18	0.23	[0.15 – 0.37]	1.87
Crude IRR			2.56	[0.64 – 10.26]		1.10	[0.59 – 2.04]	
Adjusted IRR[1]			2.61	[0.65 – 10.35]		0.91	[0.50 – 1.65]	

[1] Adjusted for age group and sex

Table 17. Reported malaria and EVD infections, morbidity rates and Design effect, mortality studies Bo District

Area	N of cases		Malaria/fever morbidity rate (*)			EVD morbidity rate (*)		
	Malaria/ fever	Ebola	Rate	[95% CI]	Design Effect	Rate	[95% CI]	Design Effect
Overall	358	29	1.74	[1.36 – 2.22]	5.47	0.17	[0.07 – 0.42]	7.01
Rural	163	18	1.72	[1.25 – 2.35]	6.72	0.19	[0.06 – 0.55]	8.21
Urban	195	11	1.86	[1.39 – 2.48]	2.07	0.11	[0.05 – 0.21]	0.62

(*) Rate per 10,000 persons per Day

Table 18. Challenges, proposed, methods and design improvements and considerations

Challenges observed	**Proposed alternative methods and/or improvements of two-stage cluster survey**	**Requisites & considerations**
Selection of a large number of unaffected clusters and households resulting in underestimation of the outbreak burden/impact	Use surveillance list or a purposeful selection of clusters as a sampling frame instead of the list of villages.	Access to surveillance data. Previous knowledge of geographical distribution of health characteristics under investigation. Potential limited representativeness of the results (i.e, estimates only relevant for the purposively selected areas, potentially over-estimate the burden if estimated are inferred a to the whole area).
	Designing a capture-recapture study using different sources (e.g. surveillance line list, burial data, EVD vaccination list, local key stakeholders).	Access and/or creation of at least three independent lists.
Sampling urban/rural	Differences in the distribution of population characteristics across urban and rural areas.	Different sampling methodology according to settings.
Precision of estimates, sample size, and geographical variability	Consider additional variables for stratification influencing DEFF: age (e.g. under 5 years old vs 5 and over) and other social/epidemiological parameters influencing transmission. Consider sampling methods used in veterinary epidemiology that use the variance partition coefficient (VPC), which measures the clustering of infection/disease for individuals with a common risk profile (e.g. animals in the same herd). Sample size estimates are obtained separately for those groups that exhibit significantly different heterogeneity.	Knowledge of social and epidemiological factors (e.g. review of chain of transmission, social factors/traditions that could influence patterns of transmission)
	Increase the number of clusters to increase geographical variability	Large logistical and human resources
Identification of EVD-affected households	Involve community chiefs, community health workers, and/or key local stakeholders to support identification of affected EVD households; Consider non-probabilistic methods (e.g. snowball approach) to identify additional affected households. If the intension is to is to find all affected households in a location and use whole population as denominator.	Breach in confidentiality and possible increase of stigma for affected households.

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5.5 Supplementary material

The detailed study protocol is publicly available on the MSF research platform at this link:

https://remit.oca.msf.org/studies/159

Chapter 6: Discussion and conclusion

This chapter summarises the research presented in this thesis, and gaps identified, including: i) putting the thesis findings in context of research published since the publication of thesis papers 1 and 2, and writing paper 3; and ii) includes discussion of how field experiences of the EVD response in Sierra Leone have contributed to the evolution of the MSF-OCA approach to community consultations. This has led to the development of an 'epi-anthro' approach during outbreak investigations and the design of new guidance and a protocol to explore open in-depth conversations with different community groups from the outset of an outbreak. The MSF-OCA Community Guidance on COVID-19 and the design and implementation of a qualitative study carried out in Sierra Leone in May 2020 are summarized in this chapter as a tangible example of this change.

6.1 Discussion

The work presented in this thesis consolidates perspectives and evidence from EVD outbreaks and emphasises the essential role of communities as frontline, and the importance of contextually adapted and compassionate control measures (1). A common theme throughout the thesis was that the epidemiological chains of transmission reveal health and social inequalities. The findings underline that outbreaks do not occur in a vacuum and that the understanding of social dynamics and dialogues with heterogeneous community stakeholders are crucial steps to designing more equitable and inclusive models of public health interventions (2).

Overall, the thesis addressed 3 main knowledge gaps; these are summarised in Table 19 together with the main findings and the areas for further research

Table 19. Overview of evidence gaps, key findings and priorities for future research

Evidence gap	PhD Objective	Methods	Key findings	Clinical or policy implication	Future research	Chapter and research paper
Lack of knowledge of the factors that influence EVD transmission dynamics and community compliance with control measures over time.	Investigate drivers of EVD transmission and community perspective toward EVD control in Kailahun District, Sierra Leone.	Mixed-methods study in a rural village that experienced prolonged EVD transmission. Exhaustive cross-sectional survey to reconstruct transmission dynamics and semi-structured interviews to explore community views.	Outbreak was controlled after prolonged transmission and a high death toll. Transmission was maintained by a small number of large households. All cases and deaths detected were spatially clustered. Reasons for non-compliance with public health guidelines included burials in plastic bags, without female attendants or prayer, perceived as dishonourable, and perceptions of a moral duty to provide care to relatives. Low EMC survival rates, poor communication with EMC, and loss of livelihoods due to quarantine further contributed to low uptake with control measures. Early understanding of social norms and experiences and the ability to link this to localised strategies and adapted health interventions is essential.	To inform responses to future EVD outbreaks.	Need to develop novel sampling methods appropriate for estimating mortality for highly clustered diseases. Evaluating how population interaction with control measures can lead to an interdependent solution.	Chapter 3; Paper 1

Evidence gap	PhD Objective	Methods	Key findings	Clinical or policy implication	Future research	Chapter and research paper
Investigation of concerns about the performance of WHO EVD case definitions and lack of evaluation of its performance.	Assess performance of the WHO EVD case definitions and other screening scores, to support surveillance and admission testing decisions at EMCs.	Systematic review and meta-analysis of studies published between June 13, 1978, and Jan 14, 2020.	WHO EVD case definitions perform sub-optimally to identify cases at both community level and during triage at health facilities. The performance of fever as a symptom varied depending on the cut-off used to define fever. The most sensitive symptom was intense fatigue. History of contact was a key predictor for the WHO case definitions and for risk scores. Gaps related to the EVD case definition for the paediatric and pregnant population. Gaps on validating externally risk score for Ebola virus infection.	To guide communities and public health practitioners to improve the effectiveness and efficiency of identification and management of suspected cases during EVD response.	Evidence is needed on EVD screening tools for the paediatric and pregnant population Need to use consistent thresholds (e.g., for fever) To externally validate risk scores for Ebola virus infection. To explore relationship between viraemia and symptoms at admission	Chapter 4; Paper 2

The first study of this thesis (Objective 1, Chapter 3) used a mixed-method approach which allowed in-depth exploration of the factors that influenced protracted EVD transmission combined with epidemiological data. The study was conducted in a village where MSF had been unsuccessfully engaging since the outset of outbreak. In the village transmission lasted for several weeks and was maintained by a small number of large households with all cases and deaths spatially clustered.

The exhaustive design of this study allowed us to capture all the cases and deaths that would otherwise not have been identified using standard sampling methods. The study provided evidence that the risks that contributed to EVD transmission are often hidden in the details of community life, part of coping mechanisms and family dynamics. Further, the study highlighted that the patterns of transmission and the high community death toll were the outcomes of poor communication and the failure of response organizations to adapt to community i.e., that households and community withdrawal was a consequence of the failure of the response to orientate itself according to the needs and values of the community. Ebola is a disease that poses the greatest risk to family members whose generosity drives them to look after each other while honouring ancestral practices. Household members became infected themselves in a brave effort to look after their loved ones, and to bury their dead honourably. Strict infection control procedures in Ebola isolation centres and during safe burial hampered family proximity and grieving, and, as observed somewhere else, were ultimately perceived as a violation of customary and religious rituals (3).

Since the publication of this thesis chapter, other authors have i) documented how Ebola isolation centres were perceived over time in Sierra Leone, and highlighted the need for better communication links between communities and Ebola facilities (4), and ii) tried to rebuild local transmission chains and explore why in some communities in Sierra Leone the transmission was sustained, with consequent devastating death tool compared to others where transmission was timely controlled (5). As with our study, the authors documented that chains of transmission were maintained by intrafamilial behavioural, social events and external public health factors (i.e. delay in detection of cases, misdiagnosis, nosocomial transmission, lack of guidance on proper PPE procedures, poor communication with communities). The authors highlighted how the initial EVD WHO case definition emphasising haemorrhagic signs was misleading for most of the communities not experiencing these signs. In their study, families referred to Ebola as 'bondawote–"family turn around" with control achieved in the most affected communities when all the family had been 'turned around'. Control in other non-affected community was achieved by timely implementation of local strategies (i.e. cooperation with affected families, strict controlling of entrance to and exit from the community), with some of those coping mechanisms similar to those once set up at the time of the war (5).

Following our study, others have also used similar mixed-method approaches to understanding transmission history and community coping mechanisms during the EVD outbreak in one village in

Sierra Leone (6). The authors documented the complex relationships between colonial heritage, social and spiritual dimensions of EVD, and the role played by local communities in acting collectively to control the outbreak themselves in an attempt to save their loved ones and honour their souls. In this study, empirical observations on how EVD was transmitted helped the community to define localised control strategies (e.g. separate the ill from the healthy and setup village burial teams) (6). This was in part similar to localised strategies implemented by the community in the village included in our study (e.g., stopping babies being delivered in the community, preventing children from playing contact games together, and not visiting other households). In their study, the military and a punitive fine system that were used by authorities to control the outbreak acted as a further deterrent to the community engaging with response agencies (6).

We found that community attitudes toward control measures changed once the health messages given to the community were consistent with what households observed in their lives (i.e., "When we saw that people touched sick people and got sick, we could see the communication of it and realised that it is real."). Similarly, perceptions toward the EMC and safe burial improved when survivors returned to the village and when burial prayer was permitted. These helped changing perceptions of contact tracing as invading privacy or “selling” people to enable community safety. Quarantine was understood and compliance improved when food support was given to affected households (i.e. “We had no food at the start. They should have given us food like they did in other households at the end.”). This shows that vertical outbreak control can act as an additional barrier to access to care and the humanization of public health response can enhance compliance, demonstrating that the use of force was an unnecessary and had a detrimental impact by further alienating community.

There has also been limited acknowledgement of the profound contribution of local communities to the generation of evidence on pharmaceutical interventions for EVD. For instance, evidence on the impact of investigational drugs on reducing mortality would not be available if households were not, bravely, delegating care of loved ones to Ebola treatment centres, despite these being places where traditional practices have been compromised by the strict biomedical approach. Likewise, evidence of safety and efficacy of experimental vaccines are a tangible result of the trust that local populations and health staff have placed in the research community (7). This contribution has provided clinicians and public health workers with new tools, altering patient outcomes and preventing transmission at community level. (see section 1.7 Treatments and vaccines).

During the validation process of our study, we shared study findings in a narrative form with the village. This was perceived by participants as a step towards a collective healing processes and reconciliation between MSF and the affected community. Recently other authors have documented how, for EVD survivors, engaging in community EVD control mechanisms was perceived as a positive coping

mechanism to heal post-traumatic distress and accelerate their acceptance and reconciliation with the local community (8).

Our study also identified key gaps in how to safely incorporate accepted local social norms from the outset of intervention, making them more acceptable for communities and therefore effective; also the clustered distribution of cases and deaths indicated the need for a new methodology to benchmark the burden for diseases with a clustered transmission pattern like EVD.

The second paper in the thesis, (Objective 2, Chapter 4) used a review and meta-analysis approach to explore the performance of EVD WHO case definitions, a critical public health tool to identify EVD cases in communities and Ebola centres.

The study showed that WHO EVD case definitions perform sub-optimally to identify cases at both the community level and during triage at health facilities. We estimated that the EVD case definition has a specificity of 36% (thus 64% of people identified as EVD suspected have potentially other diseases) and a sensitivity of 81% % (thus 19% of patients with EVD do not meet the case definition and would be otherwise missed). Discordance on the use of WHO EVD case definitions can contribute to unnecessary admissions to Ebola treatment centres with consequent community disengagement, delayed access to care, and delay in outbreak control. Since the publication of this thesis chapter, others have reported that the WHO case definition specificity varies according to stage of the outbreak, with 57% of suspect cases correctly identified in the early stages and 35% correctly identified in the late stage (9). This corroborates our results and underlies how the performance of WHO case definitions depends on a number of context-specific and epidemiological factors.

In our analysis, the performance of fever as a symptom varied depending on the cut-off used to define fever, with intense fatigue identified as the most sensitive symptom, and conjunctivitis, unexplained bleeding, difficulty swallowing as the most specific. Since the publication of our study, another systematic review and metanalysis has explored what symptoms and signs best predict EVD (10). This study confirmed that the symptoms that best predict EVD are those presenting late in the diseases (i.e., conjunctivitis) when it is likely that transmission would have already occurred in the community and the prognosis for individual patients would be more severe. In our study the reported history of contact was a strong predictor for both the paediatric and adult populations, often performing better than many of the clinical symptoms and signs. However, reporting of contact history requires communities to feel safe enough to identify themselves as being at risk, and share information on risks and behaviour with responding organization. Therefore, the performance of WHO case definitions depends on key aspect of relationship_with community identified in Objective 1. Our study identified key gaps in exploring the performance of EVD case definitions for paediatric and pregnant populations along with external validation of the reviewed risks score.

The third paper in the thesis, (Objective 3, Chapter 5) used two retrospective population surveys conducted in urban and rural areas to benchmark mortality rates (EVD and non EVD-specific) at the end of the EVD outbreak in Bo district. The study offered a complementary approach to show how the EVD localised risk of transmission can contribute to a high degree of clustering (high design effect), leading to imprecise mortality estimates. A high clustering distribution of EVD was also identified in Objective 1. By using different methodologies and approaches, the work presented here highlights how the within-household and village interaction influenced the distribution patterns of EVD, with implication on robustness of mortality estimates to benchmark the disease burden and guide response. The study provides empirical estimation of the design effect that has not been reported before by similar studies conducted in West Africa during the EVD outbreak. Furthermore, the study prompts reflection on the utility of using retrospective mortality studies for highly clustered diseases and proposes several methodological recommendations to improve similar studies in the future.

To date, no other study using this methodology has been published on EVD since the West Africa outbreak. Other authors have recently used a seroprevalence study to benchmark the outbreak burden in one village in Guinea, confirming localised transmission patterns and mortality higher than previously documented (11). Other authors have recently used ethnographic methodologies in Sierra Leone to explore which coping, cultural imperatives and social elements influenced the heterogeneity in exposure to infection, with some households and communities heavily affected with an aggregated pattern of infection distribution and others that instead better controlled transmission. This study further underlines the importance of a conjugate epidemiological with anthropological investigation when measuring transmission (5).

Overall, using different methodologies and approaches, the work in the thesis highlights that outbreak patterns are inevitably linked to community and that community is linked to social and cultural environments, influencing key public health practices (i.e. surveillance, timely patient isolation, contact tracing, reporting of deaths, and definition of rings for EVD immunization).

6.2 Implication of findings for outbreak control

The research and field work presented in this thesis have strongly influenced the foundation of the MSF-OCA “epi-anthro” approach on EVD response. This approach recognises that the risks that contribute to EVD transmission and prevention are intrinsically linked to family/community dynamics, and external public health factors. In this approach, epidemiologists, anthropologists and social scientists work in tandem to investigate chains of transmission, social dynamics and family relationships that might influence transmission, including coping mechanisms that could explain health-seeking behaviour, access and barriers to care and use this info to adapt response.

The “epi-anthro” approach has been further framed in the recent MSF-OCA Community Guidance on the COVID-19 response[10]. This Guidance outlines the core principles on how to empathetically support communities during outbreaks and co-design local adapted responses. The practical application of these principles has resulted in the design of a qualitative study protocol focused on a community consultation, aiming to ensure that community voices and experiences are included in the early design of MSF/MOHS COVID-19 control measures. In May 2020, the qualitative community consultation study was implemented in Sierra Leone[11], in one of the areas where MSF-OCA provide maternal and child health care. The study found that previous experiences (e.g. war, EVD) have fostered fears of separation and of infection from contact with strangers and health workers/facilities. The EVD experience had facilitated the understanding of COVID-19 isolation centres as an important control measure, but for the community proximity to the homestead and communication with families remain crucial. Vertical COVID-19 control measures such as lockdown were perceived to have a negative impact on essential daily activities (especially food availability) and increasing the risks of domestic and sexual violence and adolescent/child abuse. This finding is corroborated by another study that found reduction of household income as a consequence of COVID-19 lockdown in Sierra Leone (12).

Communities suggested that MSF should prioritise access to family planning and sexual and gender-based violence (SGBV) programming, along with the identification of an organization that could support safe farming activities (i.e., farming assistance and rice subsidies). In this community there was a clear desire for self-management of contact tracing and transmission prevention activities by communities, through peer supervisors/CHW/local leaders and developing a buddy system for the design and implementation of activities (MSF provides technical support, but community members must have ownership and lead the surveillance, sensitisation and implementation). The study has further highlighted that this is a community that is not naive to epidemics, and there is a need of recognising community experience and prioritising ongoing programming according to community priorities. This is consistent with another national survey on knowledge, perceptions and practices around COVID-19 in Sierra Leone, which highlighted how previous experience of EVD might have highlighted preventive practices also for COVID-19 and which observed a strong association between infection knowledge and practices (13). Our findings are consistent with another study recently conducted in Sierra Leone in two communities that had different experiences with EVD burden (14). The study explored community preference for a disease with low infectivity but high mortality (i.e., EVD) versus an infection with high infectivity but low mortality (i.e., Covid-19). Both communities showed strong ability to conceptualise decisions regarding disease risks and both showed awareness of utility of personal action and local regulations to influence outbreak controls (14).

[10] Community Approach Guidance: Maintaining health and resilience during novel coronavirus (COVID-19) transmission, MSF-OCA interim guideline April 2020

[11] Exploring the perceptions of key community leaders toward the implementation and impact on their and community lives of COVID-19 control measures Tonkolli, Sierra Leone. May 2020

6.3 Future research priorities

The work presented in this thesis identified several gaps and priorities for future research. The first study of this thesis (Objective 1, Chapter 3) and the evidence published since its publication indicated how the control of transmission during outbreaks requires shifts in behaviour and a more robust community action plan model that includes partnership with the community since the outset of a crisis. Existing community knowledge, value systems, moral decision-making, structural barriers, local leadership, coping mechanisms influence how communities anticipate, adapt to diseases and contribute to mitigate the impact of the outbreak. Future research is needed to evaluate how populations' interactions with control measures can lead to an interdependent solution. Incorporating local knowledge and health priorities will allow designing interventions that are authentic and culturally relevant, ultimately informing a more equitable and inclusive model of public health interventions. The study also identified a need to develop novel sampling methods that are appropriate for estimating mortality for highly clustered diseases.

The second study of this thesis (Objective 2, Chapter 4) identifies important gaps related to the EVD case definition for the paediatric and pregnant population. Along with research to externally validate reviewed risk scores for Ebola virus infection; and to explore relationship between viraemia and symptoms at admission. Furthermore, the study highlights the need to use consistent thresholds for fever.

The third study of this thesis (Objective 3, Chapter 5) prompt reflection on the utility of using retrospective mortality studies to generate robust estimates for highly clustered diseases such as EVD. Possible limitations on sampling model for clustered diseases were suggested from data gathered in Objective 1, but further elaborated using empirical data collected in Objective 3. Future studies should assess the validity of alternative survey methods when measuring clustered diseases to inform response.

6.4 Conclusion

This thesis highlights that community plays a critical role on EVD control by influencing transmission dynamics, uptake of control measures, performance of case definition, and death reporting. Mitigation of risks will require a respectful and compassionate approach to affected households and an understanding of social norms and experiences and will link these to localised strategies to adapted community-lead health interventions. EVD control is the outcome of a chain of trust from local communities towards public health, which requires a synergistic model of public health in close partnership with community. Each research objective addressed specific practises and research gaps

and identified additional areas of operational research to improve practises during outbreak response and benchmark the disease burden.

Finally, the research presented in this thesis and field work informed the development of the MSF-OCA Community Guidance on COVID-19 response and the Covid-19 qualitative study conducted in Sierra Leone, which are a first step toward a more synergistic model of close partnering with community that needs further evaluation.

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The factors affecting household transmission dynamics and community compliance with Ebola control measures: a mixed-methods study in a rural village in Sierra Leone

Grazia Caleo[1,7*], Jennifer Duncombe[2], Freya Jephcott[3], Kamalini Lokuge[1,4], Clair Mills[2], Evita Looijen[2], Fivi Theoharaki[1], Ronald Kremer[2], Karline Kleijer[2], James Squire[5], Manjo Lamin[5], Beverley Stringer[6], Helen A. Weiss[7], Daniel Culli[1], Gian Luca Di Tanna[8] and Jane Greig[1]

Abstract

Background: Little is understood of Ebola virus disease (EVD) transmission dynamics and community compliance with control measures over time. Understanding these interactions is essential if interventions are to be effective in future outbreaks. We conducted a mixed-methods study to explore these factors in a rural village that experienced sustained EVD transmission in Kailahun District, Sierra Leone.

Methods: We reconstructed transmission dynamics using a cross-sectional survey conducted in April 2015, and cross-referenced our results with surveillance, burial, and Ebola Management Centre (EMC) data. Factors associated with EVD transmission were assessed with Cox proportional hazards regression. Following the survey, qualitative semi-structured interviews explored views of community informants and households.

Results: All households ($n = 240$; 1161 individuals) participated in the survey. 29 of 31 EVD probable/confirmed cases died (93·5% case fatality rate); six deaths (20·6%) had been missed by other surveillance systems. Transmission over five generations lasted 16 weeks. Although most households had ≤5 members there was a significant increase in risk of Ebola in households with > 5 members. Risk of EVD was also associated with older age. Cases were spatially clustered; all occurred in 15 households.

EVD transmission was better understood when the community experience started to concord with public health messages being given. Perceptions of contact tracing changed from invading privacy and selling people to ensuring community safety. Burials in plastic bags, without female attendants or prayer, were perceived as dishonourable. Further reasons for low compliance were low EMC survival rates, family perceptions of a moral duty to provide care to relatives, poor communication with the EMC, and loss of livelihoods due to quarantine. Compliance with response measures increased only after the second generation, coinciding with the implementation of restrictive by-laws, return of the first survivor, reduced contact with dead bodies, and admission of patients to the EMC.

(Continued on next page)

* Correspondence: grazia.caleo@london.msf.org
[1]Manson Unit, Médecins Sans Frontières (MSF), London, UK
[7]MRC Tropical Epidemiology Group, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK
Full list of author information is available at the end of the article

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Conclusions: Transmission occurred primarily in a few large households, with prolonged transmission and a high death toll. Return of a survivor to the village and more effective implementation of control strategies coincided with increased compliance to control measures, with few subsequent cases. We propose key recommendations for management of EVD outbreaks based on this experience.

Keywords: Ebola virus disease, Transmission dynamics, Community perception

Background

The first case of Ebola virus disease (EVD) in Sierra Leone is believed to have occurred in mid-May 2014, in a remote village of Kailahun District (estimated population 465,048) [1, 2]. On 12th June 2014, the President of Sierra Leone declared a state of emergency in the district [3]. The last case was recorded in Kailahun in mid-December 2014 and the Ministry of Health and Sanitation (MoHS) declared Kailahun District free from human-to-human transmission on 22nd January 2015, following 42 continuous days without a confirmed case [1]. Médecins sans Frontières (MSF) opened an Ebola Management Centre (EMC) in Kailahun on 26th June 2014 to support the district MoHS [4]. The MSF EMC was the only functioning Ebola management centre in the district, responsible for isolating 63·0% of confirmed cases. In total, the district MoHS reported 565 confirmed EVD cases in the population of Kailahun (attack rate 0·12%), including 287 deaths (case fatality rate [CFR] 51·0%) [5].

Evidence-based interventions for EVD control include early detection of cases through effective surveillance and contact tracing, admission of symptomatic cases to EMCs where staff adhere to high standards of infection control procedures, and safe burials by trained teams [6, 7]. Quarantine measures were also widely implemented [8], and by-laws imposed that included travel restrictions and penalties for hiding suspected cases [9].

The transmission dynamics of the West Africa EVD epidemic have, so far, been reconstructed from EMC and surveillance data, and mathematical modelling [4, 10–12]. However, poor surveillance systems and limited EMC capacities are likely to have resulted in underestimation of the true extent of the outbreak, limiting the ability to understand the dynamics and experience of the epidemic at community level, in particular in Sierra Leone, the country most affected by the West Africa EVD outbreak [13, 14].

Little is known of the factors that influence EVD transmission dynamics and community compliance with control measures over time. Such understanding is essential if interventions are to be effective, particularly in areas like Sierra Leone with no previous local EVD experience. In order to address this knowledge gap and inform future responses, we conducted an in-depth mixed-methods study in a rural village in Kailahun District that experienced prolonged EVD transmission during the outbreak.

Methods

To enable assessment of behaviour adaptation over time, we used data from MSF EMC patient registers to select a village in the district that had experienced a very protracted EVD outbreak. We then conducted a mixed-methods study combining data gathered via a cross-sectional survey and semi-structured interviews in this selected village. The cross-sectional survey data were used to reconstruct the dynamics of transmission. Semi-structured interviews were used to document community perception, resistance, and adaptation to response strategies. Survey and interview data were triangulated with data from the safe burial and MoHS surveillance databases to verify the reconstruction of the EVD transmission, and explain changes in transmission and behaviour over time.

Cross-sectional survey

All consenting households in the village were included in the cross-sectional household survey. A trained MSF team, using a validated instrument for household mortality studies and EVD case investigation forms, collected demographic data from household heads on household members, births, arrivals, departures, deaths, illnesses (including signs and symptoms compatible with the EVD case definition), and history of contact with individuals symptomatic for EVD [15, 16]. Verbal consent for participation was obtained from the head of each household after a briefing about the aim of the survey, the questions and duration of the questionnaire, and the option to end the interview at any time if wished.

The household survey was conducted in April 2015, with a recall period for responses between May 2014 (date of the first reported EVD case in the district) and the date of the survey. A local events calendar was developed to aid recall. MSF-EMC patient registers were used to verify the date of admission, symptoms, laboratory confirmation of EVD, and outcomes of patients admitted to the EMC. Each household in the village was enumerated and listed; from this list we randomly selected the households for the semi-structured interview.

Geographic positioning system (GPS) data were used to map the village layout and location of all households. Data were de-identified and entered into a password-protected electronic database.

Semi-structured interviews

At the end of the cross-sectional survey, semi-structured interviews were conducted with key community informants and selected households. Households were divided into two groups based on whether they had experienced at least one EVD case or no EVD cases. Ten households were randomly selected for interview from each group (total of 20 interviews).

A purposive approach was used to select key community informants: traditional healers; biomedical healthcare providers; and community leaders including tribal authorities, heads of community groups, and religious leaders. The heads of the selected households and key community informants were interviewed after verbal consent to participate was obtained. Participants were briefed about study objectives, questions and duration of interview, and the option to leave the study at any time. All interviews were semi-structured, took place in a private space, and were conducted by a trained MSF team.

Interviews were conducted in the local language using an interpreter to translate and back translate to English. The local events calendar developed for the household survey was also used in the semi-structured interviews. Topic guides directed interviewers to explore changes over time in perceptions of EVD and perspectives related to EVD response activities including contact tracing activities, the MSF EMC, the safe burial team, and quarantine. Interviews explored how these EVD control strategies were implemented and how these accorded with cultural beliefs. The topic guide was the same for household and key informant groups except for an additional section in the key informant guide, regarding how the outbreak started in the village. After initial data analysis had been completed, a summary narrative was compiled and shared with the village in the format of a story. Participant validation was achieved in this way in order to refine findings [17].

Case definitions

World Health Organization (WHO) EVD case definitions were used to define suspect, probable, and confirmed cases [16]. A suspect case was defined as: any person, alive or dead, suffering or having suffered from sudden onset of high fever and having had contact with a suspect, probable, or confirmed EVD case or with a dead or sick animal; any person with sudden onset of high fever and at least three relevant symptoms (headaches, vomiting, anorexia/loss of appetite, diarrhoea, lethargy, stomach pain, aching muscles or joints, difficulty swallowing, breathing difficulties, hiccup); any person with inexplicable bleeding; or any sudden, inexplicable death. A confirmed case was defined as anyone with a positive quantitative reverse transcription polymerase chain reaction (PCR) result. PCR cycle threshold (Ct) results were used as indicators of viral load. The lower the Ct value the higher the viral load [18]. A probable EVD case was defined as anyone who met the clinical case definition and had a history of contact with a person with confirmed EVD, but who did not have a confirmed laboratory test result [16].

Data analysis

Cox proportional hazards regression models were fitted to estimate hazard ratios (HRs) and 95% confidence intervals (95% CI) for the association between EVD (probable and confirmed cases) and covariates previously documented to be associated with EVD, including household size, sex, and age [19, 20]. Events were dated by epidemiological week and used as the time parameter in the Cox model. Cox shared frailty models were used to allow for within-household correlation.

The crude mortality rate (CMR) and EVD-specific mortality rate were estimated as deaths during study period/(mid-period population at risk x duration of period), where mid-period population at risk accounted for births, deaths, arrivals, and departures during the recall period [21]. Mortality rates were expressed as deaths per 10,000 per day. The attributable risk percent (AR%) and population attributable risk percent (PAR%) were used to estimate excess mortality risk due to EVD in the exposed households and village level, respectively.

The proportion of EVD cases isolated by admission to the EMC and the proportion of people who died from EVD and received safe burial were assessed by comparing cases reported in MoHS surveillance, EMC, and burial team data with cases (confirmed and probable) identified through the household survey.

Transmission dynamics were constructed using contact history, and described using transmission chains. Relationships between individuals were categorised as nuclear (immediate family), extra nuclear (extended family), and social (neighbours and friends).

Statistical analyses were carried out using Stata 14.0 (Stata Corporation, Texas-USA); maps were generated using QGIS™ software (version 2.14, https://qgis.org/en/site/). Participant responses from all semi-structured interviews were translated and transcribed at the time of the interview. Key community informants and household interview data were analysed separately using an inductive framework approach via an iterative process of coding and categorization (using ©NVivo 10) leading to the identification of emerging themes. The former contributed to the description of initial phase of the outbreak along with documenting the village experience over time, and the latter to exploring affected and unaffected household experience.

Results

Study population

The village consisted of 240 households (1161 individuals); all heads of households gave consent to participate. The median age of villagers was 18 years (interquartile range [IQR] 7–34 years), with 44·4% (n = 515) younger than 15 years old. Approximately half the villagers were female (52·7%). Household size ranged from 1 to 17 people, with a median size of 5 (IQR 3–6).

Transmission dynamics

Overall, 31 EVD cases (15 confirmed, 16 probable) were identified, giving an overall attack rate in the village of 2·7%. The index case was an adult male who was resident in a city that was a known EVD hotspot in June–July 2014. In late July 2014, while symptomatic, he travelled back to his village of origin and died 1 week after his return. Table 1 details the possible routes of EVD transmission that were reported by his household and key informants. There was no record of the index case being tested for EVD, although he was reportedly taken to a holding centre for testing.

Following death, the index case was buried in an unsafe manner by community members, many of whom had unprotected contact with the body. It is believed that this may have started the chain of person-to-person transmission in the village. Transmission lasted for 16 weeks, with 30 cases arising over five transmission generations: 11 cases in the 1st generation, seven in the 2nd, five in the 3rd, four in the 4th, and two in the 5th. For the one remaining case, a traditional birth attendant, a clear source of infection and transmission generation was not established (Fig. 1). The time from exposure to symptom onset was ≤2 weeks for all cases with known exposure. The first survivor came back to the village in week 35 (late August), after 7 weeks of transmission, when most of the cases in the village had already occurred.

Amongst the secondary cases with known exposure: 38·0% (11/29) had, as sole exposure, contact with a symptomatic person who was a probable/confirmed case; 10·3% (3/29) had a history of attending a funeral; and almost half (14/29; 48·2%) had history of both contact with a symptomatic person and a funeral exposure.

Table 1 Possible sources of infection for the index case

Contact with EVD patient(s) in the course of his work as a pharmacist
Contact with EVD patient(s) when he had a tooth extracted at a local Government Hospital, which was, at that time, a major hotspot of EVD
Contact with a traditional healer, who reportedly came from Guinea to treat the index case, who may have been infected
Contact with EVD patient(s) at a holding centre he was taken to for testing because he was symptomatic following the tooth extraction
Unknown source of infection (e.g. community)

The proportion of cases exposed via a funeral decreased over time from 90·9% (10/11) in the 1st generation to 71·4% (5/7) in the 2nd, 40·0% (2/5) in the 3rd, 25·0% (1/4) in the 4th, and none in the last. Contact with a symptomatic person increased from 72·7% (8/11) in the first to 100·0% in the following generations. Among the 30 secondary cases, 28 died (93·3%) and two survived (6·7%).

There was strong evidence of clustering of EVD (p < 0·0001), with all cases occurring in 15 of the 240 households (Fig. 2). Thirty-two percent of cases occurred in two households, in which cases occurred over three- and four-generation chains.

Most secondary cases were exposed via the nuclear (57·6%; 17/30) or extended family (30·0%; 9/30). Affected households had a median of seven members (IQR 6–8), and non-affected households a median of three (IQR 2–4) (p < 0·0001).

Factors associated with EVD

EVD was associated with older age and household size in unadjusted analysis; these associations became stronger after adjustment for both variables and sex (Table 2). The rate of EVD was similar by sex (aHR 1·03; 95% CI 0·49–2·17 for females vs males), but was greater among those aged 15–54 years (aHR 23·04; 95% CI 3·06–173·12) and ≥55 years (aHR 57·28; 95% CI 7·03–466·33) compared with those aged 5–14 years, and among those living in larger (> 5 members) (aHR 56·53; 95% CI 19·64–162·73) compared with smaller households (Table 2).

Mortality

Of the 31 cases (index case plus 30 secondary cases), 29 died (CFR 93·5%; 95% CI 78·6–99·2%). Thirteen of 15 confirmed cases and all 16 probable cases died. About half (55·2%) of EVD deaths were among females; three were pregnant and miscarried at home.

The community reported five non-EVD deaths during the recall period. The CMR for all causes of death (EVD and other) was 0·97 per 10,000 per day. EVD-specific CMR was 0·83 per 10,000 per day and the non-EVD CMR was 0·14 per 10,000 per day.

The AR% for death associated with EVD was 99·5% (95% CI 98·6–99·8) among the exposed households, while the PAR% for death associated with EVD in the whole village was 84·5%.

Admission to the MSF EMC

In mid-August 2014, cases started to be admitted to the EMC. Of the 31 cases, 15 were admitted to the EMC and had Ebola infection confirmed by PCR testing. Twelve cases had an exact date of symptom onset recorded, with a median time from first onset of symptoms to admission of 4·0 days (IQR 3–5). The median time to admission was 5·0 days in the first generation

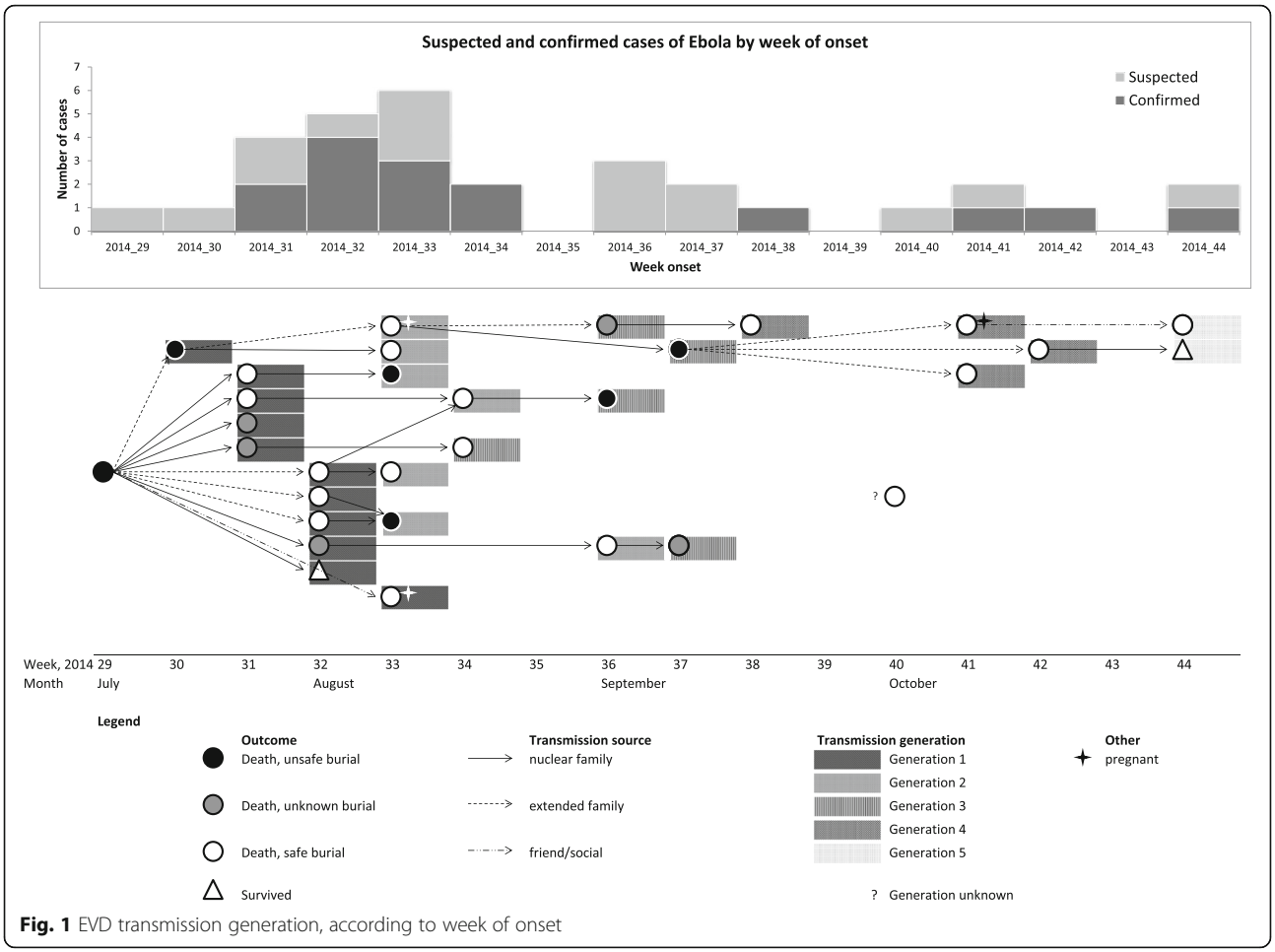

Fig. 1 EVD transmission generation, according to week of onset

(IQR 4–7), falling to 1·0 day in the last generation (IQR 0–1). The mean Ct value at admission was 21·8 (SD 4·5). Among the confirmed cases at EMC, 12 (80·0%) presented at admission with at least one wet symptom (diarrhoea, vomiting, or bleeding).

Burial, quarantine, and contact tracing

Of the 29 EVD deaths, 13 (44·8%) occurred within the EMC; five deaths in the community then had a safe burial by the burial team. Six deaths (20·6%) were captured during the survey but were not listed in the EMC, MoHS surveillance system and/or safe burial database. A further five people who died were reported by families to have been transported to an MSF or local Government hospital, however, there was no record of those patients in the EMC database. Contact tracing was reported to have occurred starting in late July; one in five village households reported they had been under contact tracing and quarantine measures. However, in August 2014, when 18 secondary cases had already occurred, the entire village was put under restriction of movements.

Community perception, resistance, and adaptation to response activities

Semi-structured interviews were conducted with 38 participants: 10 households reporting EVD cases (affected households (AH)), 10 households with no cases (UH), and 18 key community informants (CI).

Introduction of EVD in the village

When discussing how EVD had been introduced to the village, all participants referred to a single member or index case in the family or community, ranging from a family visitor to a health worker.

> "The man [index case] brought Ebola here. He used to treat people in [city] that was a hotspot at the time. When he got sick, he came here to see traditional healers and a herbalist came from Guinea to treat him using traditional herbs." – (CI09_m)

> "An ambulance came to collect him and take him to [XX] holding centre. It was anecdotally reported that he tested negative, so some relatives went to pick him

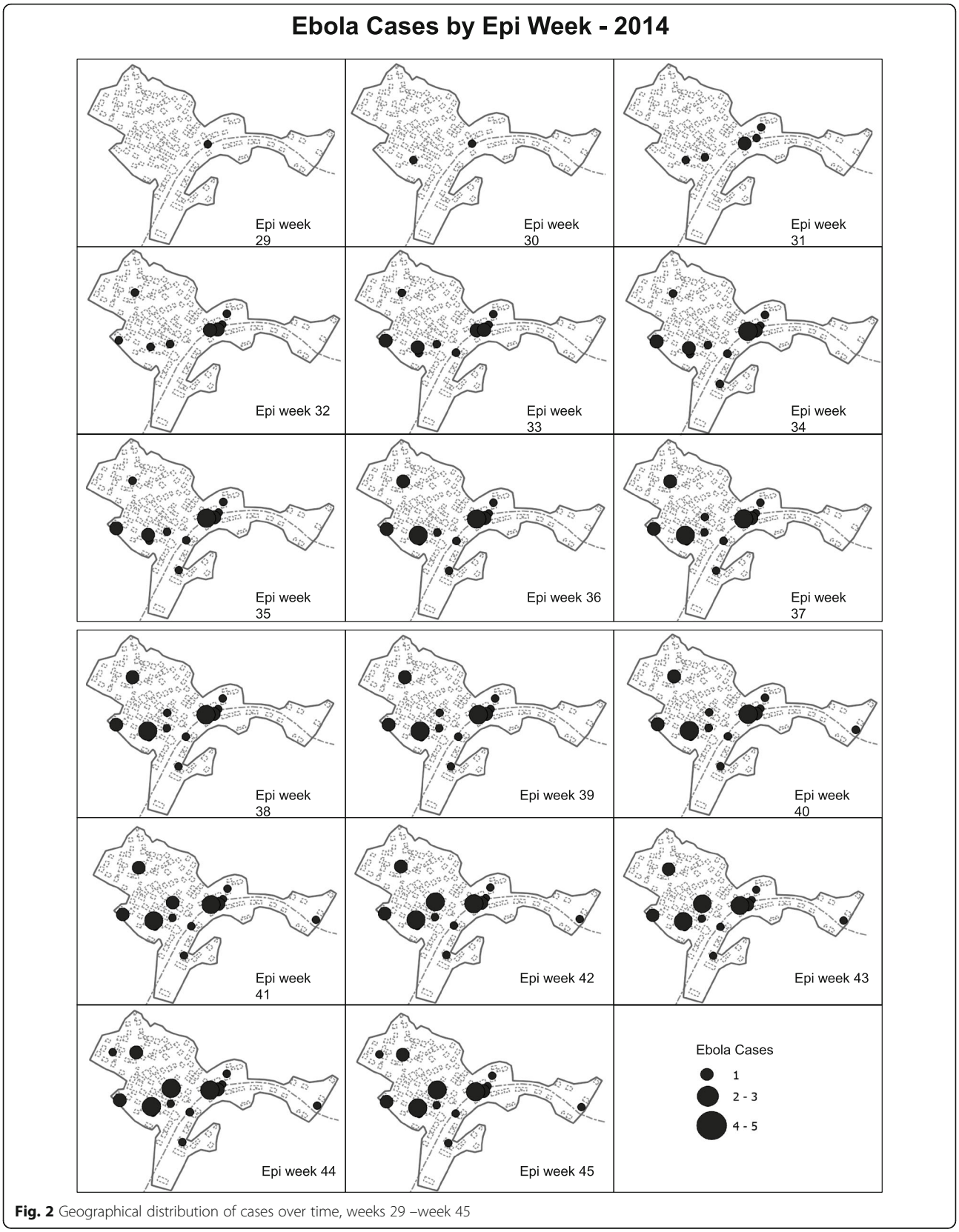

Fig. 2 Geographical distribution of cases over time, weeks 29 –week 45

Table 2 Demographic characteristics of the study participants and risk factors for EVD

	Entire village N	EVD infected N (% of village)	Crude hazard ratio	95% CI	p	Adjusted hazard ratio	95% CI	p	Adjusted hazard ratio from shared frailty Cox	95% CI	p
Sex											
Male	549	13 (2·4%)	ref			ref			ref		
Female	612	18 (2·9%)	1·24	0·61–2·54	0·55	1·03	0·49–2·17	0·92	1·19	0·52–2·73	0·68
Age group (years)											
< 5	174	4 (2·3%)	7·87	0·88–70·48	0·0001	6·02	0·66–54·39	< 0·0001	6·10	0·63–58·63	0·12
5–14	341	1 (0·3%)	ref			ref			ref		
15–54	552	18 (3·3%)	11·27	1·50–84·45		23·04	3·06–173·12		20·26	2·48–165·09	0·005
≥ 55	94	8 (8·5%)	31·57	3·94–252·48		57·28	7·03–466·33		53·06	5·89–477·66	< 0·0001
Household size											
≤ 5 members	973	4 (0·4%)	ref			ref			ref		
> 5 members	188	27 (14·4%)	37·15	12·99–106·19	< 0·0001	56·53	19·64–162·73	< 0·0001	56·08	16·38–191·92	< 0·0001

up. People were very happy, so they came to greet him/celebrate." - (CI04_m)

Misgivings toward Ebola

Initially, it was difficult for villagers to believe that infection could spread through everyday person-to-person contact. This perception was compounded by a climate of mistrust of authorities, fear of death, and lack of understanding of complex health messages such as the importance of isolation of those infected.

"We had never seen a sickness like this before, where you touch someone and you die." - (CI12_m)

"It seemed like someone had poisoned our village; many, many, many people died. It was similar to other diseases [e.g. malaria]." – (CI16_m)

"We thought it was a curse; some people thought that it was some kind of traditional medicine that was being thrown on them." - (CI13_f)

"People thought it was a conspiracy between the President and the westerners, who needed blood. They thought that if you go to the EMC, you will die." - (CI03_m)

"People didn't believe it: like war, we didn't believe it could come here. There was lots of arguing - some people thought Ebola wasn't real. They thought it was something sent by God." - (CI04_m)

"People were hiding symptoms and deaths because they were scared of the camp [EMC]; by the time they were found and the ambulance called, they were already dead." - (CI11_m)

"Early on, people were hiding if they were sick. By the time we knew they were sick, they weren't alive long enough to send them to the EMC (1-2 days)." - (UH05_m)

"We beat the contact tracers - we thought they were responsible for our relatives' deaths because they went for training at the same time [end of July] XX [index case] got sick." - (CI16_m)

"At the start, people hated the contact tracers - they beat them. One man in particular was beaten almost to death." - (CI17_f)

"The man [index case] came with a letter that said he should be isolated for 21 days. But we didn't understand what 'isolation' meant." - (CI16_m)

Change in perception

The perception of EVD held by the villagers changed when information received from contact tracers and the MSF health promotion team was consistent with what villagers observed in their lives at the community level. Implementation of the by-laws on travel and penalties for not reporting cases supported the understanding of the severity of the outbreak by villagers, and helped them accept that control measures were intended to protect and help the community.

"When we saw that people touched sick people and got sick, we could see the communication of it and realised that it is real." - (CI13_f)

"Sensitisation from different sources [MSF/MoHS/radio] started to make sense; symptoms in our loved ones were exactly the same as they were telling us." - (CI11_m)

"We realised that no contact was good, after a while, we saw the benefit." - (CI15_m)

"But we had to follow the law we had to pay 500,000 Leones if there was a sick person found in the house." - (CI12_m)

"It was for our own safety - to avoid touching bodies. To help them to stop the spread of Ebola. The word 'safe' equals 'help'." - (CI16_m)

Behaviour adaptation

Understanding of the route of transmission, and observing survival of cases admitted to the EMC supported changes in behaviour and adaptation by the community. This mainly occurred in late August coinciding with the return of a survivor, reduced contact with dead bodies, restriction of movements and isolation of patients.

"When we heard about people surviving people's attitude changed." - (CI03_m)

"We would go far away from the person and inform contact tracers who will call an ambulance to remove them to the camp [EMC]." - (UH04_f)

"Initially, it [burial team] was not good but when we saw that the deaths increased, we knew it was for our own safety." - (AH02_m)

The village implemented a number of local measures to prevent spread between households.

"During the outbreak, some people even devised their own preventive measures, like stopping children from playing football so they don't have contact with each other, and stop visit other households." - (CI09_m)

"Traditional birth attendants stop doing deliveries." - (CI17_f)

Understanding control strategies and constraints

All strategies such us MSF/EMC, MSF health promotion, contact tracing, burial practices, quarantine/restriction of movements were understood by the community as helping to control EVD. However, resistance to specific practices that were perceived as offensive to socio-cultural norms was reported; this resistance continued until the value of such practices was understood.

MSF/EMC

The EMC was understood to help people survive:

"Without the camp [EMC] - we would have no survivors." - (CI04_m)

However, communication regarding the status of admitted patients was perceived as poor:

"We received no information while they were still alive. When they died, a nurse who worked at the camp [EMC] told us." - (CI14_f)

"When the ambulance went with XX to the camp [EMC], some family members went to visit and they learned that he had died." - (CI07_m)

The MSF health promotion team were perceived as empowering the community:

"It gave the Community Health Workers a zeal to call ambulances; they empowered us. They sensitised us about preventive methods and no touch." - (CI06_m)

"Helped to decrease cases." - (CI12_m)

"We learned not to touch other people, and use water and soap." - (CI15_m)

Contact tracing

Contact tracing was perceived as a mechanism to remove people from the community who were thought to be a risk, which initially created mistrust. This gave contact tracers a reputation for invading privacy and disrupting family and community life and sending people to their deaths.

"There was no sensitisation about why contact tracers were here. They would just call the ambulance and collect people to the EMC." - (CI01_m)

"We didn't like the contact tracers; called them murderers." - (CI02_f)

"Invasion of privacy - it was not their business to investigate our household." - (CI04_m)

"We didn't like the fact that they were involving themselves in our affairs, we thought contact tracers

were selling us to other people and that they were too inquisitive." - (CI17_f)

However, contact tracers were valued once people understood that they were trying to protect people and prevent the spread of Ebola:

"It is our culture to touch people when they are sick, so if you don't take people out of the village, people will touch them." - (CI11_m)

"Without contact tracers we would have continued touching people. Instead, sick people were collected to the camp [EMC]." - (CI16_m)

"Otherwise we would have far more deaths." - (CI14_f)

"Contact tracers should be empowered with training to stop the spread." - (CI13_f)

Burial practices

The value of safe burials was understood:

"Without the burial team, the disease would have spread because touching dead bodies is bad." - (CI02_f)

However, burials were initially seen as lacking honour in terms of how they were performed, specifically the use of plastic bags, and the lack of burial clothes and prayers. Respondents also commented on the lack of women in the burial team and on the arrival of the teams in the village already dressed in personal protective equipment (PPE).

"Plastic bags are not traditional - there is no honour when you bury people this way." - (CI03_m)

"Praying was not allowed." - (CI09_m)

"Sometimes, in dreams, my husband appears and says 'I have no clothes'." - (AH06_f)

"Men burying women is not good; women should be part of the burial team." - (CI17_f)

"We weren't happy about it. Before the outbreak, if a chief dies or a special person dies, they are buried by other special people. Now, we can't do that. There is no clothes, no dressing - and men are burying women, which is a problem for us." - (CI11_m)

"People were afraid of the burial team when they came dressed in full protective clothes. They thought they were ghosts." - (CI03_m)

In October, the burial procedures were improved to incorporate greater respect for local tradition:

"We couldn't pray before, either, but now we can." - (CI03_m)

"Now they [burial team] dress in protective clothes in the village." - (CI11_m)

Quarantine/restriction of movements

The community understood the value of quarantine:

"Because of quarantine, we couldn't spread Ebola to other households." - (AH07_m)

However, people were also angry about quarantine:

"It destroyed many things, especially farming, our crops were destroyed and there is no food available now." – (CI15_m)

In September, quarantine measures were improved by incorporation of food supply to quarantined households:

"We had no food at the start. They should have given us food like they did in other households at the end." - (AH06_f)

Affected versus unaffected households

Both affected and unaffected households were sensitive to law enforcement and were in favour of stricter methods to control Ebola in the future. The consequences of quarantine, in terms of financial and emotional impact and stigma, were harsher in affected households compared with non-affected households, since non-affected households were only directly impacted when the entire village was quarantined.

"Seven members of my family were taken to EMC. They all died there. Everyone would yell at us, 'you brought Ebola here!' I didn't - my brother did. But I still felt guilty." - (AH03_m)

Affected households provide some insight into factors that led to continued transmission in some homes but not in others, and why within-household transmission continued even when between-household transmission was reducing:

"We could not abandon sick people – we must care for [them]." - (AH05_f)

"People didn't come around - it was like the devil was here." - (AH04_m)

Discussion

Our study provides a comprehensive description of EVD in one village in Kailahun District, Sierra Leone that experienced sustained EVD transmission during 2014. We attempt to capture the complexities of the social context influencing outbreak control in this specific epidemic. We documented that immediate family members of large households were at greater risk of being infected, and because of the larger number of inhabitants, these households were more likely to maintain transmission. This finding corroborates insights from other studies. This may imply that future responses to an EVD outbreak could justify prioritization of affected large households and their immediate family members, in particular when human resources are insufficient to address the scale of the outbreak [19, 22].

Within affected households, transmission was maintained by the need to provide care for sick relatives, with cases continuing to occur over several generations. Compliance with response measures increased only after the second generation, coinciding with the return of a survivor, and strict implementation of other components of the EVD response, such as restriction of movements, reduced contact with dead bodies, and isolation of cases. However, this changing context only occurred after 7 weeks of transmission, when most of the cases in this outbreak had already died.

In particular, return of survivors to the village after treatment prompted a shift toward belief in Ebola and increasing acceptance of control measures. Late return of survivors prevented teams from building trust within the community. At the time that survivors returned, the village was experiencing a peak in case numbers, the MSF EMC was reaching the limit of its capacity (100 beds), and communication with households was primarily to inform of deceased loved ones, thus contributing to community fear and despair. People reported avoiding the MSF EMC because of poor survival rates, which reinforced the community perception of the EMC as a place where people die. One approach to improving community understanding and uptake of EMC services in future could include developing the role of an EMC-village liaison, whose role would be to support timely communication with communities about the status of relatives throughout admission. Use of EMC-village liaisons could acknowledge the gap in understanding of health system workers as to why patients may undermine control measures when faced with the need to look after their loved ones. Contact tracers could potentially play this liaison role, and therefore have the potential to be seen as providing something positive to the community rather than just reporting and tracing cases.

Reduced misgivings and doubt about Ebola were crucial to influencing attitudes toward control measures. This change likely occurred once the health messages given to the community mirrored their reality. Once Ebola transmission was understood, the perceptions of contract tracing changed from invading privacy, selling people, to working collectively toward community safety. The community then participated in control measures by setting up a number of local strategies such as stopping babies being delivered in the community, preventing children from playing contact games together, and not visiting other households. These strategies contributed to outbreak control, as observed by other authors [23]. Our findings emphasised the importance of the community having a role in tailoring outbreak responses. Following a localised governance approach may permit incorporation of accepted local social norms from the outset of intervention efforts, making them more acceptable and therefore effective.

Clear communication of complex health messages was challenging, but played a role in the acceptance of EVD control measures. It was essential that the community understood there was a 21-day incubation period, the importance of EMC isolation (both self-imposed and institutional), and that a single negative test result could not rule out disease during the incubation period. Other authors described similar issues for messaging in Sierra Leone and in previous outbreaks [24, 25].

Similar to the rest of the country, the age structure of the village was young, with those under age 15 accounting 44% of the population. The limited life experience of youth, and particularly collective experience with death from exposure to body fluids (e.g. "touch someone and you die") or with infection prevention and control concepts (e.g. "we didn't understand what 'isolation' meant") may have contributed to delays in understanding and adoption of the necessary responses, rather than villagers being deliberately uncooperative. However, we documented that regardless of age, the population in general suffered an overwhelming level of inexperience toward this disease and its impact. Response agencies must acknowledge community demographic structure and perspectives on the presence of EVD in parallel with launching control measures cognisant of their baseline understanding.

Our study findings show nuanced perceptions toward quarantine as both a way to control the spread of Ebola and a cause of social and livelihood disruption, which challenged compliance, as reported by other researchers [26].

This argues for such social disruption to be taken into account when planning how best to protect affected people and control transmission.

Safe burial using plastic bags, lack of burial clothes, and the absence of women in the burial team were described as showing a lack of honour for the deceased. Burials were described as being more compliant to control measures when practices such as community prayer were permitted. In addition, the burial team started to dress in PPE after arrival in the village as now recommended by WHO Guidelines [27]. Additional measures that can be implemented without compromising safe burial, such as including female members in the burial team, and safe alternatives to plastic burial bags, would further enhance community acceptance compliance, and should be included in EVD control guidelines.

The comprehensive design of this study enabled every household in the village to be surveyed, and therefore a number of deaths were captured by our survey that were not identified by MOHS surveillance, EMC, or burial data. All cases and deaths detected were spatially clustered; this is a key finding since traditional methods to estimate mortality rely on cluster sampling approaches, which in this case could have generated either an under- or over-estimation of EVD, depending on whether the limited number of affected households was randomly selected. This is an important element to take into account while trying to benchmark the burden of highly clustered diseases like EVD. Even in a highly affected community, clustering of disease means that household sampling is likely to miss many households unless an appropriate estimate of intra-cluster correlation is available. It is noted that it would not have been feasible to carry out exhaustive studies on the wider population in the middle of the EVD outbreak. In future, we recommend developing alternative methods of sampling to estimate disease and mortality that account for the highly clustered nature of diseases such as EVD.

Strengths and limitations

A major strength of this study is its mixed methods design, which provides a deeper understanding and explanation of the social reactions to dealing with EVD at community level. Half of the EVD cases in this study were not confirmed by PCR. However, they met the suspected case definition, died, had clear epidemiological links with a confirmed case, and some generated secondary cases, some of which were confirmed EVD. The number of deaths may have been under-reported, as villagers may have feared a penalty for not adhering to the mandatory notification by-law. However, it should also be noted that the study was well perceived by villagers, as demonstrated by the participation of the entire village, their help in documenting the transmission chains, and their willingness to tell the story of the village outbreak. We cannot exclude underestimation of the burden of EVD infection in the village by missing mild or asymptomatic cases. We also collected data on morbidity at the time of the outbreak, and three living people reported history of symptoms compatible with EVD, and a history of exposure, but they were never tested or isolated and thus not included in the analysis. If they were true cases, our EVD mortality may be overestimated, however, when we did include these cases in the analysis it did not change our findings significantly. The true EVD infection rate could be known only via a serological study [28].

Incorrect recall of the timing of deaths may have occurred, but the impact of Ebola makes this less likely, and the use of a local community calendar of events aided recollection of timing. In addition, we validated dates and symptoms for cases admitted to the Kailahun EMC, MOHS surveillance, and buried by the burial team. We were able to rebuild accurate dates for the events of each case we identified, validated across multiple data sources.

For the qualitative part of the study, we acknowledge it was more difficult to definitively link community behaviour change with specific measures or events. Furthermore, we recognise that those are reported perceptions recollected at the time of the outbreak, however, these were consistent among the different people interviewed and suggested a shift in the way the community expressed their ideas of EVD. We acknowledge that perception of changes in the village may have been influenced by the differing roles played by community informants, and in relation to the differing experiences of affected vs. unaffected households.

It is also important to note that our observations were based on a single, high-burden village. Our findings are therefore likely to be generalizable to similar rural settings with high levels of transmission. However, it is possible that the outbreak and response would be different in villages with lower levels of transmission, as experience of the disease was an important driver of behavioural change.

Finally, the main limitation of our qualitative work was that questions regarding burial practices seemed to provoke a limited depth of response in particular among affected households. This may have been because respondents were still affected by their loss.

Conclusion

In this high-burden village, transmission was maintained by a small number of large households; the outbreak was controlled in this community only after prolonged transmission and a high death toll. A key recommendation emerging from these findings is to ensure that large

households and immediate family members are prioritized in control and prevention activities. There is also a need to develop novel sampling methods appropriate for estimating mortality for highly clustered diseases like EVD.

Our findings provide practical information on how future interventions could be implemented more humanely and effectively. We emphasise the following factors: recognising the role of communities for their contribution in controlling outbreaks; identifying community liaison roles which can keep families informed of their relatives' progress in the EMC; ensuring survivors are engaged to increase community trust to delegate care to EMCs; conveying complex health messages around incubation periods and infectivity clearly to the community; using appropriate alternatives to burial in plastic bags; including women in burial teams; and compensating quarantined households and communities to ensure they can maintain and re-establish livelihoods.

Factors underlying delays in implementing control measures included community belief or otherwise in the presence of EVD, lack of trust, and the toll imposed by interventions such as safe burial procedures and the social disruption of quarantine. Early understanding of social norms and experiences and the ability to link this to localised strategies and adapted health interventions would be essential.

Including these findings in future recommendations for outbreak control policy could help to improve the accuracy of mortality estimates and avoid unnecessary deaths and protracted suffering in future outbreaks.

Abbreviations

AR%: Attributable risk percent; CFR: Case fatality rate; CI: Confidence intervals; CMR: Crude mortality rate; Ct: Cycle threshold; EMC: Ebola Management Centre; EVD: Ebola virus disease; GPS: Geographic positioning system; HRs: Hazard ratios; IQR: Interquartile range; MoHS: Ministry of Health and Sanitation; MSF: Médecins sans Frontières; PAR%: Population attributable risk percent; PCR: Polymerase chain reaction; PPE: Personal protective equipment; WHO: World Health Organization

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Availability of data and materials

Data are available under the MSF data sharing policy. Requests to access data can be made to data.sharing@msf.org.

Authors' contributions

GC, JG, CM, and FJ conceived the idea; JD and EL implemented the study; and GC wrote the first and final drafts. GC, BS, FT, DC, and GDT contributed to the analysis and interpretation. JG, KL, RK, KK, JS, ML and HW reviewed early drafts. KL, JG, BS, and HW reviewed the late draft. All authors have given signed or electronic approval to be authors on the manuscript. All authors read and approved the final manuscript.

Authors' information

GC, JG, FT, DC, KL are associated with the Manson Unit, Médecins Sans Frontières (MSF).
KL is associated with the National Centre for Epidemiology and Population Health, Research School of Population Health, Australian National University.
JD, CM, EL, RK, KK are associated with Médecins Sans Frontières (MSF) Amsterdam.
FJ is associated with the Department of Veterinary Medicine, University of Cambridge.
JS, ML are associated with the District Health Management Team, Ministry of Health and Sanitation, Kailahun, Sierra Leone.
BS is associated with Médecins Sans Frontières (MSF) London.
HW is associated with MRC Tropical Epidemiology Group, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine.
GDT is associated with the Centre for Primary Care and Public Health, Queen Mary University of London.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Review Board of MSF, the Internal Review Board of the Sierra Leone MoHS, and The London School of Hygiene & Tropical Medicine (LSHTM). Verbal consent for participation was obtained from the head of each household after a briefing about the aim of the survey, the questions and duration of the questionnaire, and the option to end the interview at any time if wished.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author details

[1]Manson Unit, Médecins Sans Frontières (MSF), London, UK. [2]MSF, Amsterdam, The Netherlands. [3]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK. [4]National Centre for Epidemiology and Population Health, Research School of Population Health, Australian National University, Canberra, Australia. [5]District Health Management Team, Ministry of Health and Sanitation, Kailahun, Sierra Leone. [6]MSF, London, UK. [7]MRC Tropical Epidemiology Group, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK. [8]Centre for Primary Care and Public Health, Queen Mary University of London, London, UK.

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Clinical and epidemiological performance of WHO Ebola case definitions: a systematic review and meta-analysis

Grazia Caleo, Foivi Theocharaki, Kamalini Lokuge, Helen A Weiss, Leena Inamdar, Francesco Grandesso, Kostas Danis, Biagio Pedalino, Gary Kobinger, Armand Sprecher, Jane Greig, Gian Luca Di Tanna

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Manson Unit, Médecins sans Frontières, London, UK (G Caleo MD, K Lokuge PhD, J Greig PhD); **Centre for Longitudinal Studies, University College London, London, UK** (F Theocharaki MSc); **National Centre for Epidemiology and Population Health, Research School of Population Health, Australian National University, Canberra, ACT, Australia** (K Lokuge); **MRC Tropical Epidemiology Group, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK** (G Caleo, Prof H A Weiss PhD); **Public Health England, Leeds, UK** (L Inamdar MD); **Epicentre, Paris, France** (F Grandesso MSc); **Santé Publique France, Saint-Maurice, France** (K Danis PhD); **TEPHINET, Task Force for Global Health, Decatur, GA, USA** (B Pedalino DrPH); **Istituto Superiore Di Sanita, Rome, Italy** (B Pedalino); **Département de Microbiologie-Infectiologie et d'Immunologie, Université Laval, Quebec City, QC, Canada** (Prof G Kobinger PhD); **Médecins sans Frontières, Brussels, Belgium** (A Sprecher MD); **and The George Institute for Global Health, University of New South Wales, Sydney, NSW, Australia** (G L Di Tanna PhD)

Correspondence to: Dr Grazia Caleo, Manson Unit, Médecins sans Frontières, London EC4A 1AB, UK **grazia.caleo@london.msf.org**

Summary

Background Ebola virus disease case definition is a crucial surveillance tool to detect suspected cases for referral and as a screening tool for clinicians to support admission and laboratory testing decisions at Ebola health facilities. We aimed to assess the performance of the WHO Ebola virus disease case definitions and other screening scores.

Methods In this systematic review and meta-analysis, we searched PubMed, Scopus, Embase, and Web of Science for studies published in English between June 13, 1978, and Jan 14, 2020. We included studies that estimated the sensitivity and specificity of WHO Ebola virus disease case definitions, clinical and epidemiological characteristics (symptoms at admission and contact history), and predictive risk scores against the reference standard (laboratory-confirmed Ebola virus disease). Summary estimates of sensitivity and specificity were calculated using bivariate and hierarchical summary receiver operating characteristic (when four or more studies provided data) or random-effects meta-analysis (fewer than four studies provided data).

Findings We identified 2493 publications, of which 14 studies from four countries (Sierra Leone, Guinea, Liberia, and Angola) were included in the analysis. 12 021 people with suspected disease were included, of whom 4874 were confirmed as positive for Ebola virus infection. Six studies explored the performance of WHO case definitions in non-paediatric populations, and in all of these studies, suspected and probable cases were combined and could not be disaggregated for analysis. The pooled sensitivity of the WHO Ebola virus disease case definitions from these studies was 81·5% (95% CI 74·1–87·2) and pooled specificity was 35·7% (28·5–43·6). History of contact or epidemiological link was a key predictor for the WHO case definitions (seven studies) and for risk scores (six studies). The most sensitive symptom was intense fatigue (79·0% [95% CI 74·4–83·0]), assessed in seven studies, and the least sensitive symptom was pain behind the eyes (1·0% [0·0–7·0]), assessed in three studies. The performance of fever as a symptom varied depending on the cutoff used to define fever.

Interpretation WHO Ebola virus disease case definitions perform suboptimally to identify cases at both community level and during triage at Ebola health facilities. Inclusion of intense fatigue as a key symptom and contact history could improve the performance of case definitions, but implementation of these changes will require effective collaboration with, and trust of, affected communities.

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Introduction

Ebola virus disease case definition is a crucial surveillance tool to detect suspected cases for referral and as a screening tool for clinicians to support admission and laboratory testing decisions at Ebola health facilities. However, there have been long-standing concerns about the poor performance of the WHO Ebola virus disease case definitions, including the inability to distinguish Ebola virus disease from common diseases such as malaria and typhoid fever.[1–3]

The scale of the 2014–16 west African Ebola epidemic further challenged the operational use and validity of the WHO case definitions in detecting suspected cases at the community level and allocating patients appropriately to high-risk or low-risk wards for testing at specialised isolation centres.[4] Consequently, during and since this epidemic, organisations involved in the Ebola virus disease response have estimated the sensitivity and specificity of the WHO case definitions and its constituent symptoms and signs, and developed alternative definitions and risk scores to identify clinical and epidemiological factors that could predict infection under outbreak conditions.[5,6] Discordance on the use of WHO Ebola virus disease case definitions with consequent delay on outbreak control and community disengagement have been reported in west Africa and, in the current outbreak, in the Democratic Republic of the Congo along with its bordering countries.[7–9]

However, the operational use and performance of those definitions and risk scores has not been rigorously evaluated. Such an evaluation is needed to guide communities and public health practitioners to improve the effectiveness and efficiency of identification and management of suspected cases during Ebola virus disease responses.

Research in context

Evidence before this study

There have been long-standing concerns about the poor performance of WHO case definitions for Ebola virus disease, including their inability to distinguish Ebola virus infection from common tropical diseases. We did a systematic search of the scientific literature using PubMed, Scopus, Embase, and Web of Science, without regional restrictions, for research articles published in English between June 13, 1978, and Jan 14, 2020. We used the search terms "Ebola", "EVD infection", "case definition", "admission symptoms", "sensitivity", "specificity", "likelihood", "score", "classification", "validity" and "performance". We also contacted relevant experts. We found that different organisations have attempted to assess the performance of WHO Ebola case definitions and developed alternative definitions and risk scores. However, there has been no systematic and rigorous evaluation of those studies. Such an evaluation is needed to guide communities and public health practitioners to improve the effectiveness and efficiency of identification and management of suspected cases during an Ebola virus disease outbreak.

Added value of this study

To our knowledge, this study is the first systematic review and meta-analysis that assesses the performance of the WHO Ebola virus disease case definitions, and other clinical and epidemiological characteristics such as symptoms and signs at admission and contact history, against the reference standard (laboratory confirmation of Ebola virus infection). Our analysis provides the most comprehensive evidence on the limitations of WHO case definitions and its constituent symptoms and signs, and predictive risk scores. We show that the WHO case definitions perform suboptimally to identify cases at both the community level and during triage at general and specialist health facilities. The performance of fever as a symptom varied depending on the cutoff used to define fever. The most sensitive symptom was intense fatigue. History of contact was a key predictor for the WHO case definitions and for risk scores. This study identifies important gaps related to the paediatric and pregnant population and highlights the need to use consistent thresholds (eg, for fever) to explore viraemia and symptoms at admission, and to externally validate risk scores for Ebola virus infection.

Implications of all the available evidence

Inclusion of intense fatigue as a key symptom could improve the sensitivity, the primary requirement for community-based screening, of WHO and alternative case definitions. Inclusion of contact history will improve specificity, resulting in a lower number of false positives and thus a lower number of unnecessary admissions to Ebola health facilities. These improvements will contribute to reduced isolation from family, fear of being stigmatised, delay to appropriate care, and community mistrust in response activities.

We aimed to assess the performance of the WHO Ebola virus disease case definitions and other clinical and epidemiological characteristics, such as symptoms and signs at admission and contact history, as the index test or test under assessment, against the reference standard of laboratory-confirmed Ebola virus infection.

Methods

Search strategy and selection criteria

For this systematic review and meta-analysis, we searched PubMed, Scopus, Embase, and Web of Science, without regional restrictions, for studies in English published between June 13, 1978 (when the first Ebola virus disease outbreaks were reported on), and Jan 14, 2020.[10,11] We also endeavoured to capture data on the current outbreak of Ebola virus disease in the Democratic Republic of the Congo by contacting relevant people involved in the response.

The search terms included "Ebola", "EVD infection", "case definition", "admission symptoms", "sensitivity", "specificity", "likelihood", "score", "classification", "validity", and "performance" (appendix pp 5–6).

We included observational retrospective studies that estimated the sensitivity and specificity of WHO Ebola virus disease case definitions and other clinical and epidemiological characteristics (symptoms and signs at admission and contact history) against the reference standard (laboratory confirmation of Ebola virus infection), and studies that developed, or externally validated, predictive risk scores (based on a combination of symptoms and signs, and epidemiological information) to predict the risk of being positive for Ebola virus. We also included studies looking at sensitivity and specificity of WHO case definitions for Ebola or Marburg virus infections because they belong to the same family of viruses (Filoviridae) and share the same case definitions, and the reference standard is laboratory confirmation of infection.[12] We excluded studies on the sensitivity and specificity of diagnostic tests, animal and vaccine studies, studies of survivors of Ebola virus disease, and studies on predictors of outcomes or severity of Ebola virus disease, community surveillance, and outbreak and clinical management. Studies specifically on frequency of symptoms at admission were also excluded as a previous review exists.[13]

Two reviewers (GC and FT) independently screened all titles and abstracts to identify those meeting the selection criteria, and a third author (LI) arbitrated for studies without consensus. A full-text review was then done for these articles, and their bibliographies were assessed for other eligible studies. We extracted data on author, year of publication, country, virus, period of data collection, study design, study objective, outcomes measured, setting in which data were collected (eg, Ebola treatment centres), age of population included in the study, study size including number of patients who were negative and positive for Ebola virus, diagnostic method, limitation of

See Online for appendix

	WHO case definitions (August, 2014) all ages[12]	WHO case definition (December, 2014) all ages in Sierra Leone[15]	Late 2014 WHO case definition for paediatric population in Sierra Leone[15]
Suspected	Any person, alive or dead, suffering or having suffered from sudden onset of high fever and having had contact: • a suspect, probable, or confirmed Ebola virus disease case • with a dead or sick animal (for Ebola) • a mine (for Marburg); OR any person with sudden onset of high fever and at least three of the following symptoms: • headaches • lethargy • anorexia or loss of appetite • aching muscles or joints • stomach pain • difficulty swallowing • vomiting • difficulty breathing • diarrhoea • hiccups; OR any person with inexplicable bleeding; OR any sudden, inexplicable death	Any person having had contact with a clinical case and presenting with acute fever (>38°C); OR having had contact with a clinical case (suspected, probable, or confirmed) and presenting with three or more of the symptoms below; OR presenting with acute fever and presenting with three or more of the symptoms below: • headache • nausea or vomiting • loss of appetite • diarrhoea • intense fatigue • abdominal pain • generalised or articular pain • difficulty in swallowing • difficulty in breathing • hiccups • miscarriage; OR any person with unexplained bleeding or miscarriage; OR any unexplained death	Any child with fever and either one symptom (in children younger than 5 years), two symptoms (in children aged 5–12 years), or more than three symptoms (in children older than 12 years); for children younger than 1 years old, maternal history is very important
Confirmed	Any suspected or probable cases with a positive laboratory result; laboratory-confirmed cases must test positive for the virus antigen, either by detection of virus RNA by RT-PCR, or by detection of IgM antibodies directed against Marburg or Ebola	Any person with a positive PCR test for Ebola or Marburg virus	Any person with a positive PCR test for Ebola or Marburg virus
Probable	Any suspected case evaluated by a clinician; OR any deceased suspected case (where it has not been possible to collect specimens for laboratory confirmation) having an epidemiological link with a confirmed case	A suspect case that is known to have had contact with a known case (suspected, probable, or confirmed); OR any person who is, on clinical or epidemiological grounds, very likely to have Ebola or Marburg	Not further specified

Figure 1: **WHO Ebola virus disease case definitions for all ages and the paediatric population**

individual studies, and performance of the WHO Ebola virus disease case definitions, and individual symptoms and signs, and epidemiological links or contact history with known patients with Ebola virus disease.

Performance data extracted included sensitivity, specificity, predictive values and risk score, and area under the receiver operating characteristic (ROC) curve (AUC). We developed a spreadsheet to compile extracted data based on the Cochrane data tool.[14] The primary data extracted from each article were checked by a second researcher (FT). No protocol was developed for this study.

WHO Ebola virus disease case definitions were used to define suspected, probable, and confirmed cases, which varied by context and period of outbreak. In 2014 in Sierra Leone, WHO included miscarriage as an additional symptom (eg, abdominal pain) or sign (eg, vaginal bleeding) to the existing definitions.[12,15] For paediatric populations, the modified WHO case definition used in Sierra Leone was evaluated (figure 1).[15]

Data analysis

We derived the numbers of true positive, false negative, true negative, and false positive cases in each study using data provided in each article for each symptom and sign, and WHO Ebola virus disease case definition. Sensitivity and specificity are correlated, and univariate measures of heterogeneity, such as I^2, are not suitable to report heterogeneity in diagnostic test accuracy reviews.[16] We used bivariate and hierarchical summary ROC (HSROC) models for meta-analysis.[17,18]

The bivariate model provides estimation of a summary of sensitivity and specificity, whereas the HSROC model provides the estimation of a summary curve from studies that have used different thresholds, the 95% confidence region for the summary point, and the 95% prediction region. The prediction region graphically illustrates between-study heterogeneity as well as the bivariate relationship between sensitivity and specificity.[19] Only studies that used comparable thresholds, symptoms and signs, or definitions were combined using these methods.

Given that HSROC models cannot be fitted when there are data from fewer than four studies, for some symptoms and signs we did a random-effects meta-analysis to calculate pooled estimates for sensitivity and specificity.[20] Compared with bivariate and hierarchical models, pooled estimation from random-effects meta-analysis could slightly overestimate point estimation, so

	Country	Virus	Period of data collection	Design	Objective	Outcomes	Setting of data collection	Age of study population	Patients positive for Ebola virus/ total patients	Method (reference standard) and timing of Ebola virus confirmatory testing	Limitations
Roddy et al (2010)[22]	Angola	Marburg	March–July, 2005	Observational retrospective study of data at admission	Evaluate the diagnostic validity of individual patient clinical and epidemiological characteristics and WHO-recommended case definitions for Marburg haemorrhagic fever, and develop a data-derived diagnostic algorithm for Marburg haemorrhagic fever that improves the WHO-recommended definitions	Sensitivity and specificity of WHO case definition, WHO case subdefinitions, symptoms at admission, and epidemiological link; and risk score	Screening at one hospital	All ages	41/102	Quantitative PCR on admission	Small sample; only saw patients at admission; data only captured Marburg haemorrhagic fever; hospital-based data collection; detailed data not available for all Marburg haemorrhagic fever cases; only presenting symptoms were recorded; highlights the necessity of collecting high-quality clinical and epidemiological data during outbreaks; over-representation of individuals with more serious symptoms that required hospital admission; no reported validation (external or internal)
Kuehne et al (2015)[23]	Liberia	Ebola	August, 2014–March, 2015	Observational retrospective study of data at admission and clinical results	Study the discriminative accuracy (sensitivity, attributable frequency, diagnostic test odds ratio, area under the receiver operating characteristic curve) of clinical signs, contact history, and combinations thereof	Sensitivity and specificity of WHO case subdefinitions, symptoms at admission, and epidemiological link; and risk score	One Ebola treatment centre	All ages	1235/1832	Quantitative PCR on admission	Reporting bias; poor data quality; conference poster and abstract data (Kuehne A, Epicentre, Paris, France, personal communication); no reported validation (external or internal)
Levine et al (2015)[29]	Liberia	Ebola	September, 2014–January, 2015	Observational retrospective study of data at admission	Develop a clinical prediction model that can help to guide care for patients with suspected Ebola virus disease, provide specific parameters for isolation and admission to treatment centres, and maximise resource use	Sensitivity and specificity of WHO case definition, symptoms at admission, and epidemiological link; and risk score	One Ebola treatment centre	All ages	160/382	Quantitative PCR on admission	Data collected only at admission, different stages of disease process; data might not be representative of all patients with Ebola virus disease; poor data quality; small sample; patients pre-screened by Ebola treatment units (ambulance travel); only assessed 14 variables; no reported external validation, only internal validation
Lado et al (2015)[5]	Sierra Leone	Ebola	May, 2014–December, 2014	Observational retrospective study of data at admission	Identify clinical characteristics that were predictive of Ebola virus disease diagnosis and assess the accuracy of suspected Ebola virus disease case definitions	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link	One Ebola holding unit	All ages	464/724	Quantitative PCR on admission	Small sample; poor accuracy on reporting of symptoms and history; no access to patients who chose not to present to hospital or did not have access; no reported validation (external or internal)

(Table 1 continues on next page)

	Country	Virus	Period of data collection	Design	Objective	Outcomes	Setting of data collection	Age of study population	Patients positive for Ebola virus/ total patients	Method (reference standard) and timing of Ebola virus confirmatory testing	Limitations
(Continued from previous page)											
Arranz et al (2016)[30]	Sierra Leone	Ebola	December, 2014–March, 2015	Observational retrospective study of data at admission	Compare the clinical characteristics of confirmed cases (patients with Ebola virus disease) and non-confirmed cases (patients without Ebola virus disease), assess the diagnostic validity of initial symptoms used in WHO case definition to diagnose Ebola virus disease in a low-incidence situation	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link	One Ebola treatment centre	All ages	31/75	Quantitative PCR on admission	Only data at admission; poor data quality; retrospective design; small sample; no reported validation (external or internal)
Loubet et al (2016)[31]	Guinea	Ebola	December, 2014–February, 2015	Observational retrospective study of data at admission	Identify epidemiological, sociodemographic, and clinical variables associated with Ebola virus disease diagnosis and to create, based on these variables, a predictive score for identification of confirmed Ebola virus disease	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link; and risk score	One Ebola treatment centre	All ages	76/145	Quantitative PCR on admission	Data collected only at admission; poor data quality; retrospective design; patients included might have been reluctant to come to the Ebola treatment centre, and thus were more likely to present severe clinical presentation with late symptoms; temperature taking might be affected by several factors; small sample size; anorexia and temperature (the factors that in that study were associated with an increased likelihood of Ebola virus disease) are not easy to measure and interpret; no reported external validation, only internal validation
Hartley et al (2017)[32]	Sierra Leone	Ebola	December, 2014–November, 2015	Observational retrospective study of data at admission	Construct an easy-to-use and highly accurate triage scoring system that discriminates Ebola virus infection risk in a malaria-sensitive manner and improve the predictive accuracy for Ebola virus disease and malaria	Risk score	One Ebola virus treatment centre	All ages	158/566	Quantitative PCR on admission; rapid diagnostic malaria test (histidine-rich protein-II antigen rapid diagnostic kits were used)	Only the most prevalent symptoms at admission were included in the score; poor data quality; did not fully cover all the malaria season because the Ebola treatment centre was opened from December to June; recall bias
Fitzgerald et al (2017)[26]	Sierra Leone	Ebola	August, 2014–March, 2015	Observational retrospective study of data at admission	Refine the case definition and describe outcomes of admitted children	Sensitivity and specificity of WHO case subdefinitions	11 Ebola holding units	Paediatric population (younger than 13 years)	309/1006	Quantitative PCR on admission	Only included children younger than 13 years; oral plenary abstract; no reported external validation, only internal validation

(Table 1 continues on next page)

	Country	Virus	Period of data collection	Design	Objective	Outcomes	Setting of data collection	Age of study population	Patients positive for Ebola virus/ total patients	Method (reference standard) and timing of Ebola virus confirmatory testing	Limitations
(Continued from previous page)											
Ingelbeen et al (2017)[27]	Guinea	Ebola	March, 2014–September, 2015	Observational retrospective study of data at admission	Describe the burden of non-cases in relation to the phase of the outbreak; determine the duration of their stay at the Ebola treatment centre and (potential) subsequent nosocomial infections; compare characteristics, outcome, and risk factors for death in confirmed cases and non-cases to improve the selection, diagnosis, and care of people with suspected Ebola virus disease	Sensitivity and specificity of WHO case subdefinitions and symptoms on admission	One Ebola treatment centre	All ages	822/2362	Quantitative PCR on admission; Xpert Ebola Assay (Cepheid GeneXpert, Sunnyvale, CA USA) on admission	The Ebola treatment centre for part of the outbreak was located within one hospital but then moved to another area in July; could not assess possible drivers for the large proportion of non-cases; no reported validation (internal or external)
Oza et al (2017)[33]	Sierra Leone	Ebola	November, 2014–March, 2015	Observational retrospective study of data at admission	Develop two Ebola risk scores to supplement the broad WHO case definition by further separating triaged patients based on their likelihood of being positive for Ebola virus	Risk score	One Ebola treatment centre	All ages	252/424	Quantitative PCR on admission; biochemistry laboratory tests with the Piccolo Xpress (Abaxis, Union City, CA, USA) and i-STAT (Abbott Point of Care, Princeton, NJ, USA) device	Only one treatment centre; investigated 14 commonly recorded symptoms; small amount and poor quality of patient data; excluded exposure as a potential predictor because of large amount of missing data or poor data quality; patients might not be representative of the overall population of suspect Ebola cases; no reported external validation, only internal validation
Hsu et al (2018)[24]	Guinea	Ebola	March–October, 2014	Observational retrospective study of surveillance data	Assess the diagnostic performance of the WHO suspected case definition by using epidemiological surveillance and diagnostic test	Sensitivity and specificity of WHO case definition, WHO case subdefinition, symptoms at admission, and epidemiological link	National surveillance line list	All ages	1304/2847	Quantitative PCR (on admission and for deceased patients at the community level)	Unknown how representative the database was for all patients with Ebola virus disease; only 1412 patients had complete data to assess and analyse the WHO case definition; possible overestimation of performance of WHO definition because only common symptoms were recorded in the early stage of the outbreak; poor data quality; no reported validation (internal or external)

(Table 1 continues on next page)

	Country	Virus	Period of data collection	Design	Objective	Outcomes	Setting of data collection	Age of study population	Patients positive for Ebola virus/ total patients	Method (reference standard) and timing of Ebola virus confirmatory testing	Limitations
(Continued from previous page)											
Fitzgerald et al (2018)[25]	Sierra Leone	Ebola	August, 2014–March, 2015	Observational retrospective study of data at admission	Develop a predictive score that could be used to tailor the paediatric case definition for suspected Ebola virus disease according to the clinical and epidemiological setting	Sensitivity and specificity of WHO case definition and risk score	11 Ebola holding units	Paediatric population (younger than 13 years)	309/1006	Quantitative PCR on admission	Only included children younger than 13 years; poor data quality; no data on the true Ebola status of people who did not meet the WHO case definition and were not admitted; no reported validation, only internal validation
Ingelbeen et al (2018)[28]	Guinea	Ebola	March, 2014–September, 2015	Observational retrospective study of data at admission	Validate risk score by Oza and colleagues[13]	Risk score	One Ebola treatment centre	All ages	805/2311	Quantitative PCR on admission; Xpert Ebola Assay (Cepheid GeneXpert) on admission	Did not propose another algorithm; letter; no reported external validation, only internal validation
Huizenga et al (2019)[6]	Sierra Leone	Ebola	September, 2014–November, 2015	Observational retrospective study of data at admission	Evaluate the pre-existing health-care infrastructure during the Ebola virus disease outbreak, and assess the provided health care and safeguard functionality of a health-care system for all patients not suspected to have or diagnosed with Ebola virus disease	Sensitivity and specificity of WHO case subdefinitions	Screening at one hospital	All ages	22/1556	Quantitative PCR on admission	Scant description of data; poor data quality; no reported validation (external or internal)

Table 1: **Overview of articles included in the systematic review and meta-analysis**

estimates from the random-effects model are provided for completeness.

We summarised, without any further re-analysis, studies that developed or externally validated risk scores for predicting Ebola virus infection. Scores were used to identify individuals with a higher or lower risk of Ebola virus infection during screening at Ebola health facilities. To obtain the risk scores, these studies used the regression coefficients of independent risks obtained by multivariable logistic regression against Ebola virus infection and then converted regression coefficients into an integer-based point-scoring system. Reviewed studies assigned positive and negative risk scores with calculated AUC to epidemiological, demographic, and clinical characteristics. Positive values indicated higher risk of Ebola virus infection and negative values indicated higher risk of another infection such as malaria or typhoid.

Values assigned to the risk score varied by study; therefore, a meta-analysis of risk scores was not done, but instead evidence was systematically reviewed. For comparability, we reclassified the risk scores reported in the included studies into categories, from very low risk to very high risk (appendix p 7). STATA 15 was used for statistical analysis.

PRISMA guidelines for Diagnostic Test Accuracy Studies (PRISMA-DTA) were followed (appendix pp 2–4).[21]

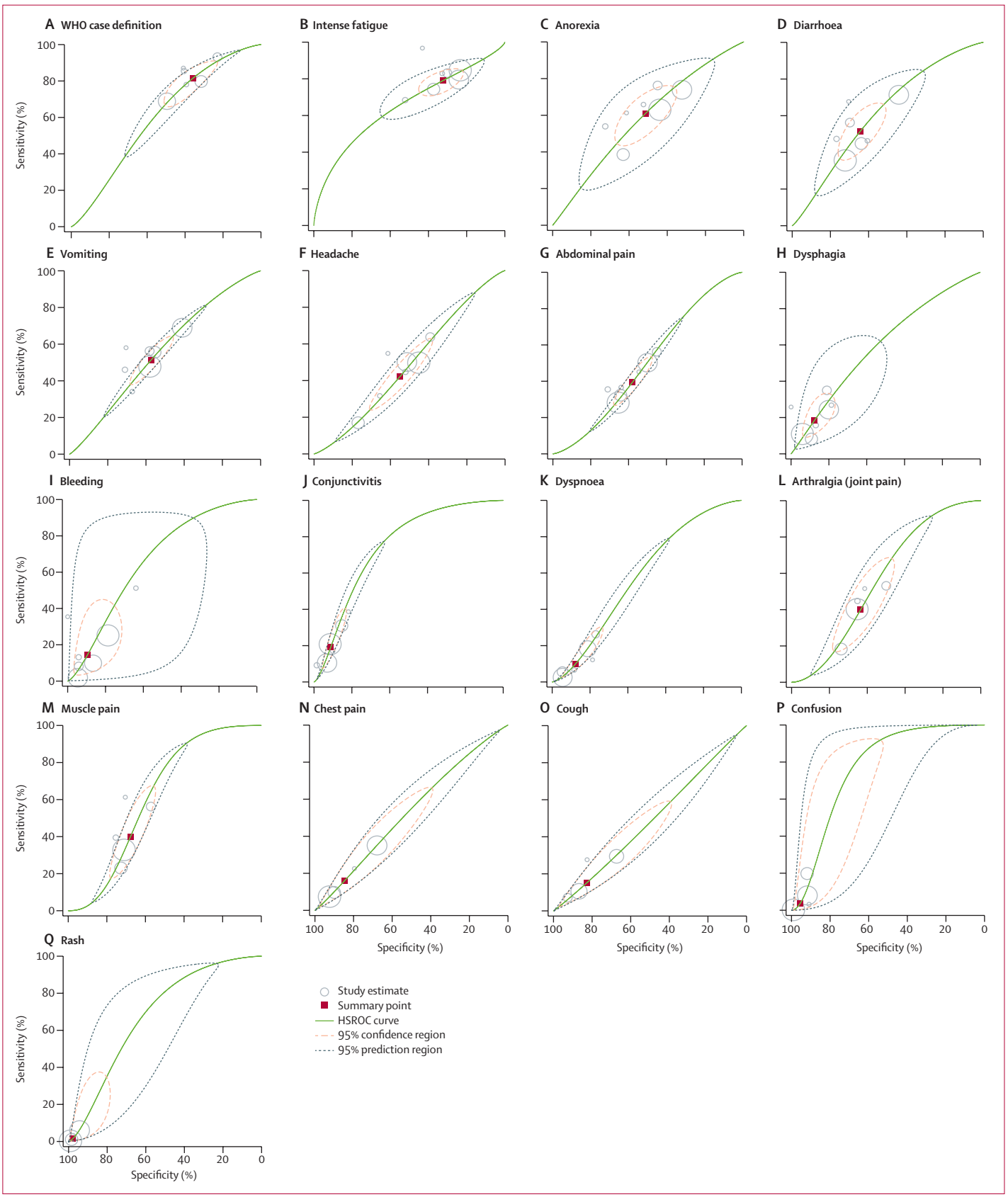
Role of the funding source

GC, KL, AS, and JG were employed by the funder, and participated in planning the study, carrying out the research, and writing the report. The funder of the study had no further 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

Of the 2493 studies initially screened using the article title, 143 were deemed to be potentially eligible on the basis of the abstract, and their full-text articles were assessed. Of these studies, 18 met the inclusion criteria, but three were excluded because data on sensitivity and specificity could not be extrapolated (appendix p 8). One was excluded because it is yet unpublished (FG). Of the 14 included studies, 11 were full manuscripts,[5,6,22,24,25,27,29–33] one a letter,[28] one an oral plenary abstract,[26] and one a conference poster[23] (the author of this poster was also contacted and they provided an abstract with additional data [Kuehne A, Epicentre, Paris, France, personal communication]; table 1). 13 studies were published between 2015 and 2019 and assessed Ebola virus disease in the west Africa outbreak

Figure 2: **HSROC summary of sensitivity and specificity**
HSROC=hierarchical summary receiver operating characteristic.


A WHO case definition
B Intense fatigue
C Anorexia
D Diarrhoea
E Vomiting
F Headache
G Abdominal pain
H Dysphagia
I Bleeding
J Conjunctivitis
K Dyspnoea
L Arthralgia (joint pain)
M Muscle pain
N Chest pain
O Cough
P Confusion
Q Rash
Sensitivity (%)
Specificity (%)
100
80
60
40
20
0
Study estimate
Summary point
HSROC curve
95% confidence region
95% prediction region

	WHO subdefinition	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Huizenga et al (2019)[6]	WHO definition, with the difference that fever with sudden onset is not a mandatory criterion	100·0%	42·5%*	2·4%*	100·0%
Fitzgerald et al (2017)[26]	Contact alone, fever (in children older than 2 years) OR fever and conjunctivitis (in children younger than 2 years)	94·0%*	35·0%*	Not provided	Not provided
Roddy et al (2010)[22]	Epidemiological link or a combination of myalgia or arthralgia and any haemorrhage	79·0% (64·0–91·0)	73·0% (60·0–84·0)	Not provided	Not provided
Loubet et al (2016)[31]	WHO subdefinition 2 (temperature ≥37·5°C plus risk factor†)	75·0% (63·5–83·9)	62·3% (49·8–73·5)	Not provided	Not provided
Roddy et al (2010)[22]	WHO case definition (clinical criteria only‡)	73·0% (57·0–86·0)	43·0% (30·0–56·0)	Not provided	Not provided
Roddy et al (2010)[22]	Fever plus three or more symptoms§	68·0% (52·0–82·0)	46·0% (33·0–59·0)	Not provided	Not provided
Loubet et al (2016)[31]	Temperature ≥38·5°C plus risk factor†	68·4% (56·6–78·3)	82·6% (71·2–90·3)	Not provided	Not provided
Arranz et al (2016)[30]	Contact and three symptoms§	67·7% (51·3–84·2)	81·8% (70·4–93·2)	72·4% (56·1–88·7)	78·3% (66·3–90·2)
Loubet et al (2016)[31]	WHO subdefinition 3 (temperature ≥37·5°C plus clinical symptoms§)	67·1% (55·2–77·2)	76·8% (64·8–85·8)	Not provided	Not provided
Loubet et al (2016)[31]	WHO subdefinition 1 (risk factor plus clinical symptoms§)	63·2% (51·3–73·7)	66·7% (54·2–77·3)	Not provided	Not provided
Lado et al (2015)[5]	Three or more major symptoms¶	57·8% (52·1–61·4)	70·8% (64·7–76·4)	77·9% (73·1–82·3)	47·5% (42·3–52·7)
Arranz et al (2016)[30]	Fever and three symptoms§	58·1% (40·7–75·4)	50·0% (35·2–64·8)	45·0% (29·6–60·4)	62·9% (46·8–78·9)
Hsu et al (2018)[24]	Clinical criteria§	57·2%*	62·0%*	66·4%*	52·5%*
Ingelbeen et al (2017)[27]	WHO case definition (clinical criteria only‖)	56·9%*	46·4%*	36·3%*	66·8%*
Roddy et al (2010)[22]	Epidemiological link and two or more general symptoms§	54·0% (37·0–70·0)	91·0% (80·0–97·0)	Not provided	Not provided
Roddy et al (2010)[22]	Epidemiological link and three or more general symptoms§	54·0% (37·0–70·0)	93·0% (83·0–98·0)	Not provided	Not provided
Arranz et al (2016)[30]	Contact plus fever	48·4% (30·8–66·0)	77·3% (64·9–89·7)	60·0% (40·8–79·2)	68·0% (55·1–80·9)
Roddy et al (2010)[22]	Fever plus haemorrhage	44·0% (28·0–60·0)	72·0% (59·0–83·0)	Not provided	Not provided
Ingelbeen et al (2017)[27]	Three major signs**	27·7%*	79·1%*	41·5%*	67·2%*
Fitzgerald et al (2017)[26]	Contact, fever, and conjunctivitis OR contact, fever, anorexia, and two of abdominal pain, diarrhoea, or male sex (older than 2 years)	23·0%*	97·0%*	Not provided	Not provided
Kuehne et al (2015)[23]	History of contact, gastrointestinal symptoms†† and illness duration of >3 days	20·0%*	94·4%*	Not provided	Not provided
Hsu et al (2018)[24]	Unexplained death	14·2%*	92·8%*	72·0%*	45·2%*

*95% CI not provided in the original paper. †For example, being a health worker, have attended a funeral, and having contact with a relative suspect of having Ebola virus. ‡Fever plus three other symptoms or fever and haemorrhage. §Symptoms or criteria not specifed in original paper. ¶Three or more symptoms among the following: intense fatigue, confusion, conjunctivitis, hiccups, diarrhoea, or vomiting. ‖Acute fever and presenting three or more of the following: headache, anorexia or lack of appetite, lethargy, muscle or joint pain, breathing difficulties, vomiting, diarrhoea, stomach ache, difficulty swallowing, and hiccups; or any person with unexplained bleeding. **As proposed by Lado and colleagues.[5] ††Diarrhoea, vomiting, and anorexia or loss of appetite.

Table 2: **Sensitivity and specificity of WHO Ebola virus disease subdefinitions against reference standard of laboratory-confirmed Ebola virus infection, in decreasing order of sensitivity**

(seven in Sierra Leone,[5,6,25,26,30,32,33] four in Guinea,[24,27,28,31] and two in Liberia[23,29]). The remaining article was published in May, 2010, assessing Marburg virus in Angola.[22]

Overall, 12 021 people with suspected disease were included, of whom 4874 were confirmed as positive for Ebola virus infection. Study populations varied from 75 to about 2847 (table 1). All studies, apart from the national surveillance study, included patients who presented alive to health facilities for assessment. The national surveillance study included all cases (suspected, probable, and confirmed), including patients both alive and deceased, identified in both the community and health facilities. Eight studies' data were from single Ebola treatment centres,[23,27–33] with the remaining using a national surveillance list,[24] three from Ebola holding units,[5,25,26] and two from hospitals screening patients for

	AUC (95% CI) on own study database	AUC (95% CI) of Levine et al algorithm[29] on Hartley et al[32] database	AUC (95% CI) of Oza et al algorithm[33] on Ingelbeen et al[28] database	Epidemiological link	Referral (4–9 days)	Days since first symptom	Duration of illness >3 days	Gastrointestinal symptoms*	Male sex	Age (≥2 years)	Age (<2 years)	Diarrhoea	Conjunctivitis	Fever (>38·0°C)	Unexplained bleeding	Nausea or vomiting	Fever (≥38·5°C)
Hartley et al (2017)[32]	89% (86–93)	NA	NA	6	3	Y	NA	NA	NA	NA	NA	3	4	1	2	Y	NA
Oza et al (2017)[33]	83% (79–86)†	NA	58% (56–61)	NA	NA	NA	NA	NA	NA	NA	NA	2	2	Y	Y	1	NA
Loubet et al (2016)[31]	82% (77–87)	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	Y	NA	Y	NA	3
Fitzgerald et al (2018; paediatric population)[25]	80%‡	NA	NA	2	NA	Y	NA	NA	1	2	Y	1	2	1	Y	Y	NA
Levine et al (2015)[29]	75% (70–80)	76%‡	NA	2	NA	NA	NA	NA	NA	NA	NA	1·5	NA	Y	Y	Y	NA
Kuehne et al (2015)[23]	53–59‡	NA	NA	+	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA
Roddy et al (2010)[22]	‡	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	Y	Y	Y	+	Y	NA

(Figure 3 continues on next page)

Ebola virus disease while still functioning as general health facilities.[6,22] All studies covered distinct patient groups from different periods and geographical areas, except for two studies from Guinea.[24,27] Although these two studies covered overlapping patient groups, they reported on different clinical and epidemiological characteristics (WHO case definition performance *vs* symptom performance).[24,27]

All selected manuscripts analysed all ages combined, except one author who assessed, in two different studies, the sensitivity and specificity of 2014 WHO Ebola case definitions and also developed a risk score specifically for the paediatric population (younger than 13 years).[25,26]

Six studies explored the performance of a WHO case definition in non-paediatric populations.[5,22,24,29–31] In all of these studies, suspected and probable cases were combined and could not be disaggregated for analysis. The following results therefore apply to this combined group of suspected and probable cases. The pooled sensitivity was 81·5% (95% CI 74·1–87·2) and pooled specificity was 35·7% (28·5–43·6; figure 2). One study assessed WHO 2014 case definitions for a paediatric population (younger than 13 years old); the sensitivity was 98·0% (95% CI 95·0–99·0) and specificity was 5·0% (3·0–7·0).[25]

When WHO subdefinitions were assessed, history of contact and symptoms had high specificity compared with clinical symptoms alone, ranging from 62·3% (95% CI 49·8–73·5) to 94·4% (95% CI not provided in original paper; table 2). The highest sensitivity (100·0%) was documented for the WHO subdefinitions in which fever was not mandatory. Among studies using clinical symptoms and signs alone, the definition including three or more symptoms (intense fatigue, confusion, conjunctivitis, hiccups, diarrhoea, and vomiting) had the highest specificity (79·1% [95% CI not provided in original paper]). Unexplained death had high specificity (92·8% [95% CI not provided in original paper]) but the lowest sensitivity (14·2% [95% CI not provided in original paper]; table 2).

For children, the highest specificity (97·0% [95% CI not provided in original paper]) was with a case definition of contact, fever, and conjunctivitis, or contact, fever, anorexia, and two of abdominal pain, diarrhoea, or male sex (older than 2 years; table 2).[26]

Seven articles developed a risk score,[22,23,25,29,31–33] and among those five[25,29,31–33] did an internal validation (using bootstrap or test and training methods) and one assessed a risk score according to outbreak prevalence in a paediatric population.[25] An eighth study[28] externally validated the score developed by Oza and colleagues[33] without developing an alternative score. Of the 44 potential predictors of Ebola virus infection included across

	Joint pain	Anorexia or loss of appetite	Muscle pain	Difficulty swallowing	Abdominal pain	Rash	Headache	Difficulty breathing	Fatigue, weakness, or asthenia	Hiccups	Cough	Diarrhoea or vomiting	Epigastralgia	Anuria	Haematuria	Disorientation	Hepatomegaly	Haemoptysis	Malaria infection	ORL haemorrhage	Dehydration	Haematochezia	Joint or muscle pain	Bleeding at injection site	Bloody gingivitis	Jaundice	Non-menstrual vaginal bleeding	Bloody diarrhoea	Haematemesis	Epistaxis
Hartley et al (2017)[32]	NA	Y	–2	2	Y	Y	Y	Y	Y	Y	NA	NA	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA
Oza et al (2017)[33]	NA	–1	NA	Y	Y	Y	–1	–1	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	NA	NA	NA	NA	NA	NA	NA
Loubet et al (2016)[31]	Y	2	Y	Y	Y	NA	Y	Y	Y	NA	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fitzgerald et al (2018; paediatric population)[25]	Y	1	Y	–1	1	–2	–1	–1	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Levine et al (2015)[29]	Y	1	1	1	–1	NA	Y	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kuehne et al (2015)[23]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Roddy et al (2010)[22]	+	Y	+	Y	Y	NA	Y	Y	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	Y	Y	Y	Y	Y	Y

Figure 3: **Overview of risk score by symptoms and epidemiological characteristics**
Predictive scores (numeric or + symbol) are shown in shaded cells (blue indicates positive scores and light pink indicates negative scores). Y indicates that the characteristic was assessed, but not used. AUC=area under the receiver operating characteristic curve. NA=not assessed. ORL=otorhinolaryngology. *Diarrhoea, vomiting, or anorexia or loss of appetite. †95% CI is taken from Ingelbeen et al (2018)[28] because, although Oza and colleagues do not report 95% CIs in their manuscript, Ingelbeen and colleagues have externally validated Oza and colleagues' score and they do report the 95% CI. ‡95% CI, AUC, or both AUC and 95% CI not given in original paper.

the seven studies that develped risk scores, 20 were found to be positive or negative predictors (figure 3). The score system ranged from very low to very high risk, with intermediate categories varying across studies (appendix p 7).

One study created a malaria sensitive score aiming to discriminate between Ebola virus infection and malaria infection, which indicated a predictor power of 89·6% (95% CI 86–93) to discriminate Ebola virus positive versus negative, reaching a discrimination power of 98·5% (95% CI not provided in original paper) during the malaria season.[32] The same study obtained similar results (AUC 76·8% [95% CI not provided in original paper] *vs* 75·0% [70·0–80·0]), when externally validating the scores developed by Levine and colleagues.[29,32]

The study validating Oza and colleagues' algorithm found poorer performance in their cohort (AUC 58% [95% CI 56–61] *vs* 83·0% [79–86]).[28,33]

The highest performing score was developed by Hartley and colleagues,[32] a key difference being referral time (figure 3). For the adult population (six studies[22,23,29,31–33]), a positive risk score for infection was associated in more than one study with each of the following five characteristics: epidemiological link (eg, history of contact), diarrhoea, conjunctivitis, unexplained bleeding, difficulty swallowing (also called dysphagia; figure 3). Fever was assessed at different thresholds (>38·0°C or ≥38·5°C), and inclusion of fever in the final predictive score was only reported by two studies[31,32] (figure 3). Discordant values were assigned across studies (either positive or negative) for anorexia or loss of appetite, muscle pain (also called myalgia), and abdominal pain.

For the paediatric population (one study[25]), positive predictors were age (2 years or older), sex (male), epidemiological link, diarrhoea, conjunctivitis, fever (>38·0°C), anorexia or loss of appetite, and abdominal pain. Negative predictors were difficulty swallowing, rash, headache, and difficulty breathing (also called dyspnoea; figure 3). The same study compared two different time periods over the Ebola virus disease 2014–16 outbreak in Sierra Leone (high prevalence in October, 2014 [77% of suspected cases testing positive], and low prevalence in March, 2015 [4% of suspect cases testing positive]): a low cutoff for the risk score (with high sensitivity) performed better at periods of high prevalence transmission, and a high cutoff with high specificity performed better during low prevalence.[25] Similarly, the positive predictive value decreased from 93% to 31%, and the negative predictive value increased from 23% to 90% when comparing high (early) to low (late) transmission periods in the Ebola virus disease outbreak in another study in Liberia in an all ages population.[23]

	Variable	Sensitivity (95% CI)	Specificity (95% CI)
Fever cutoff			
Loubet et al (2016)[31]	≥38·5°C	80·2% (69·2–88·2)	82·6% (71·2–90·3)
Loubet et al (2016)[31]	≥38·0°C	88·2% (78·2–94·1)	72·5% (60·2–82·2)
Loubet et al (2016)[31]	≥37·5°C	93·4% (84·7–97·5)	50·7% (38·5–62·9)
Kuehne et al (2015)[23]	History of fever	85·3%*	26·4%*
Lado et al (2015)[5]	≥37·5°C or referred	85·9% (82·4–89·0)	16·4% (12·0–21·6)
Arranz et al (2016)[30]	≥38·0°C or referred	61·3% (44·1–78·4)	29·5% (16·1–43·0)
Roddy et al (2010)[22]	>38·0°C	85·0% (71·0–94·0)	20·0% (11·0–32·0)
Levine et al (2015)[29]	>38·0°C	85·0% (79·0–91·0)	21·0% (16·0–27·0)
Ingelbeen et al (2017)[27]	>38·0°C	71·5%*	30·5%*
Pooled analysis†	>38·0°C	80·0% (69·0–90·0)	25·0% (17·0–33·0)
Epidemiological link			
Hsu et al (2018)[24]	Contact with infected persons or body fluid, handling of bushmeat, attending the funeral of a patient with Ebola virus disease	74·7%*	67·1%*
Roddy et al (2010)[22]	Epidemiological link‡	67·0% (50·0–81·0)	86·0% (74·0–94·0)
Arranz et al (2016)[30]	History of contact with a person with confirmed Ebola virus disease	100·0%	59·0% (43·5–74·4)
Levine et al (2015)[29]	Sick contact§	65·0% (58·0–73·0)	61·0% (54·0–67·0)
Loubet et al (2016)[31]	Health worker or having had contact with a person with suspected Ebola virus disease or having attended funerals	81·5% (44·0–60·7)	29·0% (19·0–41·3)
Kuehne et al (2015)[23]	Contact to case	47·3%*	71·2%*
Lado et al (2015)[5]	Travel to an Ebola virus disease hotspot area, health-care work, funeral attendance, or contact with an ill family member or friend¶	21·6% (17·9–25·6)	84·6% (79·6–88·8)

Optimal performance is the definition that achieved best balance between maximising sensitivity versus maximising specificity. *95% CI not provided in original paper. †The pooled analysis was used for the studies that had the same cut-off for fever (>38°C).[22,27,29] ‡Epidemiological link was defined as direct contact with an individual potentially infected with Marburg haemorrhagic fever or his or her body fluids or direct contact during funeral practices. §Direct or indirect contact with a patient with suspected or confirmed Ebola virus disease in the previous 21 days, including living in the same household or providing direct care for the patient. ¶A contact is any person who comes into contact with a case or suspected case by sleeping in the same household within the past month; direct physical contact with the case (dead or alive); touching his or her linens or body fluid; or attendance at a funeral of a person with confirmed or suspected Ebola virus disease.

Table 3: **Sensitivity and specificity of fever, epidemiological link, or contact history, ordered by optimal performance**

Eight studies measured sensitivity and specificity of individual symptoms at admission, assessing a total of 35 symptoms.[5,22–24,27,29–31] The pooled sensitivity per symptom ranged from 79·0% (95% CI 74·4–83·0) for intense fatigue (seven studies) to 1·0% (0·0–7·0) for pain behind the eyes (three studies). By contrast, the pooled specificity ranged from 98·0% (95% CI 91·0–100·0) for pain behind the eyes to 32·3% (25·8–39·4) for intense fatigue (appendix p 9).

Haemorrhagic symptoms and signs were the most specific indicator of infection. Other symptoms and signs with high specificity included confusion, coma, hiccups, rash, and sore throat with specificity ranging from 92·0% (95% CI 91·0–94·0) for hiccups to 97·8% (95·2–99·0) for rash (appendix p 9). Performance of fever was assessed by seven studies, but each one used a different definition of fever.[5,22,23,27,29–31] The optimal performance (definition that achieved best balance between maximising sensitivity *vs* maximising specificity) for fever was a threshold at ≥38·5°C (sensitivity 80·2% [95% CI 69·2–88·2]; specificity 82·6% [71·2–90·3]; table 3).[31] In the random-effects analysis, a threshold at greater than 38·0°C (three studies[22,27,29]) gave a pooled sensitivity of 80·0% (95% CI 69·0–90·0) and specificity of 25·0% (17·0–33·0; table 3).

Seven studies assessed sensitivity and specificity of an epidemiological link.[5,22–24,29–31] Across these studies, the sensitivity of an epidemiological link ranged from 21·6% (95% 17·9–25·6) to 100·0% and specificity ranged from 29·0% (95% CI 19·0–41·3) to 86·0% (74·0–94·0). The most sensitive definition was history of contact with a person with confirmed Ebola virus infection (100·0%; table 3). The most specific definition was direct contact with an individual potentially infected with Marburg virus or his or her body fluids, or direct contact during funeral practices.[22]

Discussion

Our results indicate that, for all ages combined, the WHO case definitions have a sensitivity of 81·5% and a specificity of 35·7%. The sensitivity is not high enough to achieve acceptable false negative rates, particularly in low-prevalence settings, the primary requirement for community-based screening. The low specificity results in high numbers of false positives and thus potentially unnecessary admissions to Ebola treatment centres, with associated risk of nosocomial transmission and costs of managing suspected cases.[1] As a consequence, a large number of people who do not have Ebola virus disease will experience unnecessary invasive procedures, risk of

being infected with Ebola virus, isolation from family, fear of being stigmatised, and delay to appropriate care, and community mistrust in response activities will increase.

In our meta-analysis, fever had low specificity (25·0%), except for when defined as a threshold at 38·5°C or more (82·6%), and the WHO case subdefinition had 100% sensitivity only when fever was not a mandatory criterion. In the risk score systematic review, the association of fever with Ebola virus infection was not consistent across studies, with only two studies including it in the final predictive score. Presence of fever is likely to be related to the stage of infection at admission, with previous studies reporting absence of fever in a large proportion of suspected cases at admission.[34] This finding is consistent with a recent Ebola seminar reporting that fever was absent in at least 10% of the cases in the west Africa outbreak.[35]

Therefore, exclusion of fever from the case definition at the community level is likely to increase the sensitivity of the case definition. Intense fatigue was the most sensitive symptom (79·0%) that could be used at the community level to facilitate early referral of suspected cases and prevent community transmission.

The meta-analysis did not identify any individual symptom or sign having an optimal trade-off between sensitivity and specificity. Conjunctivitis, unexplained bleeding, difficulty swallowing, and diarrhoea were individual symptoms and signs with the best discriminatory accuracy in the studies that explored risk score for the all-age population and with the exception of diarrhoea all had high specificity (>80%) in the studies that explored their performance. However, these symptoms and signs could also be a proxy for late-stage disease when the virus infects endothelial cells, compromising vascular integrity, with massive tissue injury resulting in disseminated intravascular coagulopathy with risk of thrombosis, bleeding, and damage to the adrenal glands and gastrointestinal system.[36–38] These symptoms and signs could enable health practitioners to prioritise patients for admission to an Ebola treatment centre when resources are scarce but are less useful at the community level because they appear at a late stage of the disease when transmission risk is the highest.

None of the studies assessed miscarriage, despite it being included in the December, 2014, WHO case definition.[15] History of miscarriage and other associated pregnancy complications (eg, stillbirth) could help to identify cases that can be a major source of nosocomial transmission in general health facilities.[39]

Although only one study focused on a paediatric population, this study used data from 11 Ebola holding units and included a large population of children (1006), providing useful guidance for this age group.[26] The WHO paediatric definition had very high sensitivity (98·0%) but very poor specificity (5·0%). When the same authors assessed a WHO subdefinition (including contact, fever, and conjunctivitis, or contact, fever, anorexia, and two of abdominal pain, diarrhoea, or male sex [older than 2 years]), the sensitivity dropped markedly to 23·0% but the specificity improved to 97·0%. The optimal fever temperature cutoff for the paediatric population was not explored. However, in another study of a paediatric population of patients with confirmed Ebola virus disease admitted to one Ebola treatment centre in Sierra Leone, 25% of children aged 5 years and younger were afebrile.[40] This difference might be due to several factors: how fever was assessed (either reported in their history or measured at admission), age groups included (younger than 13 years *vs* younger than 5 years), period of data collection (August–March, 2015, *vs* June–Dec, 2014) when seasonality of other febrile illnesses could have influenced fever prevalence, background Ebola virus transmission rates, and viraemia at admission and time since onset of symptoms.

The paediatric analysis did not explore sensitivity and specificity of individual symptoms and signs at admission for children. Alongside the fact that they might have different clinical presentations compared with adults, children are more likely to experience adverse outcomes from Ebola virus disease and are less able to report symptoms and history of contact.

Similarly, pregnant women with non-Ebola virus disease-related complications usually present with symptoms (such as bleeding and abdominal pain) that mimic Ebola virus infection.[39] As suggested elsewhere, the paediatric and pregnant women populations might require adaptation of case definitions that take into account their specific characteristics.[41–43] None of the selected manuscripts explored the performance of WHO Ebola case definitions among pregnant women. Therefore, further evidence specifically applicable to children and pregnant women is required to develop appropriate tools for screening for Ebola virus disease in these populations.

Reported history of contact was a strong predictor for paediatric and adult populations, often performing better than many of the clinical symptoms included in accepted case definitions, as also reported by other studies.[44] However, it is likely that this is an underestimate of the potential performance of actual contact history in screening for Ebola virus disease.

Levels of disclosure of self-reported clinical information and contact history depend on community engagement with intervention strategies, including trust in the health-care provider. Therefore, to improve WHO case definition performance, effective and trusted collaboration with communities is essential to ensure reliable understanding and reporting of such crucial epidemiological information. Equally, it is the responsibility of response agencies to understand the underlying pattern of Ebola virus transmission, local traditions, coping mechanisms, and family dynamics in order to identify people at risk of infection. Genetic sequencing has also been put forward as a tool for identifying chains of transmission when contact history is unknown.[45] One of the limitations in interpreting

the results of this meta-analysis is that all the evidence reviewed, apart from the national surveillance study, came from patients triaged at health facilities or Ebola isolation centres. Thus, this meta-analysis might represent only cases with severe symptoms, limiting generalisability to the performance of these screening criteria at the community level and in early stages of disease. Second, there was significant heterogeneity between selected studies, and considerable variation in the quality of data on clinical symptoms and recollection of patients' history, with different variables and thresholds used in each study, and limited data on co-infection. For example, fever is a key symptom in the WHO case definitions, but different temperatures were used to define fever, which could explain the between-study heterogeneity. Inconsistency on thresholds for fever and the decision to include fever or not have been reported in the Democratic Republic of the Congo and in four neighbouring countries.[9]

For the two studies with overlapping patient populations, performance of WHO case definitions was assessed only using national surveillance data, with Ebola treatment centre data for these patients being assessed for only individual symptoms or WHO subdefinitions. These two studies were therefore not included together in pooled estimations, so the cohort overlap would not have affected results. Individual studies mentioned small sample size and poor quality of data as part of their limitations.

A range of contextual factors related to study setting will affect the performance of Ebola virus disease case definitions, including seasonally occurring diseases such as malaria and Lassa fever, which have a similar clinical presentation to Ebola virus disease. Such factors will affect the generalisability of our findings to other settings. In addition, only two of the recommended risk scores were externally validated,[28,32] limiting the generalisability of those scores because performance appears to vary across outbreak periods and populations.

Finally, there is potential for publication language bias because we considered only studies in English. However, for Guinea, a French-speaking country, we included data from national surveillance and two major Ebola treatment centres; therefore, we consider that bias due to language restrictions was minimised in our results. We included peer-reviewed abstract and poster data to capture data on paediatric populations and additional evidence for all age cohorts, and we sought unpublished evidence from French-speaking countries.

This systematic review is relevant to inform public health practitioners in the current Ebola virus disease outbreak in the Democratic Republic of the Congo, in which only 8% of suspected cases isolated are confirmed, possibly because of inconsistent use of WHO case definition at community and health facility levels.[46]

In conclusion, this first systematic review and meta-analysis of the strengths and limitations of the WHO Ebola virus disease case definitions highlights the need for further studies to assess consistent thresholds for fever, to explore viraemia and symptoms and signs at admission, and to externally validate risk scores for Ebola virus infection. The sensitivity and specificity of WHO Ebola case definitions could be improved by excluding fever and instead including both intense fatigue and history of contact. However, reliable disclosure of reported symptoms and history of contact requires effective collaboration with, and the trust of, affected communities. To achieve this trust and collaboration, responding organisations must recognise the paramount role of communities in controlling transmission and ending outbreaks. We also identified important gaps related to the paediatric and pregnant population, which must be addressed through future research.

Contributors

GC, KL, and HAW conceived the idea of this study. GC and FT undertook the literature review and extracted the data with help from LI. GC wrote the first and final drafts of the manuscript. GC, KL, HAW, and GLDT contributed to the analysis and interpretation of the data. KL, HAW, FG, KD, BP, GK, AS, JG, and GLDT reviewed early and late drafts of the manuscript, and all authors have given signed or electronic approval to be authors on the manuscript.

Declaration of interests

We declare no competing interests.

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