

Epidemiological Analysis of Long-Term Sequelae of Ebola Virus Disease Survivors in Sierra Leone: A Cross-Sectional Study Six to Eight Years Post Recovery

By Brayden Schindell

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Dedication

This thesis is dedicated to my parents Janet and Bruce Schindell. Mom and Dad, I could not have done this without you. You have supported me through all my academic adventures no matter where they took me and no matter what I wanted to pursue in life. Your constant and unwavering support even when I was questioning myself and my own decisions are more than a small reason for why this was possible. Love you forever and always.

And to my grandparents, Larry and Pearl Schindel, and Bill and Eve Buhr who are no longer with us, I want to thank you for all your love and support making me the person I am today. This would not have been possible without your encouragement of my endless questions and curiosity.

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over the years with a few laughs and a lot of advice on how to push towards to next milestone. The beers we have shared over the years have helped too I suppose.

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Abstract

The unprecedented number of survivors from the 2013-2016 West African Ebola virus disease (EVD) epidemic have raised questions about the long-term mental health impacts of EVD survivorship and the capacity to meet these needs. Sierra Leone was the most affected country during this epidemic, given this large survivor population, understanding the long-term health effects and future medical needs of survivors is a public health priority. This thesis explored one overarching hypothesis, that there are continued negative effects on the overall health, reproductive health, stigma and mental health experienced by EVD survivors. This was assessed through a cross-sectional study of 595 EVD survivors and 403 close contacts ($n=998$) from Sierra Leone via survey between November 2021 to March 2022. It involved simultaneous recruitment and surveying across all affected districts.

Findings from this study showed most overall health symptoms were no longer elevated compared to controls. However, frequent urination, eye discomfort, and ear pain persisted. Female EVD survivors reported significantly different menstrual patterns compared to close contacts with association of EVD survival to irregular periods and decreased interest in sexual intercourse. While minimal associations with EVD survival existed for male EVD survivors. A majority of EVD survivors reported that they continued to experience stigma and females were disproportionately affected. EVD-associated post-traumatic stress disorder (PTSD) was reported by about half of EVD survivors. With all mental health outcomes higher than baseline rates for the region of Africa.

Concerningly, several overall health symptomologies, female reproductive health, stigma and mental health disorders remain present six to eight years after recovery for EVD

survivors in Sierra Leone. These findings highlight the need for improving access to clinical care and mental health care for EVD survivors.

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List of Abbreviations

Abbreviation	Definition
AIDS	acquired immunodeficiency syndrome
AOR	adjusted odds ratio
AUDIT	alcohol use disorders identification test
BDBV	Bundibugyo virus
BMI	body mass index
BOMV	Bombali virus
CatB	cathepsin B
CatL	cathepsin L
CFR	case fatality ratio
CI	confidence interval
CNS	central nervous system
COVID-19	Coronavirus disease 2019
CSF	cerebrospinal fluid
DAST-20	drug abuse screening test
DC	dendritic cell
DC-SIGN	dendritic cell-specific ICAM-3-grabbing non integrin
DRC	Democratic Republic of the Congo
DSM-5	Diagnostic and Statistical Manual Fifth Edition
EBOV	Ebola virus
ENT	ear, nose and throat
ER	endoplasmic reticulum
ETC	Ebola treatment centre
ETU	Ebola treatment unit
EVD	Ebola virus disease
FSAD	female sexual arousal disorder
FSFI	female sexual function index
GP	glycoprotein
HIV	Human immunodeficiency virus
IB	inclusion body
IFN	interferon
IFN-I	interferon type-1
IgG	immunoglobulin G
IIEF	international index of erectile dysfunction
IQR	interquartile range
IR	intergenic region
IRF-3	interferon regulatory factor 3

Abbreviation	Definition
L	Filoviral RNA-dependant RNA polymerase
L-SIGN	liver/lymph node-specific ICAM-3 grabbing non-integrin
MARV	Marburg virus
MD	major depression
MHPSS	mental health and psychosocial support
MVD	Marburg virus disease
NGO	Non-governmental organization
NIRV	non-retroviral integrated virus
NP	nucleoprotein
NPC1	Neimann Pick C1 receptor
PALM	Pamoja Tulinde Maisha
PCL-5	post-traumatic stress disorder checklist
PHQ-9	patient health questionnaire
PLHIV	people living with human immunodeficiency virus
PREVAIL	partnership for research on Ebola vaccines in Liberia
PS	phosphatidylserine
PTSD	Post-traumatic stress disorder
RdRp	RNA-dependant RNA polymerase
REB	research ethics board
RESTV	Reston virus
RNA	Ribonucleic acid
RNP	ribonucleoprotein
RT-PCR	reverse transcription polymerase chain reaction
rVSV-EBOV	recombinant vesicular stomatitis virus-Zaire ebolavirus
sGP	soluble glycoprotein
ssGP	small soluble glycoprotein
STAT1	signal transducer and activator of transcription 1 α / β
SUDV	Sudan virus
TAFV	Taï Forest virus
TB	tuberculosis
TIM-1	T-cell immunoglobulin and mucin domain
UTR	untranscribed region
VP 30	viral protein 30
VP24	viral protein 24
VP35	viral protein 35
VP40	viral protein 40
WHO	World Health Organization

Author Contributions

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Author contributions are as follows: JK and JBK conceived the study, while BGS, JBK, SYS, and JK designed the study. JBK lead the recruitment and in-country support in Sierra Leone. BGS BKT SYS analysed the data. BGS prepared the manuscript. JK supervised the study, analysis and writing process. All authors contributed to the intellectual content of the manuscript and read and approved the final version of the manuscript.

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Chapter 1: Introduction

1.0 Introduction

1.1 A perspective on Ebola Virus

EBOV is a member of the *Filoviridae* virus family which comprises a growing group of single-stranded, negative sense (-) ribonucleic acid (RNA) viruses within the *Mononegavirales* order (figure 1.1)¹⁻³. The viruses classified as part of the family *Filoviridae* are contained within the order *Mononegavirales* and related to the families *Paramyxoviridae* including Measles and Mumps viruses and more distantly to *Rhabdoviridae* including Rabies virus^{4,5}. Among the *Filoviridae*, viruses originating from two genera pose the most relevant threat to individual and public health: orthoebolaviruses and orthomarburgviruses³. Sudan virus (species *Orthoebolavirus sudanense*) (SUDV), Bundibugyo virus (species *Orthoebolavirus bundibugyoense*) (BDBV), and Taï Forest virus (species *Orthoebolavirus taiense*) (TAFV) can cause disease in humans¹⁻³. Meanwhile, Reston virus (species *Orthoebolavirus restonense*) (RESTV) is considered apathogenic in humans⁶, while no human infections with Bombali virus (species *Orthoebolavirus bombaliense*) (BOMV) have been reported (rev. in⁷). Although considered endemic in Equatorial Africa⁸, Ebola virus (species *Orthoebolavirus zairense*) (EBOV) is targeted for public health action due to its pathogenicity and virulence⁹.

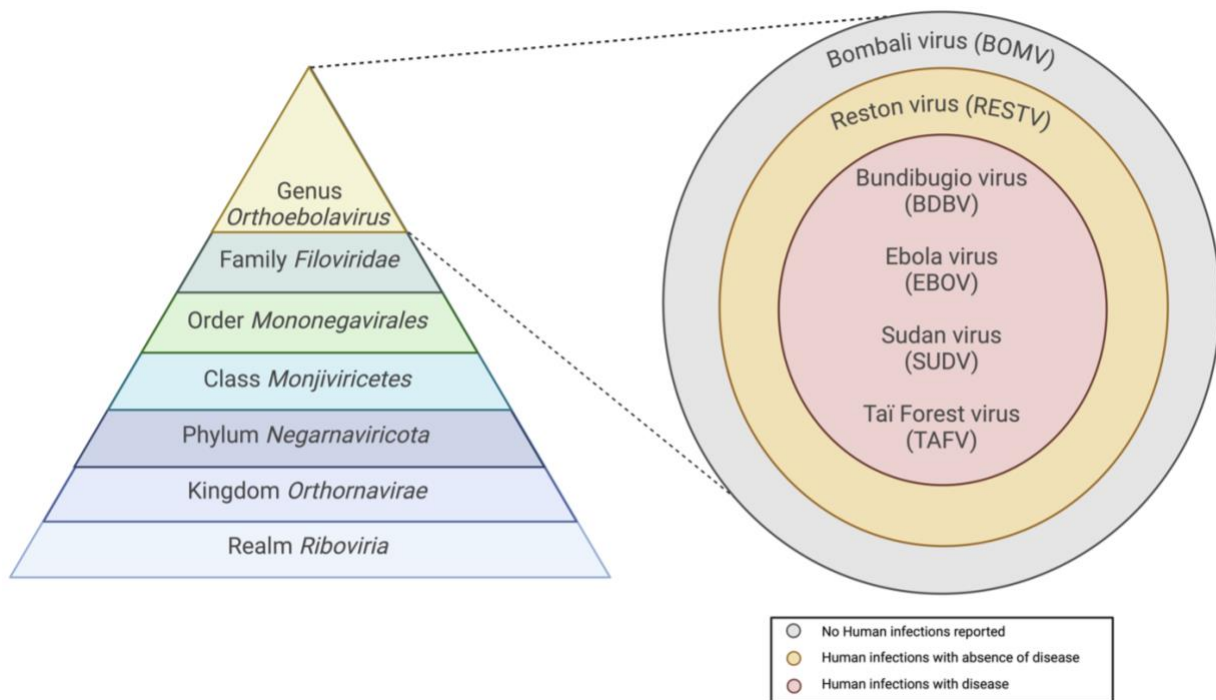


Figure 1.1 Phylogeny of *Orthoebolavirus* genus and the known infective range of *Orthoebolavirus* species within the genus. Figure legend, grey, no known human infections. Yellow, human infection with no signs of disease. Red, human infection with disease. Adapted from Jacob et al. with changes⁷. Reproduced with permission from Springer Nature. Created with BioRender.com

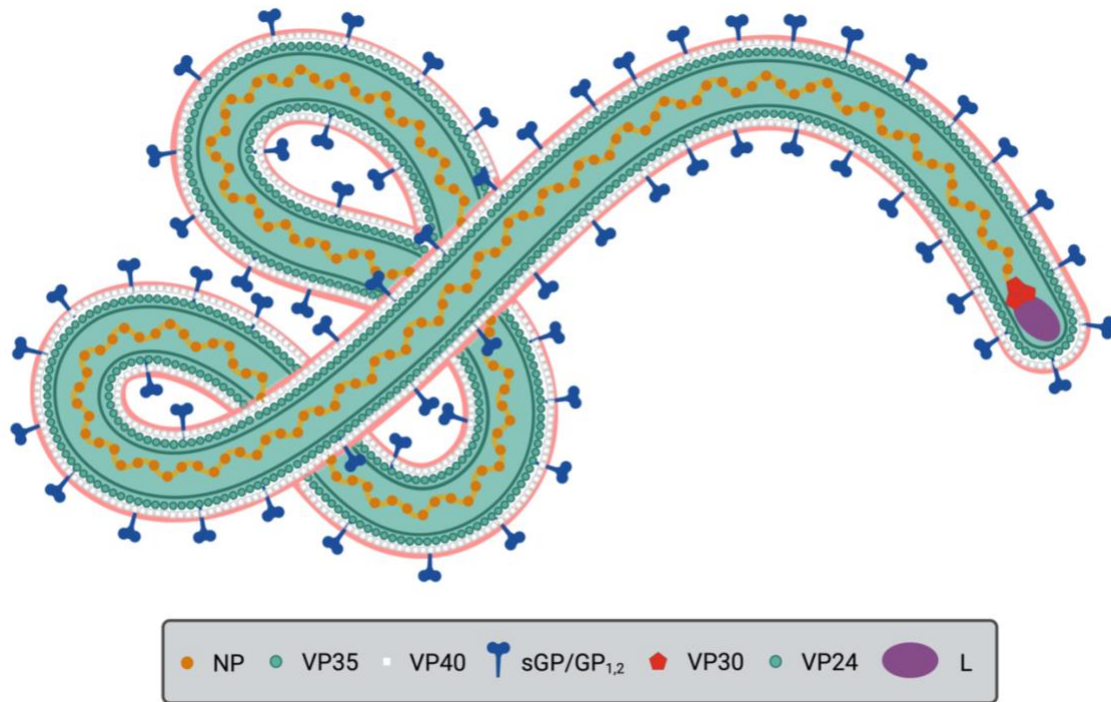
The EBOV species is subdivided into two viral strains; EBOV-Mayinga, the reference virus isolated from the original outbreak in the Democratic Republic of Congo (formerly Zaire) and EBOV-Makona, the strain which caused the 2013-2016 epidemic^{4,10}. The phylogenetic classification of *Filoviridae* has largely been based on differences and similarities of the glycoprotein (GP) sequence^{11,12}. Within the *Ebolavirus* genus, GP sequence can vary from 37-40% while the amino acid sequence varies from 34-43%¹³. TAFV has been associated with a single non-fatal human infection and RESTV results in asymptomatic infections in humans¹⁴⁻¹⁶. RESTV is the only *Ebolavirus* species to have emerged outside of Africa and has been associated with multiple epizootics in captive Philippine macaques^{17,18}. Mean case fatality rates of EBOV, SUDV and BDBV in human outbreaks have ranged from 30-50%¹⁹. Ebola virus disease (EVD) causes a mild to severe systemic illness with an exceptionally high case fatality ratio (CFR) of approximately 45% averaged across all outbreaks in human populations (rev. in⁷).

1.2 Ebola Virus Genome Structure

EBOV is a negative stranded non-segmented RNA virus^{4,10-12,20}. The EBOV genome comprises 18.96 kb and encodes seven genes for the expression of nine proteins and one peptide (figure 1.2) (rev. in²¹⁻²³). *Orthoebolavirus* and *Orthomarburgvirus* have 7 genes encoded by their genome, as these are negative stranded viruses their genes are arranged in the 3' to 5' direction (figure 1.2)^{4,10,11,20}. The genes of the EBOV genome in order are nucleoprotein (NP), viral protein 35 (VP35), VP40, GP, VP30, VP24 and the RNA-dependant RNA polymerase (RdRp) (L) (figure 1.2)^{4,5,10}. The (-)-genome is synthesized as a continuous RNA molecule from an (+)-antigenome template, while the sequential mRNA transcription and gene

expression nearly linearly decrease across the genome (NP>VP35>VP40>GP>VP30>VP24>L) (rev. in^{21,22}). These 7 genes encode nine known proteins with functions related to viral transcription, replication, and particle assembly^{4,5,10}. Surrounding the genes on the ends of the genome are untranscribed regions (UTRs) that are highly conserved among all members of *Filoviridae*^{4,10,11,20}. These UTR regions contain promoter sequences as well as packaging signals for packaging the genome into nucleocapsids (the protein case surrounding the genome and polymerase complex)^{4,10,11,20}. The 3' UTR (i.e. the "leader") encodes a promoter for viral encoded RdRp binding and the 5' UTR (i.e. the "trailer") encodes a genome replication promoter for the production of anti-genome (positive strand RNA running 5'-3') that acts as a template for genome replication^{5,10}. The ends of the EBOV genome comprise a 3' leader and 5' trailer sequence, while the open reading frames of the single genes are separated by non-coding, untranslated regions which regulate initiation and stop of the transcriptional units (rev. in²¹). Notably, these regions are of variable size and overlap between the adjacent genes^{21,22}. Viral mRNA is further 5'-capped and poly-adenylated for downstream translation in the cytoplasm or rough endoplasmic reticulum (ER) (rev. in^{21,22}).

A



B

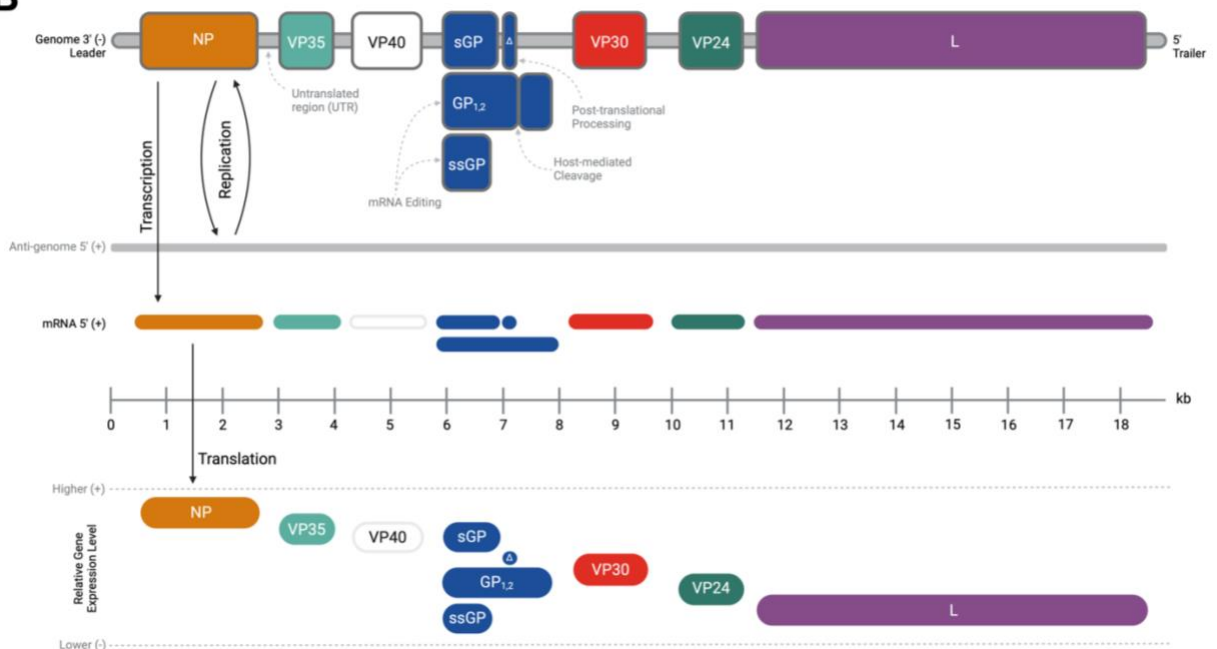


Figure 1.2 Viral structure, genome organization, transcription, translation and associated post processing. (A) Viral particle structure with associated viral proteins labelled and colour coded accordingly. (B) Genome organization, with RNA processing outlined for mRNA production, followed by replication antigenome, processed mRNA, ruler for genome length, processed proteins arranged by genome position and relative abundance. Created with BioRender.com

When the genome is transcribed for protein production each protein is transcribed into a separate mRNA due to intergenic regions (IRs)^{4,5,11}. These IR regions encode a polyadenylation signal which is a five uracil region that makes it difficult for the polymerase to stay on to transcribe the next gene⁵. These unstable IR regions make it more likely that the RdRp will fall off after each gene and less likely to continue to transcribe the next gene, resulting in less transcription of each subsequent gene⁵. This stop start transcription of the individual genes due to the IRs creates a gradient of protein produced, NP at the 3' end is the most produced and L at the 5' end is the least produced gene, with some variation in the middle genes (figure 1.2)^{5,10}. A capping signal is also encoded in the IR for the mRNA, this processing along with the polyadenylation signal allow the virus to trick the host ribosomes to produce the viral proteins for viral production^{4,5,10,11}.

1.3 Viral Proteins

Each of the viral proteins have multiple functions due to EBOV only encoding seven essential proteins for the viral particle including: protein production, genome replication and viral escape and entry⁵. These proteins/peptides (figure 1.2) include the NP, VP 24, 30, 35, and 40, L as well as multiple transcriptionally and post-transcriptionally edited forms (figure 1.2) GP, soluble glycoprotein (sGP), ssGP and delta-peptide) (rev. in^{22,24,25}). NP forms the nucleocapsid around the viral genome and the proteins associated with the genome^{4,10,11}. Along with NP viral proteins L, VP30 and VP35 also associate with the viral genome to form the ribonucleoprotein complex^{4,10,11}. In addition to forming the nucleocapsid, NP also plays a role in promoting genome replication by binding the genomic RNA to protect it from degradation as well as allowing full genome transcription^{4,5,10}. VP35 is an important cofactor

for the L RNA dependent RNA polymerase, and together with L they form the polymerase complex^{4,10,11}. VP30 is a transcription activator for primary transcription as well as an activator for mRNA editing of viral transcripts produced^{5,10}. L is responsible for transcribing mRNA and replication of the viral genome through an anti-genome transcript that acts as a template for replication^{5,10}. When L produces enough genome copies, assembly of NP into the nucleocapsid and budding of the virus particles from host cells is activated^{5,10}. VP24 and VP40 are the minor and major matrix proteins, responsible for connecting the nucleocapsid to the viral envelope acting like bridges; VP24 and VP40 produce the long filamentous form characteristic of the *ebolaviruses*^{4,10,11}. VP24 assists in mediating nucleocapsid assembly providing scaffolding for NP and is a protein unique to the *Filoviridae* family (figure 1.2)^{4,10,11}. The major matrix protein of EBOV, VP40 is the mediator of budding of the nucleocapsid into the GP coated envelope of the viral particle for complete progeny formation^{4,10}. GP is the transmembrane glycoprotein on the surface of the *filoviridae* viral envelope; GP has a large amount of N and O linked glycosylation and GP is the mediator of binding and entry into host cells⁵. After transcription, GP can be processed through RNA editing to create 3 possible products in *Orthoebolaviruses*, similar activity is not seen in *Orthomarburgvirus*^{4,5}. Full length GP is inserted into the envelope, but soluble GP lacks the transmembrane segment and are released into the host system alongside more truncated small soluble GP these play a role in host inflammation^{4,5}. Membrane-bound GP is integral in host cell recognition and entry most commonly through macropinocytosis^{4,20}. The high degree of glycosylation characteristics of EBOV GP facilitates recognition on multiple cell receptors⁴. Recognized receptors include lectins dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) and liver/lymph node-specific ICAM-3 grabbing non-integrin (L-SIGN), TIM-1 (T-cell immunoglobulin and

mucin domain), with NPC1 (Neimann Pick C1 receptor) acting as the primary receptor^{4,10,11,20}. Which in turn allows the virus to infect a wide range of cells in the host organism through this wide range in general receptors recognized.

1.4 Ebola Virus Structure

Filoviruses have a thread-like appearance (figure 1.2) and is the origin of the family name, filum, the Latin word for thread (rev. in²³). The particles have a diameter of ~80nm and a length of ~1 μm ²³. The genome of EBOV is 19 kilobases long making up 1% of the total viral particle mass showing how large the previously mentioned filamentous viral particles are^{4,10-12,20}. The viral RNA is encapsidated by NP to form a left-handed helix with a diameter of 50 nm, and a periodicity of 24 NP per turn of the helix^{23,26}. Each NP binds six nucleotides of the helix and oligomerize with the N-terminal domain of neighbouring NPs, while the NPs of neighbouring helices interact with the N-terminal β -sheets through the C-terminal α -helix^{23,26}. A repeating unit of viral proteins VP24 and VP35 forms on the outer layer of the Nucleocapsid (two VP24 and one VP35 per two NP molecules), it is unknown how L interacts with the nucleocapsid and VP30 is believed to be in the outer layer of the nucleocapsids^{23,26}. There is then a matrix layer between the nucleocapsid and the viral membrane which contains VP40 dimers that are in a linear arrangement surrounding the nucleocapsid^{23,27}. GP_{1,2} is randomly distributed on the surface of the viral membrane with concentrations near the VP40 dimers within the matrix^{23,27}.

1.5 Ebola Virus Life Cycle

In its primary target cells, EBOV establishes a productive infection which culminates in the release of infectious viral progeny (Figure 1.3). The infection of a cell with EBOV is initiated by the attachment of the virion to host factors, including C-type lectin receptors and phosphatidylserine (PS) receptors such as DC-SIGN and TIM-1, among others with final attachment to NPC1²⁸⁻³⁴. Here, the trimeric EBOV GP_{1,2}, which is embedded in the viral envelope, engages with the respective attachment receptors, mediating internalization via the macropinocytotic pathway (figure 1.3)^{32,35-39}. Upon arrival in late endolysosomes, the local acidification facilitates the precursor GP_{1,2} being cleaved by the host proteases cathepsin B (CatB) and cathepsin L (CatL) into GP_{CL}, whereby the mucin- like domain and glycan cap of GP₁ are removed (figure 1.3)⁴⁰⁻⁴³. Conclusively, GP_{CL} can subsequently bind to the endosomal cholesterol transporter NPC1, inducing a conformational change enabling the GP₂-mediated fusion of the virion with the endosomal membrane (figure 1.3)⁴⁰⁻⁴³. The following formation of a pore finally leads to the liberation of the viral ribonucleoprotein (RNP) into the host cytoplasm⁴⁰⁻⁴⁴. Further determinants of a successful fusion seemingly comprise an adequately low pH, enhanced Ca²⁺ concentration as well as disassembly of the VP40 viral matrix (figure 1.3)^{41,43,45}.

In the host cytoplasm, the EBOV genome transcription takes place, whereby a complex formed by the L polymerase, NP, VP30 and VP35 is established (figure 1.3) (rev. in^{37,46}). Here, the phosphorylation status of VP30 has been identified as a determinant for the switch between viral genome replication versus transcription⁴⁷⁻⁵⁰. In case VP30 is phosphorylated, replication occurs, while transcription is blocked^{48,49}. Accordingly, dephosphorylation by host phosphatases, such as protein phosphatase 1, must take place to initiate viral

transcription⁴⁷⁻⁵⁰. Transcription of the EBOV genome further requires a multitude of host factors and finally enables translation and expression of viral proteins (rev. in^{37,51-56}). Viral protein translation, either in the cytoplasm or the rough ER (GP_{1,2}), is mainly driven by the host machinery, although suppression of host restriction factors like protein kinase R by VP35 further enables the process (figure 1.3) (rev. in^{37,56}). Viral genome replication, upon phosphorylation of VP30 by host kinases⁴⁷⁻⁵⁰, takes place in perinuclear inclusion bodies (IBs), where the genome is amplified and packed in progeny RNP⁵⁷⁻⁵⁹. EBOV IBs comprise liquid organelles consisting of oligomerized NP, further enriched with viral RNP proteins and host factors relevant to replication and assembly^{57,58,60,61}. Concluding progeny formation, viral proteins and RNP are trafficked to the host membrane (figure 1.3) for their egress, whereby the host cytoskeletal transport network including the endosomal sorting complexes required for transport machinery and host exocytosis recycling machinery are exploited (rev. in^{54,62-67}).

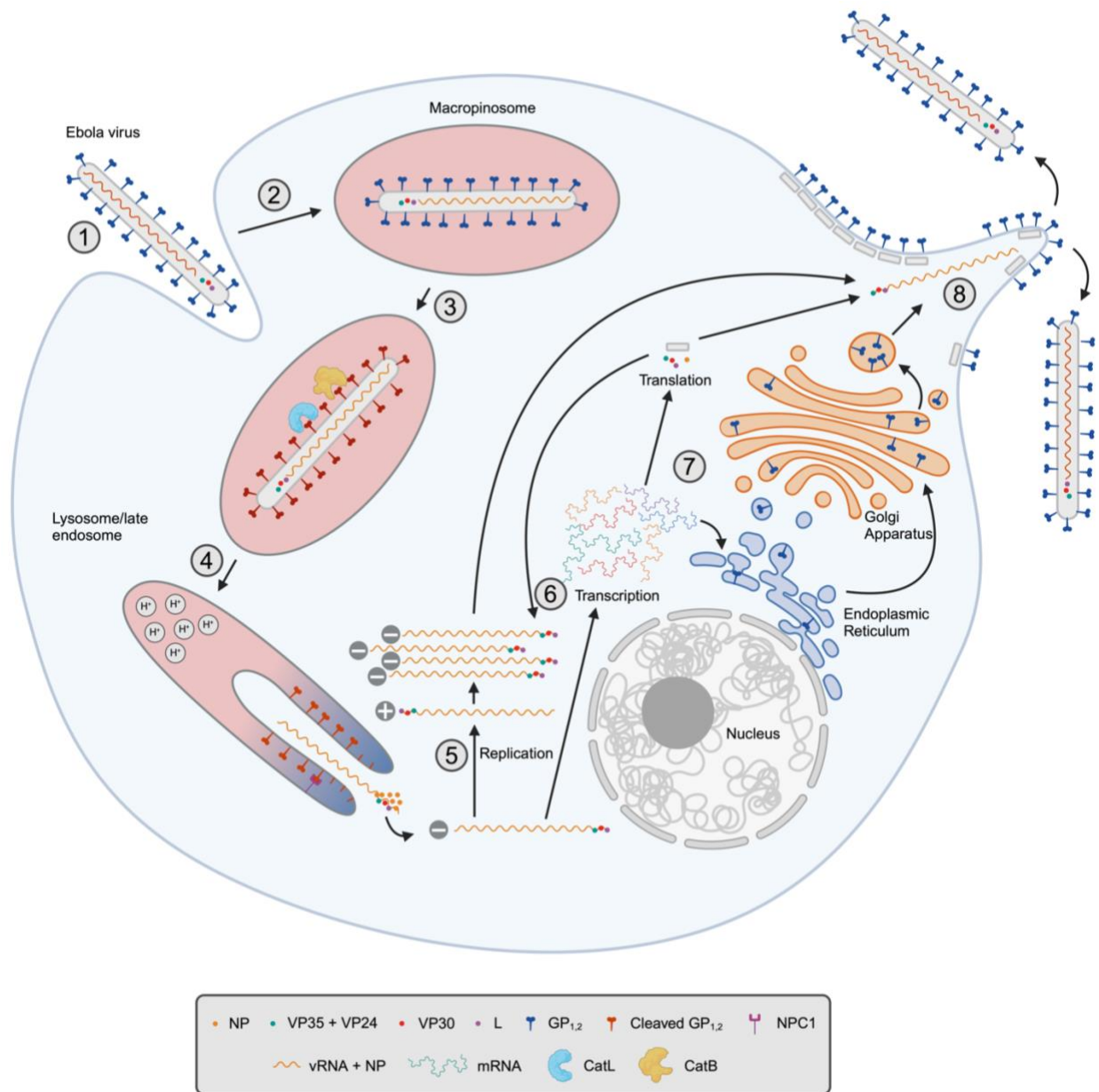


Figure 1.3 EBOV life cycle within a human host cell. (1) EBOV attachment to host cell receptors. (2) Macropinocytosis, is the main way of internalization mediated by cellular attachment. (3) Cleavage of GP mediated by CatL and CatB occurs allowing binding to NPC1. (4) Late endosome, acidification of endosome further facilitates membrane fusion for release of viral genome and proteins. (5) Replication of genome occurs. (6) Transcription of viral mRNA. (7) Translation of viral mRNA to viral proteins. (8) Budding and release of new viral particles occurs. Adapted from Jacob et al. with changes⁷. Reproduced with permission from Springer Nature. Created with BioRender.com

Viral budding takes place at designated PS-enriched areas at the host membrane (lipid rafts), where VP40 oligomerization and RNP protrusion into the host membrane occurs (figure 1.3)^{62,68-72}. During the budding, the EBOV GP is incorporated into the viral envelope, while host scramblases interact further with VP40 to flip PS in the particle, which is subsequently beneficial for re-infection^{62,68-72}. The final release of the virion from the membrane requires interference with host restriction factor activity such as Tetherin, which traps egressing particles to the cell surface⁷³⁻⁷⁵. As an alternative route to budding, EBOV employs multiple cell-to-cell transmission pathways, whereby intercellular connections allow the transfer of RNP and virions⁷⁵⁻⁷⁸. Here, GP enables transmission via cellular fusion in an actin and TIM-1-dependent manner^{75,76}. Syncytia formation has also been observed^{76,77,79}. Furthermore, EBOV seemingly exploits the tunneling nanotubules network for intercellular spread and translocation of RNP between cells⁷⁸. Here, long-range actin-formed cell connectors, which form a continuous membrane between neighboring cells, seemingly allow for a novel mechanism of EBOV dissemination within a tissue⁷⁸.

1.6 Ebola Outbreaks

Although infectious to humans, EBOV has yet to have its wild reservoir identified. It has been suspected that certain bat species may serve as a reservoir for this virus (rev. in⁸⁰) as other filoviruses have been shown to replicate in wild bats (figure 1.4)⁸¹⁻⁹¹. Further, with the discovery of paleoviral EBOV non-retroviral integrated virus (NIRV) sequences in bats suggest a close relationship between EBOV and its suspected reservoir⁹²⁻⁹⁴. Importantly, Angolan free-tailed bats have been shown to actively replicate and shed EBOV, possibly also vertically transmitting the virus to their offspring⁸⁰. Bats tolerate the infection with EBOV

and do not develop morbidity⁸⁰. In contrast, other animals susceptible to infection by EBOV (chimpanzees, duikers, gorillas, etc.) have been found to suffer severe disease caused by EBOV (figure 1.4)^{84,95-97}.

Historically, EVD outbreaks in humans had started with a single spillover event from a wild-life reservoir or animal host to the originating human host (figure 1.4) (rev. in⁷). EBOV subsequently establishes robust human-to-human transmission via fomite and direct contact with contaminated bodily fluids, spreading via complex transmission chains, often diversified by superspreaders^{84,98}. Especially at early stages of human outbreaks, EBOV disseminates in vulnerable settings involving close bodily contact (figure 1.4). This leaves treatment unit personnel and caring family members or participants of traditional burial practices at particularly high risk of transmission (rev. in⁷). These transmission events have led to 25 outbreaks of EBOV between 1976 and the time of writing this in 2024 with details provided below on what is known about these outbreaks^{7,99}.

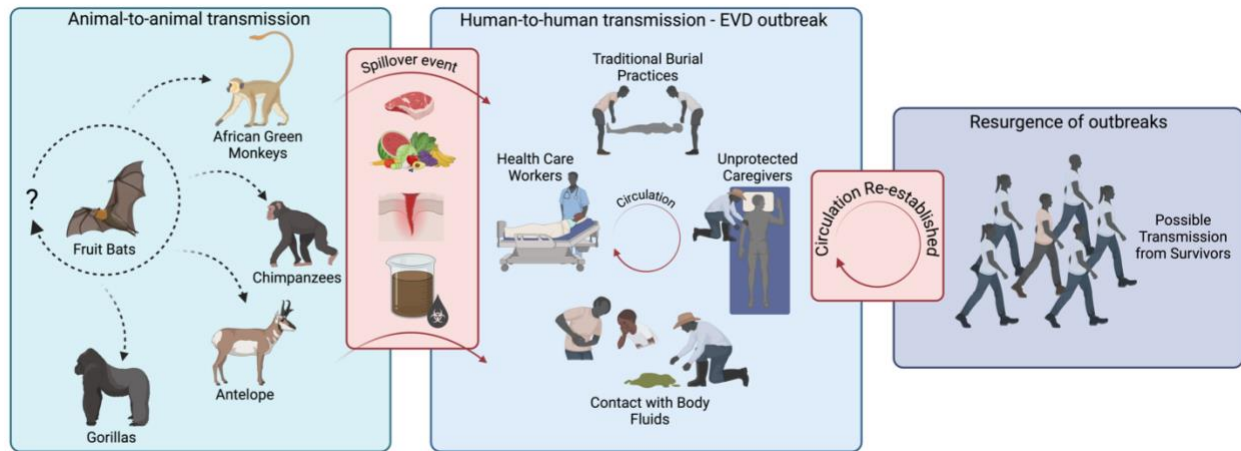


Figure 1.4 Transmission chain for EBOV from animal reservoirs to humans, human to human spread, and resurgence of outbreaks. Question mark and dotted lines indicate the uncertainty that still exists around reservoir and dead end/intermediate hosts within the transmission chain. Spillover events listed include contaminated bushmeat, contaminated fruits and vegetables, bites, and fecal contamination. Created with BioRender.com

1.6.1 First outbreak of 1976 in Democratic Republic of the Congo

The first known filoviral outbreak occurred in 1967 with Marburg virus disease (MVD) was first recognized in 1967 in West Germany and Yugoslavia following several infections transmitted from African green monkeys imported from Uganda¹⁰⁰⁻¹⁰³. EVD was characterized 1976 when EBOV and SUDV emerged in Democratic Republic of the Congo (DRC) (formerly Zaire) and South Sudan (formerly southern Sudan), respectively¹⁰⁴⁻¹⁰⁶. The EVD outbreak was the first to identify the “Marburg-like virus” and was later confirmed to be a distinct virus named after the Ebola River which the village of Yambuku sits on⁹⁹. The initial case was identified as possible malaria infection, and the infection spread through the hospital via contaminated needles and syringes and later by close contact with infected community members⁹⁹. There were 38 confirmed cases, with 318 reported cases of infection respectively with an 88% fatality rate⁹⁹.

1.6.2 Outbreaks from 1977-2012

Between 1977 and 2012, EBOV has caused multiple, sporadic EVD outbreaks in Equatorial Africa, specifically DRC, Gabon and Republic of Congo (rev. in⁷). Notably, these remained geographically limited but nevertheless culminated in 1073 reported cases and a CFR of 46.9% to 90.9% (rev. in⁷).

1.6.3 2013-2016 West African Ebola Virus Epidemic

Between 2013 and 2016 the largest recorded EVD epidemic occurred in West African countries, originating in Guinea and subsequently spreading to Liberia, Mali, Senegal, and Sierra Leone, whereby cases were further exported internationally (rev. in^{7,84}). Here, 28,652 cases of EVD were confirmed with a CFR of 39.5 % (rev. in⁷). The first reported case was in Meliandou, Guinea where Ebola virus is believed to have emerged for the West African EVD

epidemic⁹⁹. In Sierra Leone, there were 14,124 cases⁹⁹. the most of all affected countries during this outbreak. Sierra Leone has 3,466 registered survivors; however, it has been estimated the total number of survivors could be upwards of 5,000¹⁰⁷. During the 2013 to 2016 EVD epidemic, novel, atypical transmission chains of EBOV variants, previously thought to be contained, re-emerged in geographically distinct locations. This was later attributed to the persistence of EBOV in survivors and subsequent transmission to naïve individuals¹⁰⁸⁻¹¹². Indeed, evidence for sexual transmission of EBOV up to 500 days after disease was confirmed by viral sequencing, strongly suggesting EVD outbreak dynamics to be affected by flare-ups from convalescent survivors (figure 1.4)¹⁰⁸⁻¹¹². In 2021, a transmission chain from the 2013-2016 EVD outbreak resurged, indicating at least five years of persistence of EBOV in an unknown site in an EVD survivor¹¹³. Taken together, a novel mode for the start of EVD outbreaks has been established, whereby humans may serve as reservoirs for subsequent virus transmission¹¹³.

1.6.4 Outbreaks from 2017-2024

Since the end of the West African epidemic (March, 2016) EVD continues to pose an important public health threat in sub-Saharan Africa, with nine EVD outbreaks resulting in 3,713 cases and 2,408 deaths⁹⁹ with eight in DRC and one in Guinea⁹⁹. Multiple smaller outbreaks in DRC and Guinea and particularly the 2018 to 2020 DRC outbreak in North Kivu and Ituri provinces developed into the largest and most complex in the country's history, and 2,287 deaths, for a fatality rate of 66%⁹⁹ (rev. in⁷). The lone outbreak outside of the DRC since the West African EVD epidemic of 2013-2016 occurred in Guinea in February 2021 with a cluster of 23 cases (with 12 deaths) following the funeral of a nurse, where sequencing demonstrated it was a re-emergence of the strain that caused the West African EBOV

epidemic, suggesting a survivor was persistent for 5-7 years due to the minimal substitutions observed much lower than would be expected for that time period for circulating virus^{114,115}. Finally, the most recent EVD outbreak occurred in DRC (the 16th outbreak in the country beginning in 2020) and was declared over in September 2022, resulting in one fatal case (figure 1.4)⁹⁹.

1.7 Ebolavirus Pathogenesis

In the past, the spontaneous emergence and inaccessible geographic locations of filovirus outbreaks hindered direct studies of viral pathogenesis in humans^{116,117}. Cultural considerations and biosafety concerns (as well as jurisdictional difficulties) have impeded biological sample collection during filovirus outbreaks^{118,119}. As a result, current understanding of the host and viral molecular factors involved in filovirus pathogenesis has been based primarily on animal model and *in vitro* studies as well as limited biological samples collected from infected and deceased patients¹²⁰⁻¹²². Complete reviews of the current state of understanding of filovirus cellular tropism and pathogenesis are available elsewhere¹²³⁻¹²⁵. Briefly, filovirus pathogenesis begins with targeted infection of the immune system, which causes widespread immune dysregulation^{126,127}. Widespread immune dysregulation caused by conflicting pro-inflammatory and anti-inflammatory regulators allows filoviruses to infect a range of susceptible tissues, including the spleen and kidneys, liver, lungs, and gastrointestinal tract¹²³. As the infection progresses, barrier function of endothelial cells is impaired¹²⁵. At some point during the late stages of infection, filoviruses infiltrate sensitive tissues such as the eye, brain, and reproductive tract^{122,123,128}. Aside from supportive medical care, the differentiation between fatal and non-fatal disease largely

depends on the poorly understood interaction between filoviruses and the host immune system.

EBOV inhibits both adaptive and innate immune responses to infection providing an environment for efficient replication of viral particles and accelerating spread¹²⁹⁻¹³¹. These delayed responses are attributed to viral-mediated inhibition (rev. in^{7,132}) of inflammatory immune responses and interferon (IFN) signalling pathways¹³³. Normally, invading viruses are detected by the innate immune system and a signalling cascade culminates in upregulation of antiviral genes in infected and neighboring cells¹³⁴. The best studied inhibitor is VP35, which also inhibits type I interferon (IFN-I) responses^{135,136}. VP35 can interfere with IRF-3 (IFN regulatory factor 3) phosphorylation (suppressing IFN-I production) and suppresses post-translational regulation (suppresses micro-RNA silencing)^{7,132}. VP 24 is another viral factor that suppresses the antiviral response of cells by inhibiting nuclear translocation of STAT1 (signal transducer and activator of transcription 1 α/β)¹³⁷. VP40 is involved with exosomes that can kill host immune cells. Besides virions, EBOV-infected cells release sub-viral particles such as extracellular vesicles containing VP40 or GP, as well as sGP by itself, all of which have been implicated in modulating the host cellular environment and immune response^{25,138,139}.

1.8 EVD Clinical Presentations

1.8.1 Ebola virus Incubation

Transmission likely occurs through mucosal surfaces, skin abrasions or parenteral introduction following direct contact with symptomatic patients or deceased patients^{106,140-142}. Data on the time from exposure to onset of symptomologies are limited, an analysis of all

available human exposure data to determine the mean incubation period of known EVD cases is 6.22 days +/- 1.57 days¹⁴³. After surpassing initial physical barriers (skin and mucosal membranes), EBOV primarily infects immune cells such as macrophages and dendritic cells (DC), before disseminating to its target organs via the lymphatic system for amplification and final spread through the circulatory system (figure 1.5)^{7,84}.

1.8.2 EVD onset and early illness

During early infection in the first one to three days, the illness presents as a nonspecific febrile illness prior to detectable viremia accompanied by fever, malaise, fatigue, headache, muscle weakness myalgia and rash^{101,144-147}. Filovirus infections are difficult to diagnose during the early stages of disease because the associated symptoms are common for many infectious agents present in the same geographic region (e.g. malaria, respiratory viruses, arboviruses, leptospirosis and enteric bacterial pathogens)⁴. With the onset of these clinical signs detectable viremia begins to occur around 6-10 days after exposure⁷. At sites of amplification (liver, spleen, and kidney), inflammation, damage, and necrosis occur (rev. in⁸⁴). Clinically, acute EVD initially manifests as systemic febrile illness with subsequent severe gastrointestinal signs (nausea, vomiting and high-volume diarrhea) (figure 1.5)⁷. These may progress towards multi-organ failure with hematological abnormalities and overt inflammation (cytokine storm), ultimately leading to the death of the individual if not contained by the host response or treatment in the terminal phase of illness (days 7-12) (rev. in⁷).

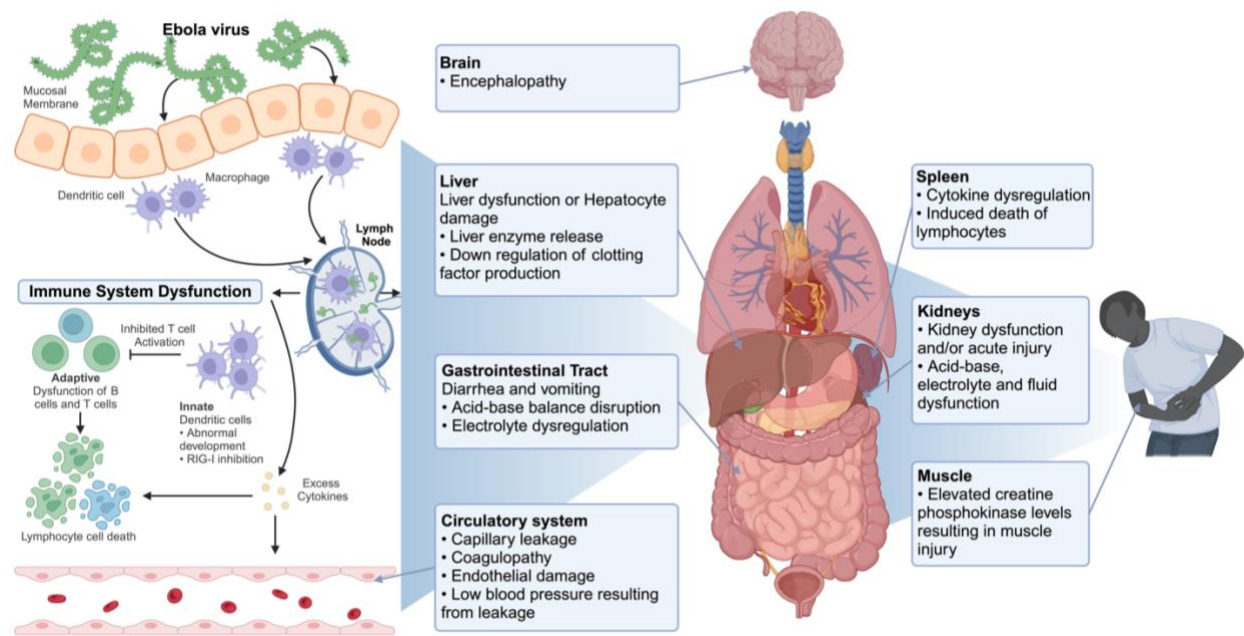


Figure 1.5 Early to late clinical presentation of acute EVD infection. Left, overview of initial infection of the lymphatic system and immune cells. Right, possible late stage EVD clinical presentations by organ system. Adapted from Jacob et al. with changes⁷. Reproduced with permission from Springer Nature. Created with BioRender.com

1.8.3 EVD peak illness

Severity of disease typically increases as the viral load increases. Thrombocytopenia, leukocytosis, elevated liver enzymes and coagulation defects (including disseminated intravascular coagulation, prolonged prothrombin time and partial thromboplastin time) are commonly associated with fatal illness (figure 1.5)^{101,144,145,147-150}. Patient data from fatal and non-fatal EVD cases suggests that while the initial onset and increase in viremia is similar between both groups, higher peak viremia and slower rates of viral decline are often associated with fatal disease¹⁵¹⁻¹⁵⁴. Although orthoebolaviruses have traditionally been considered as hemorrhagic fever viruses, hemorrhage is found in less than half of EVD patients¹⁵⁵. The development of severe hypotension and shock also appear to differentiate fatal from non-fatal disease^{101,106,117,142,156}. Hemorrhagic manifestations are predominantly localized to mucosa and conjunctivae tissues and may be associated with persistent bleeding from venipuncture sites^{101,117,145,157-160}. Petechiae and mucosal hemorrhage result in altered fluid distribution, hypotension, and aberrant coagulopathy during peak illness¹⁶¹⁻¹⁶⁴. Only in rare cases, do hemorrhagic events occur, although EBOV has classically been grouped as a viral hemorrhagic fever virus. More commonly severe disease leads to gastrointestinal fluid loss and hypotension from dehydration leading to kidney injury resulting from abnormalities in electrolyte concentrations (sodium and potassium) with multiorgan failure resulting from dysregulated inflammation⁷. It is also possible to develop neurological manifestations including meningoencephalitis directly mediated by EBOV^{20,165}.

1.8.4 EVD recovery

More recently, serological studies and case reports have shown asymptomatic infection is also possible (rev. in^{7,166}). There are reports of asymptomatic and pauci-symptomatic

infection with no clinical manifestations of EVD following exposure which testing positive for EBOV GP IgG (immunoglobulin G)¹⁶⁷. Factors influencing human survival have been suggested but are mostly based on low-resolution clinical data and animal models. Nevertheless, putative influencers of survival include viral determinants like exposure route, virus variant, and viral load (viremia)^{168,169} but also host-related factors such as age, sex, genetics, nutrition, and co-infections¹⁷⁰ as well as immune status and recognition or treatment of EVD (rev. in⁷).

1.9 Treatment for Ebola Virus

Efforts to develop countermeasures for patients with EVD has resulted in the development of several experimental therapeutics (rev. in⁷). To assess these therapeutics in humans has proven tricky as the sporadic nature of EVD outbreaks has limited access and changed the normal clinical trials-based system for approval (in both numbers of patients and randomness of outbreaks). This has resulted in many being tested in an outbreak setting through Emergency Use Authorizations to use these unapproved medications in public emergencies, the sporadic use and loosely controlled distribution of these experimental therapeutics has limited the ability to draw conclusions from their use as these were in many cases non-randomized clinical trials and uncoordinated efforts⁷.

During the 2018 outbreak in DRC, the case numbers and duration of the outbreak allowed for the design of a randomized controlled trial to test three of the most promising experimental therapeutics. The Pamoja Tulinde Maisha trial (PALM) was a large consortium therapeutics trial with the primary objective of looking at the 28-day mortality of investigational therapeutics (mAb114, remdesivir, REGN-EB3) compared with ZMapp as the

control arm^{171,172}. The study was designed to enroll patients equally stratified among all 4 arms who were confirmed to be Ebola polymerase chain reaction positive and began enrollment in November 2018¹⁷¹. The study was monitored by an independent data safety and monitoring board, and on August 8, 2019, after reviewing the data, the board recommended stopping enrollment and continuing an extension phase with mAb114 and RGN-EB3¹⁷¹. The final analysis of 673 eligible patients demonstrated that 76.5% were at least 18 years old, 56% were female (17 pregnant) at time of EVD diagnosis, and average duration of symptoms prior to enrollment was 5.5 days¹⁷¹. The 28-day mortality was 50% (84 of 169) for ZMapp, 53% (93 of 175) for remdesivir, 35% (61 of 174) for mAb114, and 34% (52 of 155) for RGN-EB3¹⁷¹. Patients who received mAb114 or RGN-EB3 cleared serum viremia faster than those who received ZMapp¹⁷¹. In 2020, PALM-trial efforts culminated in the approval of monoclonal antibody therapies REGN-EB3 (*Inmazeb*) and mAb114 (*Ebanga*)^{171,172}. Despite targeted efforts, robust symptomatic treatment and aggressive supportive care are needed¹⁷¹.

1.10 Ebola Virus Vaccine

Quick implementation of Merck's recombinant vesicular stomatitis virus–Zaire ebolavirus (rVSV-ZEBOV) vaccine at the start of the North Kivu–Ituri EVD outbreak was instrumental in preventing a larger emergency on the African continent. The single-dose vaccine protects against *Zaire ebolavirus* and is a modified vesicular stomatitis virus vector loaded with the Ebola glycoprotein¹⁷³. The vaccine was initially deployed during the 2018 Équateur Province EVD outbreak in northwest DRC that ended 7 days prior to declaration of the North Kivu–Ituri outbreak. Because appropriate regulatory approvals had been

obtained, ring vaccination campaigns were initiated almost immediately after the outbreak was declared¹⁷³.

In April 2019, WHO (World Health Organization) released an interim data analysis of the vaccine's effectiveness, announcing that it demonstrated a 97.5% protection rate¹⁷³. More than 90,000 individuals had been vaccinated at the time, with 71 going on to develop EVD¹⁷³. Of those patients, 56 developed EVD fewer than 10 days after vaccination, indicating they had not gained full immunity from the vaccine¹⁷³. None of the individuals who developed EVD 10 days or more after vaccination died of the disease¹⁷³. Based on these results, the vaccine received conditional marketing authorization by the European Commission on November 11, 2019, and Federal Drug Administration approval on December 20, 2019, under the name Ervebo¹⁷³. The DRC, Ghana, Zambia, and Burundi have agreed to license the vaccine, which will allow Merck to stockpile and distribute the vaccine in countries at risk of EVD outbreaks¹⁷³. More recently, the CDC's Advisory Committee on Immunization Practices voted unanimously that healthy US adults 18 years or older at occupational risk of Ebola be vaccinated with Ervebo¹⁷⁴. To date, over 290,000 individuals in the DRC have received this vaccination¹⁷³.

The other candidate vaccine is Ad26.ZEBOV/MVA-BN-Filo, developed by Janssen pharmaceutical and administered as 2 doses given 56 days apart¹⁷⁵. This vaccine is modified to induce production of Ebola virus proteins and trigger an immune response¹⁷⁵. Administration of the Janssen vaccine to at-risk populations in areas without active disease transmission; was introduced in Goma, the capital of North Kivu, in November 2019, with over 16,000 individuals vaccinated¹⁷⁵. A follow up study assessing the humoral response

found it to be robust in a Phase 2 trial two years after initial vaccination and boosted at year one and year two¹⁷⁵.

1.11 Long term clinical health effects

Symptoms experienced by survivors were first reported in Kikwit, DRC following the 1995 outbreak, where reports of arthralgia, myalgia, ocular pain, photophobia, hyperlacrimation and loss of visual acuity reported by survivors¹⁷⁶. It is now known many EVD survivors deal with sequelae following recovery, including long term clinical symptoms with over half of survivors continuing to experience sequelae at least seven years after discharge¹⁷⁷. Through the establishment of large EVD survivor cohorts in the aftermath of the West African EVD epidemic it has become clear that many survivors experience long-term sequelae. These range from general symptoms across many body systems including respiratory, gastrointestinal, or musculoskeletal abnormalities as well as skin changes, involvement of the urinary and reproductive tracts, sensory issues including eyesight and hearing as well as a large group of neurological symptoms (figure 1.6)^{7,20,178-180}. The most commonly reported sequelae in the available literature are fatigue, joint pain, headache, muscle pain, abdominal pain, ocular complications, anorexia, memory loss, hearing loss and alopecia^{176-178,181-192}.

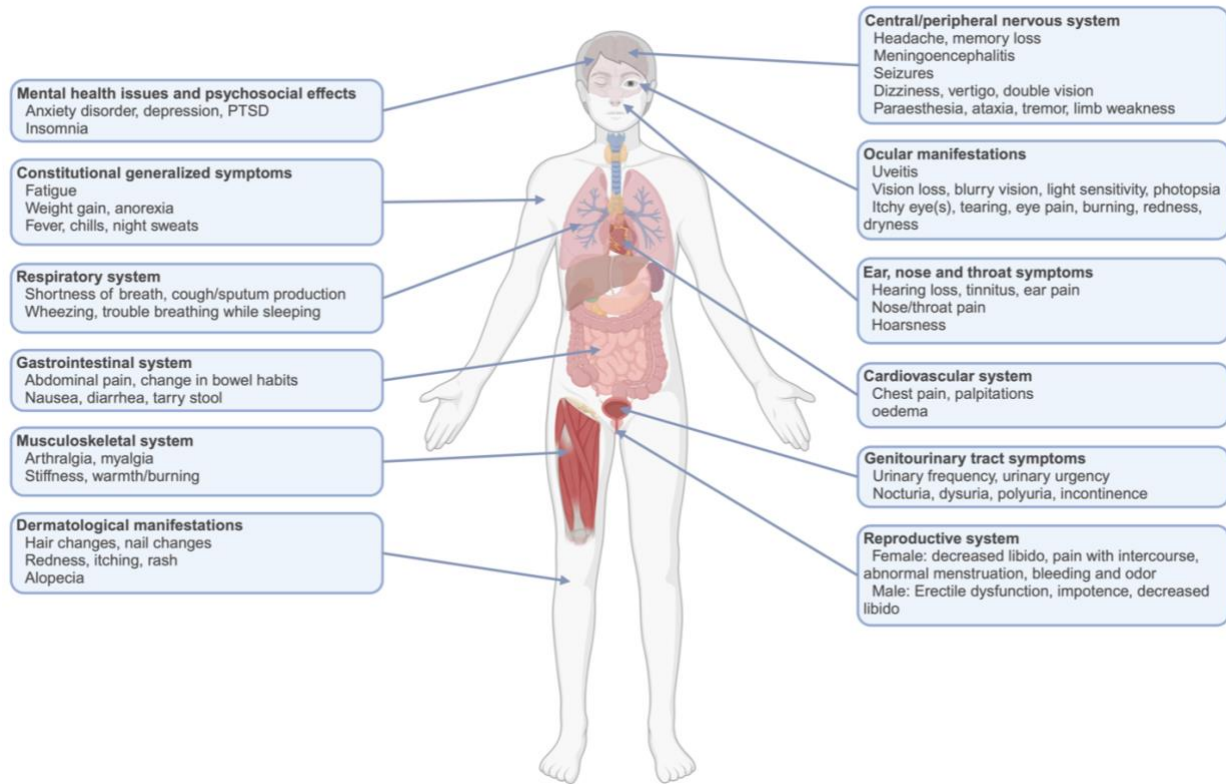


Figure 1.6 Somatic symptomology previously reported to be experienced by EVD survivors. Overview by organ system of reported symptomology available in the literature about the survivor experience in the aftermath of recovery. Adapted from Jacob et al. with changes⁷. Reproduced with permission from Springer Nature. Created with BioRender.com

Ocular complications have been among the most reported symptom groups experienced by EVD survivors. Specifically, uveitis has been commonly reported with several studies identifying survivors 33.3%^{178,184}, another study found 61.7% reported uveitis, with no improvements in follow-up¹⁸² and a further study found 63% reported ocular symptoms¹⁸⁷. Skin symptomology has also been reported with alopecia reported by approximately one in ten EVD survivors¹⁹³. Mobility limitations have been reported to be a common post-Ebola disability¹⁹³, a previous study in Sierra Leone has found that EVD survivors had significant limitations in walking and climbing stairs a year after recovery, and musculoskeletal pain was a contributing factor¹⁹⁴. Neurological symptomology in survivors include but are not limited to abnormal reflexes, tremors, balance abnormalities, speech, and cranial nerve abnormalities, or cognitive symptoms such as memory loss and insomnia^{178,187,195,196}. Moreover, difficulties concentrating, changed moods, peripheral paresthesia or dysesthesia, dizziness, migraines, and tiredness as well as seizures and strokes have been reported^{20,178,191,195-198}. Etiology of post-EVD health issues remains unclear, with no associations with inflammatory markers identified so far¹⁹⁹. It is believed reservoirs of persistent virus could be sources of antigen that stimulate immune responses triggering post-EVD symptoms²⁰⁰. No statistically significant associations between inflammatory markers and symptoms have been found to date¹⁸⁵.

A study found in the 15 months between baseline and endline, the proportion of EVD survivors seeking care at a health facility for post-Ebola health issues increased from 89 to 96% and just under one-third were referred to a higher-level facility to receive appropriate care¹⁰⁷. EVD survivors who were older, did not go to school, or were widowed reported increased numbers of post-Ebola symptoms¹⁹³. An increase in the number of post-Ebola

symptoms was correlated with participants' lower physical health¹⁹³. Another study found lower physical health scores, suggested that EVD survivors may face limitations in performing basic daily physical activities¹⁹³. Most symptoms were reported to be highly interfering with everyday life by survivors¹⁸⁵. The persistent symptoms experienced by survivors makes it challenging for them to return to their previous lives¹⁹¹, for example most survivors of the Sudan Ebola virus outbreak in Gulu Uganda reported an inability to return to work a year after infection²⁰¹. While 70% of survivors in Kikwit indicated capacity to work was worse 21 months following infection compared to before¹⁹⁰. A lack of consistent follow up on management of sequelae and limited access to healthcare services exacerbated the effects of sequelae²⁰². Some survivors were unable to get medications or follow up care with the withdrawal of aid agencies and NGOs (Non-governmental organizations) in 2018 when funding for international initiatives for survivors ran out²⁰².

1.12 Ebola virus persistence

EBOV persistence research has been ongoing to varying degrees since the 1995 Kikwit outbreak in the DRC^{203,204}. Case reports have been published on EBOV infections and persistence since the emergence of filoviruses. Rare cases of mother to child transmission suggested the potential for viral persistence in breast milk^{205,206}. Further highlighting the risks of female persistence was a case of potential in-utero transmission from a mother to a stillborn infant²⁰⁷, and detection of EBOV RNA in vaginal fluid 30 days after disease recovery^{208,209}. Varkey *et al.* describe a case in which a survivor developed ocular EBOV persistence²¹⁰. Ten weeks after discharge, the convalescent individual reported symptoms of uveitis which worsened over a four-week time course²¹⁰. EBOV RNA and infectious virus

were detected in the aqueous humor and confirmed via sequencing^{210,211}. However, no evidence of EBOV RNA has been detected in EVD survivors 19 to 34 months after disease²¹².

Severe recrudescent inflammatory disease in EVD survivors may further affect the CNS (central nervous system) with EBOV shown to persist in CSF (cerebrospinal fluid) long after clearance of EBOV from the serum as described by Jacobs *et al.*²¹³. In one well known case of the “Scottish nurse”, Pauline Cafferkey, who contracted EBOV while volunteering in Sierra Leone and was diagnosed in the United Kingdom on her return home showed no signs of neurological complications²¹³. Nine months after release from hospital tests showed she had EBOV RNA in her CSF and had relapsed with meningitis²¹³. Likewise, Sagui *et al.* reported a patient who developed seizures and tested positive for EBOV RNA in the CSF²¹⁴. Chertow *et al.* describe a case, where a male patient upon multi-organ failure presented with signs of meningoencephalitis with lesions in the brain and the spinal cord resolving after 7 months²¹⁵. Howlett *et al.* describe a patient where at day 40 after disease onset, the CSF tested positive via RT-PCR(reverse transcription polymerase chain reaction)²¹⁶.

Notably, only one case of a true relapse of EVD has been confirmed so far to result in a new transmission chain²¹⁷. The patient relapsed at 171 days after the initial disease, with symptoms of acute EVD²¹⁷. Clinical testing revealed markers of multi-organ failure and organ dysfunction, which culminated in the patient’s death shortly after²¹⁷. EBOV was detected in serum and oral swab using RT-PCR confirming a true relapse given the evolutionary conservation of the variant²¹⁷. The relapse resulted in 91 subsequent transmissions. The site of persistence remained undetected²¹⁷. Further research is crucial to determine how EBOV establishes and maintains persistence in certain sites.

First detected in 1968 following the MVD (Marburg virus disease) outbreak, filovirus testicular persistence has grown from an anomaly to a major public health concern based on observations from the West African EVD epidemic^{218,219}. Longitudinal studies have focused on the duration of viral persistence (Rev. ²²⁰)^{128,205,211,221}. In early case reports, EBOV and MARV (Marburg virus) were identified in sperm cells or seminal fluid similar to that observed during the recent West African EVD epidemic^{122,203,218,222-225}. Despite demonstrated risks of sexual transmission, little is understood about the mechanisms of persistence^{111,205,226-230}. When viral RNA is isolated from semen samples EBOV shows a reduction in evolutionary rate (six to 27 times slower)^{109,111} compared to circulating virus. The five autopsies performed to date (due to the high level of risk for pathologists), have shown EBOV is present in the seminiferous tubules, endothelium and in fibrin clots in blood vessels with no significant inflammation observed^{12,231}.

An epidemiological survey in Sierra Leone identified a chain of transmission in Kambia stemming from a persistently infected male 52 days after discharge with matching EBOV genome sequences between acute blood sample (July 2015) and seminal sample (September 2015)²²¹. A US healthcare worker infected in Sierra Leone following recovery had a viral load 10000-fold higher than peak viremia in the blood with no genome diversification¹²⁸. EBOV has also been found in semen for far more extended periods including 531 days post-disease onset with a 10-fold reduction in evolutionary rate¹¹¹. There have been 11 seminal/testicular persistence longitudinal cohort studies since 1995 that have followed a total of 628 persistent male survivors with 9 studies conducted during the West African EVD epidemic^{122,178,203,205,229,232-236}. With persistence recorded from a cohort of patients from Liberia examined from July 2016 to January 2017 had 13 participants that tested positive for EBOV

genome material for >600 days post-EVD onset and up to 965 days²²⁹. A separate study found positive samples up to 40 months post recovery (3.3 years)¹⁷⁸. In February 2021 a cluster of 23 cases (with 12 deaths) followed the funeral of a nurse, where sequencing demonstrated it was a re-emergence of the strain that caused the West African EBOV epidemic, suggesting 5-7 years of viral persistence in a survivor due to the minimal substitutions observed¹¹³.

1.13 Sexual Transmission of Ebola Virus

Since the first MVD outbreak in 1967 there have been 9 filovirus sexual transmission events (Rev. ²²⁰). Although much rarer than persistent testicular infections, similar trends are observed with the range of filovirus sexual transmission events occurring from 2-15 months post-disease^{111,221,229,234}. Sexual transmissions resulting in new chains of disease transmission were observed in the West African EVD epidemic^{227,237} thus highlighting the importance for the consideration of these events during future outbreaks as will be discussed in the following sections. The first confirmed case of filovirus sexual transmission occurred during the West African EVD epidemic in Liberia involving a male survivor who had been persistent for 179 days^{227,238}. This sexual transmission event was confirmed through contact tracing and viral genome sequencing analysis of the male patient's acute blood and semen samples compared to an acute blood sample from the female patient ^{227,238}. Most recently in February, 2021 a cluster of 16 cases (with 12 deaths), were linked by sequencing to a strain from the West African EVD Epidemic and suggested a survivor persisted for 60-84 months due to the reduced evolutionary rate observed in the sequence^{113,114}. It has also been found that there are long term reproductive health effects associated with EVD survival and reproductive persistence of EBOV.

1.14 Long term reproductive health effects

A study conducted in 2016 in Montserrado county, Liberia found that 16.2% of male EVD survivors surveyed reported testicular pain or erectile dysfunction, while 19.7% of female EVD survivors reported having menstrual problems including amenorrhoea and other menstrual irregularities¹⁹¹. Another study found half of female survivors reported a decrease in libido after recovery²³⁹. This has been previously observed; male survivors are reported to have decreased libido and erectile dysfunction following recovery from EVD^{239,240}. Another study conducted in Bombali District, Sierra Leone found that 2.2% of female EVD survivors reported a menstrual disorder and 3.4% of male EVD survivors reported erectile dysfunction²⁴¹. A study conducted in Liberia between 2016 and 2017 including 111 female participants from Monrovia, reported 25.7% of female participants had irregular menstruation of unknown cause, all of which experienced regular menstruation prior to EVD²⁴². Of reported irregular menstruation 81.5% of reports were for amenorrhea or oligomenorrhea while 14.8% were for dysmenorrhea and/or menorrhagia²⁴². Another study found 46% of female survivors reported a change in menstruation²³⁹. The same group reported in a qualitative study including 69 women, that 50% of female survivors from Liberia had reported decreased sexual libido following recovery with several women also reporting pain during intercourse, and 46% of participants reporting some of menstrual irregularity²³⁹. Most were single prior to EVD however 5 that were married are now single (3 partners died, 2 divorced)¹⁸⁶.

There have been reports of spontaneous abortion following release from ETU (Ebola treatment unit) with fetus, placenta and amniotic sac testing positive for EBOV¹⁸⁰. Another

study found 56.5% of pregnancies with an average of 709 days after discharge resulted in either spontaneous abortion (47.8% n=11) or still birth (8.7% n=2), at higher than the estimated rates for Sub-Saharan Africa (28.5% for still births and spontaneous abortions)²⁴². Sustained immune activation and/or dysregulation might contribute to the incidences of failed pregnancies, dysregulation is strongly associated to spontaneous abortion in the general population²⁴². While irregular menstruation among EVD survivors could be related to several underlying factors including weight loss and stress, persistent inflammation may also play a role in irregular menstruation due to menstruation being driven by interactions between ovarian hormones and immune system²⁴². Relationships of EVD survivors have also been affected with one study reporting of 62 participants in an intimate relationship prior to EVD, 44% reported relationship ended either due to death or abandonment by partner²³⁹. As many as 28% of participants reported abandonment by their partners (in Bong County)²³⁹. 37% of participants who lost their partner did not find a new relationship following recovery with reported reasoning being people fear them as Ebola survivors²³⁹.

1.15 Stigma

The current definition is, stigma exists when the four components of labelling, stereotyping based on this label, separation and status loss and discrimination converged in an environment where differences in power allow stigma to arise²⁴³. Current research into stigma emphasizes psychological and social aspects of stigma, where the experiences of those stigmatized can be broken down into two broad categories: “enacted stigmatization”, which encompasses social manifestations including prejudice and discrimination that are directed towards individuals with the stigmatizing attributes; and “internalized stigma”,

which is centred around internal perceptions of others, and results in feelings of guilt, shame, and/or worthlessness.²⁴⁴

1.15.1 History of stigma and infectious diseases

Stigma surrounding infectious diseases was first documented with infectious diseases in historical cultures dating back to the ancient Greek and Egyptian empires, later in the Roman empire visible diseases like leprosy which were associated with moral failure and impurity and was understood to be sexually transmitted²⁴⁵. Later with the pandemics of plague in the Middle Ages in particular the Black Death in the 14th century led to mass stigma of both the infected and survivors of plague²⁴⁶. Entire communities, including Jews, Roma, and foreigners, were blamed for spreading the disease, often leading to violent persecution and social isolation²⁴⁷. The stigmatization of outsiders during the plague shows how disease-related stigma can exacerbate social tensions and lead to violent discrimination^{246,247}. Syphilis manifested a new fear and associated stigma given its virulence²⁴⁵. The length of illness and visual manifestation gave it an association with being “unclean” making it the most stigmatized disease in Europe for 500 years until treatments were developed in the 1940s²⁴⁵. Around the same time as syphilis, in the 18th and 19th centuries tuberculosis (TB) was also heavily stigmatized. TB, in particular, was associated with poverty, poor living conditions, moral weakness and was generally considered a disease of the poor, despite the fact all people in society were equally susceptible^{245,248}. Jewish people were heavily stigmatized in regards to TB, as they were falsely thought to be immune and natural carriers of TB as a result of 2000 years living in poverty²⁴⁵.

The HIV (Human immunodeficiency virus)/AIDS (acquired immunodeficiency syndrome) epidemic in the 1980s is perhaps the most infamous example of modern

infectious disease stigma²⁴⁹. Terms like “gay plague” became widely used homophobic stigma, contributing to the delay of a comprehensive public health response²⁵⁰. Anxiety around HIV/AIDS revolved around its incurability and the sexual origins similar to other STDs²⁴⁵. This fueled pre-existing social prejudices and reinforced the stigmatization of these marginalized populations²⁴⁵. Even after it became clear that HIV could affect anyone, the stigma persisted, leading to discrimination in healthcare, housing, and employment for people living with HIV/AIDS (PLHIV)^{251,252}. Despite advances in treatment and prevention, the stigma surrounding HIV/AIDS continues to be a major barrier to care in many parts of the world²⁵³.

The COVID-19 (Coronavirus disease 2019) pandemic saw an early association of COVID-19 with China, where the virus was first identified, which led to widespread stigmatization and discrimination of individuals of Asian descent^{254,255}. Terms like “Wuhan virus” and “Chinese virus,” used by political figures and media outlets, contributed to a rise in xenophobia and hate crimes against Asians in many parts of the world^{254,255}. Healthcare workers and survivors of COVID-19 were also stigmatized, facing social isolation and discrimination, even after recovering²⁵⁶. Public health measures such as quarantine, mask mandates and vaccinations became politicized^{256,257}. The stigmatization of those infected with COVID-19 hindered efforts to control the pandemic, as many individuals delayed testing and treatment for fear of being ostracized²⁵⁷. Stigmatization has been associated with infectious diseases throughout human history as a defense mechanism to protect from disease even though it is largely unwarranted in many cases resulting in more harm than good.

1.15.2 Stigma of Ebola Survivors

Stigmatization has been reported by EVD survivors in all regions that have experienced high burdens of EVD infections, including DRC, Guinea, Liberia, and Sierra Leone^{192,258,259}. Community fears around EVD in the aftermath of the West African epidemic resulted in lasting emotional, physical and social impacts on EVD survivors (figure 1.7)²⁶⁰. Upwards of 95% of community members reported some discriminatory attitudes early in the outbreak²⁶¹ and 39% had stigmatizing attitudes that persisted in 2017²⁶². Increases in stigma coincided with reemergence events of Ebola around possibilities of transmission by survivors, as this study identified spikes in stigma reports around the times of reemergence events²⁶³. Survivor stigma has also been observed to correspond with news of survivor associated re-emergence events due to fear of, for example, sexual transmission of EVD²⁶³. It is widely recognized that stigma existed and continues to exist towards EVD survivors in Sierra Leone^{261,263-267}. In Sierra Leone one study found 83.2% of EVD survivor participants reported experiencing at least one form of stigma (figure 1.7)²⁶⁰. Verbal abuse was the most experienced form of stigma (77.1%) followed by social isolation (74.3%) and health care neglect (70.7%)²⁶⁰. Another follow-up in 2018 found that female EVD survivors experienced more enacted (experienced) stigma than male EVD survivors¹⁹³. Many EVD survivors deal with sequelae following recovery, including long term clinical symptoms and mental health complications (figure 1.7)²⁶⁴. Sequelae among EVD survivors has been shown to be compounded by stigma and psycho-social effects (figure 1.7)²⁶⁴. Stigma has also been linked with mental health diagnoses including PTSD (figure 1.7)²⁶⁸.

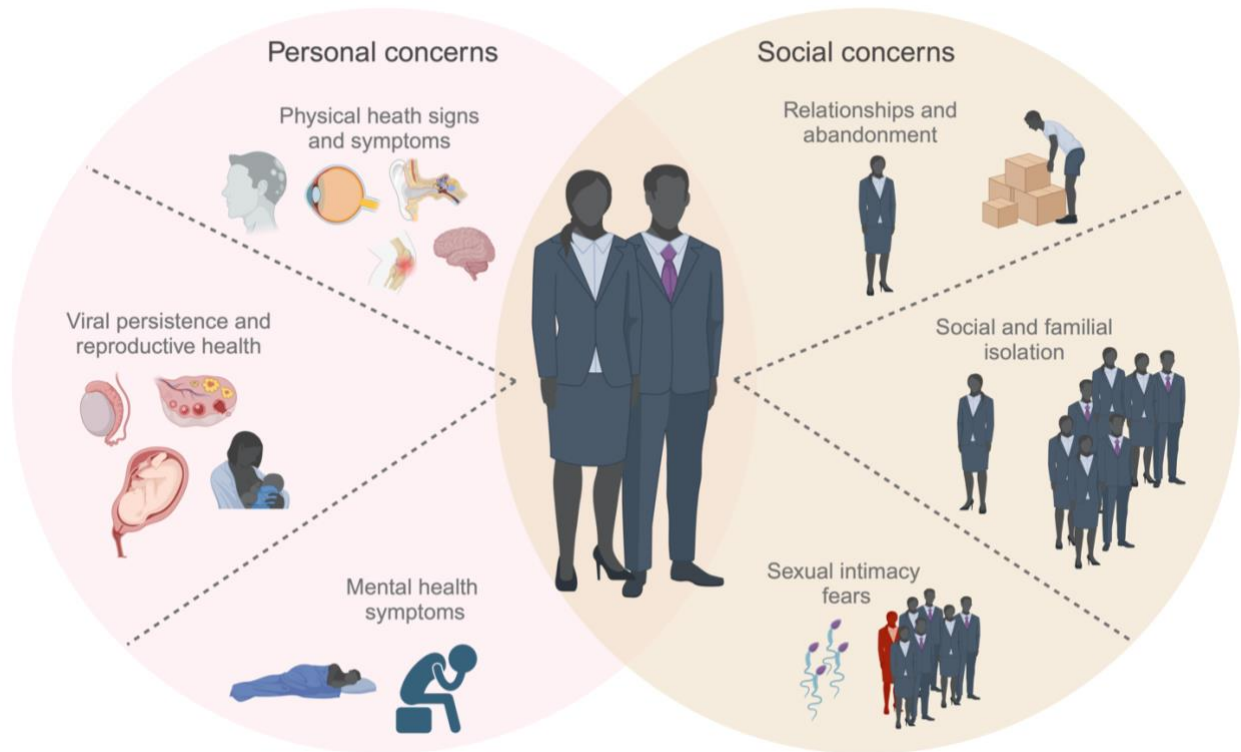


Figure 1.7 Summary of the lived experience of EVD survivors. Personal concerns, are the physical health sequelae they experience, the fears around reproductive persistence and healthy pregnancy and the mental health issues they experience as a result of their survival and day to day experience with others. Social concerns, are the relationship issues they experience due to stigma, the isolation by family and friends because of stigma and employment discrimination, and the fears around sexual intimacy due to their survival. Created with BioRender.com

1.16 Long term mental health effects

Previous investigations of mental health effects of EVD across Liberia, Guinea, and Sierra Leone found a high prevalence of symptoms consistent with depression (12-75%), anxiety (28-84%), and psychological distress (46%)²⁶⁹. Sierra Leone had the highest prevalence of depression, with 22.0% of participants meeting the criteria for a tentative diagnosis of depression, compared with 20.2% in Liberia and 13.0% in Guinea²⁷⁰. Recent Sierra Leonean studies have shown that anxiety, depression, post-traumatic stress disorder and stigma are common among EVD survivors^{260,270,271}. A recent study in Sierra Leone found close to half (47.2%) of Ebola survivors interviewed show possible case of depression²⁷¹. This was higher than the prevalence reported among survivors in eastern Sierra Leone (35%) that were assessed 4 months post-ETC (Ebola treatment centre) discharge¹⁸⁹. More specifically one study found 27% (95% CI 25.8% to 28.8%) met levels of clinical concern for PTSD (post-traumatic stress disorder) and 16% (95% CI 14.7% to 17.1%) met levels of probable PTSD diagnosis and among all respondents, 47% (95% CI 44.2% to 47.4%) reported both symptoms of anxiety and depression, and PTSD²⁶¹. Another report found four times as many survivors (23%) met criteria for major depressive disorder (MDD) than uninfected control participants (6%) and post-traumatic stress disorder (PTSD) criteria for survivors (24%) and uninfected control participants (17%) was four times higher than the comparisons (6%)²⁷². In addition, suicide attempts among survivors were six times higher than uninfected control participants²⁷². There was a gender disparity in respect to reports of mental health with reporting being female associated with higher depression scores in both Sierra Leone and Guinea²⁷⁰. There have been associations found with the acute EVD experience, where

EVD survivors with an increase in the time spent in the Ebola treatment centre were likely to report a decrease in mental health¹⁹³. Low physical and mental health assessments indicated that EVD survivors may have suffered from poor physical and mental health¹⁹³. In line with previous Sierra Leonean studies, which show that EVD survivors continue to experience poor physical and mental health more than two and half years after discharge from the Ebola treatment centre^{181,270,271}, they continue to suffer from psychological distress due to grief resulting from losing loved ones, social exclusion, and community stigmatisation (figure 1.7)^{264,273}.

1.17 Gaps in knowledge

Questions remain regarding the long-term implications on physical health effects. Moreover there is disagreement over the duration and extent of sequelae, with some studies indicating symptoms waning over time while others show persistent symptoms²⁷⁴. There is little information regarding the long-term impact on reproductive health of survivors following recovery. Furthermore, there were and still are several health needs that have been ignored or overlooked in this community of survivors with early reports of long-term complications in survivors suggest adverse pregnancy outcomes and irregular menstruation in female survivors, while reproductive health in male survivors are understudied^{242,275}. To date, there have been limited studies comprehensively examining EVD-related stigma, making it difficult quantify the extent of these experiences among survivor communities^{262,263}. Most studies examining stigma have been conducted in the immediate aftermath of EVD outbreaks^{192,258,263,276,277}. Moreover, there is limited knowledge of the sociodemographic factors related with psychosocial sequelae^{264,269}. Few studies have assessed the mental

health of EVD survivors^{240,261,270,278}. Psychosocial effects associated with EVD have been understudied, with little known about demographic associations to mental health experiences of EVD survivors including whether sex plays a role in those experiencing mental health issues²⁶². No studies have followed up on the mental health outcomes beyond three years after the epidemic was declared over leaving a gap in knowledge on the current state of mental health disorders among the survivor population in Sierra Leone as well as what the long-term mental health outcomes of EVD survival are. Varying levels of reported mental health symptoms by EVD survivors suggest that further investigations are required to better understand the specific mental health impact of the epidemic²⁶¹.

1.18 Study rationale

An Amnesty International report found that EVD survivors were still struggling with a range of physical and mental health symptoms and that the COVID-19 pandemic had brought back distressing memories, with some survivors speaking of a “persistent fear of death”²⁷⁹. Past studies have shown that physical symptoms (e.g., joint pain, reduced hearing, and blurry vision) are associated with significant mental health problems and stigma among survivors^{258,280}. Nearly all published reports on survivor somatic symptomology have occurred within the first two years of the post recovery period except for two studies at 4 years^{185,274} and one at 7 years¹⁷⁷. Given the lack of epidemiological study on long-term health outcomes of EVD survivors and our access to survivor associations in Sierra Leone we used this access determine somatic symptomology, reproductive health, stigma and mental health of EVD survivors. Availability of validated question sets for Sierra Leone and EBOV allowed for efficient development while producing quality results. Utilization of *REDCap* allowed the

continuation of the study in the face of a global pandemic with intuitive easy to use interface and remote usage by participants in country with limited internet access.

1.19 Hypothesis

I hypothesize that, *Ebola virus has negatively affected health, reproductive health, stigma and mental health of EVD survivors, with symptomology continuing to persist six to eight years later.*

1.20 Objectives

Aim 1) Determine somatic symptomology, reproductive health, stigma and mental health experienced by EVD survivors six to eight years post recovery. **Aim 2)** Determine the issues that are most affecting EVD survivors six to eight years post recovery

In **Aim 1**, to assess the effects of EVD survival on long term somatic symptomology, reproductive health, stigma and mental health a study on long term consequences of EVD was conducted with a cohort of EVD survivors in Sierra Leone. We had a target to recruit 350 male survivors/close contacts and 355 female survivors/close contacts between 18 and 50 yoa²⁸¹⁻²⁸³. Close contacts are an accepted control in EVD survivor studies as they are more likely to be retained since they have a vested interest in the health of survivors²²⁶. This study utilizes collaborations with Dr. Jia Kangbai who ran the surveying in country (Kenema, Sierra Leone), Dr. Boghuma Titanji is providing consulting support and medical expertise and is currently an Associate Professor (Emory University), Dr. Souradet Shaw who is an epidemiologist and Associate Professor (Department of Community Health Sciences, University of Manitoba), Dr. Kaarina Kowalec an epidemiologist and Associate Professor

(College of Pharmacy, University of Manitoba), Dr. Bev Fredborg a clinical psychologist and assistant professor (Department of Psychology, University of Winnipeg) and Dr. Anne Rimoin an epidemiologist and Professor (University of California, Los Angeles). The survey covers demographics, stigma, somatic symptomology, mental health and reproductive health issues in relation to disease recovery, with standardized question sets relevant to mental health, substance abuse and sexual dysfunction. To ensure cultural sensitivity, the survey followed validated questionnaires for Sierra Leone^{284,285} and for EVD survivors²²⁶ respectively. Administration through the online secure health survey platform REDCap allows for the anonymous entry of data protecting participants' privacy and health information in compliance with PHIA, FIPPA and participant safety in regard to the COVID-19 pandemic.

In **Aim 2** using the data collected and analyzed in **Aim 1**, it was determined through statistical analysis (utilizing *STATA* statistical software) what symptomology for somatic symptomology, reproductive health, stigma and mental health were most prevalent at the endpoint of the cross-sectional study in comparison to the close contact participants. This involved standardization of the data set for relevant parameters depending on what was being assessed (e.g. for somatic symptomology anyone reporting symptoms in line with acute illness was excluded). Associations to EVD were assessed through logistic regression analysis and presented in the form of odds ratios to represent association and significance of the association to EVD. This data was used to inform interpretation of the ongoing experiences of EVD survivors as a whole in Sierra Leone.

Chapter 2: Materials and Methods

2.0 Materials and Methods

The full protocol submitted to and approved by the Research Ethics Boards (REBs) can be viewed in Appendix 2 along with the consent provided to participants and the ethics approvals.

2.1 Sample size

This was cross-sectional study of EVD survivors and their close contacts conducted in Sierra Leone between November 2021 and March 2022. Recruitment target of participants was based on estimates of the national EVD survivor population in the range of 3-5,000 in Sierra Leone, and to obtain 95% confidence intervals a sample size of 715 was determined to be appropriate for the study format using the Fleiss with continuity correction method of sample size determination for cross-sectional studies²⁸⁶. This recruitment target was increased to 1,000 to account for non-responses and to ensure the minimum sample size was met for analysis.

2.2 Participant Recruitment

To obtain the desired national representation, a multistage sampling approach was used, where known survivors provided by the Sierra Leone Association of Ebola Survivors, were stratified by district and gender followed by random sampling within each district. This was utilized for recruitment across all EVD-affected districts (Bo, Bombali, Falaba, Kailahun, Kambia, Karene, Kenema, Koinadugu, Kono, Moyamba, Port Loko, Pujehun, Tonkolili, Western Area Rural, and Western Area Urban)¹⁰⁷. The recruitment team utilized this multistage sampling method starting in the South and moving counterclockwise around the

country ending in the Western region. Due to a lack of reliable internet access in many areas, our recruitment team surveyed participants after recruitment where they accommodated translation to Krio and assisted those who were unable to read. Recruitment was concentrated within the Eastern region (Bo, Kailahun, Kenema, and Kono) and additional proportional representation to case numbers from the remaining districts of the country.

Inclusion and exclusion criteria for the participants required Survivors to provide an EVD discharge card, or certificate from an ETU in Sierra Leone from between March 2014 and March 2016 as proof of EVD survival. Close contacts were required to provide proof they were a family member of an EVD survivor or a deceased EVD patient by providing an EVD discharge card, certificate, or a death certificate for their deceased EVD patient relative. These close contacts were included as a representative group similar to previous studies conducted¹⁷⁸.

2.3 Participant consent

Data were collected from November 2021 to March 2022 and managed via a fully anonymous survey hosted by and, developed using REDCap and securely stored on servers at the University of Manitoba.^{287,288} The University of Manitoba Research Ethics Board [HS24515 (H2020:538)] and the Office of the Sierra Leone Ethics and Scientific Review Committee, Government of Sierra Leone, granted ethical approval for this study (can be viewed in Appendix 2). This study was also supported by the local EVD survivor association (SL Ebola Survivor Association) through presentation to the association heads by co-investigator Jia Kangbai (JBK). Through this association information about this study was disseminated to

the members, including the purpose, requirements, risks and benefits of this study if they have decided the study is beneficial.

EVD survivors were informed about the scope, expected outcomes of the study, and their ability to end participation at any time without any repercussions via a consent disclosure statement, with continuation only occurring for those participants who consented to participate. Consent to participate in the study was interpreted by checking yes on the consent to participate box following the consent disclosure statement at the beginning of the survey. Survivors who agreed to participate were further asked to recruit at least one close contact (who has never had confirmed EVD) to participate in the study with them, meeting the above-mentioned inclusion and exclusion criteria for our close contact control group. At the time of identifying the close contact, they were provided contact information for our co-investigator to answer any further questions of prospective study participants regarding types of questions.

The study materials describing the purpose, requirements, risks and benefits for this study were shared with the close contacts if they have decided the study was beneficial. Participants were also encouraged to read through the consent disclosure statement at the top of the anonymous survey at their own pace with as much time as they needed. Participants could discuss the consent disclosure statement with whoever they need prior to continuing to the survey. The participant had the opportunity to ask any questions that they may have had through the contact information provided for the investigators of the study. Consent was obtained by agreeing to continue with the questionnaire. Participants were not obligated to complete the questionnaire and participation was completely voluntary. Participants were only encouraged to answer what they were comfortable with.

Consent was documented by participants continuing with the questionnaire after the consent disclosure statement. Translations of study materials in local languages of Sierra Leone (Kiro and Mende) were provided to accommodate participants where English was not their primary language.

Participants were informed that they can receive up to \$10 upon completion of the online questionnaire through a confidential and anonymous prompt that will not retain personal or financial information. They would obtain one third of the total amount for each of the three sections of the survey completed before submission (regardless of the number of questions completed in each of the sections). This has become standard practice with survivors in Sierra Leone as early research on EVD survivors and patients resulted in a lot of exploitation of this community by researchers.

2.4 Study data use and access

Both consent and data were captured electronically when service allowed, surveyors had backup hard copies of the survey when this was not possible and recorded both consent and data for upload to REDCap when and where internet service allowed. All research staff who were involved in the collection and analysis of study data had training on ethical conduct for research involving humans and applicable personal and health privacy legislation.

Name, telephone number and e-mail address were only used for the recruitment portion of the study. These were crucial during the time of conduction due to the ongoing COVID-19 pandemic, provided that the gathering of groups in person with the SL Ebola Survivor Association was not allowed and morally a poor decision for the health and safety of researchers and potential participants. Biological sex, number of children and age were

collected for determining the effects/previous ability to have children or what types of sexual dysfunctions may have been present. This was important for the determination of long-term health effects. Health conditions were sampled to determine potential confounding variables that may need to be accounted for during data analysis. Ethnicity and district of residence were important for data breakdown to inform where and who was most affected by the results obtained from this study. It is important to note the anonymous nature of this survey ensured that none of this information can be linked back to any identifiers obtained in the recruitment process or any specific individual as the data will be presented together for all participants for each data point collected.

The findings of the survey were and will be kept confidential and anonymous. All participants will remain anonymous through the completion of the fully anonymous online survey. The indirect identifiers will never describe individual participants and will only be described in any publications, reports or presentations as a representative number of the participant cohort. Likewise, the survey system will not record e-mail addresses or IP (Internet protocol) addresses. The anonymous questionnaire data collected will be stored in a computer in the Department of Medical Microbiology and Infectious Diseases. Accessible on password-protected computer by only study personnel through individual logins and stored in a locked room when not in use for analysis. The computer will be ensured to be fully PHIA compliant before uploading from *REDCap* platform in consultation with University of Manitoba ICT.

Direct identifiers that were used in the recruitment process (restricted to name, telephone number and e-mail address) were kept separate from all study data and only accessible by the principal investigator (PI) and co-investigator for recruitment purposes in

a password-protected and encrypted file only accessible in circumstances that meet these requirements. The encrypted file was stored on a password-protected computer requiring a user account and password separate from the file in question. The computer was stored in a locked cabinet when not in use due to the sensitive nature of the list of identifiers in question. This computer will/was ensured to be fully PHIA compliant before collection of recruitment information from SURVIVORS association.

Questionnaire direct results will be deleted 12 months after completion of this study and all other data will be retained for 10 years. Personally identifying information will only be retained for the duration of the study. Disposal will be completed by erasing all relevant files from the hard drive of the associated computer. The PI, co-investigator and the study personnel had access to the data at all stages of active collection (study personnel only had access to the survey data not recruitment data), processing and analysis. The Bannatyne Campus Research Ethics Board, The Sierra Leone Ministry of Health Ethics and Scientific Review Committee and University of Sierra Leone may be asked to review research records for audit purpose. Study personnel and their tasks were maintained in study files.

2.5 Survivor survey

The completed survey can be found in Appendix 1 for a more detailed overview of the questions included and asked of the participating EVD survivors and close contacts.

2.5.1 Participant demographics

Socio-demographic factors were assessed in the context of EVD-related stigma to represent and describe participants' lived experiences following EVD recovery. Socio-demographic and behavioral characteristics included age, sex assigned at birth, education, employment,

survivor status, marital/relationship status prior to and since EVD, sexual history prior to and since EVD, religious affiliation, district/province of residence prior to and since EVD, place of residence, and change of residence, BMI (body mass index) at the time of the survey. The inclusion of marital/relationship and sexual history data allowed for stratified analyses examining intimate partnerships participants have had and assessing the impact any changes EVD survival had on those partnerships.

2.5.2 Somatic symptomology

Somatic symptomology was assessed through a symptom screen in the survey where participants self-reported symptoms experienced in the past two weeks at the time of the survey. This symptom screen assessed chronic medical conditions, infections, ocular symptoms, ear, nose and throat (ENT) symptoms, neurological symptoms, constitutional symptoms, cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms, genitourinary symptoms, musculoskeletal symptoms and dermatology symptoms (a full breakdown can be found in Appendix 1). Participants were asked if they had currently or recently experienced a symptom from a particular organ system and at what frequency, assessed on a 6-point Likert scale from 1 (monthly) to 6 (constantly). They were also asked whether they sought medical care and were currently taking medication for it. Both EVD survivors and close contacts reported symptoms experienced.

2.5.3 Reproductive health

2.5.3.1 Female reproductive health and sexual activity

Female participants were assessed through the female sexual function index (FSFI) which is a 19-item questionnaire that assesses six dimensions of female sexual health: desire, arousal, lubrication, orgasm, satisfaction and pain²⁸⁹. This questionnaire was designed to assess the

multidimensional nature of female sexual health. Items were scored on either a 5-point Likert scale from 1 (Very High) to 5 (Very low/none) or a 6-point Likert scale from 0 (No sexual activity) to 5 (Almost always/always) over the last four weeks, with scores ranging from 4 (Severe female sexual dysfunction) to 95 (Normal female sexual function). Female sexual arousal disorder (FSAD) is a tentative diagnosis a score of 57 or less is obtained from the question set.

2.5.3.2 Male reproductive health and sexual activity

Male participants were assessed through the international index of erectile dysfunction (IIEF) which is a 15-item questionnaire that assesses five different domains of male sexual health: erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall satisfaction²⁹⁰. Items were scored on either a 5-point 1 (Very High) to 5 (Very low/none) or a 6-point Likert scale from 0 (No sexual activity) to 5 (Almost always/always) over the last four weeks, with scores ranging from 6 (Severe male sexual dysfunction) to 75 (Normal male sexual function). Providing tentative diagnoses for male sexual dysfunction and erectile dysfunction if scoring 36 or less for the IIEF and 13 or less on the erectile function section respectively.

2.5.4 Stigma

This study used an adapted version of The Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) III EVD study Stigma index, a tested and validated index for Sierra Leone based on consultations with one of our in-country collaborators (JBK). The PREVAIL III Stigma Index was adapted from the PLHIV Stigma Index, which has been used more than 100,000 times in more than 90 countries since 2008¹⁷⁸. The PLHIV Stigma Index was adapted for EVD stigma in consultation with EVD survivors, and mental health professionals familiar

with using the PLHIV Stigma Index in Liberia to determine relevant items, utilize language relevant to EVD survivor experiences, and posed in a way to comprehensively capture any experience of stigma while also not using portions that were deemed not applicable to EVD.²⁶⁵ The eight-item Stigma Index is listed in Table 2b. Two items were used to measure stigma and discrimination from other people; three items measured stigma through work, health, and education and three items were selected to capture internalized stigma. Binary (yes/no) responses were used with an option to not answer to determine the main outcomes of EVD-related stigma.¹⁷⁸ Thus, the EVD Stigma Index represents the cumulative sum of the eight items, with values ranging from zero to eight. Internal consistency of the index was high (Cronbach's α : 0.911).

2.5.5 Mental Health

2.5.5.1 Patient Health Questionnaire (PHQ-9).

The PHQ-9 is a nine-item self-administered questionnaire to evaluate depressive symptoms during the two preceding weeks. These nine items were directly based on the nine diagnostic criteria for major depressive disorder in the Diagnostic and Statistical Manual Fifth Edition (DSM-5). This instrument can serve dual purpose in that it may both screen for the presence of depressive disorder and screen for symptom severity²⁹¹.

Each item of the index is scored on a 4-point Likert scale from 0 (not at all) to 3 (nearly every day) with cumulative scores ranging from 0 (no depressive symptoms) to 27 (severe depressive symptoms). Major depression (MD) is considered a tentative diagnosis. This index had a Chronbach's α of 0.76 demonstrating good internal reliability for use in this study for EVD survivors and close contacts. This instrument has been previously used and validated for use in sub-Saharan Africa²⁹¹.

2.5.5.2 PTSD Checklist (PCL-5).

The PCL-5 is a 20-item self-reported index that assesses the presence and severity of PTSD symptoms during the previous month. The 20 items directly correspond with the DSM-5 PTSD criteria: quantifying and monitoring symptoms over time, screening individuals for PTSD, and assisting in making a provisional diagnosis of PTSD. Each item of the index is scored on a 5- point Likert scale from 0 (Not at all) to 4 (Extremely) with total symptom severity scores ranging from 0 to 80. A cut-off score of 33 was used to screen participants for a tentative diagnosis of PTSD. The index trauma used for PTSD in this study was the participant's EVD infection. This index had a Chronbach's α of 0.97 demonstrating excellent internal reliability for use in the EVD survivor population. This instrument has been previously used in Sierra Leone and other West African populations.^{292,293}

2.5.5.3 Alcohol Use Disorders Identification Test (AUDIT).

The AUDIT is a 10-item self-reported index to assess alcohol use during the past year. The AUDIT index was developed by the WHO in 1989 through a study with a bank of 150 questions assessed (10 included in final version were strongest performing questions) across all socioeconomic, and geographic regions ensuring no country dominated the dataset allowing it to be a robust international instrument. This index had a Chronbach's α of 0.89 demonstrating good internal reliability for use in this EVD survivor population. The AUDIT assesses three domains of alcohol use: alcohol intake, dependence on alcohol, and experience with alcohol-related harm. Each item is scored on a 5-point Likert scale from 0 (Never) to 4 (Daily or almost daily) with total frequency scores ranging from 0 to 40. A cut-off score of 8 was used as the assessment point for harmful drinking behavior by participants.

2.5.5.4 Drug Abuse Screening Test (DAST-20).

The DAST-20 is a 20-item self-reported index to assess drug involvement excluding alcohol during the preceding year. Each item of the index is scored on a binary (yes/no) scale producing a total drug use score between 0 (No drug use) and 20 (Severe drug use). A cut-off score of 6 was used to screen for participants with tentative drug use. This index had a Chronbach's α of 0.95 demonstrating high internal reliability for use in this EVD survivor population. The DAST-20 was developed by Dr. H. A Skinner.

2.6 Statistical analysis

2.6.1 Descriptive statistical analysis, processing and presentation

Descriptive statistics were used for reporting continuous (mean/standard deviation or median/IQR (interquartile range)) and categorical variables (frequency/percentages). Statistically significant associations between independent variables (e.g., sociodemographic variables, such as age, and health related variables, such as eye pain) and dependent variables (e.g., Ebola, stigma, PTSD) For bivariate descriptive analyses, tests of statistical significance used were Student's t-test for continuous variables, and Fisher's exact test, or the Chi-square test of association for categorical variables, where appropriate. Additionally, sex-based stratification was conducted where appropriate to determine if sex-based differences existed between independent and dependent variables.

2.6.1.1 Mental health descriptive statistical analysis, processing and presentation

For univariable, bivariate and multivariable analyses, depression, PTSD, substance use disorder and alcohol use disorder were converted into binary outcomes based on their

respective cut-off scores (PHQ-9 $\geq$ 10, PCL-5 $\geq$ 33, AUDIT $\geq$ 8, DAST-20 $\geq$ 6) and presented in comparison to close contacts.

2.6.2 Logistic regression analysis, processing and presentation

Logistic regression analysis results are presented as adjusted odds ratios (AORs) with associated 95% confidence intervals (CI). Independent variables with $p \leq 0.05$ in the bivariate models were included in the multivariable logistic regression model determining which factors independently associated with the outcomes unless otherwise stated. A priori, age, sex assigned at birth, province of residence, and BMI (BMI included for somatic symptomology analysis only) were selected to control for associations between symptomologies and EVD survival status. Missing data was minimal, however when needed for variable analysis in the multivariate logistic regression models pairwise deletion was used to ensure the dataset for assessed variables were complete.

2.6.2.1 Somatic symptomology logistic regression analysis, processing and presentation

For somatic symptomology, independent (e.g. demographic, health-related variables) and dependent variables (e.g. EVD survival) were assessed by Firth multivariable logistic regression models. For the purposes of this study, statistical significance in regression models was defined as a q-value of 0.05 or less after controlling for multiple testing using the Benjamini-Hochberg procedure.

2.6.3 Statistical analysis software

Stata version 17.0 (College Station, TX: StataCorp LLC; 2021) was used to perform all analyses.

Chapter 3: A Cross-sectional study of health and social status of EVD survivors in Sierra Leone

3.0 A Cross-sectional study of health and social status of EVD survivors in Sierra Leone 6-8 years after recovery.

The 2013-2016 West African EVD epidemic was the largest in history, resulting in 28,652 recorded cases and 11,325 deaths⁹⁹. Sierra Leone was the most affected country with 14,124 cases^{99,182} reported. As of 2020, 3,466 EVD survivors were registered in Sierra Leone, though estimates suggest true numbers may exceed 5,000¹⁰⁷. Given this large survivor population, understanding the long-term health effects and future medical needs of survivors is a public health priority¹⁷⁶. This national cross-sectional study was conducted in Sierra Leone from November 2021 to March 2022. It involved simultaneous recruitment and surveying across all affected districts²⁹⁴. A sample size of 715 participants was determined to provide 95% confidence intervals for our outcome variables, as previously described for this population²⁹⁴. To account for potential non-responses and ensure a minimum sample size for analysis, the recruitment target was increased to 1,000 participants, with a total recruitment of 998 participants outlined in figure 3.1. Where there were 1247 respondents, however 249 were removed from the study, for nonresponse, lack of consent, ineligibility due to age, or their response missing for survivor status.

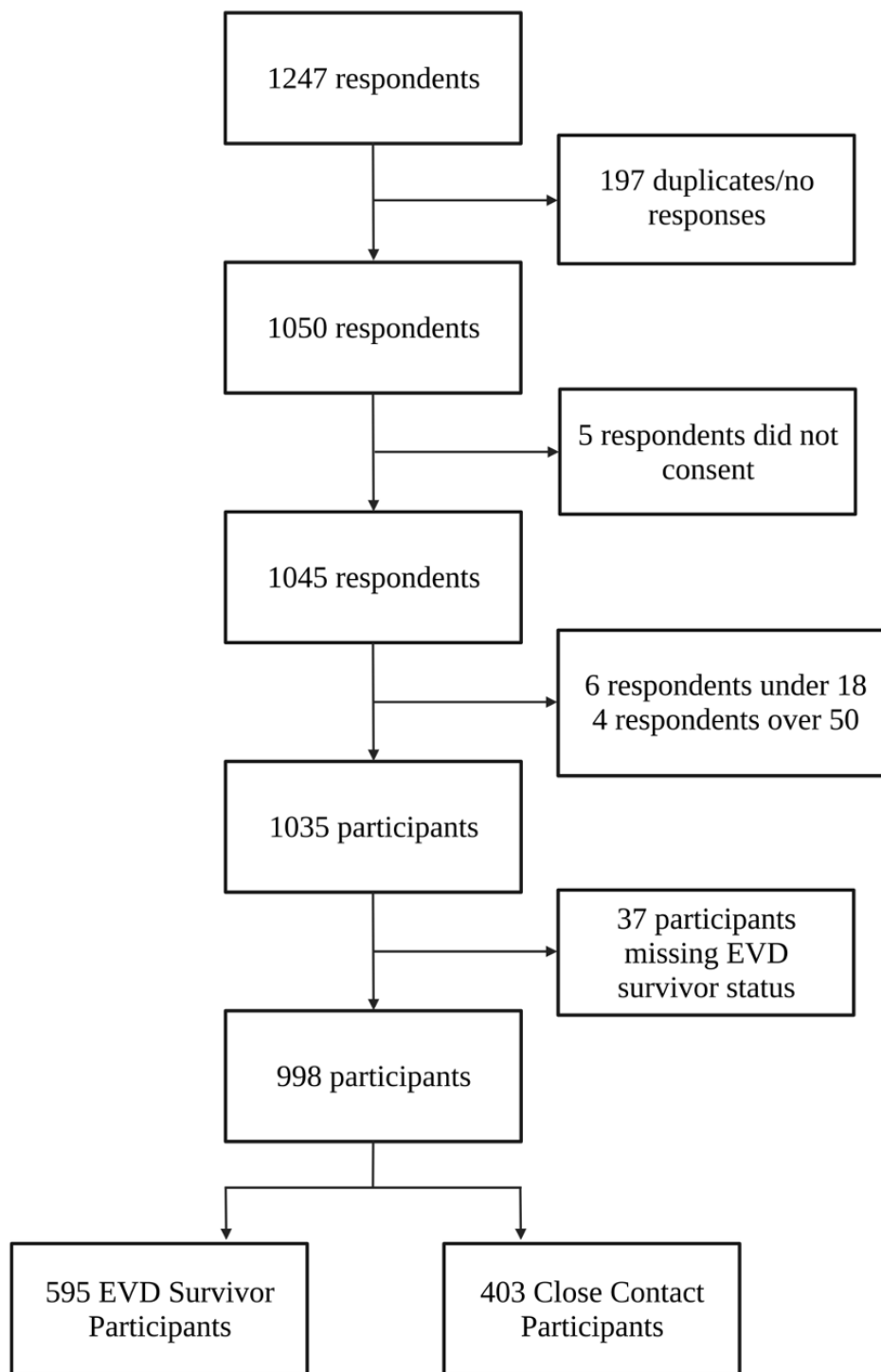


Figure 3.1 Recruitment and participant breakdown flowchart. Flowchart of those who have been excluded through the recruitment, data validation, and analysis stages of the study. Created with BioRender.com

3.1 Cohort Description

A total of 595 EVD Survivors (59.6%) and 403 close contacts (40.4%) were included in this study, as shown in table 3.1 with demographics outlined recruited between November 2021 and March 2022. The geographic distribution of EVD survivors is shown in Figure 3.2, briefly the majority of survivors were recruited in the Eastern ($n=238$, 40.3%), and Southern ($n=136$, 23.0%) provinces, followed by the Western province ($n=87$, 14.7%), Northern Western province ($n=67$, 11.3%) and Northern province ($n=63$, 10.7%).

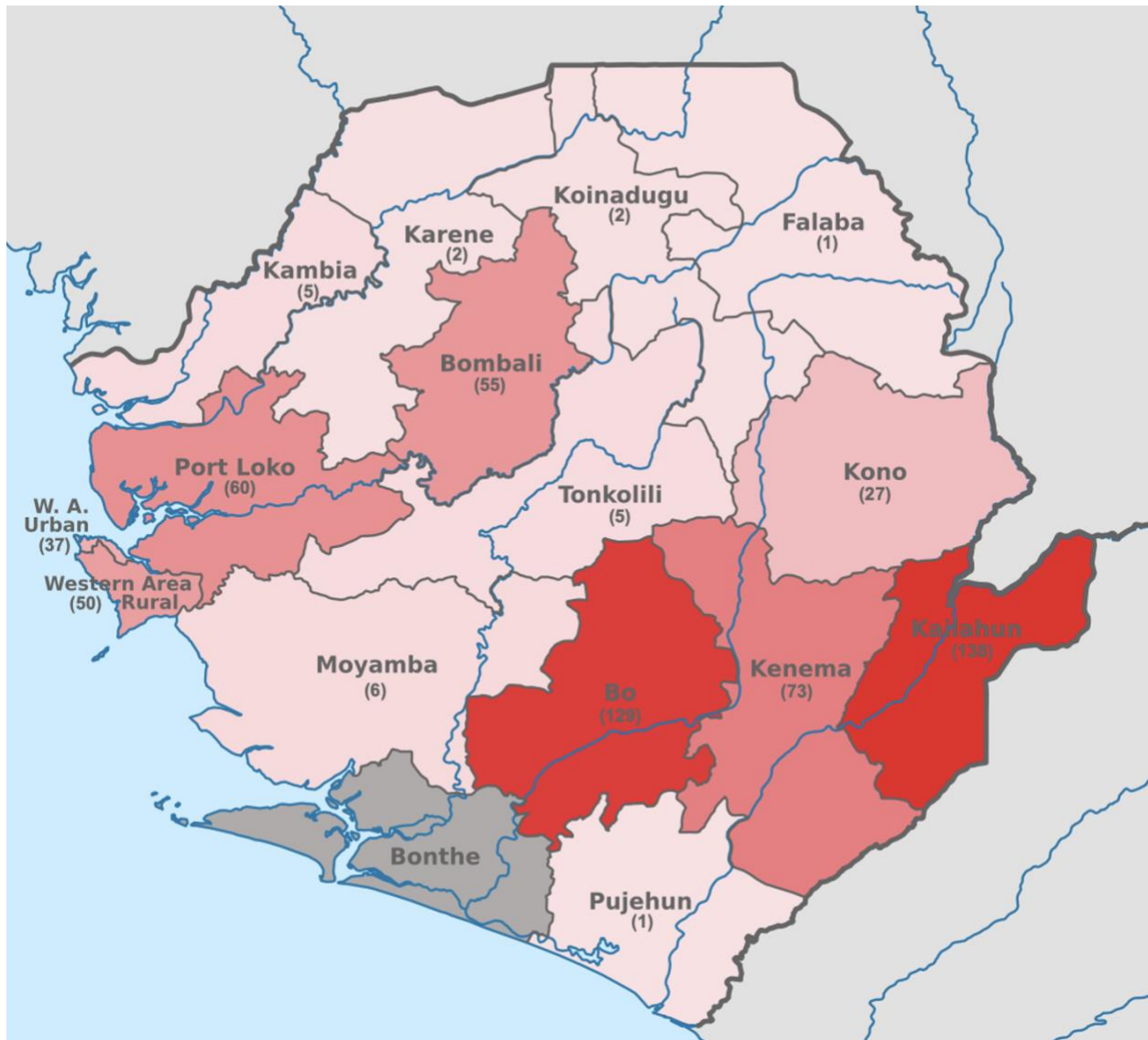


Figure 3.2 Survivor cohort distribution by district in Sierra Leone. Parentheses indicate enrolled survivors in district. Heat mapping tied to proportion of survivor cohort by district (Bright red=highest proportion; light red=lowest proportion). Grayed out=No recorded survivors during epidemic in Bonthe district.

Sociodemographic characteristics of participants described in table 1, demonstrated 52.9% ($n=315$) of EVD survivors were female while 37.2% of close contacts were female ($n=150$, $p< 0.001$). The average age of participants was not statistically different ($p=0.327$) between close contacts (mean = 32.6 years, SD = 8.2 years) and EVD survivors (mean = 32.1 years, SD = 8.9 years). Most survivors reported they were unemployed at the time of the study ($n=494$, 84.0%) which was significantly higher ($p<0.001$) than close contacts surveyed ($n=156$, 38.8%). There was no significant difference ($p=0.433$) observed for education between EVD survivors ($n=228$, 38.9%) and close contacts ($n=166$, 41.4%). There was no significant ($p=0.221$) difference in ethnicity between close contacts and EVD survivors observed, while the most common ethnicity was Mende, (close contacts $n=201$, 49.9%; EVD survivors $n=306$, 52.2%) respectively. The most frequently reported religion among close contacts was Christianity ($n=204$, 53.0%) while EVD survivors it was Islam ($n=323$, 58.3%) marking a significant difference ($p=0.033$) between the two groups. Most EVD survivors ($n=252$, 46.6%) and close contacts ($n=175$, 52.4%) reported being single at the time of survey, followed by marriage (close contacts $n=123$, 36.8%; EVD survivors $n=188$, 34.8%), while significantly ($p=0.007$) more EVD survivors ($n=166$, 41.4%) reported being in a romantic relationship than close contacts ($n=166$, 41.4%). Sociodemographic differences of EVD survivor participants and close contacts highlight differences in current life experiences and the potential role of EVD survival contributing to these differences.

Table 3.1: Participant demographics based on Ebola virus survivor status

	Close Contact		EVD Survivor		p-value
Biological Sex					<0.001
Male	253	62.8%	280	47.1%	
Female	150	37.2%	315	52.9%	
Age Group					0.469
<20	25	6.2%	51	8.6%	
20-24	43	10.7%	81	13.6%	
25-29	73	18.1%	108	18.2%	
30-34	97	24.1%	119	20.0%	
35-39	82	20.3%	86	14.5%	
40-44	39	9.7%	76	12.8%	
45+	44	10.9%	74	12.4%	
Education					0.433
No	235	58.6%	358	61.1%	
Yes	166	41.4%	228	38.9%	
Employment					<0.001
No	156	38.8%	494	84.0%	
Yes	246	61.2%	94	16.0%	
Ethnicity					0.221
Creole	3	0.7%	2	0.3%	
Fullah	19	4.7%	9	1.5%	
Kono	25	6.2%	25	4.3%	
Limba	24	6.0%	71	12.1%	
Loko	16	4.0%	39	6.7%	
Mandingo	31	7.7%	18	3.1%	
Mende	201	49.9%	306	52.2%	
Sherbo	18	4.5%	7	1.2%	
Temne	66	16.4%	88	15.0%	
Kissi	0	0.0%	20	3.4%	
Kerio	0	0.0%	1	0.2%	
Provinces					<0.001
Northern	13	3.2%	63	10.7%	
Eastern	153	38.2%	238	40.3%	
Southern	80	20.0%	136	23.0%	
Western	92	22.9%	87	14.7%	
Northwest	63	15.7%	67	11.3%	
Religion					0.033
Christian	204	53.0%	225	40.6%	
Muslim	169	43.9%	323	58.3%	
Traditional	11	2.9%	0	0.0%	
Kissi	0	0.0%	4	0.7%	
None	1	0.3%	2	0.4%	
Relationship status					0.007
Single	175	52.4%	252	46.6%	
Married	123	36.8%	188	34.8%	
Relationship	36	10.8%	101	18.7%	

N=998

3.2 Acute EVD

There were 40% ($n=208$) of survivors who reported their acute EVD infection lasted for over 21 days, with the average length of acute EVD of 19 days (IQR=9-24) [table 3.2]. While shorter acute illness was less commonly reported with 22.7% ($n=118$) of survivors who reported illness less than 7 days. Most EVD survivors (65.2%, $n=388$) reported being treated in an ETC followed by treatment in a hospital (30.3%, $n=180$) [table 3.3]. The vast majority of EVD survivors in this cohort reported acute infections with between 1 and 5 symptoms (54.5%, $n=324$) and 6-10 symptoms (39.3%, $n=234$) [Table 2].

Table 3.2: Clinical characteristic associations with EVD survivor stigma*

Duration of Acute EVD		
<7 Days	118	22.7%
7-14 Days	67	12.9%
15-21 Days	127	24.4%
>21 Days	208	40.0%
Acute EVD Symptoms		
No Symptoms	4	0.7%
1-5 Symptoms	324	54.5%
6-10 Symptoms	234	39.3%
11 to 14 Symptoms	33	5.5%

*N=595

**Table 3.3: EVD treatment location
reported by EVD survivors***

Ebola Treatment Center			
No	207	34.8%	
Yes	388	65.2%	
Hospital			
No	415	69.7%	
Yes	180	30.3%	
Home			
No	580	97.5%	
Yes	15	2.5%	
Traditional healer			
No	577	97.0%	
Yes	18	3.0%	

*N=595

Table 3.4 describes the reported acute EVD symptomology by EVD survivor participants in the study cohort. The most commonly reported acute EVD symptoms were, headache (79.8%, $n=475$), fever (76.1%, $n=453$), lost appetite (44.7%, $n=266$), diarrhea (44.2%, $n=263$), nausea/vomiting (40.3%, $n=240$), and joint pain (36.8%, $n=219$). Symptoms associated with more severe disease, bleeding/hemorrhage (8.6%, $n=51$), breathing issues (11.9%, $n=71$) and shortness of breath (12.6%, $n=75$) were much less commonly reported from this cohort and in line with what is known about severity of disease determining the outcome of illness⁷.

**Table 3.4: Acute EVD symptomology
reported by EVD survivors***

Headache	475	79.8%
Fever	453	76.1%
Sore Throat	183	30.8%
Hiccups	109	18.3%
Lost Appetite	266	44.7%
Abdominal Pain	173	29.1%
Nausea/Vomiting	240	40.3%
Diarrhea	263	44.2%
Bleeding/Hemorrhage	51	8.6%
Muscle Pain	127	21.3%
Joint Pain	219	36.8%
Shortness of Breath	75	12.6%
Breathing Issues	71	11.9%
Red Eyes	190	31.9%

*N=595

Chapter 4: Long term somatic symptomology among EVD survivors in Sierra Leone.

The results presented in this chapter is from a second revision of the manuscript text in preparation for submission to the Journal *Clinical Infectious Diseases*.

The abstract and a brief introduction and discussion respectively are included in this manuscript, otherwise the introduction, methods, general discussion, and conclusions have been added to the corresponding chapters of this thesis.

4.0 Somatic symptoms experienced by Ebola virus disease survivors eight years post recovery: a cross-sectional study in Sierra Leone.

Brayden G. Schindell¹, Boghuma K. Titanji², Anne Rimoin³, Souradet Y. Shaw⁴, Jia B. Kangbai⁵, Jason Kindrachuk¹

¹Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

²Division of Infectious Disease, Emory University, Atlanta, United States of America

³Center for Global and Immigrant Health, Fielding School of Public Health, University of California Los Angeles

⁴Department of Community Health Sciences, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

⁵Department of Public Health, Njala University, Sierra Leone

4.1 Abstract

We studied 490 Ebola survivors and close contacts in Sierra Leone (2021–2022) to assess long-term health effects. Most symptoms were no longer elevated compared to controls. However, frequent urination, eye discomfort, and ear pain persisted, highlighting the need for targeted clinical care for Ebola virus disease survivors.

4.2 Introduction

Post-EVD health complications, often described as post-Ebola syndrome are characterized by chronic clinical symptoms that may reflect ongoing immune dysfunction¹⁹⁹. In the

immediate aftermath of the epidemic, studies identified a wide-range of commonly reported symptoms including fatigue, joint pain, muscle pain, abdominal pain, chest pain, ocular complications, memory loss, hearing loss, and frequent urination^{176-178,181-192}. These findings underscored the substantial morbidity faced by survivors in the months and years following recovery. However, the duration and persistence of these symptoms remain poorly understood. There is discordance in the reported duration of sequelae in the literature, with some studies reporting that symptoms wane over time^{177,185} while others indicate longer term persistence of symptoms²⁷⁴. To address this gap in the literature, we conducted a national cross-sectional survey of EVD survivors in Sierra Leone six to eight years after the West-African epidemic. We aimed to assess the prevalence of self-reported somatic symptoms among survivors compared to controls, providing new insight into the chronic health burden associated with EVD survival.

4.3 Results

4.3.1 Participant Characteristics

The total recruitment for the study was 998 participants (403 close contacts and 595 EVD survivors), here we controlled for any symptoms of acute illness. All participants who reported signs of acute illness (fever, congestion, hoarseness, sputum, vomiting and/or diarrhea) screened out resulting in a study population of 490 (255 close contacts and 235 EVD survivors). A total of 490 participants were included: 255 close contacts and 235 EVD survivors (Table 4.1). The sample was 58.8% male (n=288) and 41.2% female (n=202) participants. The mean age was similar between groups (survivors: 30.6 years, SD = 9.3; contacts: 32.1 years, SD = 8.2; $p = 0.059$). However, body mass index (BMI) differed

significantly ($p < 0.001$), with survivors having a lower mean BMI (18.3, SD = 5.6) compared to contacts (21.3, SD = 11.8).

Table 4.1: Demographic characteristics of participants by EVD survival status

	Close Contact (N=255)		EVD Survivor (N=235)		Total (N=490)	
Biological Sex						
Male	167	65.5%	121	51.5%	288	58.8%
Female	88	34.5%	114	48.5%	202	41.2%
Age Group						
<20	17	6.7%	29	12.3%	46	9.4%
20-24	31	12.2%	45	19.1%	76	15.5%
25-29	47	18.4%	38	16.2%	85	17.3%
30-34	66	25.9%	46	19.6%	112	22.9%
35-39	46	18.0%	23	9.8%	69	14.1%
40-44	21	8.2%	26	11.1%	47	9.6%
45+	27	10.6%	28	11.9%	55	11.2%
BMI Classification						
Underweight	179	70.2%	168	71.5%	347	70.8%
Normal Weight	48	18.8%	51	21.7%	99	20.2%
Overweight	5	2.0%	11	4.7%	16	3.3%
Obese - Class I	0	0.0%	3	1.3%	3	0.6%
Obese - Class II	1	0.4%	0	0.0%	1	0.2%
Obese - Class III	22	8.6%	2	0.9%	24	4.9%
Provinces						
Northern	7	2.7%	38	16.2%	45	9.2%
Eastern	97	38.0%	106	45.1%	203	41.4%
Southern	45	17.6%	26	11.1%	71	14.5%
Western	68	26.7%	40	17.0%	108	22.0%
Northwest	38	14.9%	25	10.6%	63	12.9%

N=490

4.3.2 Persistent somatic symptomology reported 6-8 years post recovery

Analyzing the frequency of frequently previously reported symptomologies experienced by EVD survivors (table 4.2) found limited significant differences in prevalence compared to close contact participants. Significant differences were found in the genitourinary tract, eyes, and ears. Specifically for the genitourinary tract frequent urination (21.3%, $n=50$) was significantly ($p=0.001$) more reported compared to close contacts (10.2%, $n=26$). For eye/vision symptomology, we found EVD survivors (10.2%, $n=24$) reported eye pain significantly ($p<0.001$) more than close contacts (0.4%, $n=1$), and the same was observed for itchy eyes (12.3%, $n=29$, $p<0.001$) in comparison to close contacts (2.4%, $n=6$). For ear/hearing symptomology reduced hearing (3.8%, $n=9$) was significantly ($p=0.002$) more frequent when compared to close contacts (0.0%, $n=0$), with the same observed for ringing in ears (2.1%, $n=5$, $p=0.019$) compared to close contacts (0.0%, $n=0$) and ear pain (5.5%, $n=13$, $p=0.001$) compared to close contacts (0.4%, $n=1$).

Table 4.2: Frequency of commonly reported somatic symptomology by EVD survival status

	Close contact (N=255)		EVD survivor (N=235)		p-value
Frequent urine					0.001
No	229	89.8%	185	78.7%	
Yes	26	10.2%	50	21.3%	
Fatigue					0.117
No	230	90.2%	221	94.0%	
Yes	25	9.8%	14	6.0%	
Joint pain					0.344
No	239	93.7%	215	91.5%	
Yes	16	6.3%	20	8.5%	
Muscle pain					0.119
No	248	97.3%	233	99.1%	
Yes	7	2.7%	2	0.9%	
Abdominal pain					0.486
No	249	97.6%	227	96.6%	
Yes	6	2.4%	8	3.4%	
Eye pain					<0.001
No	254	99.6%	211	89.8%	
Yes	1	0.4%	24	10.2%	
Light sensitivity					0.150
No	254	99.6%	231	98.3%	
Yes	1	0.4%	4	1.7%	
Itchy eyes					<0.001
No	249	97.6%	206	87.7%	
Yes	6	2.4%	29	12.3%	
Memory loss					0.071
No	255	100.0%	232	98.7%	
Yes	0	0.0%	3	1.3%	
Chest pain					0.344
No	249	97.6%	226	96.2%	
Yes	6	2.4%	9	3.8%	
Reduced hearing					0.002
No	255	100.0%	226	96.2%	
Yes	0	0.0%	9	3.8%	
Ringing in ears					0.019
No	255	100.0%	230	97.9%	
Yes	0	0.0%	5	2.1%	
Ear pain					0.001
No	254	99.6%	222	94.5%	
Yes	1	0.4%	13	5.5%	

The most prevalent symptomology experienced by EVD survivors in the immediate aftermath of the West African EVD epidemic were assessed for their continued excess prevalence among EVD survivors in comparison to close contacts. From these symptomologies Firth logistic regression models identified four somatic symptoms that continued to be significantly associated with EVD survival status (Table 4.3): frequent urination (AOR 2.73, 95% CI 1.59–4.67; $q < 0.001$), eye pain (AOR 26.9, 95% CI 4.73–153.06; $q < 0.001$), itchy eyes (AOR 7.56, 95% CI 2.98–19.18; $q < 0.001$), and ear pain (AOR 11.8, 95% CI 2.06–67.57; $q = 0.025$). Other symptoms previously reported among survivors, including fatigue, joint pain, muscle pain, and memory loss, were not significantly elevated in this cohort at the time of the survey. Only a single somatic symptom had a significant AOR with biological sex, specifically itchy eyes (AOR 0.27, 95% CI 0.10–0.74; $q = 0.035$) [table 4.4]. Upon further analysis this was due to more male EVD survivors (13.2%, $n=16$) reporting itchy eyes than female EVD survivors (11.4%, $n=13$). The limited nature of biological sex associations indicate it has not played a significant role in the somatic symptomology experienced by EVD survivors.

Table 4.3: Associations with EVD survival by symptom – Crude and Adjusted Odds Ratios (AORs) and 95% Confidence Intervals (95% CI), Firth Logistic Regression Models

	AOR	95% CI	q-value
Frequent urination	2.73***	[1.59,4.67]	<0.001 [#]
Fatigue	0.60	[0.30,1.22]	0.384
Joint pain	1.68	[0.79,3.59]	0.334
Muscle pain	0.49	[0.11,2.19]	0.538
Abdominal Pain	1.51	[0.50,4.56]	0.613
Eye pain	26.9***	[4.73,153.06]	<0.001 [#]
Light sensitivity	4.16	[0.60,28.71]	0.310
Itchy eyes	7.56***	[2.98,19.18]	<0.001 [#]
Memory loss	9.59	[0.43,213.80]	0.327
Chest Pain	1.46	[0.50,4.29]	0.643
Reduced hearing	19.0*	[2.73,22.22]	0.156
 ringing in ears	16.5	[0.84,324.69]	0.197
Ear pain	11.8**	[2.06,67.57]	0.025 [#]
Observations	490		

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Adjusted for sex, age, BMI, province of residence.

[#] Indicates significant q value

Table 4.4: Associations with sex of EVD survivors by symptom – Crude and Adjusted Odds Ratios (AORs) and 95% Confidence Intervals (95% CI), Firth Logistic Regression Models

	AOR	95% CI	q-value
Frequent urination	1.07	[0.52,2.18]	0.937
Fatigue	2.24	[0.62,8.06]	0.314
Joint pain	1.38	[0.47,4.03]	0.744
Muscle pain	0.07	[0.00,2.01]	0.227
Abdominal Pain	0.62	[0.14,2.84]	0.716
Eye pain	1.69	[0.65,4.41]	0.407
Light sensitivity	0.07	[0.00,1.50]	0.226
Itchy eyes	0.27*	[0.10,0.74]	0.035 [#]
Memory loss	0.13	[0.01,2.89]	0.310
Chest Pain	1.24	[0.31,5.03]	0.871
Reduced hearing	0.75	[0.19,3.03]	0.847
Ringing in ears	1.86	[0.26,13.46]	0.719
Ear pain	1.97	[0.53,7.30]	0.412
Observations	235		

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Adjusted for sex, age, BMI, province of residence.

Indicates significant q value

4.4 Discussion

It has previously been reported that the persistence of EVD-related sequelae diminishes over time^{177,178,274}, our findings align with this pattern as we observed that most symptoms commonly reported in earlier stages of recovery were no longer significantly reported among survivors six to eight years after recovery. However, symptoms related to vision (eye pain and itchy eyes), hearing (ear pain) and genitourinary (frequent urination) remained significantly associated with EVD survival. These findings are consistent with prior report of long-term EVD complications^{183,274} and suggest that while many post-infectious symptoms may resolve over time, certain organ-specific impairments persist well beyond the acute phase. The persistence of these symptoms may reflect their chronic nature leading to continued interference in the daily lives of EVD survivors. Vision and hearing disturbances, as well as urinary issues, can significantly impair functioning and quality of life, potentially making them more memorable or more likely to be reported. Alternatively, these symptoms may be indicative of lasting inflammation, tissue damage, or unresolved immune dysregulation following EVD, as suggested by prior studies of post-EVD immunologic dysfunction^{177,178,181,185,199}. Only one other study has reported outcomes beyond five years post-infection. In a longitudinal cohort in Liberia Wohl et al.¹⁷⁷, found a reduction in the adjusted odds ratios for many symptoms over time when comparing EVD survivors to a control population 5 years after discharge from an ETU. Notably, several symptoms frequently reported in earlier studies such as fatigue, joint pain, muscle pain, abdominal pain and memory loss^{176-178,181-192} were not significantly elevated among survivors in our cohort. This may reflect actual symptom resolution over time, underreporting, or recall decay, as the assessment relied on symptoms experienced within two weeks of the survey date.

Chapter 5: Long term reproductive health effects experienced by EVD survivors in Sierra Leone.

The results presented in this chapter is from a first revision of the manuscript text in preparation for submission to *Clinical Infectious Diseases*.

The abstract and a brief introduction and discussion respectively are included in this manuscript, otherwise the introduction, methods, general discussion, and conclusions have been added to the corresponding chapters of this thesis.

5.0 Reproductive and sexual health of Ebola virus disease survivors eight years post recovery: a cross-sectional study in Sierra Leone.

Brayden G. Schindell¹, Boghuma K. Titanji², Souradet Y. Shaw³, Jia B. Kangbai⁴, Jason Kindrachuk¹

¹Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

²Division of Infectious Disease, Emory University, Atlanta, United States of America

³Department of Community Health Sciences, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

⁴Department of Public Health, Njala University, Sierra Leone

5.1 Abstract

We studied 875 participants and close contacts in Sierra Leone to assess the long term reproductive and sexual health effects of EVD survival six to eight years after recovery. This was conducted through a cross-sectional study conducted between November 2021 and March 2022. Female EVD survivors reported significantly different menstrual patterns compared to close contacts with association of EVD survival to irregular periods (AOR: 3.07, 95% CI: 1.75 – 5.36, $p \leq 0.001$). Significantly more female EVD survivors ($n=56$, 19.7%, $p \leq 0.001$) expressed decreased interest in sexual intercourse. While minimal associations with EVD survival existed for male EVD survivors. These results demonstrate there are reproductive and sexual health differences observed in EVD survivors and warrants further investigation into female reproductive health.

5.2 Introduction

Among the post-EVD complications that have been observed is the long-term persistence of Ebola virus in reproductive tracts and have led to reports of sexual transmission, and in some cases renewed outbreaks²²⁰. While, more is known about the persistence mechanisms and the chains of transmission that occur in the immediate aftermath of the sexual transmission events, little has been studied on the long term effects on reproductive health of EVD survivors regardless of if they were involved in documented sexual transmission or not^{220,295}. Furthermore, little is understood about the effects and mechanisms affecting female reproductive health with very limited reports indicating possible effects could include menstrual effects and reduced sexual desires^{239,242}. To address these gaps in the literature, we conducted a national cross-sectional survey of EVD survivors in Sierra Leone six to eight years after the West-African epidemic. With the aim of assessing self-reported reproductive health symptoms and screening for sexual dysfunction, providing new insights into the reproductive and sexual health of EVD survivors.

5.3 Results

Of the 875 participants used in this reproductive and sexual health study with 46.3% female participants ($n=405$) and 53.7% male participants ($n=470$). Likewise, 61.8% ($n=541$) participants were EVD survivors compared to close contacts (38.2%, $n=334$).

5.3.1 Female reproductive and sexual health

Menstruation reported by EVD survivors over the year preceding the survey found significantly ($p \leq 0.001$) different results between the EVD survivor participants and the close contacts (table 5.1). Where 67.2% of close contacts ($n=86$) reported regular menstruation 42.6% of EVD survivors ($n=120$) reported regular menstruation. Irregular menstruation, the inconsistent and sporadic occurrence of menstruation was most commonly reported by EVD survivors (49.6%, $n=140$) in the year prior to the survey while 26.6% of close contacts ($n=34$) reported irregular menstruation.

Table 5.1: Menstruation of EVD survivors compared to close contacts

	Close Contact		Ebola Survivor		Total		p-value
Menstruation							≤ 0.001
Regular Periods	86	67.2%	120	42.6%	206	50.3%	
Irregular Periods	34	26.6%	140	49.6%	174	42.4%	
No Periods	8	6.2%	22	7.8%	30	7.3%	

Amongst female participants, significantly more EVD survivors ($n=56$, 19.7%, $p\leq 0.001$) expressed decreased interest in sexual intercourse (table 5.2) than close contacts ($n=6$, 5.0%). While no significant ($p=0.390$) difference was observed in the frequency of EVD survivors (51.8%, $n=29$) and close contacts (33.3%, $n=2$) who met the criteria for sexual dysfunction. Logistic regression analysis was performed for female reproductive health (table 5.3). EVD survival among female participants was significantly associated with several reproductive health variables, including sexual intercourse (AOR: 5.53, 95% CI: 3.04 – 10.07), decreased interest in sexual intercourse (AOR: 3.64, 95% CI: 1.34 – 9.86), irregular periods (AOR: 3.07, 95% CI: 1.75 – 5.36) and no recent pregnancies (AOR: 3.53, 95% CI: 1.69 – 7.34).

Table 5.2: Female Sexual Dysfunction in study population by EVD survival status

	Close Contacts		EVD Survivors		Total		p-value
Decreased Interest in Sexual Intercourse							
No	115	95.0%	228	80.3%	343	84.7%	≤0.001
Yes	6	5.0%	56	19.7%	62	15.3%	
Female Sexual Function Index							0.390
Female Sexual Dysfunction	2	33.3%	29	51.8%	31	50.0%	
No Female Sexual Dysfunction	4	66.7%	27	48.2%	31	50.0%	

Table 5.3: Associations Female Reproductive and Sexual Health Associations with EVD survival – Crude and Adjusted Odds Ratios (AORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

AOR	
Sexual Intercourse in the Last Year	
No	5.53*** [3.04,10.07]
Yes	1 [1.00,1.00]
Decreased Interest in Sexual Intercourse	
No	1 [1.00,1.00]
Yes	3.64* [1.34,9.86]
Menstruation in the Last Year	
Regular Period	1 [1.00,1.00]
Irregular Period	3.07*** [1.75,5.36]
No Period	1.56 [0.57,4.27]
Pregnant in the Last Year	
No	3.53*** [1.69,7.34]
Yes	1 [1.00,1.00]
Given Birth	
No	1 [1.00,1.00]
Yes	1.28 [0.74,2.20]
Observations	384

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Adjusted for sex, age, BMI, province of residence.

5.3.2 Male reproductive and sexual health

For male participants there was no significant increase of EVD survivors ($n=24$, 9.3%, $p=0.086$) reporting more decreased desire for sexual intercourse compared to close contacts ($n=11$, 5.2%) [table 5.4]. Likewise, there was no significant ($p=0.478$) difference observed between the frequency of male EVD survivors (41.7%, $n=10$) and close contacts (54.5%, $n=6$) meeting criteria for sexual dysfunction. For male participants the only significant association observed for EVD survivorship was reporting no conceived pregnancies with their partner in the last year (AOR: 2.73, 95% CI: 1.29-5.81) [table 5.5].

Table 5.4: Male Sexual Dysfunction in study population by EVD survival status

	Close Contacts		EVD Survivors		Total		p-value
Decreased Interest in Sexual Intercourse							0.086
No	202	94.8%	233	90.7%	435	92.6%	
Yes	11	5.2%	24	9.3%	35	7.4%	
Male Sexual Function Index							0.478
Male Sexual Dysfunction	6	54.5%	10	41.7%	16	45.7%	
No male Sexual Dysfunction	5	45.5%	14	58.3%	19	54.3%	

Table 5.5: Associations Male Reproductive and Sexual Health Associations with EVD survival – Crude and Adjusted Odds Ratios (AORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	AOR	
Sexual Intercourse in the Last Year		
No	1.46	[0.93,2.29]
Yes	1	[1.00,1.00]
Decreased Interest in Sexual Intercourse		
No	1	[1.00,1.00]
Yes	1.75	[0.75,4.10]
Conceived child with partner in the Last Year		
No	2.73**	[1.29,5.81]
Yes	1	[1.00,1.00]
Fathered a child		
No	1	[1.00,1.00]
Yes	1.22	[0.74,2.01]
Observations	463	

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Adjusted for sex, age, BMI, province of residence.

5.4 Discussion

In this study, we present the most prevalent clinical symptoms experienced by EVD survivors compared to their close contacts, 6-8 years after the West Africa EVD outbreak. This study was conducted between 1.6 and 3.3 years after discharge from ETCs suggesting that symptomology rates have remained elevated and stable over the subsequent 3-5 years from the initial recovery period to the 6–8-year follow-up time point reported in our study. Similar findings to those presented here have previously been observed in West Africa regarding reproductive health of female and male EVD survivors. A study conducted from January to April 2016 in Montserrado county, Liberia found that 16.2% of male EVD survivors surveyed reported testicular pain or erectile dysfunction, while 19.7% of female EVD survivors reported having menstrual problems including amenorrhoea and other menstrual irregularities¹⁹¹. Another study conducted in February 2015 in Bombali District, Sierra Leone found that 2.2% of female EVD survivors reported a menstrual disorder and 3.4% of male EVD survivors reported erectile dysfunction²⁴¹. A study conducted in Liberia between 2016 and 2017 including 111 female participants from Monrovia, reported 25.7% of female participants had irregular menstruation of unknown cause²⁴². The same group reported in a qualitative study including 69 women, that 50% of female survivors from Liberia had reported decreased sexual libido following recovery with several women also reporting pain during intercourse, and 46% of participants reporting some of menstrual irregularity²³⁹. We made similar observations in our study (tables 5.1, 5.2 and 5.3) with female survivors also reporting menstrual changes and changes in sexual libido. Reproductive health changes experienced by EVD survivors remain understudied.

Chapter 6: Stigmatization of EVD survivors in Sierra Leone 6-8 years after recovery

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The abstract and a brief introduction and discussion respectively are included in this manuscript, otherwise the introduction, methods, general discussion, and conclusions have been added to the corresponding chapters of this thesis.

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University for Health Sciences

6.0 Stigmatization of Ebola virus disease survivors in 2022, a cross-sectional study of Survivors in Sierra Leone

Brayden G. Schindell¹, Jia B. Kangbai², Souradet Y. Shaw^{1,3}, Jason Kindrachuk¹

¹Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

² Department of Public Health, Eastern Technical University of Sierra Leone, Kenema, Sierra Leone

³Department of Community Health Sciences, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

6.1 Abstract

Evidence has demonstrated a high proportion of EVD survivors experienced stigma related to EVD. This study sought to understand the longer-term effects of stigma encountered by survivors of the 2014-2016 EVD epidemic living in Sierra Leone. This was a cross-sectional study of 595 EVD survivors and 403 close contacts ($n=998$) from Sierra Leone. Assessments were conducted using a three-part survey between November 2021 to March 2022. This study sought to explore the socio-demographic factors associated with stigma experienced by EVD survivors. 50,6% ($n=301$) of EVD survivors reported that they continued to experience at least one aspect of stigma the index. Females were disproportionately affected by stigma, with 45.2% of females reporting isolation from friends and family compared to 33.9% of men ($p=0.005$). Multivariable logistic regression models revealed those aged 40-44, living rurally, and reporting an acute infection longer than seven days was associated

with stigma related to EVD at the time of survey. This study demonstrates stigma is still prevalent among people who survived EVD in 2022. It also identified socio-demographic factors associated with stigma that can be used for targeting interventions. Importantly, this highlights the continued need for EVD survivors to access mental healthcare and social support systems well after disease recovery.

6.2 Introduction

Stigmatization has been reported by EVD survivors in all regions that have experienced high burdens of EVD infections, including DRC, Guinea, Liberia, and Sierra Leone.^{192,258,259} Stigmatization is a social construction that leads to feelings and experiences of blame, shame, worthlessness, loneliness, isolation, and discrimination.²⁹⁶ Research into stigma emphasizes psychological and social aspects of stigma, where the experiences of those stigmatized can be broken down into two broad categories: “enacted stigmatization”, which encompasses social manifestations including prejudice and discrimination that are directed towards individuals with the stigmatizing attributes; and “internalized stigma”, which is centred around internal perceptions of others, and results in feelings of guilt, shame, and/or worthlessness.²⁴⁴ Stigma in the context of infectious diseases often stems from a fear response towards an infection, which has been observed in relation to EVD survivors.²⁷⁶ Community fears around EVD in the aftermath of the West African epidemic resulted in lasting emotional, physical and social impacts on EVD survivors.²⁶⁰ Survivor stigma has also been observed to correspond with news of survivor associated re-emergence events due to fear of, for example, sexual transmission of EVD.²⁶³

To date, there have been limited studies comprehensively examining EVD-related stigma, making it difficult quantify the extent of these experiences among survivor communities.^{262,263} Moreover, there is limited knowledge of the sociodemographic factors related with psychosocial sequelae.^{264,269} These potential associations are important to determine when considering the design of EVD survivor interventions and other advocacy initiatives. Most studies examining stigma have been conducted in the immediate aftermath of EVD outbreaks.^{192,258,263,276,277} To the best of our knowledge, only a single study of EVD survivor health has been conducted beyond two years following disease recovery.¹⁷⁷ This study had limited follow-up time, and only characterized clinical sequelae. Using results from a cross-sectional study of EVD survivors in Sierra Leone, the objective of the analyses in this study was to investigate stigma associated with EVD survivors in Sierra Leone six to eight years after discharge from Ebola treatment units.

6.3 Results

6.3.1 Stigma of EVD survivors

The prevalence of stigma among EVD survivors was 50.6% ($n=301$) where survivors reported experiencing at least one item of the EVD related stigma index (Table 6.1) with an average of 2.5 (IQR=0-5) items reported per participant. There were several demographic differences when survivors were stratified by stigma in table 6.2. A greater proportion of female survivors reported stigma (59.2%, $n=189$) than males 40.8% ($n=130$). Participants who reported stigma were older (33.9 years, IQR=28-40), than those who reported no stigma (30.1 years, IQR=22-36; Table 6.3).

**Table 6.1: Total stigma
experienced by EVD survivor
participants**

	Total Stigma	
1 or more	301	50·6%
2 or more	274	46·1%
3 or more	242	40·7%
4 or more	218	36·6%
5 or more	182	30·6%
6 or more	118	19·8%
7 or more	59	9·9%
8	37	6·2%

Table 6.2: Demographics of EVD survivors by stigma experience

	No		Stigma Yes		Total		p-value
Biological Sex							0.001
Male	150	54.3%	130	40.8%	280	47.1%	
Female	126	45.7%	189	59.2%	315	52.9%	
Age Group							<0.001
<20	34	12.3%	17	5.3%	51	8.6%	
20-24	55	19.9%	26	8.2%	81	13.6%	
25-29	48	17.4%	60	18.8%	108	18.2%	
30-34	57	20.7%	62	19.4%	119	20.0%	
35-39	30	10.9%	56	17.6%	86	14.5%	
40-44	23	8.3%	53	16.6%	76	12.8%	
45+	29	10.5%	45	14.1%	74	12.4%	
Education							0.003
No	149	54.6%	209	66.8%	358	61.1%	
Yes	124	45.4%	104	33.2%	228	38.9%	
Provinces							<0.001
Northern	44	16.1%	19	6.0%	63	10.7%	
Eastern	108	39.4%	130	41.0%	238	40.3%	
Southern	39	14.2%	97	30.6%	136	23.0%	
Western	69	25.2%	18	5.7%	87	14.7%	
Northwest	14	5.1%	53	16.7%	67	11.3%	
Duration of Acute EVD							<0.001
<7 Days	105	43.9%	13	4.6%	118	22.7%	
7-14 Days	40	16.7%	27	9.6%	67	12.9%	
15-21 Days	42	17.6%	85	30.2%	127	24.4%	
>21 Days	52	21.8%	156	55.5%	208	40.0%	
Ebola Treatment Center							<0.001
No	162	58.7%	45	14.1%	207	34.8%	
Yes	114	41.3%	274	85.9%	388	65.2%	
Hospital							<0.001
No	130	47.1%	285	89.3%	415	69.7%	
Yes	146	52.9%	34	10.7%	180	30.3%	
Home							<0.001
No	276	100.0%	304	95.3%	580	97.5%	
Yes	0	0.0%	15	4.7%	15	2.5%	
Traditional healer							<0.001
No	276	100.0%	301	94.4%	577	97.0%	
Yes	0	0.0%	18	5.6%	18	3.0%	

Table 6.3: Continuous demographic characteristic associations with EVD survivor stigma

	Stigma					
	No		Yes		Total	
	Mean	IQR	Mean	IQR	Mean	IQR
Age	30.1	22 - 36	33.9	28 - 40	32.1	25 - 40
Ebola duration	14.4	4 - 21	22.8	21 - 27	19.0	9 - 24

A lower proportion of survivors with stigma reported formal education (33.2%, $n=104$), compared to survivors with no stigma (45.4%, $n=124$, $p=0.003$) [table 6.1]. Survivors with stigma reported were more likely to reside in the Eastern, Southern, and Northwestern Provinces. The length of acute EVD for survivors reporting stigma (mean=22.8 days, IQR=21-27) was longer than those reporting no stigma (mean=14.4 days, IQR=4-21, $p\leq 0.001$). Similarly, where survivors were treated for EVD also has an association with stigma. EVD Survivors that reported stigma were more likely to have been treated at an ETC (85.9%, $n=274$) when compared to those who reported no stigma ($p\leq 0.001$). Survivors reporting stigma were similarly, less likely to be treated in hospital (10.7%, $n=34$) when compared to those reporting no stigma ($p\leq 0.001$).

The distribution of items comprising the stigma index is reported in table 6.4. Briefly, the most commonly reported items were: *Isolation from family and friends* (39.9%, $n=236$), *Lost partner/spouse due to fear of infection* (39.5%, $n=231$), and *Deprived of social/community gatherings for fear of infection* (39.3%, $n=232$). Multivariable logistic regression models (table 6.5) adjusted for age, sex assigned at birth, education province of residence, duration of acute EVD, and whether participants were treated in ETC or hospital, revealed that ages 40-44 (AOR: 7.32, 95% CI: 2.30-23.3), compared to 20-24 age group, living in the Eastern (AOR: 18.3, 95% CI: 7.90-42.1), Southern (AOR: 21.3, 95% CI: 7.87-57.6), or North Western (AOR: 51.1, 95% CI: 13.6-192.4) provinces compared to the Western province, and an acute infection of 7-14 days (AOR: 9.78, 95% CI: 2.87-33.3), 15-21 days (AOR: 36.3, 95% CI: 10.4-126.8), or over 21 days (AOR: 34.8, 95% CI: 11.2-107.8) compared to less than 7 days were all associated with stigma related to EVD.

Table 6.4: Reported Stigma from EVD-related stigma assessment by item.

		Stigma					
		No		Yes		Prefer Not To Answer	
Ebola Stigma and Discrimination Question Set							
Have you been prevented from getting more education due to your Ebola diagnosis?	489	83.2%	86	14.6%	13	2.2%	
Have you lost job or income source due to your Ebola infection?	480	82.1%	99	16.9%	6	1.0%	
Have you lost your partner/spouse due to fear of being infected?	345	59.0%	231	39.5%	9	1.5%	
Have you been deprived from attending social/community gatherings for fear of infecting others?	349	59.2%	232	39.3%	9	1.5%	
Have you been forced to change residence due to alienation from family and/or friends?	451	75.9%	134	22.6%	9	1.5%	
Have you withdrawn from school/training or passed on opportunity for school/training due to fear of what others might think?	382	65.4%	189	32.4%	13	2.2%	
Have you isolated yourself from family and/or friends?	351	59.3%	236	39.9%	5	0.8%	
Are you afraid that someone would not want to be sexually intimate with you knowing that you are an Ebola survivor?	360	61.2%	224	38.1%	4	0.7%	

Table 6.5: Demographic associations with stigma for EVD survivors - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Age				
<20	1.06	[0.50,2.23]	0.92	[0.28,2.99]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	2.64**	[1.45,4.82]	1.31	[0.48,3.60]
30-34	2.30**	[1.28,4.15]	2.01	[0.72,5.59]
35-39	3.95***	[2.07,7.52]	2.21	[0.74,6.56]
40-44	4.87***	[2.48,9.58]	7.32***	[2.30,23.32]
45+	3.28***	[1.70,6.35]	2.29	[0.72,7.30]
Sex				
Female	1.73***	[1.25,2.40]	1.42	[0.79,2.54]
Male	1	[1.00,1.00]	1	[1.00,1.00]
Education				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.60**	[0.43,0.84]	1.03	[0.55,1.95]
Province				
Northern	1.66	[0.78,3.50]	0.79	[0.31,1.98]
Eastern	4.61***	[2.59,8.23]	18.3***	[7.90,42.14]
Southern	9.53***	[5.04,18.05]	21.3***	[7.87,57.63]
Western	1	[1.00,1.00]	1	[1.00,1.00]
North West	14.5***	[6.62,31.81]	51.1***	[13.57,192.39]
Acute EVD				
<7 Days	1	[1.00,1.00]	1	[1.00,1.00]
7-14 Days	5.45***	[2.56,11.60]	9.78***	[2.87,33.31]
15-21 Days	16.3***	[8.24,32.42]	36.3***	[10.41,126.81]
>21 Days	24.2***	[12.57,46.70]	34.8***	[11.23,107.81]
Treated in ETC				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	8.65***	[5.82,12.86]	1.55	[0.48,5.04]
Treated in Hospital				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.11***	[0.07,0.16]	0.46	[0.12,1.78]
Observations			513	

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

6.3.2 Sex-stratified analyses

Sex-based differences in stigma among EVD survivors were observed, with a greater proportion of female survivors reporting stigma as outlined in table 6.2 where 59.2% ($n=189$) of females and 40.8% ($n=130$, $p=0.001$) of males reported experiencing any stigma item(s). For example in table 6.6, females were more likely than males to report *isolating from family and friends* (female $n=141$, 45.2%; male $n=95$, 33.9%, $p=0.005$), *have a partner leave them due to their survivor status*, (female $n=145$, 46.9%; male $n=86$, 31.2%, $p\leq 0.001$), and *be excluded from social events or gatherings* (female $n=140$, 45.0%; male $n=92$, 33.0%, $p=0.003$). Statistically significant relationship variables were used to assess stigma in multivariable logistic regression models adjusted for age relationship status, change in marital status, having a relationship since recovery, and reporting more sexual encounters prior to EVD.

Table 6.6: Reported stigma from EVD-related stigma assessment by item and biological sex.

	Male		Sex Female		p-value
Been denied more education due to Ebola infection?					0.292
No	237	85.6%	252	81.0%	
Yes	36	13.0%	50	16.1%	
Prefer not to answer	4	1.4%	9	2.9%	
Lost job or income source due to Ebola infection?					0.082
No	237	85.6%	243	78.9%	
Yes	39	14.1%	60	19.5%	
Prefer not to answer	1	0.4%	5	1.6%	
Lost partner/spouse due to fear of infection?					<0.001
No	186	67.4%	159	51.5%	
Yes	86	31.2%	145	46.9%	
Prefer not to answer	4	1.4%	5	1.6%	
Been deprived of social / community gatherings for fear of infection?					0.003
No	184	65.9%	165	53.1%	
Yes	92	33.0%	140	45.0%	
Prefer not to answer	3	1.1%	6	1.9%	
Been forced to change residence due to alienation from family and/or friends?					0.535
No	216	77.1%	235	74.8%	
Yes	60	21.4%	74	23.6%	
Prefer not to answer	4	1.4%	5	1.6%	
Withdrawn from school / training or passed on opportunity for school/training due to fear of what others may think?					0.001
No	199	72.4%	183	59.2%	
Yes	70	25.5%	119	38.5%	
Prefer not to answer	6	2.2%	7	2.3%	
Isolated from family and/or friends?					0.005
No	180	64.3%	171	54.8%	
Yes	95	33.9%	141	45.2%	
Prefer not to answer	5	1.8%	0	0.0%	
Afraid someone would not want to be sexual intimate knowing survivor status?					0.006
No	186	66.7%	174	56.3%	
Yes	90	32.3%	134	43.4%	
Prefer not to answer	3	1.1%	1	0.3%	

Relationship status was associated with EVD survivor stigma (table 6.7 and 6.8), female (AOR: 9.08, 95% CI: 1.51-54.4) and male (AOR: 8.98, 95% CI: 2.21-36.44) EVD survivors who reported to be married had higher associations with stigma. While female EVD survivors who also reported a change in marital status since recovery (AOR: 12.3, 95% CI: 3.02-50.1) had similar increased associations with stigma.

Table 6.7: Relationship associations with stigma for female survivors - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Age				
<20	1.06	[0.50,2.23]	1.00	[1.00,1.00]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	2.64**	[1.45,4.82]	2.72	[0.44,16.77]
30-34	2.30**	[1.28,4.15]	2.07	[0.38,11.16]
35-39	3.95***	[2.07,7.52]	3.42	[0.59,19.81]
40-44	4.87***	[2.48,9.58]	3.03	[0.38,23.96]
45+	3.28***	[1.70,6.35]	1.05	[0.18,6.20]
Female Relationship Status				
Single	1	[1.00,1.00]	1	[1.00,1.00]
Married	2.85***	[1.59,5.11]	9.08*	[1.51,54.40]
Relationship	1.07	[0.58,1.97]	1.00	[0.17,5.92]
Change in Marital Status				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	4.04**	[1.69,9.68]	12.3***	[3.02,50.08]
Relationship Since Recovery from EVD				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.58*	[0.35,0.97]	0.07***	[0.03,0.20]
More Sexual Intercourse Prior to EVD				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	3.81**	[1.63,8.91]	40.4***	[6.19,264.04]
Observations			173	

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 6.8: Relationship associations with stigma for male survivors - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Age				
<20	1.06	[0.50,2.23]	1.00	[1.00,1.00]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	2.64**	[1.45,4.82]	2.37	[0.53,10.55]
30-34	2.30**	[1.28,4.15]	5.12	[0.80,32.64]
35-39	3.95***	[2.07,7.52]	1.53	[0.42,5.58]
40-44	4.87***	[2.48,9.58]	3.75	[0.97,14.45]
45+	3.28***	[1.70,6.35]	1.00	[1.00,1.00]
Male Relationship Status				
Single	1	[1.00,1.00]	1	[1.00,1.00]
Married	7.58***	[4.09,14.04]	8.98**	[2.21,36.44]
Relationship	2.54*	[1.21,5.36]	1.00	[1.00,1.00]
Change in Marital Status				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	2.78	[0.75,10.26]	6.04	[0.86,42.41]
Relationship Since Recovery from EVD				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1.76	[0.95,3.27]	0.54	[0.20,1.48]
More Sexual Intercourse Prior to EVD				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	4.83**	[1.57,14.89]	2.67	[0.51,14.12]
Observations			107	

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

6.4 Discussion

This investigation provides insight into the longer-term burden of stigma encountered by EVD survivors following infection. We found that six to eight years after infection that stigma was higher (50.6%) than found in reports for EVD survivors in Sierra Leone (32-39%) earlier in the post-recovery period (2014-2017).^{262,277,297} Increases in stigma such as reported here have previously been described, noting that increased levels of stigma coincided with EVD re-emergence as a fear response by the general population within communities towards EVD survivors.²⁶³ In particular this has been the case for reports of sexual transmissions. As there was a recent outbreak in neighbouring Guinea in February 2021, it is possible the news of this and what community members believe about survivors reignited high levels of stigma faced by survivors in Sierra Leone. Additionally reports of probable long term (5-7 years) persistence and sexual transmission lead to this outbreak in Guinea,²⁹⁸ furthering fear responses towards EVD survivors given the role they can play in virus emergence as previously documented.²⁶³ Older participants were associated with reports of stigma (table 6.5), older age has been associated with higher risk for severe disease which is similarly associated with stigma. The duration of acute EVD was associated with participants reporting stigma and similarly duration of EVD was significantly longer for participants who reported experiencing stigma (Table 6.3 and 6.5). This likely implies those who report stigma had more severe acute illness and therefore a more visible infection and more visible post-Ebola sequelae. Visible forms of illness have long been tied to stigma, with an early example of this being leprosy and the isolation of infected individuals in colonies, and more recently around the first cases of Human Immunodeficiency Virus with stigma remaining

prevalent today demonstrating the similar fear responses triggered by infectious diseases more broadly.

Chapter 7: Mental health of EVD survivors 6-8 years after recovery

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The abstract, summary box and a brief introduction and discussion respectively are included in this manuscript, otherwise the introduction, methods, general discussion, and conclusions have been added to the corresponding chapters of this thesis.

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7.0 The state of mental health amongst Ebola virus disease survivors through a cross-sectional study in Sierra Leone

Brayden G. Schindell¹, Jia B. Kangbai², Bev Fredborg³, Kaarina Kowalec^{4,5}, Souradet Y. Shaw^{1,6}, Jason Kindrachuk^{1*}

¹Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

²Department of Public Health, Eastern Technical University of Sierra Leone, Kenema, Sierra Leone

³Department of Psychology, University of Winnipeg, Winnipeg, Canada

⁴College of Pharmacy, University of Manitoba, Winnipeg, Canada

⁵Department of Medical Epidemiology & Biostatistics, Karolinska Institutet, Solna, Sweden

⁶Department of Community Health Sciences, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

7.1 Abstract

The West African EVD epidemic resulted in >28,000 disease cases and >11,000 fatalities. The unprecedented number of survivors from this epidemic have raised questions about the long-term mental health impacts of EVD survivorship and the capacity to meet these needs. Assess the frequency and factors associated with mental health consequences of EVD survivorship in Sierra Leone. A cross-sectional study of 595 EVD survivors and 403 close contacts ($n=998$) from Sierra Leone. Assessed via in-person survey between November 2021 to March 2022. Assessment included validated mental health screening tools (PHQ-9, PCL-5,

AUDIT, DAST-20) to indicate the presence/absence of disorder. The frequency of each disorder and factors associated with each disorder were assessed. EVD-associated PTSD was reported by 45.7% ($n=257$) of EVD survivors. Moreover, 3.9% ($n=22$) and 12.0% ($n=67$) of EVD survivors reported MD and substance use, respectively; all mental health outcomes were higher than baseline rates in the region (PTSD: 6-16%, MD: 1.1%, substance use: 2.2%). PTSD among EVD survivors was associated with acute EVD duration of ≥ 21 days (AOR: 2.24, 95% CI: 1.16-4.43), 35-44 years of age (AOR: 3.31, 95% CI: 1.33-8.24; AOR: 2.99, 95% CI: 1.09-8.24), and residential mobility (AOR: 4.16, CI 95%: 2.35-7.35). Concerningly, the levels of mental health disorders among EVD survivors in Sierra Leone remained elevated six to eight years after recovery. Results can be used to inform policy efforts and target resources to address mental health in EVD survivors.

7.2 Summary box

Research has shown that in the immediate aftermath of the West African EVD epidemic, a quarter of EVD survivors met diagnostic criteria for PTSD while 5% met criteria for major depressive disorder. However, community supports, including those for mental health provided by aid agencies and charitable organizations have all but disappeared for the survivor community in Sierra Leone. This is the first long-term follow-up on the mental health of EVD survivors, with surveys conducted in 2021 and 2022. Our findings suggest that levels of major depression remained unchanged compared to the immediate aftermath of the epidemic while the incidence of PTSD has doubled in that timeframe. Likewise, we demonstrated that rates of mental health disorders in our sample were three times higher when compared to the general population of Sierra Leone. This study identified a community

affected by a lack of effective mental health care access, trained professionals (e.g., psychiatrists, general practitioners, nurses) and culturally sensitive mental health and psychosocial support programs in partnership with community and spiritual leaders among EVD-affected communities.

7.3 Introduction

Few studies have assessed the mental health of EVD survivors^{240,261,270,278}. With little known about demographic associations to mental health experiences of EVD survivors including whether sex plays a role in those experiencing mental health issues. This is exacerbated due to the civil war in Sierra Leone from 1991 to 2002, which resulted in tens of thousands of civilian fatalities and displaced >2,000,000 people, leaving the health system decimated over the course of the civil war²⁹⁹. Today, health services remain underdeveloped and fraught with barriers to accessing care^{279,300}. While charitable organizations and aid agencies provided supports during these crises, shortages of mental health professionals from chronic underfunding of mental health services left people without continuing support in the aftermath of these crises²⁷⁹. A recent Amnesty International report found that EVD survivors were still struggling with a range of physical and mental health symptoms and that the COVID-19 pandemic had brought back distressing memories, with some survivors speaking of a “persistent fear of death”²⁷⁹. In Sierra Leone, one of the few studies that assessed the nationwide impact of the EVD epidemic found that one of every five participants reported symptoms that met the diagnostic criteria for PTSD using the Impact of Event Scale-6^{261,301}. Varying levels of reported mental health symptoms by EVD survivors suggest that further investigations are required to better understand the specific mental health impact of the

epidemic²⁶¹. No studies have followed up on the mental health outcomes beyond three years after the epidemic was declared over leaving a gap in knowledge on the current state of mental health disorders among the survivor population in Sierra Leone as well as what the long-term mental health outcomes of EVD survival are.

The aim of this study was to provide the first long term follow up on EVD survivor mental health to eight years post recovery of EVD survivors, providing insight into what the prevalence of mental health is following interventions implemented in the country of Sierra Leone and what factors continue to influence mental health outcomes and substance use among the EVD survivor population in comparison to close-contacts. Using cross-sectional data, this study provides an assessment of the long-term consequences of EVD on the mental health of EVD survivors when compared to close contacts in Sierra Leone in 2022. The main objective was to describe the prevalence of major depression, PTSD, substance use and alcohol use amongst EVD survivors at the time of assessment six to eight years after recovery. The second objective was to identify demographic and clinical health factors associated with major depression and PTSD in EVD survivors.

7.4 Results

7.4.1 Participant characteristics

Here, we included 824 participants in the study, with 470 (57.0%) EVD survivors and 354 (43.0%) close contacts, with the total participant demographics included in Table 7.1. The median age of EVD survivors was 30 years at survey administration (IQR=25-40) and the median duration of EVD was 21 days (IQR=9-24) Table 7.2. The majority of EVD survivors were treated in an ETC (68.7%, n=323), and 27.4% (n=129) were treated in hospital with a

further 3.9% (n=18) treated at other locations. Approximately half of EVD survivors reported experiencing stigma at time of survey (54.7%, n=257).

Table 7.1: Demographics of participants by EVD survival status

	Close Contacts (n=354)		EVD Survivors (n=470)		Total		p-value
Biological Sex							<0.001
Male	223	63.0%	232	49.4%	455	55.2%	
Female	131	37.0%	238	50.6%	369	44.8%	
Age Group							0.090
<20	20	5.6%	42	8.9%	62	7.5%	
20-24	36	10.2%	61	13.0%	97	11.8%	
25-29	63	17.8%	88	18.7%	151	18.3%	
30-34	88	24.9%	100	21.3%	188	22.8%	
35-39	71	20.1%	66	14.0%	137	16.6%	
40-44	37	10.5%	61	13.0%	98	11.9%	
45+	39	11.0%	52	11.1%	91	11.1%	
Education							0.572
No	207	58.2%	284	60.4%	491	59.6%	
Yes	147	41.8%	186	39.6%	333	40.4%	
Employment							<0.001
No	146	41.4%	402	85.5%	548	66.5%	
Yes	208	58.6%	68	14.5%	275	33.5%	
Provinces							<0.001
Northern	11	3.1%	46	9.8%	57	6.9%	
Eastern	130	36.9%	182	38.7%	312	37.9%	
Southern	69	19.6%	99	21.1%	168	20.4%	
Western	92	26.1%	84	17.9%	176	21.4%	
Northwest	52	14.2%	59	12.6%	111	13.4%	

N=824

Table 7.2: Demographics of EVD survivors by PTSD

	No PTSD		PTSD		Total (n=470)		p-value
Biological Sex							0.518
Male	119	47.8%	113	51.1%	232	49.4%	
Female	130	52.2%	108	48.9%	238	50.6%	
Age Group							0.130
<20	23	9.2%	19	8.6%	42	8.9%	
20-24	38	15.3%	23	10.4%	61	13.0%	
25-29	50	20.1%	38	17.2%	88	18.7%	
30-34	43	17.3%	57	25.8%	100	21.3%	
35-39	32	12.9%	34	15.4%	66	14.0%	
40-44	30	12.0%	31	14.0%	61	13.0%	
45+	33	13.3%	19	8.6%	52	11.1%	
Education							0.059
No	140	56.2%	144	65.2%	284	60.4%	
Yes	109	43.8%	77	34.8%	186	39.6%	
Employment							0.087
No	206	82.7%	196	88.7%	402	85.5%	
Yes	43	17.3%	25	11.3%	68	14.5%	
Provinces							<0.001
Northern	42	16.9%	4	1.8%	46	9.8%	
Eastern	123	49.4%	59	26.7%	182	38.7%	
Southern	43	17.3%	56	25.3%	99	21.1%	
Western	26	10.4%	58	26.2%	84	17.9%	
Northwest	15	6.0%	44	19.9%	59	12.6%	
Ebola Survivor Residence Change							0.047
No	110	44.2%	77	34.8%	187	39.8%	
Yes	139	55.8%	144	65.2%	283	60.2%	
Stigma							<0.001
No	155	62.2%	58	26.2%	213	45.3%	
Yes	94	37.8%	163	73.8%	257	54.7%	
Duration of Acute EVD							<0.001
<7 Days	102	41.0%	0	0.0%	102	21.7%	
7-14 Days	35	14.1%	27	12.2%	62	13.2%	
15-21 Days	36	14.5%	80	36.2%	116	24.7%	
>21 Days	76	30.5%	114	51.6%	190	40.4%	
Acute EVD Symptoms							<0.001
No Symptoms	2	0.8%	0	0.0%	2	0.4%	
1-5 Symptoms	103	41.4%	175	79.2%	278	59.1%	
6-10 Symptoms	134	53.8%	36	16.3%	170	36.2%	
11 to 14 Symptoms	10	4.0%	10	4.5%	20	4.3%	
Ebola Treatment Center							<0.001
No	126	50.6%	21	9.5%	147	31.3%	
Yes	123	49.4%	200	90.5%	323	68.7%	
Hospital							<0.001
No	133	53.4%	208	94.1%	341	72.6%	
Yes	116	46.6%	13	5.9%	129	27.4%	

N=470

7.4.2 Main outcomes

Mental health symptoms among the EVD survivor population and close contacts were assessed and are summarized in Table 7.3. PTSD was reported by 47.0% (n=221) of EVD survivors when EVD was assessed as the index trauma and was the most common mental health issue assessed. 3.8% (n=18) of EVD survivors reported symptoms congruent with MD, compared to 1.1% (n=4) of close contacts (p=0.017). Harmful levels of substance use (excluding alcohol) was reported by 9.8% (n=46) of EVD survivors, compared to 2.2% (n=8) in close contacts (p≤0.001). 3.8% (n=18) reported harmful levels of alcohol use, with 2.8% (n=10) close contacts (p=0.431) reporting harmful levels of alcohol use. Several demographic variables demonstrated differences in comparison to close contacts. Table 7.4 explores associations with EVD survival in comparison to close contacts when adjusted for sex, age, province of residence and employment status, demonstrating significant association with drug use (AOR: 4.43, 95% CI: 1.85-10.61, p≤0.001), but not with MDD or harmful drinking levels.

Table 7.3: Mental health of respondents by EVD survivor status

	Close Contact (n=354)		EVD Survivor (n=470)		Total		p value
PTSD							
No PTSD	N/A	N/A	249	53.0%	249	53.0%	0.017
PTSD	N/A	N/A	221	47.0%	221	47.0%	
Depression							
No Depressive Symptoms	350	98.9%	452	96.2%	802	97.3%	<0.001
Depressive Symptoms	4	1.1%	18	3.8%	22	2.7%	
Drug Use							
No Drug Use	346	97.8%	424	90.2%	770	93.4%	0.431
Drug Use	8	2.2%	46	9.8%	54	6.6%	
Alcohol Use							
Acceptable Drinking	344	97.2%	452	96.2%	796	96.6%	0.431
Harmful Drinking	10	2.8%	18	3.8%	28	3.4%	

N=824

Table 7.4: Mental health associations with participants by EVD survival status - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

Depression				
	Crude		Adjusted	
No Depressive Symptoms	1	[1.00,1.00]	1	[1.00,1.00]
Depressive Symptoms	3.60*	[1.23,10.55]	3.39	[1.00,11.54]
Drug Use				
No Drug Use	1	[1.00,1.00]	1	[1.00,1.00]
Drug Use	5.83***	[2.76,12.29]	4.43***	[1.85,10.61]
Alcohol Use				
Acceptable Drinking	1	[1.00,1.00]	1	[1.00,1.00]
Harmful Drinking	1.69	[0.80,3.94]	1.26	[0.50,3.17]
Observations	898			

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 7.1 shows demographic when EVD survivors are compared to close contacts. Notably in comparison to close contacts significantly fewer survivors were employed/had an income source (14.5%, n=68, $p \leq 0.001$) compared to their close contacts (58.6%, n=207). Table 7.5 shows that the AOR of survivors to be unemployed is 13.9 (95% CI: 9.13, 21.27, $p \leq 0.001$) when compared to close contacts and adjusted for sex, age, province of residence, education and religion. There were also significant differences in the geographic distribution of survivors when compared to close contacts with significantly more close contacts living in the urban capital region (26.1%, n=92) compared to EVD survivors (17.9%, n=84). This difference in province of residence was highlighted in Table 7.5 where EVD survivors were significantly more likely to be living in the Northern (AOR: 5.21, 95% CI: 2.16-12.61, $p \leq 0.001$), Eastern (AOR: 2.74, 95% CI: 1.66-4.53, $p \leq 0.001$), Southern (AOR: 2.57, 95% CI: 1.46-4.52, $p \leq 0.01$), and Northwestern (AOR: 2.13, 95% CI: 1.13-4.02, $p \leq 0.05$) regions of the Sierra Leone.

Table 7.5: Demographic associations with participants by EVD survival status - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Sex				
Male	1	[1.00,1.00]	1	[1.00,1.00]
Female	1.94***	[1.48,2.55]	1.57*	[1.10,2.25]
Age Group				
<20	1.11	[0.58,2.15]	1.15	[0.55,2.44]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	0.77	[0.47,1.28]	1.79	[0.94,3.43]
30-34	0.61*	[0.37,0.99]	1.65	[0.87,3.12]
35-39	0.54*	[0.32,0.90]	1.51	[0.79,2.87]
40-44	0.91	[0.52,1.59]	3.38**	[1.60,7.17]
45+	0.79	[0.45,1.38]	3.20**	[1.49,6.88]
Province				
Northern	5.12***	[2.51,10.45]	5.21***	[2.16,12.61]
Eastern	1.80**	[1.25,2.59]	2.74***	[1.66,4.53]
Southern	2.12***	[1.40,3.20]	2.57**	[1.46,4.52]
Western	1	[1.00,1.00]	1	[1.00,1.00]
Northwest	1.41	[0.88,2.26]	2.13*	[1.13,4.02]
Education				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.91	[0.70,1.20]	1.52*	[1.03,2.24]
Employment				
No	8.21***	[5.98,11.27]	13.9***	[9.13,21.27]
Yes	1	[1.00,1.00]	1	[1.00,1.00]
Religion				
Christian	0.56***	[0.42,0.73]	0.72	[0.51,1.03]
Muslim	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1	[1.00,1.00]	1	[1.00,1.00]
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.96	[0.09,10.71]	1.24	[0.54,2.83]
Observations			828	

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

In the fully adjusted model, EVD survivors who were middle-aged (35-39 AOR: 3.31, 95% CI: 1.33-8.24, $p \leq 0.05$; 40-44 AOR: 2.99, 95% CI: 1.09-8.24, $p \leq 0.05$), were from the Northwestern province (AOR: 14.2, 95% CI: 3.58-56.46, $p \leq 0.001$), or had changed their district of residence (AOR: 4.16, 95% CI: 2.35-7.35, $p \leq 0.001$) had a higher association with PTSD (Table 7.6).

EVD survivors who had acute EVD of 15 to over 21 days had a greater association with PTSD. Of participants who did not meet criteria for PTSD, the largest proportion of participants reported acute EVD lasting <7 days (41.0%, $n=102$) while most participants that met the criteria for PTSD had acute EVD lasting over 21 days (51.6%, $n=114$) table 7.2. Treatment in an ETC was more common amongst survivors that met criteria for PTSD (90.5%, $n=200$, $p \leq 0.001$) compared to in hospital (5.9%, $n=13$, $p \leq 0.001$). For survivors who did not meet the criteria for a PTSD diagnosis, 49.4% ($n=123$) reported treatment in an ETC and 46.6% ($n=116$) reported treatment in hospital.

Table 7.6: Demographic associations with PTSD for survivors - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Sex				
Male	1	[1.00,1.00]	1	[1.00,1.00]
Female	0.94	[0.67,1.31]	1.08	[0.65,1.80]
Age Group				
<20	1.83	[0.87,3.87]	1.23	[0.47,3.21]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	1.49	[0.80,2.78]	1.77	[0.77,4.07]
30-34	2.53**	[1.38,4.65]	1.83	[0.76,4.40]
35-39	2.89**	[1.51,5.53]	3.31*	[1.33,8.24]
40-44	2.11*	[1.08,4.12]	2.99*	[1.09,8.24]
45+	1.27	[0.63,2.55]	1.85	[0.69,4.99]
Province				
Northern	0.039***	[0.01,0.12]	0.33	[0.06,1.66]
Eastern	0.24***	[0.14,0.41]	0.37	[0.12,1.13]
Southern	0.55*	[0.31,0.97]	1.33	[0.41,4.35]
Western	1	[1.00,1.00]	1	[1.00,1.00]
Northwest	1.05	[0.52,2.10]	14.2***	[3.58,56.46]
Change in Residence				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	2.12***	[1.50,2.99]	4.16***	[2.35,7.35]
Education				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.68*	[0.48,0.97]	0.84	[0.46,1.53]
Employment				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.61*	[0.38,0.99]	1.08	[0.41,2.80]
Religion				
Christian	1.81***	[1.27,2.58]	1.54	[0.92,2.57]
Muslim	1	[1.00,1.00]	1	[1.00,1.00]
Employment Since Ebola				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.32***	[0.18,0.57]	0.17**	[0.06,0.49]
Observations	472			

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Associations to other mental health related symptoms were also assessed and are presented in Table 7.7 (adjusted for; sex, age group, stigma, depression, headache, memory loss, sleeping issues, fatigue, and night sweats). PTSD is associated with reported stigma (AOR: 5.20, 95% CI: 2.77-9.75, $p \leq 0.001$) and headaches (AOR: 2.27, 95% CI: 1.22-4.22, $p \leq 0.01$). Depressive symptoms were less common than PTSD symptoms, however, significantly more EVD survivors reported these symptoms that met PTSD symptoms criteria (5.9%, $n=15$) when compared to those who did not (2.3%, $n=7$, $p=0.047$).

Table 7.7: Clinical and mental health associations with PTSD for survivors - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Sex				
Male	1	[1.00,1.00]	1	[1.00,1.00]
Female	0.94	[0.67,1.31]	0.56*	[0.32,0.98]
Age Group				
<20	1.83	[0.87,3.87]	1.53	[0.49,4.85]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	1.49	[0.80,2.78]	0.55	[0.91,5.87]
30-34	2.53**	[1.38,4.65]	2.31	[0.80,32.64]
35-39	2.89**	[1.51,5.53]	1.74	[0.61,4.94]
40-44	2.11*	[1.08,4.12]	0.57	[0.20,1.66]
45+	1.27	[0.63,2.55]	0.69	[0.22,2.23]
Stigma				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	6.21***	[4.27,9.03]	5.20***	[2.77,9.75]
Depression				
No Depressive Symptoms	1	[1.00,1.00]	1	[1.00,1.00]
Depressive Symptoms	2.64*	[1.06,6.58]	1.27	[0.36,4.48]
Headache				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	4.33***	[2.92,6.43]	2.27**	[1.22,4.22]
Memory Loss				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.27*	[0.09,0.81]	0.41	[0.09,1.86]
Sleeping Issues				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	47.3***	[28.04,79.76]	43.3***	[23.63,79.23]
Fatigue				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	2.97***	[1.94,4.56]	1.09	[0.53,2.23]
Night Sweats				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1.80*	[1.06,3.07]	0.95	[0.42,2.15]
Observations	551			

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

7.4.3 Sex based associations of mental health and EVD

Among EVD survivors who reported PTSD, female EVD survivors were more associated with stigma (AOR: 6.20, 95% CI: 2.76-13.93, $p \leq 0.001$) (table 7.8) and living outside the Western Province compared to male EVD survivors (Northern province AOR: 13.8, 95% CI: 1.08-177.75, $p \leq 0.05$; Eastern province AOR: 13.0, 95% CI: 4.23-40.07, $p \leq 0.001$; Southern province AOR: 24.5, 95% CI: 7.82-76.81, $p \leq 0.001$; Northwestern province AOR: 22.9, 95% CI: 5.22-100.49, $p \leq 0.001$) compared to their male counterparts (table 7.9). There was no significant association observed among EVD survivors who reported PTSD between female survivors and MD (AOR: 2.20, 95% CI: 0.61-7.94, $p \geq 0.05$). Likewise, no significant association was observed between biological sex and PTSD (AOR: 1.16, 95% CI: 0.73-1.82, $p \geq 0.05$) when adjusted for demographics.

Table 7.8: Clinical and mental health associations with sex for survivors reporting PTSD - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Age Group				
<20	1.18	[0.37,3.77]	1.85	[0.48,7.20]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	2.36	[0.85,6.61]	1.43	[0.45,4.58]
30-34	0.85	[0.33,2.20]	0.83	[0.28,2.45]
35-39	2.16	[0.79,5.84]	1.48	[0.48,4.59]
40-44	0.89	[0.31,2.52]	0.46	[0.14,1.51]
45+	1.40	[0.45,4.35]	0.69	[0.18,2.65]
Stigma				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	5.05***	[2.60,9.82]	6.20***	[2.76,13.93]
Depression				
No Depressive Symptoms	1	[1.00,1.00]	1	[1.00,1.00]
Depressive Symptoms	1.87	[0.62,5.64]	2.20	[0.61,7.94]
Headache				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	2.22*	[1.13,4.33]	1.28	[0.59,2.75]
Memory Loss				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1	[1.00,1.00]	1	[1.00,1.00]
Sleeping Issues				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1.24	[0.53,2.93]	1.06	[0.39,2.84]
Fatigue				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1.97*	[1.14,3.42]	1.05	[0.57,1.95]
Night Sweats				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1.81	[0.88,3.74]	1.24	[0.54,2.83]
Observations			248	

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 7.9: Demographic associations with sex for survivors reporting PTSD - Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CI), Logistic Regression Models

	Crude		Adjusted	
Age Group				
<20	1.18	[0.37,3.77]	2.27	[0.44,11.77]
20-24	1	[1.00,1.00]	1	[1.00,1.00]
25-29	2.36	[0.85,6.61]	1.10	[0.28,4.42]
30-34	0.85	[0.33,2.20]	0.78	[0.20,3.12]
35-39	2.16	[0.79,5.84]	1.14	[0.35,5.77]
40-44	0.89	[0.31,2.52]	0.33	[0.07,1.47]
45+	1.40	[0.45,4.35]	0.39	[0.08,1.83]
Province				
Northern	6.25	[0.77,50.90]	13.8*	[1.08,177.75]
Eastern	8.14***	[3.40,19.50]	13.0***	[4.23,40.07]
Southern	14.5***	[5.90,35.58]	24.5***	[7.82,76.61]
Western	1	[1.00,1.00]	1	[1.00,1.00]
Northern	12.5***	[0.52,2.10]	22.9***	[5.22,100.49]
Change in Residence				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.42**	[0.24,0.72]	0.93	[0.38,2.30]
Education				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	0.75	[0.45,1.26]	0.51	[0.22,1.20]
Employment				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1.15	[0.53,2.49]	1.62	[0.43,6.05]
Religion				
Christian	0.67	[0.40,1.12]	1.52	[0.76,3.03]
Muslim	1	[1.00,1.00]	1	[1.00,1.00]
Employment Since Ebola				
No	1	[1.00,1.00]	1	[1.00,1.00]
Yes	1.30	[0.48,3.54]	0.55	[0.11,2.80]
Observations			226	

Exponentiated coefficients; 95% confidence intervals in brackets

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Differences in prevalence of mental health problems were found between sexes and amongst EVD survivors and their close contacts (table 7.10-7.12). Alcohol use was more prevalent among female EVD survivors (4.8%, n=14) compared to close contacts (0.8%, n=1, p=0.045) however, the same difference was not found between male EVD survivors (4.6%, 12) compared to close contacts (4.1%, n=9, p=0.826). While both male EVD survivors (9.7%, n=25) compared to close contacts (2.7%, n=6, p=0.002), and female EVD survivors (13.6%, n=40) compared to close contacts (1.5%, n=2, p≤0.001) reported increased prevalence of drug use.

Table 7.10: Mental health of female participants by EVD survival

	Close Contacts (n=130)		EVD Survivors (n=294)		Total		p-value
Age Group							0.851
<20	10	7.7%	24	8.2%	34	8.0%	
20-24	16	12.3%	43	14.6%	59	13.9%	
25-29	28	21.5%	58	19.7%	86	20.3%	
30-34	28	21.5%	60	20.4%	88	20.8%	
35-39	25	19.2%	44	15.0%	69	16.3%	
40-44	12	9.2%	30	10.2%	42	9.9%	
45+	11	8.5%	35	11.9%	46	10.8%	
Provinces							0.003
Northern	3	2.3%	23	7.8%	26	6.1%	
Eastern	55	42.3%	119	40.5%	174	41.0%	
Southern	32	24.6%	95	32.3%	127	30.0%	
Western	16	12.3%	12	4.1%	28	6.6%	
Northwest	24	18.5%	45	15.3%	69	16.3%	
Depression							0.165
No Depressive Symptoms	128	98.5%	280	95.2%	408	96.2%	
Depressive Symptoms	2	1.5%	14	4.8%	16	3.8%	
Drug Use							≤0.001
No Drug Use	128	98.5%	254	86.4%	382	90.1%	
Drug Use	2	1.5%	40	13.6%	42	9.9%	
Alcohol Use							0.045
Acceptable Drinking	129	99.2%	280	95.2%	409	96.5%	
Harmful Drinking	1	0.8%	14	4.8%	15	3.5%	

Table 7.11: Mental health of male participants by EVD status

	Close Contacts (n=222)		EVD Survivors (n=259)		Total		p-value
Age Group							0.068
<20	10	4.5%	23	8.9%	33	6.9%	
20-24	20	9.0%	32	12.4%	52	10.8%	
25-29	35	15.8%	45	17.4%	80	16.6%	
30-34	58	26.1%	51	19.7%	109	22.7%	
35-39	46	20.7%	37	14.3%	83	17.3%	
40-44	25	11.3%	41	15.8%	66	13.7%	
45+	28	12.6%	30	11.6%	58	12.1%	
Provinces							0.010
Northern	8	3.6%	29	11.2%	37	7.7%	
Eastern	75	33.8%	97	37.5%	172	35.8%	
Southern	37	16.7%	40	15.4%	77	16.0%	
Western	76	34.2%	73	28.2%	149	31.0%	
Northwest	26	11.7%	20	7.7%	46	9.6%	
Depression							0.116
No Depressive Symptoms	220	99.1%	251	96.9%	471	97.9%	
Depressive Symptoms	2	0.9%	8	3.1%	10	2.1%	
Drug Use							0.002
No Drug Use	216	97.3%	234	90.3%	450	93.6%	
Drug Use	6	2.7%	25	9.7%	31	6.4%	
Alcohol Use							0.826
Acceptable Drinking	213	95.9%	247	95.4%	460	95.6%	
Harmful Drinking	9	4.1%	12	4.6%	21	4.4%	

Table 7.12: Mental health reports of EVD survivors by biological sex.

	Biological Sex						
	Male (n=260)		Female (n=296)		Total		p-value
Stigma							0.011
No	135	51.9%	121	40.9%	256	46.0%	
Yes	125	48.1%	175	59.1%	300	54.0%	
PTSD							0.733
No PTSD	139	53.5%	163	55.1%	302	54.3%	
PTSD	121	46.5%	133	44.9%	254	45.7%	
Depression							0.386
No Depressive Symptoms	252	96.9%	282	95.3%	534	96.0%	
Depressive Symptoms	8	3.1%	14	4.7%	22	4.0%	
Drug Use							0.148
No Drug Use	235	90.4%	255	86.1%	490	88.1%	
Drug Use	25	9.6%	41	13.9%	66	11.9%	
Alcohol Use							1.000
Acceptable Drinking	248	95.4%	282	95.3%	530	95.3%	
Harmful Drinking	12	4.6%	14	4.7%	26	4.7%	

7.5 Discussion

This paper assesses the prevalence of, and factors associated with, mental health symptoms amongst EVD survivors in Sierra Leone. Regarding PTSD in EVD survivors, we found that the prevalence of PTSD in our sample (47.0%) was higher than reported in previous studies of EVD survivors in Sierra Leone. In a 2018 study, 27% of EVD survivors met levels of clinical concern for PTSD while 16% met levels of probable PTSD diagnosis; notably, PTSD was assessed using the IES-6 scale, which is a shorter but less robust measure of DSM-V defined PTSD and reduced sensitivity may have affected probable diagnoses^{261,302}. A more recent 2020 study of northern Sierra Leone conducted two years after the West African epidemic found a PTSD prevalence of 21.8%²⁷¹. While we found that the prevalence of MD (3.8%) was congruent with previously reported levels of MD in EVD survivor populations, though these studies tended to examine the recent aftermath of the epidemic and not at an extended time scale as reported herein and only one used the PHQ-9 while the others used the Center for Epidemiologic Studies-Depression Scale, or utilized self-reported depression^{192,270,303}. For example, a study of mental health symptoms experienced by individuals who survived EVD across Sierra Leone, Liberia, and Guinea also using the PHQ-9 found that 7.1%, 6.8%, and 3.6% of the studied population met criteria for major depressive disorder in 2018, respectively²⁷⁰. Moreover, a 2018 study using hospital outpatient records of EVD survivors in Liberia found that 13% of survivors had depressive symptoms¹⁹². Finally, results from the PostEboGui cohort of survivors in Guinea found that 11% met the criteria for depression after a clinical consultation by a psychiatrist³⁰³. There was one outlier study that found depression in Northern Sierra Leone had a prevalence of 47.2% however it should be noted this study used a brief assessment of anxiety and depression (Hospital Anxiety and

Depression scale) which cannot assess MD as described here with PHQ-9^{271,304}. Nonetheless it is notable that rates of depression have not decreased in the eight years since the epidemic started. These results suggest a gap in care for EVD survivors, and that future epidemics may also lead to similar mental health symptoms.

Chapter 8: Final Discussion, Limitations, Future Directions and Conclusion

8.0 Final Discussion, Limitations, Future Directions and Conclusion

8.1 General Discussion

The findings discussed build upon the currently available evidence, providing a broader population-level assessment of survivor health in Sierra Leone, where the burden of disease was the highest. While at an extended time interval compared to previous studies, providing much needed insight into the long-term somatic symptomology, mental health and social/stigma effects experienced on a day-to-day basis six to eight years post recovery. Here the relationship between these separate assessments is discussed along with the interplay between these separately described symptomologies and demographics. It is important to consider the influence of social, economic and psychological stressors on health among EVD survivors. As we have previously reported, in this cohort a substantial proportion of survivors continue to experience stigma (50.6%), unemployment (85.5%) and unaddressed post-traumatic stress disorder (47%). These factors likely contribute to overall morbidity and may exacerbate or prolong symptom reporting through non-biological mechanisms^{294,305}. For example, chronic stress, economic instability, and poor access to healthcare may hinder recovery, increase symptom vigilance, or limit opportunities for treatment.

8.1.1 Broad reaching effects of long-term somatic symptomology of EVD survivors

Physical health effects have been shown to be associated with mental health effects of EVD survival have been reported and experienced by EVD survivors during their acute infection³⁰⁶. Previous studies have shown that these physical symptoms are associated with major mental health problems among survivors^{258,280}. The physical symptoms associated with EVD (e.g., fever, severe headache, muscle pain, weakness, fatigue, diarrhea, vomiting,

abdominal and stomach pain, unexplained hemorrhage) are significant stressors and can contribute to mental health symptomology of affected individuals^{187,258}. Here we reported associations with both headaches and sleeping issues. Furthermore, EVD survivors with a unit increase in the time spent in the Ebola treatment centre were likely to report an increase in negative mental health symptomology¹⁹³. Our results agreed with these findings demonstrating that a prolonged duration of acute EVD increased the likelihood of experiencing PTSD.

8.1.2 Associations of long-term reproductive effects and interpersonal relationships of EVD survivors in Sierra Leone

Likewise, there are negative mental health and social effects associated with reproductive health and interpersonal relationships. There are long-term social and mental health effects, as we have previously reported in this cohort^{294,305}. Many survivors experience social isolation, with their relationship status being directly associated with stigmatization. The mental health effects are also profound, with 45.7% of EVD survivors reporting post-traumatic stress disorder related to their Ebola experience and its impact on their interpersonal relationships^{294,305}. While relationships appear to also play important roles in stigma particularly for intimate relationships where we identified that changes in these relationships can impact stigma experience (Table 6.7 and 6.8, and Table 12.3, 12.4, and 12.5 of Appendix 3) through losing a support or support network.

8.1.3 Stigmatization of Ebola virus disease survivors in Sierra Leone and the association with mental health and social effects

This study has demonstrated how stigma affects the day to day lives of survivors and the impacts that has well beyond the social interactions this stems from. A significant proportion of EVD survivors were found to experience multiple manifestations of stigma that persists for many years. Stigma has been linked to several sociodemographic risk factors for increased disease prevalence including sex assigned at birth, age, level of education, access to medical care and economic status²⁶⁵. A significantly larger proportion of female than male survivors were found to be experiencing stigma suggesting sex assigned at birth plays a role in stigma and needs to be accounted for in interventions. Other Risk factors for anxiety depression and PTSD include experience of perceptions of others, mortality, socioeconomic insecurities, stigma and discrimination, all may have been experienced in Sierra Leone by EVD survivors²⁶¹. EVD survivors who experienced a unit increase in Enacted Stigma were more likely to report a decreased level of mental health¹⁹³. We demonstrated in our results that stigma is significantly associated with experiencing PTSD²⁹⁴. EVD survivors continue to suffer from psychological distress due to grief resulting from losing loved ones, social exclusion, and community stigmatisation^{264,273}.

Employment was associated with survivor status and income opportunities (Table 12.1, of Appendix 3). limitations have led to survivors being unemployed, leading to dependence on others and a loss of self-worth particularly among those with advanced age^{186,240,264}. Among EVD survivors there was a shift in employment from trades and medical fields towards cleaning and craftsmanship which may be more accepting opportunities or could also be a move towards entrepreneurship and self-employment due to workplace

stigma (Table 12.2 of Appendix 3). We found that although unemployment within EVD survivors is not significantly associated with PTSD it is highly associated with EVD survival and likely a contributor to feelings of self-worth and internalized stigma and other rumination activities negatively affecting mental health.

Stigmatization has led to EVD survivors being evicted from their residence, divorce from their spouses, losing employment, and mocked in the community by their family and friends^{240,259,307}. There are reports of EVD survivors experiencing discrimination, prejudice, social isolation, and abandonment by family members during the West African EVD epidemic³⁰⁸. This was evident among survivors that participated in this study with, a majority of EVD survivors reported changing residence following recovery from EVD (Table 12.6 of Appendix 3). Rurally located participants also had greater associations with stigma compared to those from urban places of residence, which is likely a result of living in a close knit community where information spreads more easily and stigmatizing forces can more easily take hold. Similarly, where a survivor lived in a district was also strongly associated with experiencing being forced to change residence ($p \leq 0.001$) with demonstrated movement away from villages and towards more populated towns and cities (Table 12.7 and 12.8 of Appendix 3).

8.1.3 The need for mental health supports in Sierra Leone

There is a lot of overlap between the observed manifestations in this study with mental health and social issues that continue to persist among the EVD survivor population in comparison to close contacts. Addressing these issues will require providing access to suitable mental health services to both EVD survivors and the general population in West Africa. However, doing so remains a challenge for numerous reasons including, but not

limited to: mental health expenditure remaining low, with an estimated \$0.02 USD spent annually per person in Liberia (data is not available for Sierra Leone), and insufficient health staffing, with an estimated 2–4 psychiatrists per 10,000,000 people (population of Sierra Leone is 8.8 million) and 0.33–6.4 mental health nurses per 100,000 people across the three countries³⁰⁹. In Sierra Leone, as of 2021, there were three psychiatrists and approximately 20 mental health nurses in the public workforce for a population of seven million people²⁷⁹, with the majority of EVD survivors unaware of any psychological services, either through government health facilities or non-governmental organisations ²⁷⁹. Infrastructure to provide specialised services such as psychiatric in-patient care remains insufficient, while community stigma surrounding mental health conditions remains a barrier to both accessing and providing care^{310,311}. However, efforts are being made to address these, as all three countries have official mental health policies which call for expanded resources for and access to mental health services and psychiatric medications³⁰⁹.

The WHO Mental Health Gap Action Programme emphasises improvement of mental health services is a joint responsibility that requires collaboration from governments, health professionals, donors, civil society, communities and families³¹². Countries affected by EVD often lack mental health and psychosocial support (MHPSS) programs and trained professionals (e.g., psychiatrists, general practitioners, nurses, etc.) or do not have the resources to put them in place³¹³ resulting in most MHPSS programs in affected countries externally funded by international organizations³¹⁴. However, the King's Sierra Leone Partnership which was supported by the WHO was developed and lead by the Government of Sierra Leone, established a nurse-led MHPSS service with inpatient services and an outpatient clinic³¹³. Mental health nurses and volunteers from the King's Sierra Leone

Partnership trained fourteen general nurses in EVD-focused psychological first aid, case identification and referral pathways³¹³. The nurses offered a series of half-day well-being workshops on coping with stigma, discrimination, stress and self-care to the healthcare staff of the Connaught hospital³¹³.

8.2 Limitations

We also acknowledge several limitations of our study. In our study, close contacts were identified by EVD survivors and may have led to some bias due to the close associations of these participants to survivors. It is also acknowledged that our results may be influenced by recall bias as we relied on self-reported data. The cross-sectional design of our study also limits determination of causality between dependent and outcome variables or analyze changes over time. The end-point design of this study limits the applicability of results to the time since recovery from EVD. As our study was limited to EVD survivors in Sierra Leone, it may not be representative of EVD survivors across other geographic locations. Although our use of close contacts as a comparison group helped minimize confounding by geography, socio-economic status and environmental exposures unmeasured differences may still exist. Lastly as with any voluntary survey there is selection bias although this was mitigated by broad recruitment across multiple districts and through survivor networks. Exploratory analyses conducted may have increased the risk of type I error in this study.

8.2.1 Limitations of mental health assessments

Although data was collected in standardized validated question sets (PHQ-9 $\geq$ 10, PCL-5 $\geq$ 33, AUDIT $\geq$ 8, DAST-20 $\geq$ 6) the data was self-reported and therefore respondents may have focused on past experiences. This limitation was overcome through framing questions on

these question sets in a specific recent time-period (in the past two weeks or month). We did not directly assess the effects of civil war (1992 to 2002) on study participants and therefore cannot determine how history of PTSD in Sierra Leone may have influenced levels of clinical concern detected here. By recruiting close contacts as a control population allowed for matched experiences during the civil war and EVD epidemic as much as possible between the two populations of study. Tentative diagnoses of depression and PTSD could not be validated through clinical consultations. To overcome this limitation, valid and reliable diagnostic tools were used for their sensitivity and specificity in preliminary diagnosis of mental health and substance use²⁷⁰.

8.2.2 Limitations of somatic symptomology and reproductive health assessments

It is possible that individuals with more severe symptoms were more likely to participate, but this was mitigated through broad recruitment both in terms of the number of survivors and their geographic distribution. Symptom data were self-reported and not confirmed through clinical examination or diagnostic testing, which may introduce recall bias and misclassification. Survivors may be more attuned to their health due to the experience of severe illness, potentially inflating symptom reporting relative to close contacts. The cross-sectional design limits causal inference and prevents assessment of symptom trajectories overtime.

8.3 Future Directions

Learning from past attempts, future interventions need to include comprehensive strategies that are community led to ensure buy-in and success. Strategies that should be included in these initiatives include psychoeducation, cognitive techniques, peer support, legislative and

policy change at the local and national level all need to be explored to improve EVD survivor health, mental health and quality of life¹⁹³. Further research is needed to elucidate the biological and contextual mechanisms underlying long-term sequelae and to inform evidence-based interventions for this aging survivor population. Future studies that combine self-report data with clinical and laboratory evaluations are needed to better distinguish between persistent physiological sequelae and symptoms shaped by psychosocial or contextual factors. While work on understanding the viral and immune-related mediators may help develop targeted treatment plans to address these chronic symptoms for EVD survivors^{190,315}. Longitudinal studies could help track changes observed over time. In addition to biological factors, it is important to consider the influence of social, economic and psychological stressors on health among EVD survivors. Future studies should also consider including qualitative analysis to put collected responses in context to individual experiences within the cohort contextualizing individuals experiencing stigma and those that are not, providing expanded insights to interventions. Qualitative assessment should be included in future assessments to add context of individual experiences of mental health to help highlight barriers experienced by survivors and where improvements can be made. Given the lack of mental health assessment of EVD survivors, future studies should look to expand on the findings presented here to determine causality in the factors associated with EVD related mental health. Additionally, assessment of PTSD associated with the civil war should be determined in how it relates to EVD survivors as well as the general population of Sierra Leone. Conducting these recommendations as a longitudinal study could help track changes over time.

8.5 Conclusion

The results presented here underscore the continued somatic symptomology, stigma and mental health issues of the collective experience of EVD survivors in Sierra Leone. Six to eight years after recovery from EVD, PTSD was the most prevalent mental health issue survivors were facing. Although there have been a few programs implemented in Sierra Leone by national organizations (King's Sierra Leone Partnership) and state institutions, more needs to be done to address the clinical mental health needs of EVD survivors in Sierra Leone. Implementation of intervention programs utilizing assessments such as the PCL-5 used here are easy to implement and can decrease the burden on the health infrastructure through partnerships with community and spiritual leaders to identify individuals who would benefit from the care resources available. Such assessments of mental health and risk factors for mental illness can also support policy efforts to improve resources to address mental health and inform how resources can be deployed most efficiently in the aftermath of the West African epidemic and possible future outbreaks. Specifically, Cognitive Behaviour Therapy and Interpersonal Therapy should be explored as part of mental healthcare interventions for subsequent outbreaks and for those who have survived previous outbreaks²⁷¹. Anti-stigma interventions can further these efforts in the form of mental health literacy campaigns (implemented by government or non-governmental organisations), peer support and interventions that encourage contact between EVD survivors and the public can help to overcome existing interpersonal divide and foster positive connection and interaction resulting in community reintegration and inclusion³¹⁶.

Community engagement aimed at reducing EVD stigma and improving understanding of Ebola virus, improved access to the public health system and targeted mental health interventions for EVD survivors should be implemented as part of outbreak response given how strongly stigma persists and the impact early interventions can have. Improved funding and access in addition to public awareness and engagement campaigns could work to destigmatize conventional treatment. Stigma continues to be prevalent among the EVD survivor community with effects across multiple aspects of survivors' lives, as demonstrated by this study. These results highlight the continuing need for healthcare access and social support systems for EVD survivors that likely extends across different geographic regions and populations. This work provides novel insights into sociodemographic factors associated with EVD survivor stigma with the aim of providing a framework to better assist EVD survivors of past and future outbreaks independent of outbreak location.

On a societal level, stigmatization around infectious diseases, including EVD, is believed to be linked to poor access and availability of conventional medical treatment leading to favouring traditional medicine, which is used by 58% of people in sub-Saharan Africa and is associated with lower community-level stigma²⁶⁰. Populations with low confidence in conventional state infrastructure and institutions were more likely to ignore recommendations to control the spread of EVD resulting in increasing stigmatization because of citizens "fending for themselves"³¹⁷. While most somatic symptoms among EVD survivors declined over time, a subset particularly those affecting vision, hearing and urination remained significantly more common than among close contacts six to eight years after recovery. Factors such as lack of consistent follow up on the management of sequelae²⁰², limited access to healthcare services²⁰², inability to obtain medications or follow

up care²⁰², poor health seeking behaviour coupled with limited access to quality healthcare complicates the management of these symptoms¹⁹⁴. Given the chronic nature of EVD-associated sequelae, consideration should be given to funding and strengthening existing medical infrastructure in-country to ensure long-term follow-up and care¹⁹². Support for survivor services in West Africa should be sustained and funding for survivor follow-up should be included in planning for future outbreak responses to provide adequate care both during acute infection and post-recovery¹⁹². Taken together these findings highlight the importance of sustained, survivor-centered care and underscore the need for integrated follow-up that includes physical, psychological, and social support.

Chapter 9: References

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10 Appendix 1: Study Survey

This appendix contains the full survey as it was submitted to and approved by the Research Ethics Boards. These are the direct questions asked of participants from which the data in this thesis is derived from and analyzed from.

10.1 Study survey

Questionnaires/Scales/Instruments Appendix Cover Page

Appendix VERSION DATE: November 17, 2020

Study Protocol #: N/A

Study Title: Epidemiological Analysis of Long-Term Sequelae in Ebola Virus Disease Survivors in Sierra Leone

Title of Document	Version # (if applicable)	Version Date
Questionnaire Section 1 – General Information	V3	November 16, 2020
Questionnaire Section 2 – Clinical Health Information	V3	November 16, 2020
Questionnaire Section 3a – Female Reproduction and Sexual Health Information	V2	November 16, 2020
Questionnaire Section 3b – Male Reproduction and Sexual Health Information	V2	November 16, 2020

No.	Filters and Questions	Answers	Go to
101	START TIME OF INTERVIEW.	Hour. . . . Minutes. . . .	
102	Are you biologically male or female?	Female Male	
103	What day, month and year were you born?	Day Don't know Day Month Don't know Month . . Year Don't know year	
104	What was your age at you last birthday CORRECT 102 AND/OR 103 IF INCONSISTENCIES	Age at last full year	
105	Have you ever attended school	YES NO	108
106	What is the highest level of school you have CURRENTLY attended (Primary, Secondary or higher)?	Primary Junior Secondary . . . Senior Secondary . . . Technical/Vocational Teacher College University Postgraduate	
107	What is the highest (Grade/form/year) you have COMPLETED at this level of education? LESS THAN ONE YEAR RECORD '0'	Grade/form/year CIRCLE OPTION	
108	Have you ever been gainfully employed?	YES NO	110

No.	Filters and Questions	Answers	Go to
109	What jobs do you currently have or have had previously? CHECK ALL THAT APPLY	Teacher Tradesperson Banker Craftsperson Medical worker Day labourer Shopkeeper Other Specify for other: 	
110	What is your ethnicity?	Creole Fullah Kono Limba Loko Mandingo Mende Sherbro Temne Other Foreign	
111	What is your religion?	Christian Islam Bahai Traditional Other None	

No.	Filters and Questions	Answers	Go to
112	What district do you currently live in?	Kailahun Kenema Kono Bombali Falaba Tonkolili Kambia Karene Port Loko Bo Bonthe Moyamba Pujehun Western Rural Western Urban Koinadugu	
113	Where in the district do you live?	City Town Village	
114	Did you have Ebola?	YES NO	201
115	What symptoms did you have when sick with Ebola? CHECK ALL THAT APPLY	Headache Fever Sore throat Hiccups Loss of appetite Abdominal pain Nausea/vomiting ... Diarrhea Bleeding Muscle pain Joint pain Shortness of breath . Breathing issues Red eyes	

No.	Filters and Questions	Answers	Go to
116	Do you currently live where you were diagnosed/treated for Ebola?	YES NO	118
117	What district did you live in when you were treated for Ebola?	Kailahun Kenema Kono Bombali Falaba Tonkolili Kambia Karene Port Loko Bo Bonthe Moyamba Pujehun Western Rural Western Urban Koinadugu	
118	Where in the district did you live at the time?	City Town Village	
119	Where were you treated for Ebola? CHECK ALL THAT APPLY	Ebola Treatment Unit Transit Unit Hospital CommunityCare Centre At Home Traditional Healer ...	
120	How long were you sick with Ebola (approximate)	Days.....	

No.	Filters and Questions	Answers	Go to
121	What was your highest level of education before Ebola?	Current Level Primary Junior Secondary Senior Secondary Technical/Vocational . Teacher College University Postgraduate	
122	Were you gainfully employed when you became infected with Ebola?	YES NO	124
123	What jobs do you currently have or have had previously? CHECK ALL THAT APPLY	Teacher Tradesperson Banker Craftsperson Healthcare provider .. Other Specify for other:	
124	Are you gainfully employed since Ebola?	YES NO	126
125	What jobs do you currently have or have had previously since recovery from Ebola? CHECK ALL THAT APPLY	Teacher Tradesperson Banker Craftsperson Healthcare provider .. Other Specify for other:	

No.	Filters and Questions	Answers	Go to
126	STIGMA AND DISCRIMINATION (for over 12 years of age) 1. Have you been forced to change residence due to alienation from family and/or friends? 2. Have you lost your job or income source due to your Ebola infection? 3. Have you lost a partner/spouse due to fear of being infected? 4. Have you been deprived from attending social/community gatherings for fear of infecting others? 5. Have you isolated yourself from your family and/or your friends? 6. Have you withdrawn from school/training or passed on an opportunity for school/training due to fear of what others might think? 7. Are you afraid that someone would not want to be sexually intimate with you knowing that you are an Ebola survivor?	YES NO YES NO YES NO YES NO YES NO YES NO	

Clinical Health

No.	Filters and Questions	Answers	Go to
201	Participant vitals	Weight (kg) Height (cm)	
202	Do you or have you had any of the following medical conditions? TICK ALL THAT APPLY	None Hypertension Stroke Heart Disease Diabetes Liver cancer Prostate cancer Cervical cancer Breast cancer Other cancer Other cancer:	204
203	If any of the above apply when did they occur?	Prior to 2013 2013-2016 Since 2016	
204	Do you or have you had any of the following infections? ALL THAT APPLY	None Malaria Tuberculosis Typhoid fever HIV/AIDS Hepatitis B Hepatitis C Syphilis. Chlamydia Gonorrhoeae Other Sexually transmitted:	208
205	If any of the above apply when did they occur?	Prior to 2013 2013-2016 Since 2016	

No.	Filters and Questions	Answers	Go to
206	Are you CURRENTLY taking any medications for the above medical conditions or infections?	YES NO	208
207	What medication(s) are you taking for the above medical conditions or infections? ADDITIONAL SPACE AT END OF SURVEY		
208	Are you currently experiencing any of these VISION problems? ALL THAT APPLY	Eye pain Light sensitivity Itchy Blurry vision Redness Discharge None	210
209	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
210	Are you experiencing any of these issues with your EARS, NOSE or THROAT ALL THAT APPLY	Reduced hearing Ringing in ears Pain – ear Discharge – ear Nose bleeds Discharge – nose Hoarseness – throat . None	212

No.	Filters and Questions	Answers	Go to
211	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
212	Are you currently experiencing any of these NEUROLOGICAL issues? ALL THAT APPLY	Headache Dizziness Fainting Feeling of spinning... Seizures Double vision. Vision loss Memory loss Slurred speech and Loss of coordination . Tremor Tingling/numbness .. Loss of use In part of body Weakness in limb(s) .. None	214
213	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
214	Are you having any of the general (CONSTITUTIONAL) symptoms? ALL THAT APPLY	Fever Chills Fatigue Weight gain Weight loss Night sweats None	216

No.	Filters and Questions	Answers	Go to
215	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
216	Are you experiencing any of these heart (CARDIOVASCULAR) issues? ALL THAT APPLY	Chest pain Pounding/fluttering. . Shortness of breath. . Swelling. Orthopnea Leg pain Breathing difficulty While sleeping. None	218
217	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
218	Are you experiencing any of these breathing (RESPIRATORY) issues? ALL THAT APPLY	Wheezing Congestion Cough Sputum Shortness of breath. . Caughing up blood ... None	220
219	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	

No.	Filters and Questions	Answers	Go to
220	Are you experiencing any of the stomach (GASTROINTESTINAL) issues listed? ALL THAT APPLY	Abdominal pain Bowel habits change . Nausea Vomiting Diarrhea Severely under weight Difficulty swallowing . Vomiting blood Rectal bleeding Jaundice None	222
221	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
222	Are you experiencing any listed LYMPHATIC issues?	Swollen lymph nodes . None	224
223	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
224	Are you having any of these issues with your GENITO-URINARY tract? ALL THAT APPLY	Frequent urination. . Urgency of urination. Loss of control. Trouble starting or continuing urination Waking up to urinate. burning urination. . . Frequent large volume Blood in urine	

		None	226
No.	Filters and Questions	Answers	Go to
225	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
226	Are you experiencing any of these muscle or bone (MUSCULOSKELETAL) issues? ALL THAT APPLY	Heat Redness Stiffness Pain or tenderness ... Swelling Joint pain Deformity None	228
227	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
228	Are you currently experiencing any of these SKIN issues? ALL THAT APPLY	Itchy Rash Lumps Bruising Hair/nail change Change in skin colour None	230

No.	Filters and Questions	Answers	Go to
229	How frequently do you experience the problem(s)?	Constant Daily 2-3 days a week Weekly Biweekly Monthly	
230	Are you CURRENTLY taking any medications for the above medical conditions or infections?	YES NO	232
231	What medication(s) are you taking for the above medical Issues? ADDITIONAL SPACE AT END OF SURVEY		
232	Do you have to seek medical attention when the above marked issues occur (Q208-229)?	YES NO	234
233	What Issue(s) do you seek medical attentions for? ADDITIONAL SPACE AT END OF SURVEY		

No.	Filters and Questions	Answers	Go to
234	COVID-19		
	1. Have you been diagnosed with COVID-19?	YES NO	5
	2. If no, do you have any of the following symptoms? ALL THAT APPLY	Fever/feverish/chills. . New/ more coughing . Shortness of breath . . New sore throat Loss of taste/smell . . Vomiting or diarrhea for more than 1 day. . None	
	3. Do you have any TWO or more of the following symptoms? ALL THAT APPLY	Sore muscles. Unusual headache . . . Runny nose. Fatigue Pinkeye Unknown skin rash . . . Nausea/no appetite . None	
	4. In the last 14 days, have you: a. Had close contact with confirmed case of COVID-19 while not physically distancing? b. Had a family member diagnosed with COVID-19? c. Returned from travel outside of Sierra Leone? d. Attended a large gathering (over 50 people)? (Continue to 235)	YES NO YES NO YES NO YES NO	
	5. If you had COVID-19, when were you diagnosed?	Day/Month/Year 	
	6. Have you experienced any symptoms since recovery from COVID-19? ALL THAT APPLY	Gastrointestinal Headache Shortness of breath . . Fatigue Chest pain Joint pain	

		None	
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No.	Filters and Questions	Answers					Go to
235	ALCOHOL ABUSE DISORDER						
	Over the past <u>YEAR</u> how do the following questions apply to you?						
	1. How often do you have a drink containing alcohol?	Never 0	Monthly 1	2-4/ month 2	2-3/ week 3	>4/ week 4	
	NEVER skip to 9						
	2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1-2 0	3-4 1	5-6 2	7-9 3	>10 4	
	3. How often do you have SIX OR MORE drinks in one occasion?	Never 0	< Monthly 1	Monthly 2	Weekly 3	Daily 4	
	4. How often during the last year have you found that you were not able to stop drinking once you had started?	Never 0	< Monthly 1	Monthly 2	Weekly 3	Daily 4	
	5. How often during the last year have you failed to do what was normally expected from you because of drinking?	Never 0	< Monthly 1	Monthly 2	Weekly 3	Daily 4	
	6. How often during the last year have you been unable to remember what happened the night before because you had been drinking?	Never 0	< Monthly 1	Monthly 2	Weekly 3	Daily 4	
	7. How often during the last year have you needed an alcoholic drink first thing in the morning to get yourself going after a night of heavy drinking?	Never 0	< Monthly 1	Monthly 2	Weekly 3	Daily 4	
	8. How often during the last year have you had a feeling of guilt or remorse after drinking?	Never 0	< Monthly 1	Monthly 2	Weekly 3	Daily 4	
9. Have you or someone else been injured as a result of your drinking?	No 0		Yes not last year 2		Yes last year 4		
10. Has a relative, friend, doctor or another health professional expressed concern about your drinking or suggested you cut down?	No 0		Yes not last year 2		Yes last year 4		

	Score over 8 is indicative of harmful drinking.	
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No.	Filters and Questions	Answers	Go to
236	DRUG ABUSE DISORDER Over the past year other than alcohol how do these questions apply to you?		
	1. Have you used drugs other than those for medical reasons?	YES 1 NO 0	
	2. Have you abused prescription drugs?	YES 1 NO 0	
	3. Do you abuse more than one drug at a time?	YES 1 NO 0	
	4. Can you get through a week without using drugs?	YES 0 NO 1	
	5. Are you always able to stop using drugs when you want to?	YES 0 NO 1	
	6. Have you had blackouts or flashbacks as a result of drug use?	YES 1 NO 0	
	7. Do you ever feel bad or guilty about your drug use?	YES 1 NO 0	
	8. Does your family ever complain about your drug involvement?	YES 1 NO 0	
	9. Has drug abuse created problems between you and your family?	YES 1 NO 0	
	10. Have you lost friends because of your use of drugs?	YES 1 NO 0	
	11. Have you neglected your family because of drug use?	YES 1 NO 0	
	12. Have you been in trouble at work/school because of your drug use?	YES 1 NO 0	
	13. Have you lost your Job because of drug use?	YES 1 NO 0	
	14. Have been in fights when under the influence of drugs?	YES 1 NO 0	
	15. Have you engaged in illegal activity to obtain drugs?	YES 1 NO 0	
	16. Have you been arrested for possession of illegal drugs?	YES 1 NO 0	
	17. Have you ever experienced withdrawal symptoms after stopping drug use?	YES 1 NO 0	
	18. Have you had medical problems as a result of drug use (e.g. memory loss, hepatitis, convulsions, bleeding etc.)?	YES 1 NO 0	
	19. Have you gone to anyone for help for your drug use?	YES 1 NO 0	

	20. Have you been involved in a treatment program specifically related to drug use? Low drug use score 1-5, Intermediate drug use score 3-5, Substantial drug use score 6-8, Severe drug use score 9-10.	YES 1 NO 0	
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No.	Filters and Questions	Answers				Go to
237	DEPRESSION Over the past <u>TWO WEEKS</u> how often have you been bothered by the following 1. Have you had little interest or pleasure in doing things? 2. Have you felt down, depressed or hopeless? END if score less than 3 3. Have you had trouble falling or staying asleep, or sleeping too much? 4. Have you felt tired or had little energy? 5. Have you had poor appetite or been overeating? 6. Have you felt bad about yourself or that you are a failure or have let yourself or your family down? 7. Have you had trouble concentrating on things, such as reading the newspaper or watching television? 8. Do you move or speak so slowly that other could have noticed. OR so fidgety/restless that you have been moving around a lot more than usual? 9. Have you had thoughts of hurting yourself, OR that you would be better off dead? 5-9 Minimal symptoms 10-14 Minor Depression- Severe Major Depression- Mild 15-19 Major Depression- Moderate Over 20 Major Depression- Severe 10. If you had any of these problems, how DIFFICULT have these problems made it for you to do your work, take care of things at home, or get along with others?	Not at all 0 0 0 0 0 0 0 0 0 0 Not 0	7 days or less 1 1 1 1 1 1 1 1 1 Some-what 1	8 to 11 days 2 2 2 2 2 2 2 2 2 Very 2	More than 12 days 3 3 3 3 3 3 3 3 3 Extremely 3	237

No.	Filters and Questions	Answers					Go to
237	POST TRAUMATIC STRESS (>12 years of age) Over the past <u>MONTH</u> how much have you been BOTHERED by your experience with EBOLA?	Not at all	A little	Moderate	Quite a bit	Extremely	
	1. Repeated, disturbing, and unwanted memories of the stressful experience?	0	1	2	3	4	
	2. Repeated, disturbing dreams of stressful experience?	0	1	2	3	4	
	3. Suddenly feeling or acting as if the stressful experience was happening again? (reliving it)	0	1	2	3	4	
	4. Feeling very upset when something reminded you of the stressful experience?	0	1	2	3	4	
	5. Having strong physical reactions when something reminded you of the stressful experience? (ex. sweating, heart pounding, trouble breathing)	0	1	2	3	4	
	6. Avoiding memories, thoughts, feelings of the stressful experience?	0	1	2	3	4	
	7. Avoiding external reminders of the stressful experience? (ex. people, places, activities, objects)	0	1	2	3	4	
	8. Trouble remembering important parts of the stressful experience?	0	1	2	3	4	
	9. Having strong negative beliefs about yourself, others, or the world? (ex. Thoughts like: I am bad, Something is wrong with me, no one can be trusted)	0	1	2	3	4	
	10. Blaming yourself or someone else for the stressful experience or what happened after it?	0	1	2	3	4	
	11. Having strong negative feelings such as fear, horror, anger, guilt or shame?	0	1	2	3	4	
	12. Loss of interest in activities that you used to enjoy?	0	1	2	3	4	
	13. Feeling distant or cut off from people?	0	1	2	3	4	
	14. Trouble experiencing positive feelings? (ex. Unable to feel happiness or loving feelings for people close to you)	0	1	2	3	4	
	15. Irritable behaviour, angry outbursts or acting aggressively?	0	1	2	3	4	
	16. Taking too many risks or doing things that could cause you harm?	0	1	2	3	4	
	17. Being "superalert" or watchful or on guard?	0	1	2	3	4	
	18. Feeling jumpy or easily startled?	0	1	2	3	4	
	19. Having difficulty concentrating?	0	1	2	3	4	
	20. Trouble falling or staying asleep?	0	1	2	3	4	

Over 31-33 is an assessment of possible Post-Traumatic Stress Disorder.

No.	Filters and Questions	Answers	Go to
300	From 114 mark if YES	Ebola Survivor	
301	Have you ever given birth in your life?	YES NO	303
302	Total number of live births	Total	
303	Are you pregnant now?	YES NO	306
304	How many months pregnant are you? NUMBER OF FULL MONTHS	Months	
305	Is this the first time you have been pregnant?	YES NO	310
306	Have you ever had a pregnancy that miscarried, was an induced abortion or still birth?	YES NO	310
307	If you are comfortable sharing which of these ways did the pregnancy(/ies) ended? SELECT ALL THAT APPLY	Miscarriage Induced Abortion . . . Still birth	
308	When was the last pregnancy ending any of these ways?	Month Don't know Month . . Year Don't know year	

309

How many months pregnant were you when the pregnancy ended?

Months

NUMBER OF FULL MONTHS

No.	Filters and Questions	Answers	Go to
310	IF 300 MARKED EBOLA SURVIVOR: Since recovery from Ebola, have you given birth? NOT EBOLA SURVIVOR	YES NO 	315 330
311	How many times have you given birth since recovery from Ebola?	Number	
312	What has the outcome been of the birth(s)?	Healthy Premature – lived ... Died within 1 Month Still birth Other Specify Other:	
313	When was the most recent birth?	Month Don't know Month .. Year Don't know year	
314	What was the duration of the pregnancy? NUMBER OF FULL MONTHS	Months	
315	Have you been pregnant since recovering from Ebola?	YES NO	320
316	How many times have you been pregnant since recovery from Ebola?	Number	

No.	Filters and Questions	Answers	Go to
317	What has the outcome been for the pregnancy(ies) ended	Live birth Induced Abortion . . . Miscarriage	
318	When was the most recent pregnancy	Month Don't know Month . . Year Don't know year	
319	What was the duration of the pregnancy? NUMBER OF FULL MONTHS	Months	
320	Have you been wanting/trying to get pregnant but couldn't since your recovery from Ebola?	YES NO	324
321	Have you had unprotected sex to try and become pregnant since recovery from Ebola?	YES NO	324
322	Do you think having had Ebola has affected your ability to get pregnant?	YES NO	
323	How many weeks have you been trying to become pregnant?	Weeks	
324	How would you describe your menstrual cycle <u>1 YEAR</u> prior to becoming infected with Ebola	Regular periods Irregular periods No periods	325 327 326

No.	Filters and Questions	Answers	Go to
325	If regular periods how often was your menstrual cycle? CONTINUE TO 327	>1/month 1/month <1/month	
326	If no period why did you not have periods? CONTINUE TO 327	Pregnant Breast feeding Don't know why Menopause Medication Other Specify Other: 	

327 Please fill in the menstrual calendar to the best of your knowledge for **1 Year** prior to Ebola infection

Month	Flow			Menstrual Symptoms						
	Light	Normal	Heavy	Cramps	Headaches	Constipation	Diarrhea	Mood swings	Duration	No period
January										
February										
March										
April										
May										
June										
July										
August										
September										
October										
November										
December										

No.	Filters and Questions	Answers	Go to
328	How would you describe your menstrual cycle since Ebola recovery?	Regular periods Irregular periods No periods	329 331 330
329	If regular periods how often is your menstrual cycle? CONTINUE TO 331	>1/month 1/month <1/month	
330	If no period why did you not have periods? CONTINUE TO 331	Pregnant Breast feeding Don't know why Menopause Medication Other Specify Other: 	

331 Please fill in the menstrual calendar to the best of your knowledge since recovery from Ebola

Month	Flow			Menstrual Symptoms						
	Light	Normal	Heavy	Cramps	Headaches	Constipation	Diarrhea	Mood swings	Duration	No period
January										
February										
March										
April										
May										
June										
July										
August										
September										
October										
November										
December										

CONTINUE TO 336

No.	Filters and Questions	Answers	Go to
332	NON EBOLA SURVIVORS How would you describe your menstrual cycle in the previous <u>1 YEAR?</u>	Regular periods Irregular periods No periods	333 335 334
333	If regular periods how often was your menstrual cycle? CONTINUE TO 335	>1/month 1/month <1/month	
334	If no period why did you not have periods? CONTINUE TO 335	Pregnant Breast feeding Don't know why Menopause Medication Other Specify Other: 	

335 Please fill in the menstrual calendar to the best of your knowledge for the previous **1 Year**

Month	Menstrual Flow			Menstrual Symptoms						
	Light	Normal	Heavy	Cramps	Headaches	Constipation	Diarrhea	Mood swings	Duration	No period
January										
February										
March										
April										
May										
June										
July										
August										
September										
October										
November										
December										

No.	Filters and Questions	Answers	Go to
336	Are you currently married or living together as if you were married?	YES, Married YES, living as if married. NO	
337	Have you ever been married or lived together as if you were married	YES, Married YES, living as if Married NO	344
338	Was your partner infected with Ebola?	YES NO	340
339	If yes, did they survive?	YES NO	
340	What is your current marital status?	Widowed Divorced Separated	
341	What was the cause of this change in marital status?		
342	Have you been married or lived together as if married more than once?	More than once Once	344
343	How many have you been married to or lived with as if married?	Number	
344	Have you had sexual intercourse in the last 12 months?	YES NO	346

No.	Filters and Questions	Answers	Go to
345	How many times in the last 12 months have you had sexual intercourse?	Number	
346	When was the last time you had sexual intercourse? IF LESS THAN YEAR PICK DAYS, WEEKS OR MONTHS	Days Weeks Months Years	
347	Do you have sexual intercourse with people outside of marriage?	YES NO Not married	
348	How many sexual partners do you have?	Number	
349	When you have sexual intercourse do you have vaginal intercourse?	YES NO	
350	When you have sexual intercourse do you have anal intercourse?	YES NO	
351	When you have sexual intercourse do you have oral intercourse?	YES NO	
352	Have you had sexual intercourse with a survivor of Ebola?	YES NO	
353	IF 300 MARKED EBOLA SURVIVOR: Prior to Ebola were you married or did you live with a man as if you were married?	YES, Married YES, lived with man .	

		NO	355
	NOT EBOLA SURVIVOR		361

No.	Filters and Questions	Answers	Go to
354	Has this relationship ended since Ebola?	YES NO	356
355	Have you been married or lived with anyone as if you were married since recovery from Ebola?	YES NO	357
356	Is this due to stigma about your Ebola infection?	YES NO	
357	In the year prior to Ebola did you have more sexual encounters than you said for the last 12 months (Answer to 340)?	YES NO	360
358	If yes, is this due to the stigma surrounding your Ebola infection?	YES NO	
359	Did this relationship end due to your Ebola infection?	YES NO Elaborate if YES:	
360	Since recovery have you experienced decreased desire for sexual intercourse?	YES NO	362 END
361	NON EBOLA SURVIVORS In the last 12 months have you experienced decreased desire for sexual intercourse?	YES NO	END

No.	Filters and Questions	Answers	Go to
362	FEMALE SEXUAL DYSFUNCTION (>18 YOA) Select most applicable answers for LAST 4 WEEKS DESIRE/INTEREST (Score of <5)		
1	How often did you feel sexual desire/interest?	Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	5 4 3 2 1
2	How would you rate your level of desire/interest?	Very high High Moderate Low Very low/none	5 4 3 2 1
	AROUSAL (Score <12)		
3	How often did you feel sexually aroused during sexual activity?	No sexual activity . . . Very high High Moderate Low Very low/none	0 5 4 3 2 1
4	How would you rate your level of sexual arousal during sexual activity?	No sexual activity . . . Very high confidence High confidence Moderate confidence Low confidence Very low confidence .	0 5 4 3 2 1
5	How Confident were you about becoming sexually aroused during sexual activity?	No sexual activity . . . Very high confidence High confidence Moderate confidence Low confidence Very low confidence .	0 5 4 3 2 1
6	How often have you been satisfied with your arousal during sexual activity?	No sexual activity . . . Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	0 5 4 3 2 1

No.	Filters and Questions	Answers	
362 cont	Select most applicable answers for <u>LAST 4 WEEKS</u>		
	LUBRICATION (Score of <15)		
7	How often did you become lubricated during sexual activity?	No sexual activity . . . Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	0 5 4 3 2 1
8	How difficult was it to become lubricated during sexual activity?	No sexual activity . . . Extremely difficult . . . Very difficult Difficult Slightly difficult Not difficult	0 1 2 3 4 5
9	How often did you maintain lubrication until ending sexual activity?	No sexual activity . . . Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	0 5 4 3 2 1
10	How difficult was it to maintain your lubrication until ending of sexual activity?	No sexual activity . . . Extremely difficult . . . Very difficult Difficult Slightly difficult Not difficult	0 1 2 3 4 5
	ORGASM (Score of <10)		
11	When you had sexual activity how often did you reach orgasm?	No sexual activity . . . Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	0 5 4 3 2 1

No.	Filters and Questions	Answers	
362 cont	Select most applicable answers for <u>LAST 4 WEEKS</u>		
12	When you had sexual activity how difficult was it for you to reach orgasm?	No sexual activity . . . 0 Extremely difficult . . . 1 Very difficult 2 Difficult 3 Slightly difficult 4 Not difficult 5	
13	How satisfied were you with your ability to reach orgasm during sexual activity?	No sexual activity 0 Very satisfied 5 Moderately satisfied . . 4 Neutral 3 Moderately dissatisfied 2 Very Dissatisfied 1	
	SATISFACTION (Score of <10)		
14	How satisfied have you been with the amount of emotional closeness during sexual activity with your partner?	No sexual activity 0 Very satisfied 1 Moderately satisfied . . 2 Neutral 3 Moderately dissatisfied 4 Very Dissatisfied 5	
15	How satisfied have you been with your sexual relationship with your partner?	Very satisfied 5 Moderately satisfied . . 4 Neutral 3 Moderately dissatisfied 2 Very Dissatisfied 1	
16	How satisfied have you been with your overall sexual life?	Very satisfied 5 Moderately satisfied . . 4 Neutral 3 Moderately dissatisfied 2 Very Dissatisfied 1	
	PAIN (Score of <11)		
17	How often did you experience discomfort or pain during vaginal penetration?	Did not attempt 0 Almost always/always 1 More than ½ the time 2 ½ the time 3 Less than ½ the time . 4 Almost never/never . 5	

No.	Filters and Questions	Answers	
362 cont	Select most applicable answers for <u>LAST 4 WEEKS</u>		
18	How often did you experience discomfort or pain following (after) vaginal penetration?	Did not attempt Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	0 1 2 3 4 5
19	How would you rate your level of discomfort or pain during or following vaginal penetration?	Did not attempt Very high High Moderate Low Very low/none	0 1 2 3 4 5

END

No.	Filters and Questions	Answers	Go to
300	From 114 mark if YES	Ebola Survivor	
301	Have you ever fathered children in your life?	YES NO	303
302	Total number of children	Total	
303	Is anyone impregnated with your child now?	YES NO	307
304	What is your relationship with the mother?	Relationship:	
305	Is this the first time you have impregnated a woman?	YES NO	310
306	Have you ever had an unsuccessful impregnation? That miscarried, was an induced abortion or still birth?	YES NO	310
307	If you are comfortable sharing which of these ways did the pregnancy(ies) end? SELECT ALL THAT APPLY	Miscarriage Induced Abortion . . . Still birth	
308	When was the last pregnancy ending any of these ways?	Month Don't know Month . . Year Don't know year	
309	How many months pregnant was the mother when the pregnancy ended?	Months	

	NUMBER OF FULL MONTHS		
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No.	Filters and Questions	Answers	Go to
310	IF 300 MARKED EBOLA SURVIVOR: Since recovery from Ebola, have you fathered any children? NOT EBOLA SURVIVOR	YES NO 	315 324
311	How many times have you fathered children since recovery?	Number	
312	What has the outcome been of the birth(s)?	Healthy Premature – lived ... Died within 1 Month Still birth Other Specify Other:	
313	When was the most recent birth?	Month Don't know Month .. Year Don't know year	
314	What was the duration of the pregnancy? NUMBER OF FULL MONTHS	Months	
315	Have you impregnated a woman since recovery?	YES NO 	320
316	How many times have you impregnated a woman since recovery from Ebola?	Number	

No.	Filters and Questions	Answers	Go to
317	What has the outcome been for the pregnancy(ies) ended	Live birth Induced Abortion . . . Miscarriage	
318	When was the most recent pregnancy	Month Don't know Month . . Year Don't know year	
319	What was the duration of the pregnancy? NUMBER OF FULL MONTHS	Months	
320	Have you been wanting/trying to impregnate a woman but have been unable to since recovery from Ebola?	YES NO	324
321	Have you had unprotected sex to try and conceive since recovery from Ebola?	YES NO	324
322	Do you think having had Ebola has affected your ability to impregnate women?	YES NO	
323	How long have you been trying to conceive (in weeks)?	Weeks	
324	Are you currently married or living together as if you were married?	YES, Married YES, living as if married . NO	
325	Have you ever been married or lived together as if you were married	YES, Married YES, living as if married .	

		NO	332
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No.	Filters and Questions	Answers	Go to
326	Was your partner infected with Ebola?	YES NO	328
327	If yes, did they survive?	YES NO	
328	What is your current marital status?	Widowed Divorced Separated	
329	What was the cause of this change in marital status?		
330	Have you been married or lived with a woman as if married more than once?	More than once Once	332
331	How many women have you been married to or lived with as if married?	Number	
332	Have you had sexual intercourse in the last 12 months?	YES NO	334
333	How many times in the last 12 months have you had sexual intercourse?	Number	
334	When was the last time you had sexual intercourse? IF LESS THAN YEAR PICK DAYS, WEEKS OR MONTHS	Days Weeks Months Years	

No.	Filters and Questions	Answers	Go to
335	Do you have sexual intercourse with people outside of marriage?	YES NO Not married	
336	How many sexual partners do you have?	Number	
337	When you have sexual intercourse do you have vaginal intercourse?	YES NO	
338	When you have sexual intercourse do you have anal intercourse?	YES NO	
339	When you have sexual intercourse do you have oral intercourse?	YES NO	
340	Have you had sexual intercourse with a survivor of Ebola?	YES NO	
341	IF 300 MARKED EBOLA SURVIVOR: Prior to Ebola were you married or did you live with a woman as if you were married? NOT EBOLA SURVIVOR	YES, Married YES, lived with man . NO 	344 349
342	Has this relationship ended since Ebola?	YES NO	344
343	Did this relationship end due to your Ebola infection?	YES NO Elaborate if YES:	

No.	Filters and Questions	Answers	Go to
344	Have you been married or lived with anyone as if you were married since recovery from Ebola?	YES NO	346
345	Is this due to stigma about your Ebola infection?	YES NO	
346	In the year prior to Ebola did you have more sexual encounters than you said for the last 12 months (Answer to 340)?	YES NO	348
347	Is this due to the stigma surrounding your Ebola infection?	YES NO	
348	Since recovery have you experienced decreased desire for sexual intercourse?	YES NO	350 END
349	NON EBOLA SURVIVORS In the last 12 months have you experienced decreased desire for sexual intercourse?	YES NO	END

No.	Filters and Questions	Answers	
350	MALE SEXUAL DYSFUNCTION (>18 YOA) Select most applicable answer for LAST 4 WEEKS ERECTILE FUNCTION (Score of <15)		
1	How often were you able to get an erection during sexual activity?	No sexual activity . . . 0 Almost always/always 5 More than ½ the time 4 ½ the time 3 Less than ½ the time . 2 Almost never/never . 1	
2	When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	No sexual activity . . . 0 Almost always/always 5 More than ½ the time 4 ½ the time 3 Less than ½ the time . 2 Almost never/never . 1	
3	When you attempted intercourse, how often were you able to penetrate (enter) your partner?	Did not attempt 0 Almost always/always 5 More than ½ the time 4 ½ the time 3 Less than ½ the time . 2 Almost never/never . 1	
4	During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	Did not attempt 0 Almost always/always 5 More than ½ the time 4 ½ the time 3 Less than ½ the time . 2 Almost never/never . 1	
5	During Sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	Did not attempt 0 Extremely difficult . . 1 Very difficult 2 Difficult 3 Slightly difficult 4 Not difficult 5	

No.	Filters and Questions	Answers	
350 cont	Select most applicable answers for <u>LAST 4 WEEKS</u>		
6	How do you rate your confidence that you could get and keep an erection?	Very high High Moderate Low Very low/none	5 4 3 2 1
	INTERCOURSE SATISFACTION (Score of <7)		
7	How many times have you attempted sexual intercourse?	No attempts One or two Three or four Five or six Seven to ten Eleven or more	0 1 2 3 4 5
8	When you attempted sexual intercourse, how often was it satisfactory for you?	Did not attempt Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	0 5 4 3 2 1
9	How much have you enjoyed sexual intercourse?	No intercourse No enjoyment Not very enjoyable . . Fairly enjoyable. Enjoyable Highly Enjoyable	0 1 2 3 4 5
	ORGASMIC FUNCTION (Score of <7)		
10	When you had sexual stimulation or intercourse, how often did you ejaculate?	No sexual activity . . . Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	0 5 4 3 2 1
11	When you had sexual stimulation or intercourse, how often did you have the feeling of orgasm or climax?	Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	1 2 3 4 5

No.	Filters and Questions	Answers	
350 cont	Select most applicable answers for <u>LAST 4 WEEKS</u>		
	SEXUAL DESIRE (Score of <7)		
12	How often have you felt sexual desire??	Almost always/always More than ½ the time ½ the time Less than ½ the time . Almost never/never .	1 2 3 4 5
13	How would you rate your level of sexual desire?	Very high High Moderate Low Very low/none	5 4 3 2 1
	SATISFACTION (Score of <6)		
14	How satisfied have you been with your overall sex life?	Very satisfied Moderately satisfied . . Neutral Moderately dissatisfied Very Dissatisfied	5 4 3 2 1
15	How satisfied have you been with your sexual relationship with your partner?	Very satisfied Moderately satisfied . . Neutral Moderately dissatisfied Very Dissatisfied	5 4 3 2 1

END

11 Appendix 2: Study Ethics Protocol and Documentation

The protocol for this study is included in the following section and outlines the ethics approval provided by the University of Manitoba Research Ethics Board. In addition to the full ethics protocol is attached the Consent disclosure statement that every participant had to agree to in order to participate in this study. Finally in this appendix are the original ethics approvals provided from both the University of Manitoba and the Department of Health and Sanitation of the Sierra Leonian Government.

11.1 Protocol

1. Title:

Epidemiological Analysis of Long-Term Sequelae in Ebola Virus Disease Survivors in Sierra Leone

2. Investigators:

Principal investigator: Dr. Jason Kindrachuk; University of Manitoba; Department of Medical Microbiology, Winnipeg, MB, Canada, University of Sierra Leone;

Co-investigator: Dr Jia Kangbai; University of Munich

3. Collaborating institutions:

The University of Manitoba, University of Sierra Leone

4. Summary:

The intention of this study is to examine long-term health and reproductive health effects of Ebola virus disease survival in survivors from Sierra Leone.

5. Introduction:

The recent Ebola virus disease (EVD) epidemic in West Africa resulted in ~11,000 fatalities and more than 28,000 confirmed infections³¹⁸. Sierra Leone bore the greatest burden of all of the affected countries during the epidemic with more than 14,000 confirmed cases and nearly 4,000 fatalities³¹⁸. There remains little information regarding the long-term consequences of EVD on health and wellness in disease survivors which is more needed now than ever with 6000 registered survivors (i.e. part of a survivors rights group) and thousands of other unregistered/unstudied survivors dealing with potential long-term health complications²²⁶. The complications seen are due to the massive amounts of direct and indirect damage caused by Ebola virus (EBOV) infection reviewed in Basler *et al.*, and Rasmussen. but briefly, through first infecting innate immune cells the virus moves to rapidly spread throughout the body where necrosis can occur leading to massive inflammation, respiratory failure and renal failure^{4,37}. As EBOV infects all tissues survivors are now known

to deal with long term complications we still do not understand the extent of these complications.

EBOV is known to persist and actively replicate in the reproductive tract of up to 30% of male Ebola virus disease (EVD) survivors one year after infection^{226,229,230,234,235}. The frequency of filovirus persistence in the female reproductive tract is unknown at this point^{319,320}. Recent EVD epidemics have provided urgency to study this phenomenon finding sexual transmission by male EVD survivors has occurred up to 18 months after recovery^{111,221,227,237,238,319}. Data regarding sexual transmission by female survivors are inconclusive due to a lack of research, with only a couple studies with incomplete data and epidemiology^{319,320}. Furthermore, early reports of long-term complications in survivors suggest adverse pregnancy outcomes and irregular menstruation in female survivors, while complications for male survivors are unstudied at present with only anecdotal reports from our SURVIVORS association partners community suggesting infertility and reduced libido are experienced^{242,275}. In addition to reports of a myriad of health complications reportedly forcing 70% of survivors to remain physically unable to work over 2 years after recovery from the illness^{4,226}. There remains a lot of stigma surrounding survivors within their home communities which also remains understudied^{242,263,275}. This has been suggested to affect income of survivors, and the health and mental health going forward raising another key area for research and understanding what EVD survivors face in day to day life after survival^{242,275,321}. There has been some degree of persistent stigmatization of survivors years after recovery from EVD and appears to flare up with re-emergence of EBOV reported in the media²⁶³. Stigmatization from partners and close family members, in a study of women who survived Ebola in Liberia, over 50% reporting loss of a partner either through abandonment or death and only 60% reported to find new partners again due to stigma surrounding their Ebola infections²⁷⁵.

we need to determine what health and reproductive health issues they are facing long term.

In order to properly care for survivors of Ebola virus disease in Sierra Leone the health needs of the survivor population need to be identified to benefit survivor care. This will be addressed through investigating overlooked long-term health needs specifically

mental and reproductive health issues most affecting female and male survivor groups of Sierra Leone.

6. Study Objectives:

General aim: Determine the long term-health needs faced by EVD survivors of the 2014-2016 West African Ebola virus disease epidemic.

Specific aims: Determining the long-term mental health and reproductive health issues most affecting male and female survivors respectively of Sierra Leone.

7. Research design and methodology:

Ebola virus disease survivors (from Sierra Leone) will be recruited for participation in this study. Participants will be recruited through their participation in the SURVIVORS association of Sierra Leone. Recruitment through this organization is the standard route for recruitment of Ebola survivors and is meant to protect the survivors from exploitation and studies deemed to be not beneficial to the survivor community. Due to the ongoing COVID-19 pandemic for safety of research staff and participants the study will be conducted online through the *REDCap* platform where participants will have an opportunity to read through the consent disclosure statement at their own pace and contact the study investigators with any questions that may arise where further information can be provided from the full consent disclosure form. After consent is obtained a self-assessment questionnaire (approx. 1 hour in length) will be completed with relevant demographic information (including; ethnicity, language, district of residence, age, sex, gender, survivor status), clinical information (self-assessment of clinical health, health effects after recovery from Ebola, stigma question set, PTSD question set, depression question set and substance use question sets) and Male (pregnancy with partner before and after Ebola, Sexual activity before and after Ebola, Sexual dysfunction question set and libido question set) or female reproductive health (Pregnancy with partner before and after Ebola, Sexual activity before and after Ebola, Menstrual calendar before and after Ebola, Sexual dysfunction question set and libido question set).

It should be noted as applicable all questions have been previously validated for use in Sierra Leone and/or with Ebola survivors^{226,242,275,284,285,322}.

8. Study cohort:

To address the aims of this project, we will conduct this study among EVD survivors SURVIVOR association of Sierra Leone, both female and male survivors of Ebola virus disease and their close contacts (acting as control group, this has been used previously in major studies for Ebola virus disease survivors²²⁶).

1000 Ebola virus disease Survivors

9. Sample size

To detect the prevalence (frequency) of long-term effects within the survivor community of Sierra Leone (approx. 5500 females and 4500 males³¹⁸) with 95% confidence, and an error of +/- 5% we will need a cohort of at least 360 female and 355 male participants providing the described confidence levels). We aim to include as many survivors and their contacts in the community as possible allowing for recruitment up to 1000 participants.

10. Inclusion criteria:

All participants willing to take part in these studies must read consent disclosure statement at the beginning of the survey and must also meet the following conditions:

Major inclusion criteria for Ebola survivors are: Be a survivor of Ebola virus

1. Be over 18 years of age (being of age of majority)
2. Be under 50 years of age (being of reproductive age)
3. A registered survivor of Ebola virus disease with SURVIVORS association

Inclusion criteria for Close Contacts:

1. Be over 18 years of age (being of age of majority)
2. Be under 50 years of age (being of reproductive age)
3. No history of Ebola virus infection

4. Close family member or sexual partner of recruited survivor

11. Exclusion criteria

1. Younger than 18 years of age
2. Greater than 50 years of age

12. Recruitment/Consent

Recruitment:

Recruitment of Ebola virus disease survivors

It is established that recruitment of Ebola survivors in Sierra Leone for purposes of research the research groups must interact with the SURVIVORS association (system established to prevent exploitation of survivors). Therefore as is the standard practice in Sierra Leone Co-Investigator Jia Kangbai (who is in country) will share information about our study with the SURVIVORS association chairman and zonal heads. The chairman and the zonal heads determine based off the information we share determine if it is in the best interest of survivors to participate and weigh the benefits and harms. (We currently have preliminary support of the chairman, Patrick Kargbo). The zonal heads will then share study materials with SURVIVORS association members describing the purpose, requirements, risks and benefits for our study if they have decided the study is beneficial. Those interested can contact the mentioned co-investigator who will be able to answer any further questions of prospective study participants regarding types of questions, consent and their rights as a participant. Participation compensation will also be mentioned for their time they may have to take off work etc.

Recruitment of close contacts

Survivors who agree to participate will also be asked to recruit at least one close contact (who has never had Ebola) to participate in the study with them. Meeting the above mentioned inclusion and exclusion criteria for our close contact control group (Section 10). At the time of identifying the close contact they will be provided contact information for our Co-investigator to answer any further questions of prospective study participants regarding

types of questions, consent and their rights as a participant. Participation compensation will also be mentioned for their time they may have to take off work etc. The study materials describing the purpose, requirements, risks and benefits for our study if they have decided the study is beneficial will be shared with the identified close contacts.

Consent process and documentation

Participants who agree to take part in the study following recruitment and materials describing the purpose, requirements, risks and benefits and contact information for the co-investigator (in country) who will be able to answer any further questions of prospective study participants regarding types of questions, consent and their rights as a participant. They will also be encouraged to read through the consent disclosure statement at the top of the anonymous survey at their own pace. They will be offered as much time as they need. They can discuss the consent disclosure statement with whoever they need prior to continuing to the survey. The participant will have the opportunity to ask any questions that they may have through the contact information provided for the investigators of the study. Consent will be obtained by agreeing to continue on with the questionnaire. Participants are not obligated to complete the questionnaire and participation is completely voluntary. Participants are only encouraged to answer what they are comfortable with. Consent will be documented by participants continuing on with the questionnaire after the consent disclosure statement. We will be providing translations of study materials in local languages of Sierra Leone (Kiro and Mende) to accommodate participants where English is not their primary language.

Participants will be informed that they can receive up-to \$10 upon completion of the Online questionnaire through a confidential and anonymous prompt that will not retain personal or financial information. They will obtain one third of the total amount for each of the three sections of the survey completed prior to submission (regardless of the number of questions completed in each of the sections). This has become standard practice with survivors in Sierra Leone as early research on Ebola virus survivors and patients resulted in a lot of exploitation of this community by researchers.

13. Analysis/Methodology

Online survey:

Data acquisition

The survey will be administered through an online questionnaire administered through the secure online platform *REDCap*. This will allow us to anonymously, safely and securely administer the questionnaire to our participants which is an important adjustment given the safety concerns for research staff and study participants. Additionally the use of *RedCap* assists and streamlines the data processing pipeline for statistical analysis.

Statistical analysis

Differences in the prevalence of symptoms and abnormal findings between survivors and close contacts will be determined with generalized estimating equations to account for the relationships between survivors and close contacts. Variables will be considered for inclusion in the analysis based on existing literature regarding factors related to clinical sequelae among EVD survivors. They include duration of EVD illness; the number of post-EVD sequelae reported (e.g. vision issues, auditory issues, joint pain, etc.); and time since ETU discharge. To reduce the risk of a type I error, the analyses will focus on symptoms evaluated in both sexes for which the P value for the difference between survivors and close contacts will be determined. For symptoms, we will also require the difference in prevalence to exceed 10 percentage points. For the symptoms and abnormal findings that meet this threshold, relative differences in the prevalence of symptoms and abnormal findings will be summarized with odds ratios and 95% confidence intervals (survivors vs. close contacts) that have been adjusted for age, sex, district and relationship between survivors and close contacts. All statistical analyses will be performed with SAS software, version 9.4 (SAS Institute). All P values are two-sided. Supplementing these preplanned analyses, other, more exploratory associations, may be examined depending on results gathered from the online survey. These exploratory analyses may not be adjusted for type I error due to their exploratory nature. As an example, these exploratory analyses could include bivariate and multivariate logistic regression models to identify potential factors associated with failed pregnancy or male infertility as related to EVD survival.

14. Materials - equipment, supplies, personnel

A questionnaire will be administered to the study participants.

Investigators will be responsible for answering question and all other communications with study participants to maintain confidentiality and recruitment of study participants.

15. Procedures - field, laboratory, and quality control

Study participants will fill out the survey, and the data collected will be analyzed in the Medical Microbiology department at the University of Manitoba.

16. Sociodemographic and clinical data collected

Data will be collected using a standardized questionnaire on demographics. Information about age, gender, ethnicity and biological sex will be collected. Clinical data will be collected via self-assessments on previous and current health statuses previously used for EVD survivors as well as standardized question sets for substance abuse, PTSD, depression and sexual dysfunction.

17. Confidentiality/Security of data

Direct and Indirect Identifiers:

Direct Identifiers

Name, telephone number and email address will ONLY be used for the recruitment portion of the study, these will be crucial given the current COVID-19 pandemic as gathering groups in person with the SURIVORS association would not be allowed and morally a poor decision for health and safety of researchers and potential participants. For this reason name, telephone number and email address will be required to recruit participants through alternative means.

Indirect Identifiers

Biological sex and number of children, and age are important for determining the effects/previous ability to have children or what types of sexual dysfunctions may have been had by the participants and is important for determination of long term health effects. Health conditions is important to know for determination of confounding variables that may need

to be accounted for proper data analysis. Ethnicity and district of residence are important for data breakdown to inform where and who is most affected by the results obtained from this study. It is important to note the anonymous nature of this survey means none of this information can be linked back to any identifiers obtained in the recruitment process or any specific individual as the data will be presented together for all participants for each data point collected.

Protection of Identity:

The findings of the survey will be kept confidential and anonymous. All participants will remain anonymous through completion of the fully anonymous on-line survey. The indirect identifiers will never describe individual participants and will only be described in any publications, reports or presentations as a representative number of the participant cohort as a whole. Likewise the survey system will not record email addresses or IP (Internet protocol) addresses. Direct identifiers that may be used in the recruitment process (restricted to name, telephone number and email address) will be kept separate from all study data and only accessible by the principal investigator and co-investigator for recruitment purposes in a password protected and encrypted file only accessible in circumstances that meet these requirements. The encrypted file will be stored on a password protected computer requiring a user account and password separate from the file in question. The computer will be stored in a locked cabinet when not in use due to the sensitive nature of the list of identifiers in question.

Data Storage and Security:

Data Storage

The anonymous questionnaire data collected will be stored in a computer in the Department of Medical Microbiology and Infectious Diseases. Accessible on password protected computer accessible by only study personnel through individual logins and stored in a locked room when not in use for analysis.

Separately direct identifiers will be stored in a computer at the University of Sierra Leone by the co-investigator separate from all study data and only accessible by the principal

investigator and co-investigator for recruitment purposes in a password protected and encrypted file only accessible in circumstances that meet these requirements. The encrypted file will be stored on a password protected computer requiring a user account and password separate from the file in question. The computer will be stored in a locked cabinet when not in use due to the sensitive nature of the list of identifiers in question.

Data Storage Security

Data entered on computer (study database) are accessible only by the PI and members working on the study. All study data will be fully anonymous and not associated with any direct identifiers. Due to containing indirect identifiers the study database will be fully encrypted and password protected with the key to unlock only available to study personnel and will require a separate login and password for the computer housing the study database. The computer will be ensured to be fully PHIA compliant before uploading from *REDCap* platform in consultation with University of Manitoba ICT.

All personal identifiers used for recruitment will be stored completely separately in fully encrypted and password protected files on a separate computer from the anonymous study database only the principal investigator and co-investigator will have the password. This computer will also require a login and the principal investigator and co-investigator will be the only study personnel with a login for this computer. This computer will be ensured to be fully PHIA compliant before collection of recruitment information from SURVIVORS association.

Data Disposal

Questionnaire direct results will be deleted 12 months after completion of study and all other data will be retained for 10 years. Recruitment personally identifying information will ONLY be retained for the duration of the study. Disposal will be completed by erasing all relevant files from the hard drive of the associated computer.

Study Personnel and Data Security

All team members, in particularly the PhD student, have undergone relevant PHIA and FIPPA training, ensuring ethical conduct in terms of privacy and confidentiality in regards to study participants. Likewise only the principal investigator and co-investigator will have access credentials to the recruitment data files and computer.

Data Access

The principal investigator, co-investigator and the PhD student will have access to the data at all stages of active collection (PhD student only to the survey data not recruitment data), processing and analysis. The Bannatyne Campus Research Ethics Board, The Sierra Leone Ministry of Health Ethics and Scientific Review Committee and University of Sierra Leone may be asked to review research records for audit purpose. Study personnel and their tasks will be maintained in study files.

18. Participant Feedback

As this study is anonymous we will share both plain language and technical reports with the SURVIVOR association of Sierra Leone for distribution to the zonal heads to share with their communities. In consultation with the SURVIVORS association we will discuss sharing with local media for further dissemination to the wider community of Sierra Leone. We will also be publishing the data as a primary research article(s) in peer reviewed journal(s) to share our findings with the scientific community and present our findings at research association conference(s).

19. Study limitation:

The lack of records from Ebola treatment units documenting the clinical manifestations during acute EVD, due to the anonymous nature of this study prevents evaluation of associations between the severity of the acute illness and outcomes during convalescence. Close contacts will likely be identified by survivors. This may bias the make-up of the controls included however this can be corrected for during the statistical analysis. Finally, although we are focused on targeted conditions utilizing criteria outlined, many tests of significance may be exploratory in nature depending where the data leads, which increases the risk of type I error.

20. Personnel:

The co-investigator (and as the need arises the principal investigator) will be responsible for recruitment of study participants from within the SURVIVORS association. The study questionnaires will be administered, by a team of experienced researchers. All team

members have undergone all required research ethics training. The PhD student of this project will carry out the majority of analyses, while exercising his TCPS2: CORES, PHIA and FIPPA training.

21. Patient cost:

There is no cost to the participants to take part in this study.

22. Potential benefits from participation:

By participating in this study, the participants will be providing information on how Ebola virus affects survivors long-term including; frequency and severity of infections by other diseases, impact on mental health, and ability to conceive children and sexual health. We hope the information learned from this study will benefit individuals exposed to/or infected by Ebola virus through the published results. Through our team and the SURVIVORS association these results can affect health policy and improvement to care for current survivors in Sierra Leone as well as benefit the survivors of other countries and future outbreaks by better understanding their health and support needs.

23. Potential risks from participation:

There are no anticipated physical risks to participation in this study. The personal nature of some questions around sexual health could cause discomfort/embarassment during participation. As well as some questions surrounding experiences of illness with Ebola virus and community stigma following this could be difficult and cause emotional distress to some study participants. Given the history of stigmatization Ebola survivors face there could be social consequences from participation. To address these risks, first for the emotional risks we will encourage taking time and stepping away from the survey if any participants experience this as outlined in the consent disclosure statement. Additionally, If requiring a break from the survey *REDCap* allows this through the use of a Return Code that pops up when saving progress allowing continuation at a later time. Additionally we do not require an answer to any question the participants find difficult emotionally or otherwise to answer. For our mental health question sets regarding depression, PTSD and substance abuse as this is an anonymous survey we cannot direct specific individuals to professionals for help but

we will however have a pop up appear if they meet the requirements for immediate risk to themselves such as in the depressions question set if they meet the threshold for severe depression. As for the social risks, this should be minimized by recruiting through the SURVIVORS association as they already have a social support network in place minimizing the social consequences to participation. Strong supports in the SURVIVORS association should also alleviate mental and emotional health risks to participation as participants have a support network that can recognize signs of distress.

11.2 Consent Disclosure Statement

Epidemiological Analysis of Long-Term Sequelae in Ebola Virus Disease Survivors in Sierra Leone University of Manitoba

Thank-you for accessing The Ebola Virus Long Term Health and Wellness Survey on REDCap. This anonymous survey is being run by Dr. Jason Kindrachuk who is an assistant professor at the University of Manitoba and the University of Sierra Leone. Along with local researcher Dr. Jia Kangbai of the University of Sierra Leone. Please take your time to review this consent page and only continue if you fully understand the information disclosed below.

This research study is being conducted to study long term health and wellness complications experienced by Ebola survivors. Also, in some cases men and potentially women Ebola survivors could have virus for a long time in their genitals following recovery. Sometimes this leads to passing the virus to sexual partners. This is why, we are trying to understand how Ebola survival impacts the long-term health and wellness of survivors in Sierra Leone. This online survey will address a number of unanswered questions. For example, do survivors experience more or less frequent infections than prior to contracting Ebola virus (e.g. influenza; colds; malaria; COVID-19)? What impact has Ebola virus disease had on the ability to conceive children, the number of conceptions and the frequency of healthy or abnormal births? Do Ebola survivors have more risk for emotional and mental health disorders? We will address these larger questions by analyzing the answers to the questions in the survey.

The purpose of this study is to determine the number of survivors with complications of Ebola survival and what those complications are. To benefit survivor care by identifying the health needs directly affecting the survivor community in Sierra Leone.

Approximately 1000 participants will be asked to participate

The following are requirements to participate in this study:

1. Survivor of Ebola virus disease **or** close contact of a survivor
2. Being over the age of majority (over 18)
3. Being of reproductive age (under 50)

Your feedback will be collected through an anonymous online survey which will ask you a series of questions and should take ten minutes to one hour to complete, but you are encouraged to progress at your own pace.

Your participation in this anonymous online survey is completely voluntary. You are not required to provide any personally identifying information such as your name, address or telephone number, and you don't have to answer any questions you don't feel comfortable with in this survey. The survey system will not record your email address or IP (Internet protocol) address.

The risks of participating in this anonymous survey are low. The personal nature of some questions around sexual health could cause discomfort during your participation. As well as some questions

surrounding your experiences of your illness and community stigma following this could be difficult and cause emotional distress to some study participants. We encourage you to take your time and step away from the survey if you experience this. Additionally we do not require an answer to any question you find difficult emotionally or otherwise to answer.

Please Note: If requiring a break from the survey REDCap allows this however please record the Return Code that pops up as this is your method of continuing when you return to the survey.

Your Participation in this anonymous online survey is important to us and will help us understand how Ebola affects survivors of this disease long term including; frequency and severity of infections by other diseases, impact on mental health, ability to conceive children and sexual health. We will be sharing all the data produced from this study with the SL Ebola Survivor Association who will share it with the survivors community. Further action from the results of this study will be at the discretion of the SL Ebola Survivor Association membership. We hope the information learned from this study will benefit individuals exposed to/or infected by Ebola virus through the published results for future outbreaks of Ebola virus as well as health policy changes to care for current survivors. Through our team and the SL Ebola Survivor Association these results can affect health policy and improvement to care for current survivors in Sierra Leone as well as benefit the survivors of other countries and future outbreaks by better understanding their health and support needs.

You will be eligible to receive up to \$10 USD upon completion of the anonymous online survey. This will be based on how many sections of the survey are completed with each section equaling 33% of the total amount. This is meant to compensate your time commitment you have taken out of your day to complete.

All research team members have undergone all relevant privacy training for personal and health information ensuring ethical conduct and security of your information provided to the study.

Data collected will only be used to describe all participants and not single out any one participant.

If you have any questions about this survey study, please do not hesitate to contact either the principal investigator Dr. Jason Kindrachuk or co-investigator Dr. Jia Kangbai. For questions about your rights as a research participant, you may contact Government of Sierra Leone Office of the Sierra Leone Ethics and Scientific Review Committee. You are also free to direct any comments or concerns you have to the University of Manitoba Research Ethics Board.

This study has been approved by the University of Manitoba Health Research Ethics Board, The Sierra Leone Ministry of Health Ethics and Scientific Review Committee, and the University of Sierra Leone.

By continuing to the survey below this statement and completing the online survey you are consenting to participate in this online survey.

11.3 Research Ethics Board Approvals



GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee

Ministry of Health and Sanitation

11th May, 2019

To: Dr Jason Kindrachuk
Associate Professor, Department of Biochemistry
College of Medicine and allied Health Sciences
University of Sierra Leone

Principal Investigator

Jia Bainga Kangbai
Department of Environmental Health Sciences
Njala University

Co-Investigator

Study Title: Epidemiological Analysis of Long-Term Sequelae in Ebola Virus Disease Patients in Sierra Leone

Version: 27 April, 2021

Submission Type: Requesting approvals for
a) Amendment to study protocol approved 30 May, 2019
b) Extension of the period of study beyond 29 May, 2020

Partners: Ebola Survivors Association, SL

Funding: Canadian Institutes of Health Research

Documents reviewed:

- Cover letter of 27 April, 2021: Due to COVID-19 pandemic. In-person survey has been changed to an online questionnaire administered through the REDCap online platform
- Progress report of the study to date
- Clean and track changed versions to the protocol

Committee Action: Expedited Review

For further enquiries please contact: [REDACTED]



GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee

Ministry of Health and Sanitation

The Sierra Leone Ethics and Scientific Review Committee (SLESRC) having conducted an expedited review of the above amended study protocol and determined that it presents minimal risk to subjects, **hereby grants ethical and scientific approval for it to be conducted in Sierra Leone.** The approval is valid for the period, **29 May, 2021 – 28 May, 2022.** It is your responsibility to obtain re-approval/extension for any on-going research prior to its expiration date. The request for re-approval/extension must be supported by a progress report.

Review Comments:

- **Amendments:** Intended changes to the approved protocol such as the informed consent documents, study design, recruitment of participants and key study personnel, must be submitted for approval by the SLESRC prior to implementation.
- **Termination of the study:** When study procedures and data analyses are fully complete, please inform the SLESRC that you are terminating the study and submit a brief report covering the protocol activities. Individual identifying information should be destroyed unless there is sufficient justification to retain, approved by the SLESRC. All findings should be based on de-identified aggregate data and all published results in aggregate or group form. A copy of any publication be submitted to the SLESRC for its archive.
- **Amendment approved and period of study extended to 28th May, 2022**


Professor Hector G. Morgan
Chair



For further enquiries please contact: efoday@mohs.gov.sl



University
of Manitoba

Research Ethics and Compliance

Research Ethics Bannatyne



HEALTH RESEARCH ETHICS BOARD (HREB) CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR: Dr. Kenneth Jason Kindrachuk	INSTITUTION/DEPARTMENT: University of Manitoba and Sierra Leone - Ministry of Health and Sanitation/Medical Microbiology and Infectious Diseases	ETHICS #: HS24515 (H2020:538)
HREB MEETING DATE (If applicable):	APPROVAL DATE: December 6, 2021	EXPIRY DATE: December 14, 2022
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable): NA		

PROTOCOL NUMBER: NA	PROJECT OR PROTOCOL TITLE: Epidemiological Analysis of Long-Term Sequelae in Ebola Virus Disease Survivors in Sierra Leone
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: Canada Research Chair Funds	

Submission Date of Investigator Documents: October 15, 2021	HREB Receipt Date of Documents: October 15, 2021
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REVIEW CATEGORY OF ANNUAL REVIEW: Full Board Review ☐ Delegated Review ☒

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name (if applicable)	Version (if applicable)	Date

Annual approval

Annual approval implies that the most recent **HREB approved** versions of the protocol, Investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.

Consent and Assent Form(s):

Consent Form For Ebola Survivors

17/11/2020

CERTIFICATION

The University of Manitoba (UM) Health Research Board (HREB) has reviewed the annual study status report for the research study/project named on this **Certificate of Annual Approval** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM HREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

HREB ATTESTATION

The University of Manitoba (UM) Health Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE

The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

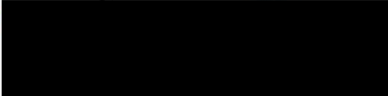
CONFLICT OF INTEREST

Any Principal or Co-Investigators of this study who are members of the UMHREB did not participate in the review or voting of this study.

CONDITIONS OF APPROVAL:

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of annual approval.** A Bannatyne Campus Annual Study Status Report must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the HREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM HREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report.**

Sincerely,


John Arnett, PhD. C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

12 Appendix 3: Appended Tables of Chapter 6

Table 12.1: Employment status before and after EVD

Employment Prior to Ebola		
No	497	85.1%
Yes	87	14.9%
Employment Following Ebola		
No	505	86.0%
Yes	82	14.0%

Table 12.2: Employment types of participants

Employment		
Teacher	12	2.0%
Trades	27	4.5%
Banker	3	0.5%
Craftsperson	10	1.7%
Medical worker	18	3.0%
Day laborer	8	1.3%
Shopkeeper	5	0.8%
Other	14	2.4%
Bike Rider	0	0.0%
Driver	0	0.0%
Farmer	5	0.8%
Interior Designer	0	0.0%
Self Employed	0	0.0%
Nurse	1	0.2%
Tailor	0	0.0%
Taxi Driver	0	0.0%
Traditional		
Healer	0	0.0%
Caregiver	3	0.5%
Cleaner	1	0.2%
Keke Rider	1	0.2%
Maid	0	0.0%

Table 12.3: EVD survivor relationship status prior to infection

	Relationship Status		
	Prior to EVD		p-value
Female			0.001
Single	172	61.0%	
Married	59	20.9%	
Relationship	51	18.1%	
Male			
Single	185	72.8%	
Married	44	17.3%	
Relationship	25	9.8%	

Table 12.4: EVD survivor relationship change

Ebola Survivor Prior Relationship				p-value
		Ended		
Female				0.060
No	66	61.7%		
Yes	41	38.3%		
Male				
No	52	75.4%		
Yes	17	24.6%		

Table 12.5: EVD survivor relationship since recovery

	Relationship Since EVD		p-value
Female			0.006
No	194	69.3%	
Yes	86	30.7%	
Male			
No	204	79.7%	
Yes	52	20.3%	

Table 12.6: Change in residence of EVD survivors by survivor status

	No		Stigma Yes		Total	
Residence Change						
No	123	45.2%	126	39.6%	249	42.2%
Yes	149	54.8%	192	60.4%	341	57.8%

Table 12.7: Province movement of EVD survivors who say they changed residence

	Provinces	
Current Residence		
Northern	16	6.5%
Eastern	100	40.5%
Southern	66	26.7%
Western	4	1.6%
Northwest	61	24.7%
Prior to EVD		
Northern	19	7.6%
Eastern	93	37.3%
Southern	62	24.9%
Western	9	3.6%
Northwest	66	26.5%

Table 12.8: Residence change at a sub-district level for those who changed residence following recovery

	District Location	
Current Residence		
City	20	8.1%
Town	136	55.3%
Village	90	36.6%
Prior to EVD		
City	17	6.9%
Town	113	45.9%
Village	114	46.3%
Do Not Remember	2	0.8%