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Ebola vaccination in the Democratic Republic of the Congo: Connections with Ebola exposure history, risk behaviors, and serology

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Ebola vaccination in the Democratic Republic of the Congo: Connections with Ebola exposure history, risk behaviors, and serology

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by

Anna Marie Bratcher

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ABSTRACT OF THE DISSERTATION

Ebola vaccination in the Democratic Republic of the Congo:

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By

Anna Marie Bratcher

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2022

Professor Anne W. Rimoin, Chair

Recent control efforts for Ebola Virus Disease (EVD) outbreaks in the Democratic Republic of Congo (DRC) employed a highly effective recombinant vesicular stomatitis virus-Zaire Ebola vaccine (rVSVΔG-ZEBOV-GP). Given the previously limited opportunities to study EVD vaccination in real-world outbreaks, little is known about this new vaccine in practice beyond its notable efficacy and safety. This dissertation aims to expand our knowledge of EVD vaccination to better understand the new landscape of EVD outbreaks which now includes effective vaccination and treatment. This dissertation focuses on studying the effects of EVD exposure history (being exposed to a family member with EVD, a close contact with EVD, a contact of a contact with EVD, or being potentially exposed to a patient with EVD in a healthcare setting) on post-vaccination transmission behaviors and vaccine immunogenicity. Additionally, this dissertation looks at how vaccination impacts occupational transmission risk

among healthcare workers affected by EVD outbreaks in the DRC. Chapter 1 is a brief introduction to Ebola virus, EVD, and Ebola vaccination with a focus on the DRC. Chapter 2 uses longitudinal data from a vaccinated Congolese cohort to show that healthcare workers have unique post-vaccination risk behavior profiles. Chapter 3 uses another Congolese cohort to show that there are few longitudinal differences between vaccinated and unvaccinated healthcare workers, with the exception that vaccinated individuals are more likely to participate in funeral rites during a period containing a resurgent outbreak. Chapter 4 uses g-computation to assess the causal structure underlying EVD exposure history, baseline antibody titer, and vaccine immunogenicity. This chapter shows that while healthcare workers have lower titers at 21 days post vaccination, this does not seem to be mediated by baseline antibody titer. However, an increased baseline titer led to a more robust immune response to vaccination, regardless of EVD exposure history. Finally, Chapter 5 discusses in detail the overall strengths, limitations, and conclusions from this dissertation.

This dissertation of Anna Marie Bratcher is approved.

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University of California, Los Angeles

2022

This dissertation is dedicated to my family and friends for their support throughout my whole career.

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Chapter 1: Introduction

Ebola virus and the disease it causes, Ebola Virus Disease (EVD), are widely known. Since its emergence in 1976, Ebola has been adopted into the pop culture pantheon of doomsday threats.[10–13] Over the decades, new Ebola outbreaks have generally received a flurry of attention from the general public that fades over time, regardless of the state of an outbreak. This pattern is unmindful of the numerous nuances and recent developments in Ebola research.

Modern Ebola outbreaks occur in an increasingly complex landscape. Recent global developments, such as the SARS-CoV-2 pandemic and ecologic change, have had dramatic effects on epidemiology in tropical Africa where EVD outbreaks occur. SARS-CoV-2 has decimated local healthcare systems and complicated health delivery.[14] Ecologic changes have drastically impacted the risk of emerging infections.[15–17] These global changes mean that Ebola outbreaks are not as rare and isolated as they once were; The risk of devastating EVD outbreaks has significantly grown over the past two decades.

Beyond global changes, there have also been Ebola-specific developments. Effective vaccines and treatments have been recently developed (discussed in detail below). Furthermore, the several, recent EVD outbreaks in North Kivu and Equateur provinces, DRC, are particularly unique from historical outbreaks. Both areas experienced recurrent outbreaks, which are outbreaks that occur in a location which has had a prior outbreak.[18] In these recurrent outbreaks, there was genetic evidence that some cases were due chains of transmission that originated from persistent shedding of virus by a survivor of the previous outbreak while other cases were a result of animal-to-human transmission to a single index case.[19] This evidence has complicated our understanding of what is possible in EVD outbreaks and resurgence.

The above changes have resulted in a field of research that looks very different from the early days of Ebola emergence. Additionally, our understanding of Ebola is still being developed at a rapid pace. This introduction will summarize the current knowledge about Ebola virus with a focus on nuances in disease transmission. It will provide a thorough background on Ebola vaccination, behaviors involved in transmission, and various types of EVD exposure, which are the focus of the proposed research.

1.1 Ebola virus and Ebola Virus Disease: Emergence and Epidemiology

Briefly, Ebola virus is a zoonotic filovirus spread through exposure to infected bodily fluids or contact with an infected human or animal.[20,21] Ebola virus causes EVD, which is characterized by flu-like illness followed by a severe stage which commonly includes hemorrhagic complications and multiple organ failure.[21] Since its discovery in 1976, there have been periodic EVD outbreaks across West and Central Africa. Given the severity of EVD, Ebola is an important public health threat in Central Africa. Additionally, the threat of an outbreak spreading beyond endemic areas and the possibility that Ebola virus could be used as for biological terrorism makes Ebola a global concern.[20,22]

Though Ebola has become a prominent disease across the world, it is also a relatively new one. The first recorded cases of Ebola virus-caused hemorrhagic fever were reported from two separate locations in 1976: southern Sudan and northern Zaire, now the DRC.[20] Recognized as a new disease at that time, Ebola virus was named for a river near the northern Zaire outbreak.[28] It was later discovered that these two outbreaks were caused by different species of Ebola virus, and therefore were independent of one another.[25]

Since its discovery in 1976, Ebola virus has continued to cause periodic outbreaks across central Africa.[23] The smallest of these outbreaks have been single cases. However, in the period before the 2010's, case counts for an EVD outbreak could reach into the four hundreds.[23] As these outbreaks continued through the decades, more species of Ebola virus were discovered in addition to those that caused the first two recorded outbreaks in Sudan and Zaire.[25]

These outbreaks were overall sporadic and unpredictable. However, some patterns emerged. EVD outbreaks tended to occur on rural, heavily forested areas.[23] Encroachment into the forest due to deforestation or an increase in wildlife hunting emerged as ecological risk factors for an EVD outbreak.[21,29–31] EVD outbreaks also seemed to become larger as the affected area became less rural. One example of this is the Kikwit outbreak in 1995, which occurred in a larger city than other outbreaks and had an official case count of 315 cases.[32] Additionally, once outbreaks were established, nosocomial transmission became a large contributing factor the the spread of many EVD outbreaks.[33]

After the year 2000, there was a notable increase in outbreaks and cases.[25] This pattern of increasing severity seemed to lead up to the 2014-2016 West Africa Ebola epidemic. This outbreak eventually totaled 28,610 confirmed cases and 11,308 (39%) deaths.[23] Since this epidemic affected such a large number of individuals for an extended period of time, there was a wealth of epidemiologic and virologic knowledge discovered during this outbreak. Much of the information presented in this introduction comes from the studies performed during the West Africa epidemic.

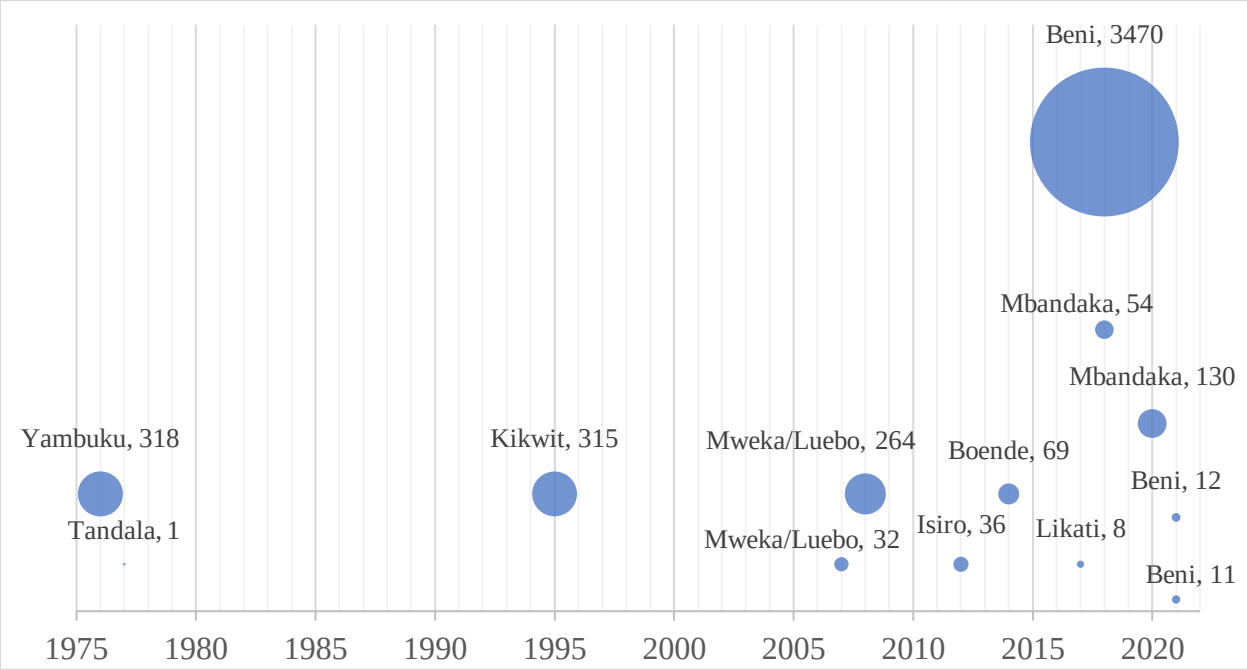
However, despite the resulting great strides in Ebola prevention and control, EVD outbreaks still occur and continue to show an increasing severity. This is evidenced by the recent 2018-2020 EVD outbreak in Eastern DRC, which reached 3,470 cases with 2,287 (66%)

deaths.[34] Additionally, there has been multiple recurrent outbreaks both in Eastern DRC and Equateur province over the past few years. These recent outbreaks demonstrate that work is still needed to ensure effective control of EVD outbreaks, particularly an improved understanding of EVD human-to-human transmission in the face of new prevention and treatment techniques.

1.2 Ebola in the Democratic Republic of the Congo

Of the many sporadic EVD outbreaks, thirteen have occurred in the Democratic Republic of the Congo (DRC), making the DRC a high risk country for EVD.[23] Not only has EVD surfaced multiple times in the DRC, but it has *consistently* surfaced. EVD was not only discovered in DRC, but the five most recent EVD outbreaks have occurred within the country.[23] Between discovery and today, EVD outbreaks have occurred throughout the decades in DRC, though occurring more frequently after the year 2000, like EVD outbreaks in general.[23] Figure 1.1 shows the years and location of EVD outbreaks in the DRC as of the time of this writing. In addition to these confirmed cases of EVD, many places in DRC show a non-zero seroprevalence of antibodies to Ebola.[35,36]

Figure 1.1 Location and years of EVD outbreaks in the DRC.[37]



Caption: Labels describe location and case count; size of bubble is proportional to case count.

These five most recent outbreaks occurred temporally close together, but on separate sides of the country. The first of these outbreaks occurred between May and August of 2018 in the North-Western part of DRC, in the Equateur province.[23] This outbreak was relatively quick and small for an EVD outbreak in the DRC, possibly due to the remoteness of the affected areas. In contrast, the second of these three outbreaks, affected over 3,000 individuals and occurred over a period of nearly two years. This outbreak affected the war-torn Eastern region of the country in 3 provinces: North Kivu, South Kivu, and Ituri.[34] This outbreak is now the second largest on record, larger than all others except for the West African epidemic.[23] Despite the use of new vaccines and therapies, EVD transmission continued at high enough levels to sustain this outbreak for a unusually long period of time.

Following these two outbreaks, there was one recurrent outbreak in Equateur and two recurrent outbreaks in North Kivu, including one currently active.[38] Hope remains that this outbreak will be resolved quickly with minimal cases.

1.3 Ecology

The sporadic periodicity outbreaks of EVD are a result of its zoonotic origins. Since Ebola virus is not constantly in the human population, the occurrence of EVD outbreaks is dependent on zoonotic emergence from a reservoir species in which the virus persists. The reservoir species for Ebola viruses that cause EVD have not yet been definitively identified (which would require the isolation of Ebola virus from naturally infected animals). [25] However, there is compelling evidence that bats may act as reservoirs.[39–43]

Apes, man, and any other mammalian species that experience disease from Ebola virus infection are considered end hosts, and are not eligible to be reservoirs.[44] Great apes in central Africa have experience devastating outbreaks alongside human outbreaks.[45,46] In some cases, these outbreaks have been severe enough to significantly reduce gorilla and chimpanzee populations.[47]

Emergence from a reservoir species would explain the sporadic occurrence of EVD outbreaks in equatorial Africa.[25] Each EVD outbreak is suspected to initially occur through direct contact between infected blood, body fluids, or tissues of an animal and the first human infected in that outbreak.[48] Exposure to the blood, body fluids, and tissues of possibly infected animal is common in Ebola affected areas due to the prevalence of wildlife hunting and consumption in these areas.[49,50] Furthermore, this zoonotic emergence also explains the increasing rate of EVD outbreaks. As deforestation and hunting due to economic reasons increases over time in

equatorial Africa, the risk of EVD outbreaks increases due to higher rates of human-animal contact.[21,29–31]

1.4 Properties of the virus

Currently, four species of ebolaviruses are known to cause EVD in humans: Zaire ebolavirus, Sudan ebolavirus, Tai forest ebolavirus (formerly Côte d'Ivoire ebolavirus), and Bundibugyo ebolavirus.[48] Two additional species have been isolated, but are not known to cause EVD in humans. The first, Reston ebolavirus, is known to have caused disease in non-human primates and pigs. The second, Bombali ebolavirus, was recently discovered in bats and has not been implicated in any animal disease as of the time of this writing.[48,51] All six of these ebolavirus species belong to the *ebolavirus* genus which, along with the genus *Marburgvirus*, forms the *Filoviridae* family.[26] Viruses in this family are commonly referred to as "Filoviruses" because of their characteristic long filamentous particles.[25] The *Filoviridae* family belongs to the order *Mononegavirales*.[26]

Ebolavirus are linear, negative-stranded RNA viruses.[25,26] These particles are generally 80nm in diameter but vary in length, and can be up to 14,000 nm long. This size makes an electron microscope necessary to view virions. The ebolavirus genome has seven genes that code for the following proteins: nucleoprotein (NP), virion protein (VP) 25, VP40, glycoprotein (GP), VP30, VP24, and RNA-dependent RNA polymerase (L).[25] Figure 1.2 shows the orientation of these proteins in an assembled virion.[52]

Figure 1.2 Ebolavirus structure and proteins

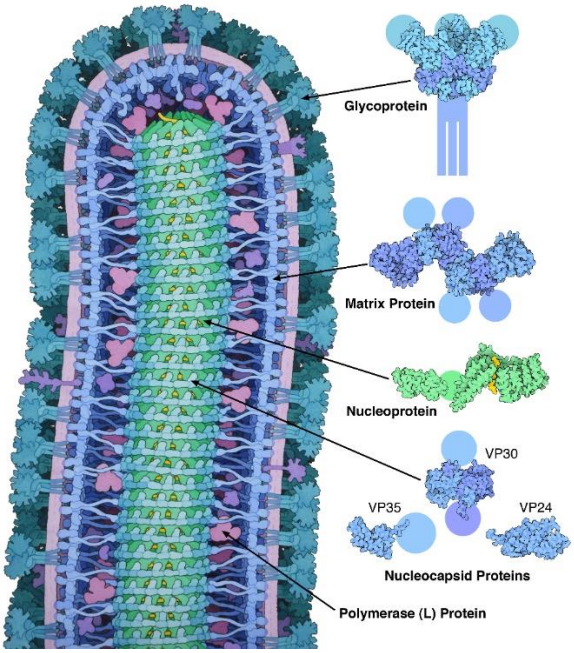


Figure from: PDB-101: Molecule of the Month: Ebola Virus Proteins. https://pdb101.rcsb.org/motm/178. Accessed April 24, 2020. [52]

1.5 Clinical characteristics of Ebola Virus Disease

Symptoms in EVD patients tend to appear 7 days after infection, but this incubation period can range from 2-21 days. The symptoms tend to happen in two phases: an initial milder stage that is characterized by "flu-like" symptoms along with vomiting and diarrhea, and a second severe stage that commonly includes hemorrhage and multiple organ failure. EVD presentation can include gastrointestinal, vascular, neurological, respiratory, and general symptoms.[25,26] A characteristic macropapular rash commonly occurs between 5 and 7 days of illness, which is followed by desquamation (peeling of the skin) in non-lethal cases.[25]

One study of EVD patients in Conakry, Guinea during the 2014-2016 West Africa outbreak thoroughly described the attack rate for common EVD symptoms.[53] This study found

the following frequencies of symptoms among 37 EVD cases confirmed through RT-PCR assays in the laboratory:

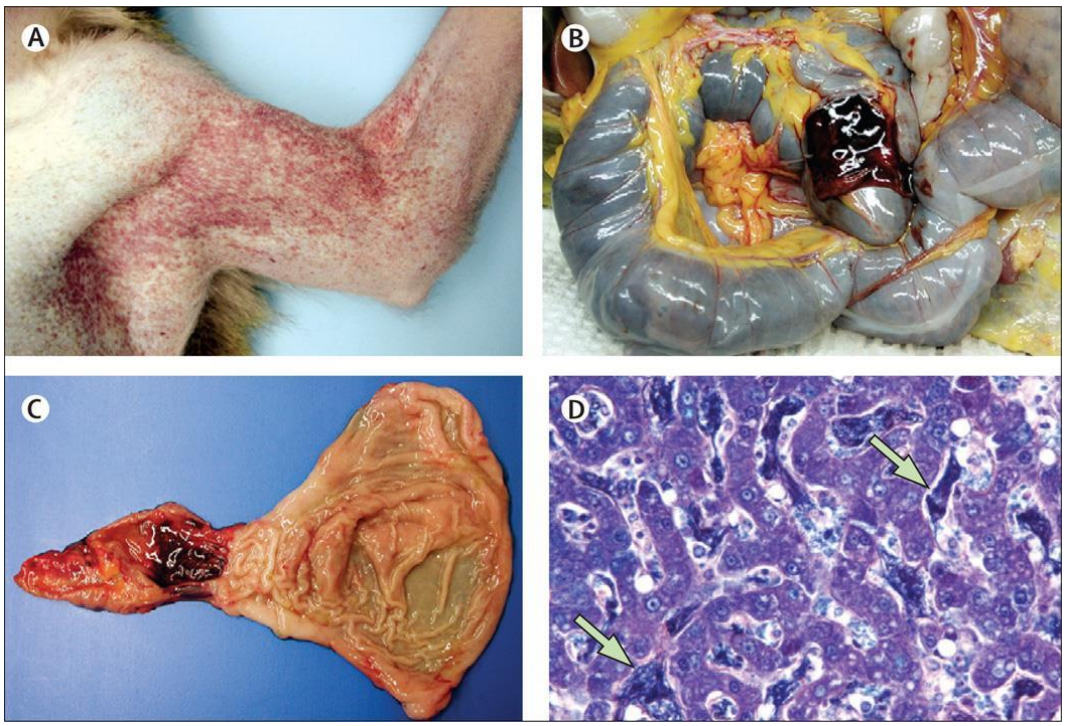
Table 1.1 Symptoms of 37 Patients with Confirmed Ebola Virus Disease (EVD), Conakry, Guinea, 2014[53]

	Count	Percent
Initial clinical stage symptoms		
Fever	31/37	84
Fatigue	24/37	65
Diarrhea	23/37	62
Headache	12/21	57
Vomiting	21/37	57
Anorexia	16/37	43
Severe clinical stage symptoms		
Mortality	16/37	43
Renal failure	2/37	5
Seizure	2/37	5
Oral candidiasis	1/37	3
Hypoxemia	1/37	3
Hemorrhage		
Any	19/37	51
Gastrointestinal	9/37	24
Subconjunctival	4/37	11
Intravenous catheter site	4/37	11
Nasorespiratory tract	2/37	55

EVD also has a severe hematological impact which occurs primarily during the peak of infection.[25] The most common cellular hematological changes are leukopenia (low white blood cell count), lymphopenia (low level of lymphocytes in the blood), and decreased neutrophil counts. It should be noted that lymphocyte die offs are not a result of direct infection of these cells by the virus, though the true mechanism for this apoptosis remains unknown.[25] These

changes occur in conjunction with an increase in liver enzymes. As EVD progresses, patients commonly develop hematological symptoms that inhibit clotting: thrombocytopenia, longer pro-thrombin and partial thromboplastin times.[26] Hemorrhages visually manifest in petechiae (tiny spots on skin caused by bleeding), ecchymoses (skin discoloration due to bleeding), bleeding from venipuncture sites, mucosal hemorrhages, and visceral hemorrhages that can be viewed post-mortem.[25] Figure 1.3 displays some of these hemorrhagic manifestations in non-human primates.[25] Coagulation issues pair with fibrin degradation products that are formed when clots degrade to cause disseminated intravascular coagulation (DIC). This clotting throughout the body contributes to the multiple organ failure seen in EVD patients.

Figure 1.3 "Haemorrhagic manifestations noted in non-human primates infected with Ebola virus" *



"Petechiae on the arm and axillary region of a Cynomolgus monkey infected with Sudan ebolavirus (A). Also shown are haemorrhages in the ileum (B) and a gastroduodenal lesion (C) from a Cynomolgus monkey infected with Sudan ebolavirus and fibrin thrombi (arrows) in sinusoids of a rhesus monkey infected with Zaire ebolavirus (D)."*

Ebola virus infects a large range of cell types, but monocytes, macrophages, and dendritic cells are preferred replication sites. These cells contribute to the dissemination of the virus to regional lymph nodes, liver, and spleen.[25] It is thought that the infection and activation of antigen-presenting cells contributes to severe late stages of disease. The release of inflammation-promoting cytokines and chemokines by these cells impairs vascular and coagulation systems in a way that resembles septic shock, causing multiple organ failure. While DIC was once thought to be the major damaging pathology of EVD, recent findings have implicated immune dysregulation, through interferon inhibition by VP35, and resulting blooms of gut flora as more destructive.

While EVD can cause severe disease in anyone, certain groups are known to have particularly poor outcomes. Pregnant women who experience EVD have been noted to be at higher risk of miscarriage.[25] Pregnant women can experience EVD symptoms that mimic labor or pregnancy complications such as vaginal bleeding and fever, and therefore present a challenge to diagnosis.[54] Additionally, children of infected mothers have a high death rate. It is suggested that this high death rate could be from mother-child transmission either through close contact or milk.[25] Furthermore, case fatality has been shown to be higher among children less than 2 years. In one cohort, children with EVD most commonly presented with weakness, fever, distress, loss of appetite, diarrhea, and cough.[55] Age also increases risk of death in EVD patients.[56,57]

* Figure title and caption quoted directly from source.[25]

If a patient with EVD recovers, clinical presentation improves as antibody response mounts.[26] These recoveries typically occurs between day 6 and 11 of disease. Non-fatal cases mount specific IgM and IgG responses and an early, strong inflammatory response that includes interleukin β, interleukin 6, and tumor necrosis factor α (TNFα).[25] Absence of this antibody response is sometimes found in lethal cases.[58] After recovery from acute disease, EVD survivors may experience long term sequalae. These long-term complications include recurrent hepatitis, myelitis, prolonged hair loss, psychosis, and uveitis.[25,59] Of particular importance is the persistence of ocular disorders as a result of uveitis after recovery from EVD, which occurs in approximately 15-20% of survivors.[60–64]

It should be noted that there is increasing evidence that not all Ebola virus infections lead to EVD.[65–67] However, the literature contains conflicting accounts for the prevalence of such asymptomatic cases surrounding EVD outbreaks. One study among individuals in EVD affected households in Sierra Leone suggested that this was not a common phenomena, as only 2.6% of close contacts with high exposure levels had confirmed asymptomatic Ebola virus infection.[68] Meanwhile, another study found evidence of Ebola virus infection among 11 of 24 (46%) close contacts without EVD symptoms.[69] There has also been research on seroprevalence of antibodies to Ebola among various populations not in close contact with EVD cases. One systematic review considered studies that found various levels of seroprevalence in asymptomatic populations, from 0-46% of a population having antibodies to Ebola virus.[35]

1.6 Mortality due to Ebola Virus Disease

For fatal EVD cases, death typically occurs 6-16 days after the onset of symptoms during the severe, hemorrhagic stage of disease. Mortality from EVD varies by species and outbreak.

Zaire ebolavirus is most lethal with historical case-fatality rates from 50-90%, though there is evidence that intense symptomatic treatment can improve outcomes.[25,26] However, these rates were found in outbreaks prior to the discovery of Ebola-specific treatments described below.

Route of disease transmission seems to play a role in the disease outcome. In the early 1976 Zaire ebolavirus outbreak, 100% of cases that were contracted through injection were ultimately fatal. This was significantly more than the 80% case-fatality rate among cases contracted through direct contact. This pattern of transmission route specific case-fatality rate has also been shown to occur in non-human primate models, where injection with Ebola virus is typically more deadly than an aerosol challenge. [26]

1.7 Treatment of Ebola Virus Disease

Treatment for EVD consisted of symptomatic and supportive care until recently.[25,26] This changed in December 2019 with the publication of results from the PALM trial, a randomized, controlled trial of therapeutics for EVD.[6] This trial tested four possible therapeutics on patients in the Eastern DRC EVD outbreak which started in August 2018. The four therapies delivered intravenously were: triple monoclonal antibody ZMapp, remdesivir (an antiviral), single monoclonal antibody Mab114, or triple monoclonal antibody REGN-EB3.[6] The ZMapp group was considered the control group for comparisons between the therapeutics.

Overall, MAb114 and REGN-EB3 performed significantly better than ZMapp in reducing deaths at 28 days. Overall, 49.7% of the ZMapp group had died by the 28-day timepoint. MAb114 showed a -14.6 percent reduction in deaths (95% CI from -25.2 to -1.7), while REGN-EB3 showed a -17.8 percent reduction in deaths (95% CI from -28.9 to -2.9) when compared to the ZMapp group.[6] Overall, this outbreak has experience an approximate 65.8% case fatality rate (using case and death counts as of April 28th, 2020).[34]

MAb114 and REGN-EB3 also showed efficacy in reducing the time until a patient returned negative on RT-PCR assay for EBOV nucleoprotein. Additionally, these treatments appeared to be more efficacious if administered closer in time to the onset of symptoms. For each day with symptoms that a treatment was delayed, a patient had a 11% increase in odds of death. While there were some adverse events, only four events in three patients (of the 681 patients in the study) experienced events that were possibly related to the treatments, as determined by an independent pharmacovigilance committee. Even so, these events could not be distinguished from complication from EVD itself.[6]

1.8 Ebola vaccines

There are currently two Ebola virus vaccines that are forefront in the field. One is a 2-dose heterologous vaccine regimen under development by Janssen Vaccines and Prevention. This vaccine involves an initial injection of Ad26.ZEBOV, which expresses Zaire ebolavirus (Mayinga variant) glycoprotein, and a MVA-BN-Filo booster, which expresses the same glycoprotein as well as Sudan ebolavirus (Gulu variant) and Marburg virus (Musoke variant) glycoproteins and Tai Forest virus nucleoprotein.[70] This vaccine has been well tolerated in studies, and has shown to be immunogenic in healthy volunteers.[70,71] However, this vaccine is still undergoing trials at the time of this writing.

The second vaccine is the a highly effective recombinant vesicular stomatitis virus-Zaire Ebola vaccine (rVSVΔG-ZEBOV-GP) developed by Merck.[3,4] This vaccine has been used in the recent EVD outbreaks in Eastern DRC, having been provided to more than 300,000 individuals in the affected area since August 2018 when the outbreak response began.[34] This vaccine was initially developed in response to the 2014-2016 West Africa EVD outbreak, and

has been tested in multiple studies and clinical trials.[72] These initial studies hinted at a high level of efficacy and safety for this vaccine.[3,4,73]

In one previous study, PREVAIL I, positive antibody responses occurred in 2.5%, 83.7%, 78.4% and 79.5% at one week, one month, six months, and 12 months, respectively.[73] This was significantly more when compared to 1.5%, 2.8%, 5.7%, and 6.8% with positive responses among the placebo groups at the same time points.[73] An antibody response was considered positive if the $\log_{10}$ titer increased by a factor of 4 from the baseline value if the baseline value was not elevated (below 607 Enzyme-linked immunosorbent assay Units (EU)/mL, as determined by 3 standard deviation above the mean antibody titer in a cohort of 92 adults in Mali that had not had a history of EVD cases).[73] In another study, Ebola ca Suffit!, cluster of EVD individuals exposed to EVD were randomized to immediate vaccination or vaccination delayed by 21 days.[4] For randomized clusters, no cases of EVD occurred between 11 and 21 days after randomization among immediate vaccinated clusters versus 16 cases in the same time period for delayed clusters. Therefore, vaccine efficacy was estimated at 100% (95% CI 68.9-100 percent).

While this vaccine has shown remarkable efficacy in the described trials, there is limited research into the effectiveness of this vaccine in real-world outbreaks. The WHO released preliminary results in April 2019 for the vaccine's performance in the current Eastern DRC outbreak.[74] In this report, 71 out of 93,965 vaccinated individuals contracted EVD after vaccination. However, only 15 of these individuals had symptom onset more than 10 days after vaccination. Of these 15 individuals, 6 were high risk contacts, 7 were healthcare workers, and 2 were contacts of a contact of an EVD case. Therefor the estimated efficacy for those with onset of illness 10+ days post vaccination was 97.5% (95% CI 92.4-99.1%).[74] Ultimately, we do not currently have a thorough knowledge of the correlates of EVD protection, and therefore cannot

make any definitive statements around the protection generated by vaccines based on antibody response alone.

1.9 Transmission and prevention

There are two broad categories of Ebola virus transmission: animal-to-human and human-to-human spread. Generally, outbreaks of EVD are initiated by an animal-to-human, or zoonotic, transmission event and then are propagated and sustained through human-to-human spread.[25]

Animal-to-human transmission has commonly been implicated as the source of EVD outbreaks.[25,45] Many outbreaks have been traced to fruit bats and non-human primates.[45,49,75] Transmission from these animals into the human population commonly occurs due the handling of infected carcasses, sometimes in preparation for human consumption.[25,45] While the cooking of meat should kill any virus within the animal, it is possible that ingestion of contaminated food could be a cause of zoonotic transmission to humans.[25]

Human-to-human transmission occurs through contact of infected body fluids with mucosal surfaces, abrasions and injuries in the skin, or by direct parental transmission.[26] In actively ill patients, the virus has been detected with RT-PCR in nearly every type of body fluid.[76] However, only blood and semen returned culture-positive results.[76] Therefore, blood and semen of actively ill patients seem to show the highest infectious risk. Furthermore, infected body fluids carry the highest viral load soon after death and are most infectious during this period. However, there is also risk of transmission from individuals who are in the convalescent phase of the disease, as Ebola virus has been found in select body fluids in affected patients. Fluids that are known to contain Ebola virus RNA during the convalescent phase are semen, aqueous humor (fluid inside of the eye), sweat, urine, vaginal secretions, conjunctival fluid,

feces, and breast milk.[77] The presence of RNA in these fluids can persist for up to two months, with the exception of semen, which can harbor viral RNA for more than two years.[77,78] Other then bodily fluids, there has been some evidence of transmission through direct contact in the absence of body fluids.[79,80] Aerosol and airborne transmission is less likely.[80–82]

As a result of these infection dynamics, there are particular behaviors that heighten risk of human-to-human EVD transmission. General community members may participate in the following risky activities: attending a funeral, having direct exposure to human remains, participating in funeral rites, receiving an injection, and attending a healthcare center. Additionally, healthcare and front line workers participate in specific activities that intensify nosocomial EVD transmission.[33,83] These healthcare specific behaviors include patient interaction, exposure to bodily fluids through means such as a needlestick or sharps, and PPE usage.

Prevention of Ebola virus infection beyond vaccination involves the cessation or reduction of transmission behaviors listed above. Additionally, the use of personal protective equipment such as gloves, face masks, and gowns can reduce risk of transmission when performing these behaviors, particularly those that occur in a healthcare environment.[84,85] In the 2014-2016 West African Ebola outbreak, safe burial practices also played a major role in halting human-to-human transmission.[86]

1.10 Ebola exposure history and risk groups

While most individuals who were able to receive the vaccine during the 2018-2020 Eastern DRC outbreak were at elevated EVD risk, reasons for being at heightened risk were varied and included: being a family member of an EVD case, a contact of an EVD case, a contact of a contact of an EVD case, or being a healthcare or frontline worker (HCW/FLW) in an

affected area.[13] Each of these types of EVD exposure, along with simply knowing if one had been exposed to EVD or not, may influence post-vaccination transmission behaviors through perceived EVD risk or knowledge of EVD prevention.[14–17] Furthermore, EVD exposure type may be predictive of who develops EVD post vaccination. HCW/FLWs are of particular interest here due to their continued intense EVD exposure post-vaccination that may lead to vaccine failure. In WHO's interim report on vaccine efficacy, this risk group composed a large proportion of those who developed EVD symptoms more than 10 days post-vaccination.[10] Therefore, it is crucial to understand how pre-vaccination EVD exposure is tied to post-vaccination transmission behavior.

While these concepts of EVD exposure history and risk group have not been directly studied in the past, the role of community and nosocomial behaviors in EVD outbreak propagation has been thoroughly studied.[33,87–90] This research has implicated specific interpersonal behaviors, such as performing funeral rites and stigmatization, are important in disease transmission. These behaviors commonly map onto social relationships, such as performing funeral rites that are primarily performed by family,[87] and as such are tied to the listed types of exposure.

The first two aims of this proposed dissertation attempt to tease apart interpersonal behaviors – community and nosocomial – and the impact of EVD exposure history in the propagation of EVD outbreaks. I hypothesize those in risk groups with the highest risk of transmission will reduce their post-vaccination behaviors that put one at risk of either acquiring or propagating Ebola virus infection. I hypothesize that this reduction in transmission behaviors will occur because of an increase in knowledge of EVD severity and a similar increase in emotional investment in the outbreak's trajectory. In the third aim of this dissertation, I will

explore the effects of EVD exposure history on vaccine response, including interaction and mediation effects between exposure type and baseline antibody titer. I hypothesize that belonging to the HCW EVD risk group will result in higher antibody titers post-vaccination due to a continued exposure post vaccination that assists immune response as opposed to a single high dose exposure, such as in close contacts of an EVD case.

If differences in behavior across various risk groups are found, this knowledge can be used to target exposed individuals in the groups with the highest frequencies of transmission behavior for either health education or vaccination. If effects on vaccination response are found, this knowledge could be used to inform vaccination campaigns or future research on vaccine efficacy. Additionally, this knowledge could inform social network modeling of EVD outbreaks by providing real world data about how connections impact transmission risk.

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Chapter 2: Ebola exposure and post-vaccination risk behaviors during the 2018 North Kivu Ebola outbreak in the Democratic Republic of the Congo

2.1 Abstract

Background: In 2018, the Democratic Republic of the Congo (DRC) declared its 9th and 10th Zaire ebolavirus (EBOV) outbreaks, in the Equateur province (May-July 2018), and in the eastern provinces including North Kivu (August 2018- June 2020). The DRC Ministry of Health deployed the rVSVΔG-ZEBOV-GP vaccine in response during both outbreaks.

Methods: A cohort of vaccinated individuals from the eastern region of DRC, including North Kivu and Ituri provinces were enrolled and followed prospectively for 18 months. Participation in Ebola transmission behaviors, such as performing funeral rites or touching dead animals, were studied over time both in general and according to Ebola exposure history prior to the baseline visit.

Results: There was a general reduction of behaviors at 6 months of follow-up, but a return to normal behaviors at the 18-month follow-up period, despite the ongoing outbreak at that time. Healthcare and frontline workers showed particularly complex behavior changes post-vaccination.

Conclusion: This analysis provides new insights into real-world relationships between Ebola vaccination and transmission behavior in an outbreak setting. Future investigations should seek to fully define risk of EVD vaccination failure and its connection with post-vaccination transmission behaviors in EVD outbreaks.

2.2 Introduction

Control efforts for the 2018-2020 Ebola Virus Disease (EVD) outbreak in the Eastern region of Democratic Republic of Congo (DRC) employed over 300,000 doses of a highly effective recombinant vesicular stomatitis virus-Zaire Ebola vaccine (rVSVΔG-ZEBOV-GP).[1–3] This deployment was the widest provision of the vaccine as an outbreak control measure to date, both in number of people vaccinated and duration of vaccination activities.[2,4] As such, this outbreak provides an opportunity to expand our knowledge of how real-world Ebola vaccination performs beyond the notable efficacy and safety observed in initial trials.[2,5–7] A thorough understanding of EVD vaccination is crucial to understanding the new landscape of EVD outbreaks which now includes effective vaccination and treatment.[8,9]

Of particular importance is how an individual's risk profile changes post-vaccination, as effective outbreak control strategies must supplement vaccination as needed to prevent further EVD transmission. While rare, there is evidence that individuals can develop EVD, and be infectious,[10] after vaccination. On the 12th of April 2019, an interim report released by the World Health Organization (WHO) stated that there were 71 individuals who had developed EVD after vaccination among 93,965 vaccinated individuals who were inoculated and followed during the 2018-2020 EVD outbreak in the Eastern region of the DRC.[11] Furthermore, vaccine failures have been known to cause large chains of transmission with many resulting cases.[12] These observations, along with the potential that the Ebola vaccine may not confer permanent immunity, make understanding post-vaccine EVD transmission risk in vaccinated individuals a priority.

A crucial component of post-vaccination EVD transmission risk is how vaccinated individuals modify their EVD transmission risk behaviors and how these behavioral changes

may follow from pre-vaccination EVD risk. Our team recently described an overall increase in EVD transmission behaviors among vaccinated individuals after the conclusion of the 2018 Mbandaka outbreak.[13] However, there are no published studies which attempt to identify variations in post-vaccination EVD transmission behavior during an active outbreak. While most individuals who were able to receive the vaccine during the 2018-2020 Eastern DRC outbreak were at elevated EVD risk, reasons for being at heightened risk were varied and included: being a family member of an EVD case, a contact of an EVD case, a contact of a contact of an EVD case, or being a healthcare or frontline worker (HCW/FLW) in an affected area.[14] Each of these types of EVD exposure, along with simply knowing if one had been exposed to EVD or not, may influence post-vaccination transmission behaviors through perceived EVD risk or knowledge of EVD prevention.[15–18] Furthermore, EVD exposure type may be predictive of who develops EVD post vaccination. HCW/FLWs are of particular interest here due to their continued intense EVD exposure post-vaccination that may lead to vaccine failure. In WHO's interim report on vaccine efficacy, this risk group composed a large proportion of those who developed EVD symptoms more than 10 days post-vaccination.[11] Therefore, it is crucial to understand how pre-vaccination EVD exposure is tied to post-vaccination transmission behavior.

This analysis will identify post-vaccination behavior changes in individuals during the 2018-2020 EVD outbreak in Eastern DRC and compare behaviors between EVD exposure types at time of vaccination. This analysis will help us understand post-vaccination transmission behaviors and resulting subsequent EVD transmission risk in a real-world setting. If associations between behaviors and EVD exposure types are identified, outbreak control could be significantly improved through targeting additional education or control measures to groups who are more likely to exhibit transmission behavior after vaccination. Ultimately, this analysis could

help provide insight for improved EVD outbreak control in the presence of a partially vaccinated population.

2.3 Methods

Study design

Cohort enrollment occurred from August 15-29, 2018, in the provinces of North Kivu and Ituri in the DRC. At the time of enrollment, the study site was experiencing an EVD outbreak that was declared on August 1, 2018.

Individuals were targeted for participation if they had received the Merck & Co. rVSVΔG-ZEBOV-GP vaccine through Expanded Programme for Immunizations (EPI)/WHO vaccination teams. Additionally, participants were eligible for the study if they were over 18 years of age and were healthy, defined as no fever (<38°C) or other self-reported acute illness at the time of enrollment. Women who reported being pregnant were not eligible for enrollment. Once enrolled, a survey was administered and blood samples were collected from consenting participants at each study visit for a total of five visits to date: baseline (day of vaccination), 21-day follow-up, 6-month follow-up, 18-month follow-up, and 2.5-year follow up. Only the baseline, 6-month and 18-month visits, which gathered data on behaviors over a 6-month period, were used in this analysis. Participants with a complete baseline visit were eligible for all follow up visits, regardless of incomplete prior follow-up visits.

Ultimately, 620 individuals were enrolled in the cohort. Of these 620 individuals, 83.4% (n=517) completed a 6-month follow-up visit and 71.5% (n=443) completed an 18-month follow-up visit. Individuals with a baseline and at least one follow-up visit (n=539) were included in this analysis.

Survey measurements

Surveys were conducted by trained interviewers in the participant's preferred local language (French, Lingala, or Swahili), and collected data on demographics, potential exposures to Ebola virus, and activities performed that increase risk of Ebola virus exposure or transmission.

At baseline, EVD exposure history was gathered through two methods. First, EVD exposure data was gathered through a "select all that are applicable" list for the participants' self-reported types of Ebola exposure, including: being an HCW/FLW in an EVD affected area, had a family member with EVD, had a close contact with EVD, or identified as a contact of a contact with EVD. Second, our questionnaire asked if the participant had been in contact with any known, suspected, or probable EVD cases. If the participant indicated that this was the case, they were further asked about the location of their exposure and their relationship with the EVD case they had been exposed to.

Respondents also self-reported behaviors that may increase risk of Ebola virus exposure or transmission, which included: traveling outside the province, frequenting markets, visiting a health facility, attending a funeral, having contact with human remains, participating in funeral rites, and touching dead animals. For each activity, the participant was asked at baseline if they had performed the activity in the 6 months prior to the outbreak. For follow-up visits, participants were asked if they had performed the activity in the past 6 months.

Analysis

Descriptive statistics on study population characteristics and demographics were obtained for the full sample: median and interquartile range for continuous variables, along with count and

percent for categorical variables. Additionally, the same statistics were obtained for individuals lost to follow-up to assess potential selection bias. The percent of the cohort that participated in behaviors of interest during each follow-up period were calculated along with 95% confidence intervals for percent behavior change obtained through generalized linear mixed models. Adjusted odds ratios (ORs) describing the associations between Ebola exposure and subsequent Ebola transmission behavior were obtained. Two groups of adjusted ORs were calculated, each representing a different classification of Ebola exposure type: self-reported exposure to an EVD case and each type of exposure as a binary variable.

Adjusted ORs were obtained through generalized linear mixed models that considered age, sex, healthcare worker status, marital status, and education level as confounders based on a priori assumptions. These models treated participant as a random effect and Ebola exposure type as a fixed effect. For all ORs, a 95% confidence interval was calculated; A 95% CI that did not cross the null value of 1.00 was considered to be evidence of an association. No corrections were made for multiple comparisons. All statistical analyses were carried out using SAS software, version 9.4 (SAS Institute, Cary, NC).

Ethical approval

Ethical approval was obtained at UCLA Fielding School of Public Health (IRB# 16-001346) and the Kinshasa School of Public Health (IRB# ESP/CE/022/2017) for all study activities. All participants provided informed consent and had the right to refuse participation at any time.

2.4 Results

Overall, our sample of 539 participants had a median age of 33 years (IQR 26, 4) and a majority of participants were male (Table 2.1). Just under a third had either finished primary school (29.9%), completed secondary school (27.5%), or attended some college/university or graduate school (30.4%) as their highest level of education, with a small remainder having none or some primary school education (12.2%). A majority of participants (58.3%) were married or cohabitating with a partner, while 39.3% reported being single. Nearly two fifths of the sample (38.0%) reported working in a healthcare setting, including traditional healers and pastors.

Table 2.1 Baseline sample characteristics of 539 rVSVΔG-ZEBOV-GP vaccine recipients from North Kivu and Ituri provinces in the Democratic Republic of the Congo, August 2018.

Demographics	Median	IQR
Age	33	26, 44

Demographics	Frequency (n)	Percent (%)
Sex		
Male	345	64.0
Female	194	36.0
Age		
18-24	103	19.1
25-34	191	35.4
35-44	127	23.6
45-54	66	12.2
55-64	28	5.2
65-82	24	4.5
Education		
None or some primary school	66	12.2
Finished primary school or apprenticeship	161	29.9
Finished secondary school	148	27.5
College/University or Graduate school	164	30.4
Marital status[a]		
Single	211	39.3

Married or living together as married	313	58.3
Divorced, separated, or widowed	13	2.4
Occupation[b]		
Farmer	120	22.6
Teacher	33	6.2
Healthcare worker	202	38.0
Merchant or artist	31	5.8
Technician	10	1.9
Caretaker	10	1.9
Office worker	24	4.5
Student	20	3.8
Driver	46	8.7
Politics or civil service	24	4.5
Unemployed	11	2.1

EVD Exposure History	Frequency (n)	Percent (%)
Self-reported contact with an EVD case[c]	157	31.7
Family member of an EVD case	90	16.7
Contact of an EVD case	173	32.1
Contact of a Contact of an EVD case	381	70.7
Health Care or Front-Line Worker	230	42.7
Number of exposure types		
0	49	9.1
1	242	44.9
2	120	22.3
3-4	128	23.8

a. 1 refused and 1 "I don't know"
b. 5 refused and 3 indecipherable write ins
c. 43 missing

Our sample showed various histories of exposure to EVD (Table 2.1). A majority (68.3%) reported that they had never had contact with a confirmed, suspected, or probable EVD case, compared to 31.7% reporting such contact. Eleven individuals (2.2%) did not know their exposure history. When considering the four specific types of EVD exposure which make an individual eligible to receive the Ebola vaccine, individuals varied in eligibility criteria, with many indicating multiple exposure categories. The most common exposure type was being a contact of a contact of an EVD case (70.7%), followed by being an HCW/FLW (42.7%), or a

contact of an EVD case (32.1%). Least common was having a family member with EVD (16.7%). Almost half of our sample had only experienced one of these exposure types, while nearly one fourth indicated having two (22.3%) exposure types. About a fourth had 3-4 exposure types (23.8%). Not reporting any exposure type was least common at 9.1% of the sample.

Those lost to follow-up (n=61) only significantly differed from those retained in the sample (Table 2.2) according to occupation. Farmers, merchants, artists, politicians, and civil servants were more likely to be lost to follow-up (p=0.01) than teachers, healthcare workers, and office workers. Retention in the study varied slightly by other demographic factors, though not significantly so.

Table 2.2 Sample characteristics by those retained in the study versus those lost to follow up.

	Retained n=539		Lost to follow up n=61		
	Median	IQR	Median	IQR	p-value
Age	33	26, 44	36	25, 43	0.78
	Frequency (n)	Percent (%)	Frequency (n)	Percent (%)	
Sex					0.81
Male	345	64.0	40	65.6	
Female	194	36.0	21	34.4	
Age					0.29
18-24	103	19.1	13	21.3	
25-34	191	35.4	13	21.3	
35-44	127	23.6	21	34.4	
45-54	66	12.2	7	11.5	
55-64	28	5.2	4	6.5	
65-82	24	4.5	3	4.9	
Education					0.17
None or some primary school	66	12.2	13	21.3	
Finished primary school or apprenticeship	161	29.9	20	32.8	
Finished secondary school	148	27.5	13	21.3	
College/University or Graduate school	164	30.4	15	25.6	
Marital status					0.32
Single	211	39.3	18	29.5	
Married or living together as married	313	58.3	41	67.2	
Divorced, separated, or widowed	13	2.4	2	3.3	
Occupation					0.01
Farmer	120	22.6	20	32.8	

Teacher	33	6.2	0		
Healthcare worker	202	38.0	21	34.4	
Merchant or artist	31	5.8	7	11.5	
Technician	10	1.9	2	3.3	
Caretaker	10	1.9	0		
Office worker	24	4.5	0		
Student	20	3.8	1	1.6	
Driver	46	8.7	3	4.9	
Politics or civil service	24	4.5	6	9.8	
Unemployed	11	2.1	1	1.6	
Has ever had contact with a confirmed, probable, or suspected EVD case*					0.95
Yes	157	31.7	12	33.3	
No	328	66.1	23	63.9	
Don't know	11	2.2	1	2.8	
Exposure type (not mutually exclusive)					
Family member of a case	90	16.7	9	14.8	0.70
Contact of a case	173	32.1	21	34.4	0.71
Contact of a Contact	381	70.7	38	62.3	0.18
Health Care Worker	230	42.7	21	34.4	0.22

*25 missing from those lost to follow-up

Percent of the sample that participated in each behavior for the 6 months prior to the outbreak, the period between the baseline visit and the 6-month follow-up visit, and the 6 months prior to the 18-month visit are displayed in Table 2.3. All behaviors except visiting a health facility saw a significant decrease in the first follow-up period compared to behavior prior to the outbreak. Given the large proportion of HCW/FLWs in this sample, this behavior could have been driven by these individuals reporting to healthcare facilities for their job, as opposed to true estimates of the population attending healthcare facilities for ailments. Alternatively, only frequenting markets displayed a significant decrease for the 18-month follow-up period compared to pre-outbreak behavior.

Table 2.3 EVD transmission behaviors in the 6 months prior to the outbreak and follow-up periods for rVSVΔG-ZEBOV-GP vaccine recipients in Beni, Democratic Republic of the Congo.

	In the 6 months prior to the outbreak (n=539)	Between baseline visit and 6 months of follow up (n=517)		Between 12 and 18 months of follow up (n=443)	
	Percent	Percent	Percent change (95% CI)	Percent	Percent change (95% CI)
Behavioral outcomes					
Attended funeral	58.4	36.8	-21.5 (-26.9, -16.2)	55.6	-2.7 (-8.5, 3.0)
Contact with human remains	28.8	12.5	-16.3 (-20.9, -11.7)	24.1	-4.6 (-9.7, 0.4)
Participated in funeral traditions	32.1	23.7	-8.4 (-13.7, -3.0)	30.2	-1.9 (-7.7, 4.0)
Touched dead animals	19.1	8.7	-10.5 (-14.3, -6.7)	19.0	-0.1 (-4.9, 4.6)
Traveled outside of province	70.9	60.0	-10.9 (-16.2, -5.6)	71.7	0.1 (-4.5, 6.1)
Frequented markets	93.5	66.6	-26.9 (-31.4, -22.5)	69.8	-23.7 (-28.6, -18.9)
Visited health facility	44.9	47.3	2.4 (-3.1,7.9)	48.7	3.8 (-2.2, 9.9)

In our sample, there was little evidence that having a known EVD exposure or unknown EVD exposure history effects transmission behaviors reported at 6 months and 18 months post-vaccination (Table 2.4), with one exception; individuals who reported a known EVD exposure had a 41% (95% CI 6-63%) reduction in odds of traveling outside the province between the baseline and 6-month follow-up compared to individuals who did not have a known exposure, holding all confounders constant. No other outcomes for known EVD exposure or unknown EVD exposure showed evidence of an association.

Table 2.4 Follow-up Ebola transmission behaviors by self-reported exposure to an EVD case for 539 rVSVΔG-ZEBOV-GP vaccine recipients in Beni, Democratic Republic of the Congo.

	Known EVD Exposure[a]		Unknown EVD Exposure[a]	
	Odds Ratio	95% CI	Odds Ratio	95% CI
Behavioral outcomes at 6 months				
Attended funeral	0.90	0.56, 1.46	1.65	0.38, 7.07
Contact with human remains	0.62	0.33, 1.17	1.29	0.24, 6.99
Participated in funeral traditions	0.91	0.57, 1.45	1.26	0.31, 5.13
Touched dead animals	1.15	0.54, 2.46	1.58	0.16, 15.36
Traveled outside of province	0.59	0.37, 0.94	0.61	0.14, 2.61
Frequented markets	0.99	0.63, 1.56	-	
Visited health facility	0.85	0.54, 1.33	2.98	0.65, 13.71
Behavioral outcomes at 18 months				
Attended funeral	1.46	0.88, 2.41	0.36	0.07, 1.79
Contact with human remains	0.70	0.41, 1.18	-	-
Participated in funeral traditions	0.94	0.59, 1.50	0.27	0.03, 2.21
Touched dead animals	0.95	0.50, 1.82	1.70	0.27, 10.86
Traveled outside of province	1.44	0.85, 2.47	0.32	0.07, 1.47
Frequented markets	1.27	0.77, 2.08	0.90	0.20, 4.11
Visited health facility	1.06	0.66, 1.70	2.44	0.51, 11.53

a. Reference category was self-reported no exposure to a known, probable, or suspected EVD case.

When looking at specific exposure types, being an HCW or FLW was associated with an increase in odds of having contact with human remains at both time points, but a decrease in odds of participating in funeral traditions, traveling outside the province, or frequenting markets

prior to the 6-month follow-up, holding confounders constant (Table 2.5). Outside of HCW/FLW effects, only the relationship between contacts of contacts and contact with human remains showed evidence of an association. Those who were a contact of a contact had 1.86 times the odds (95% CI 1.07, 3.21) of having contact with human remains in the 6 months prior to the 18-month follow-up compared to those who were not a contact of a contact, holding confounders constant.

Table 2.5 Follow-up Ebola transmission behaviors by EVD exposure types for 539 rVSVΔG-ZEBOV-GP vaccine recipients in Beni, Democratic Republic of the Congo.

	Type of exposure to Ebola			
	Family member	Close contact	Healthcare/Frontline Workers	Contact of contact
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Behavioral outcomes at 6 months				
Attended funeral	1.24 (0.69, 2.21)	0.78 (0.49, 1.23)	0.77 (0.50, 1.20)	1.02 (0.63, 1.63)
Contact with human remains	0.15 (0.02, 1.11)	0.97 (0.54, 1.73)	3.90 (2.11, 7.19)	1.09 (0.60, 1.98)
Participated in funeral traditions	1.26 (0.72, 2.20)	1.27 (0.82, 1.97)	0.64 (0.41, 0.99)	0.98 (0.61, 1.56)
Touched dead animals	0.93 (0.37, 2.34)	1.19 (0.58, 2.46)	0.78 (0.38, 1.62)	1.49 (0.65, 3.41)
Traveled outside of province	0.93 (0.53, 1.62)	0.85 (0.55, 1.32)	0.57 (0.37, 0.87)	0.87 (0.55, 1.37)
Frequented markets	1.15 (0.66, 2.01)	1.06 (0.69, 1.63)	0.64 (0.42, 0.97)	1.41 (0.91, 2.19)
Visited health facility	1.10 (0.65, 1.89)	0.82 (0.54, 1.25)	0.73 (0.49, 1.09)	1.27 (0.82, 1.96)
Behavioral outcomes at 18 months				
Attended funeral	0.94 (0.52, 1.71)	1.23 (0.76, 1.99)	0.97 (0.61, 1.54)	1.27 (0.77, 2.08)
Contact with human remains	0.57 (0.25, 1.29)	1.05 (0.64, 1.75)	3.19 (1.96, 5.20)	1.86 (1.07, 3.21)
Participated in funeral traditions	0.71 (0.39, 1.27)	1.35 (0.87, 2.10)	1.04 (0.68, 1.60)	1.47 (0.91, 2.39)
Touched dead animals	0.61 (0.27, 1.38)	1.49 (0.82, 2.70)	1.32 (0.74, 2.36)	1.71 (0.87, 3.34)
Traveled outside of province	0.62 (0.34, 1.12)	1.19 (0.72, 1.98)	1.48 (0.90, 2.43)	1.10 (0.66, 1.85)
Frequented markets	0.93 (0.52, 1.67)	1.03 (0.64, 1.64)	1.19 (0.76, 1.88)	0.87 (0.53, 1.43)
Visited health facility	1.13 (0.64, 1.98)	1.44 (0.92, 2.26)	1.23 (0.80, 1.90)	1.38 (0.87, 2.20)

2.5 Discussion

Overall, our sample generally showed a net decrease in EVD transmission behaviors at the 6-month follow-up, but not the 18-month follow-up, compared with behaviors described in the baseline survey. As both follow-up visits were gathered during the same EVD outbreak, this pattern may indicate that populations participate in prevention behaviors for only limited periods after an outbreak is declared. This limited behavior change may be due to epidemic fatigue or changing risk perception due to both natural and acquired immunity both in participants and within the community.[18,19] Given that many individuals who develop EVD after vaccination do so in the ten days after vaccination,[11] a short term reduction in transmission behavior is promising as this may prevent transmission in the case where an individual who was infected before vaccination becomes infectious immediately after vaccination. However, we saw a return to pre-outbreak behavior despite ongoing EVD risk in this population. If protection conferred by rVSVΔG-ZEBOV-GP vaccination is shown to wane over time, this pattern indicates a need for further EVD prevention among vaccinated individuals, particularly in outbreaks over one year long.

Of the populations examined, HCW/FLWs and non-HCW/FLWs displayed the most divergent transmission behaviors at follow-up. The calculated odds ratios indicated a range of effects, some that could increase and some that could decrease the risk of post-vaccination Ebola transmission among HCW/FLWs. At both 6- and 18-months of follow-up, HCW/FLWs were more likely to have contact with human remains than non-HCW/FLWs, but had lower odds of participating in funeral traditions, traveling outside of the province, and frequenting markets at six months of follow-up. Given that nosocomial transmission has historically played an important role in the propagation of EVD outbreaks,[20–22] these behavioral differences could

potentially have large effects on outbreak trajectory. For example, the reduction in funeral participation, travel, and market attendance could direct chains of transmission that include HCW/FLWs away from community propagation. Furthermore, nosocomial transmission could also be amplified by the increased contact with human remains that HCW/FLWs experience compared to non-HCW/FLWs, through increasing risk of infection among HCW/FLWs.[23,24] This combination of behavioral patterns shows that HCW/FLWs are more likely to have intense exposure to EVD, but may also be reducing behaviors that could spread the disease out in the wider community. It is possible that this pattern indicates HCW/FLWs are altruistically modifying their behavior to protect their communities. Alternatively, these individuals may have the necessary infection prevention education and EVD knowledge to motivate a change in behavior. Regardless of etiology, efforts should be made to support and show appreciation for HCW/FLWs who show behaviors that protect the wider community.

In addition to changes in behavior among HCW/FLWs, there was one other observed effect by type of exposure to EVD. Contacts of contacts were more likely to have contact with human remains in the six months prior to the 18-month follow-up than those who did not report being contacts of contacts. Since having contact with human remains carries a large risk of Ebola transmission,[25] this could indicate that the risk level of post-vaccination EVD acquisition increases as time goes on for contacts of EVD case contacts. It is unclear why this would be, particularly in the absence of increased funeral attendance or funeral rites participation. More research must identify if there is an underlying mechanism behind this finding. Meanwhile, post-vaccination EVD prevention efforts should target contacts of contacts, particularly in outbreaks that last longer than one year.

Among the other classifications of EVD exposure, we found reduced travel outside the province between baseline and the 6-month follow-up among individuals who knew they had an EVD exposure. These findings may indicate that the more aware a person is of the outbreak and their place in it, the less they travel. If so, efforts to prevent geographic spread of the outbreak cannot solely rely on self-reported exposure to EVD. Other methods such as contact tracing and rapid testing for travelers should be considered in efforts to limit geographic spread of outbreaks. Furthermore, outbreak response should incorporate education on risks of traveling during an EVD outbreak, quarantining instructions, and what to do if someone experiences EVD symptoms after traveling to a new area.

Our study is subject to a number of limitations. This study targets a specific population—vaccinated individuals – and therefore the results may not be generalizable to unvaccinated individuals. Given that our baseline data collection occurred during an active EVD outbreak in a conflict zone, it was deemed too risky to recruit unvaccinated individuals for comparison. However, there is evidence that these findings may be generalizable; previous research has shown that vaccinated and unvaccinated individuals display similar behavioral changes during EVD outbreaks.[13] Additionally, all the variables used in this analysis were self-reported and therefore may be subject to misclassification due to limitations of recall or translation errors. However, much effort was undertaken to reduce translation errors. Local staff were hired to administer questionnaires to conserve information in each translation from local languages to English and vice versa. Our sample did have some loss to follow up, which may have resulted in some selection bias. To minimize this source of bias, generalized linear mixed models, which are not sensitive to missing data, were used in the analysis. Finally, there is the possibility of residual

confounding or selection bias despite the covariate selection method used to correct for bias from these sources.

In conclusion, this analysis provides new insights into real-world relationships between Ebola vaccination and transmission behavior in an outbreak setting. Our study is the first to evaluate associations between various EVD exposures and subsequent behavior that may contribute to outbreak growth. While a short-term reduction in transmission behavior was observed, there was no apparent reduction in EVD transmission behavior during our long-term post-vaccination follow-up period. Additionally, this analysis demonstrates that HCW/FLWs have particularly complex behavior profiles post-vaccination. Given these observations, future investigations should seek to fully define risk of EVD vaccination failure and its connection with post-vaccination transmission behaviors in EVD outbreaks.

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Chapter 3: Ebola vaccination and occupational risk behaviors among healthcare workers in Equateur Province, Democratic Republic of the Congo

3.1 Abstract

*Background***:** The momentum behind several Ebola Virus Disease (EVD) outbreaks has been driven by the infection of healthcare workers, who are estimated to be 21 to 32 times as likely to contract EVD compared to the general population. This analysis will identify and compare occupational risk behaviors in vaccinated and unvaccinated healthcare workers affected by the 2018 and 2020 EVD outbreaks in Equateur province, DRC.

Methods: A cohort of vaccinated and unvaccinated healthcare workers from Equateur province, DRC was enrolled and followed prospectively for 2.5 years. Participation in occupational Ebola transmission behaviors, such as patient contact and personal protective equipment (PPE) use, over time were studied by vaccination status.

Results: Healthcare workers who did not receive vaccination during the 2018 Equateur EVD outbreak were more likely to routinely have close contact with patients at baseline, specifically through the following activities: going into patients' rooms, conversing with patients, washing patients' clothes, cleaning patients' rooms, and getting sick from patient exposure. At two and a half years of follow up, when a resurgent EVD outbreak had occurred in the area, vaccinated healthcare workers were more likely than unvaccinated healthcare workers to participate in funeral rites or rituals.

Conclusion: These results should be taken into consideration when working with both vaccinated and unvaccinated HCWs affected by EVD outbreaks, particularly in education and communication campaigns that seek to reduce workplace EVD transmission risk. Specifically,

future vaccination campaigns should make extra efforts to reach healthcare workers who routinely have close contact with patients.

3.2 Introduction

While Ebola virus disease (EVD) transmission can occur in any setting within a community, nosocomial transmission has historically been a major contributor to the propagation of EVD outbreaks.[1–5] The momentum behind several EVD outbreaks has been driven by the infection of healthcare workers (HCWs), who are estimated to be 21 to 32 times as likely to contract EVD compared to the general population.[1,5,6] Despite the recent deployment of effective and safe EVD vaccination, there is evidence that healthcare workers remain at higher risk than other members of affected communities. In a preliminary report of individuals vaccinated during the 2018-2020 EVD outbreak, HCWs were shown to constitute 7 out of 15 individuals (47%) who experienced breakthrough EVD cases 10 days or more after vaccination.[7] These vaccine failures indicate that HCWs remain at risk for Ebola infection after vaccination, though that risk is considerably lower than before vaccination. This substantial increase in risk compared to non-HCWs is thought to be a result of occupational exposure to infectious bodily fluids, despite use of personal protective equipment (PPE) and other stringent protective measures.[8]

In addition to continued infection of HCWs, there is the possibility that nosocomial transmission is exacerbated by patient-to-patient EVD transmission through transfer of the virus on fomites or a HCW's person. Routine close contact with patients, inadequate training in sanitation practices, insufficient resources for infection control, and inadequate supply of PPE all contribute to nosocomial transmission of Ebola without requiring direct infection of a HCW.[6,8] These behaviors are of particular concern among HCWs who have been vaccinated for Ebola, as perceived safety from EVD could directly influence how a HCW participates in indirect transmission behaviors.

Given continued risk among vaccinated HCWs and the potential for facilitation of patient-to-patient transmission, effective control of Ebola outbreaks relies on the understanding of occupational transmission behaviors in HCWs. Behaviors that lead to acquisition, propagation, or spread of Ebola among HCWs are all relevant to understanding continued nosocomial transmission despite Ebola vaccination among HCWs. Additionally, it is vital to understand how vaccination changes these behaviors, as it is plausible that vaccination leads to an increase in transmission behavior due to lowered risk perception.

Over 300,000 doses of the Merck & Co. rVSVΔG-ZEBOV-GP Ebola vaccine have been deployed in response to the multiple EVD outbreaks in the Democratic Republic of the Congo (DRC). Thus, the DRC is an optimal region in which to study healthcare EVD risk behavior and vaccination. One specific region of interest is the Equateur province, which contains the large city of Mbandaka with over 1 million inhabitants. This province has experienced two distinct EVD outbreaks: one in the summer of 2018 and a second outbreak in the summer of 2020. For both these outbreaks, the rVSVΔG-ZEBOV-GP Ebola vaccine was widely used as an outbreak response tool.

This analysis will identify occupational risk behaviors in vaccinated and unvaccinated individuals affected by the 2018 and 2020 EVD outbreaks in Equateur province, DRC. This analysis will assess both unadjusted prevalence of risk behaviors among vaccination status and a bias-controlled comparison of vaccinated versus unvaccinated HCWs. Additionally, this analysis will consider behaviors in the 6 months following the 2018 EVD outbreak and 6 months spanning the 2020 EVD outbreak in the same province. Unadjusted prevalence of risk behaviors will be used to make conclusions about possible risk of EVD infection in each group, considering outbreak occurrence during each follow-up period. Bias-adjusted measurements will

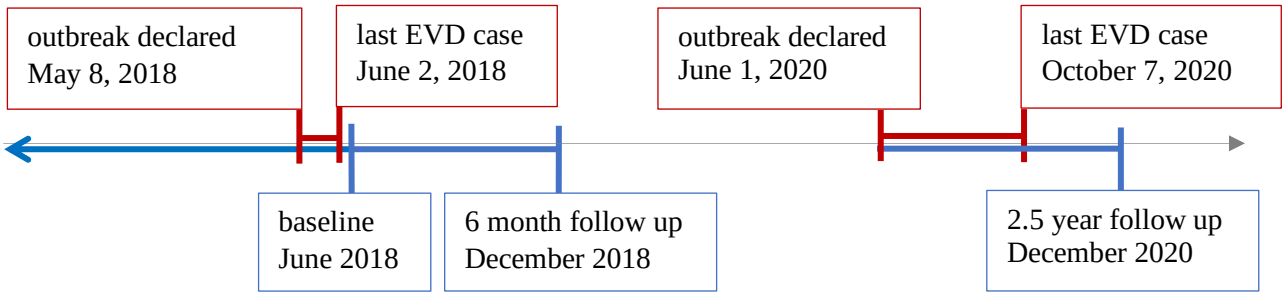
be used to develop causal hypotheses on the effect of vaccination on behavior both in between and during outbreaks. Identified patterns and associations may inform and improve outbreak control in partially vaccinated populations.

3.3 Methods

Study design

Cohort enrollment occurred in June-July 2018 in the province of Equateur in the DRC. At the time of enrollment, this region was experiencing an EVD outbreak that was declared on May 8th, 2018. The last case in this outbreak was confirmed on June 2nd, 2018, and the outbreak was officially declared over on July 24, 2018. A second EVD outbreak occurred in this area from June 1-October 7, 2020. Study visits occurred in June 2018 (baseline), December 2018 (6 month follow up), and December 2020 (2.5 year follow up). Figure 3.1 shows a timeline of when outbreaks and study events occurred in relation to each other.

Figure 3.1 A timeline of EVD outbreaks and study events in Mbandaka Democratic Republic of the Congo, 2018-2020



Blue depicts study events. Vertical blue lines show study visits; horizontal blue lines show periods for which outcomes were gathered (ever/routinely at baseline, in the past 6 months for both follow ups). Red depicts EVD outbreaks in the region.
Not to scale.

As part of a wider study on vaccine immunogenicity, vaccinated individuals were targeted for participation if they had received the Merck & Co. rVSV ZEBOV-GP vaccine through the ring vaccination strategy employed by WHO/Expanded Programme for Immunizations (EPI) vaccination teams for the first Equateur outbreak in 2018. This strategy attempted to vaccinated contacts of confirmed EBOV cases, contacts of these contacts, HCWs, and first responders. Women who reported being pregnant were not eligible for vaccination during this outbreak and therefore not enrolled in our study. Among this vaccinated cohort, only those who reported being an HCW, including traditional healers and pastors, were included in this analysis. Unvaccinated HCWs, including traditional healers and pastors, were recruited from randomly selected health facilities within three health zones in Equateur province (Wangata, Mbandaka, and Bolenge). At selected facilities, enrollment was offered to all employees as well as traditional healers and pastors practicing nearby. Both vaccinated and unvaccinated HCWs were eligible for the study if they were over 18 years of age and were healthy, defined as no fever (<38°C) or other self-reported acute illness at the time of enrollment.

Due to the second EVD outbreak in the same province in 2020, some of our unvaccinated sample was vaccinated following the 6 month follow up but prior to the 2.5 year follow up. Due to this change in exposure, 2.5-year measurements were not used for these individuals who were vaccinated outside of the 2018 EVD outbreak to avoid limitations on our conclusions for this time point.

Survey measurements

Survey data was collected from participants at baseline, 6 months of follow up, and 2.5 years of follow up. Surveys were conducted by trained interviewers in the participant's preferred

local language (French or Lingala), and collected data on demographics, HCW type, and occupational behaviors that may raise risk of EBOV exposure or transmission such as patient care activities (such as bathing a patient or performing funeral rites), personal injuries (such as needle sticks and getting sick from a patient exposure), and PPE use. At baseline, the participant was asked if they performed each patient care activity routinely at work; "Routinely" was left to interpretation by the participant. Additionally, they were asked if they had ever experienced each type of personal injury. Participants were asked if they had daily access to PPE. If so, they were asked if they used various PPE items. For follow up visits, participants were asked if they had participated in each activity, experienced each personal injury, or used various PPE items if they had daily access to in the past 6 months.

Analysis

Descriptive statistics on sample characteristics and demographics were obtained for the full sample. Additionally, percent of vaccinated and unvaccinated participants who participated in behaviors during each follow up period were obtained. Chi-square tests were used to assess statistical significance of differences between vaccinated and unvaccinated percentages at each time point. Statistical significance was not assessed for daily availability of PPE due to inestimable correlated error by health facility, due to recruitment of HCW by health facility and lack of systematically recording the health facility each participant was gathered at.

Adjusted odds ratios (ORs) describing the associations between Ebola vaccination and subsequent occupational Ebola transmission behaviors were obtained. Adjusted ORs were obtained through generalized linear mixed models that considered age, sex, marital status, patient contact, education level, self-reported EVD exposure, and behaviors at baseline where available

as confounders based on a priori identification. These models treated participant as a random effect and Ebola vaccination as a fixed effect. For all ORs, a 95% confidence interval is provided; a 95% CI that did not cross the null value of 1.00 was considered to be evidence of an association. No corrections were made for multiple comparisons. All statistical analyses were carried out using SAS software, version 9.4 (SAS Institute, Cary, NC).

Ethical approval

Ethical approval was obtained at UCLA Fielding School of Public Health (IRB#16-001346) and the Kinshasa School of Public Health for all study activities. This study was also approved by the Scientific Committee for Ebola Research during an outbreak at the National Institute of Biomedical Research (INRB) under the DRC Ministry of Health. All participants provided informed consent and had the right to refuse participation at any time.

3.4 Results

Ultimately, 572 HCWs who met study criteria were enrolled in the cohort, 197 of which were vaccinated (34%) and 375 unvaccinated (66%). Of the vaccinated individuals, 169 completed a 6 month follow up visit, though only 52% (n=103) reported still working in healthcare and had occupational behavioral data. Furthermore, 150 vaccinated individuals completed a 2.5 year follow up visit, though only 60% (n=118) were working in healthcare at that time and had available occupational behavioral data. Of the unvaccinated individuals, 326 individuals completed a 6 month follow up visit, with 62% (n=234) still working in healthcare. Finally, 298 of the unvaccinated individuals returned for a 2.5 year follow up visit; 38 of them were no longer healthcare workers and were therefore excluded from the analysis. Additionally,

144 (55%) of the remaining unvaccinated individuals' data were excluded at the 2.5-year time point analysis due to change in vaccination status during the study. This exclusion resulted in a sample size of 116 for unvaccinated individuals at 2.5 years. Figure 3.2 displays HCW status over time and retention in the study.

Figure 3.2 A flowchart depicting study retention in a cohort of (a) 197 vaccinated and (b) 375 unvaccinated healthcare workers in Equateur Province, Democratic Republic of the Congo, 2018-2020

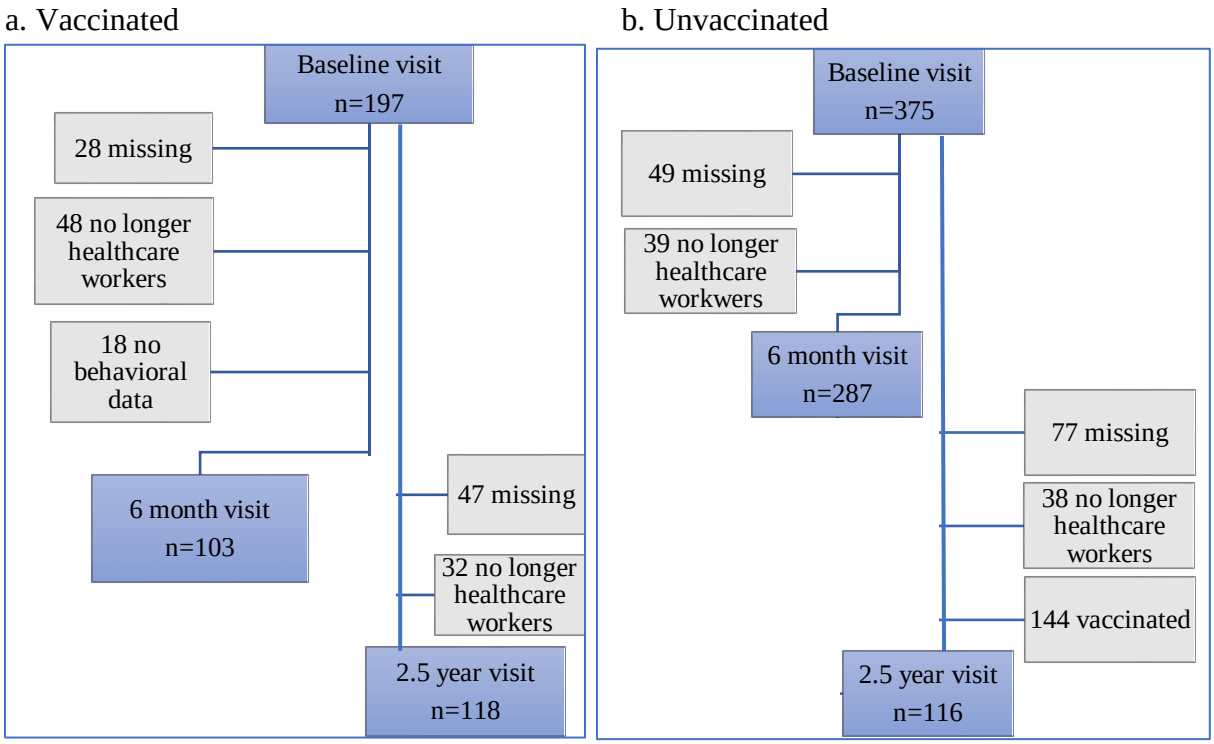


Table 3.1 displays demographics for our vaccinated and unvaccinated groups at baseline. While our unvaccinated group was nearly evenly split by gender (male 54%, female 46%), our vaccinated group was more commonly male (male 69%, female 32%). Age groups across vaccination status were similar, with a slight increase in 55–64-year-olds among our

unvaccinated groups. However, both vaccinated and unvaccinated individuals had a median age of 41 years. Individuals in both vaccination groups tended to be highly educated, married, working as nurses, and did not report ever being in close contact with an EVD case. Also in both groups, having direct contact with patients was more common than indirect or no contact with patients.

Table 3.1 Sample characteristics of vaccinated and unvaccinated healthcare workers from Mbandaka, Democratic Republic of the Congo, 2018.

	Vaccinated n=197		**Unvaccinated n=375**	
	Median	IQR	Median	IQR
Age	41	34, 51	41	34.5, 48
	Count (n)	Percent (%)	Count (n)	Percent (%)
Sex				
Male	135	68.5	173	46.1
Female	62	31.5	202	53.9
Age				
18-24	7	3.6	9	2.4
25-34	45	22.8	89	23.7
35-44	74	37.6	122	32.5
45-54	53	26.9	90	24.0
55-64	12	6.1	49	13.1
65-85	6	3.0	16	4.3
Education[a]				
None or some primary school	3	1.5	16	4.3
Finished primary school or apprenticeship	10	5.1	60	16.0
Finished secondary school	46	23.5	80	21.3
College/University or Graduate school	137	69.9	219	58.4
Marital status[a]				
Single	41	20.9	66	17.6
Married or living together as married	150	76.5	262	69.9
Divorced, separated, or widowed	5	2.6	47	12.5
Type of Healthcare worker[b]				
Nurse	73	47.1	227	62.7
Physician	29	18.7	1	0.3
Lab technician	11	7.1	17	4.7
Administrator	14	9.0	12	3.3
Room Attendant	4	2.6	33	9.1

Hygienic Service	1	0.6	31	8.6
Traditional Healer or Pastor	5	3.2	2	0.6
Red Cross Worker	7	4.5		0.0
Midwife	3	1.9	5	1.4
Maintenance		0.0	6	1.7
Community Volunteer	2	1.3	18	5.0
Other	6	3.9	10	2.8
Contact with patients[b]				
Direct	118	76.1	239	66.0
Indirect	23	14.8	102	28.2
No contact	14	9.0	21	5.8
Has been in close contact with a suspected, probable, or confirmed Ebola case?				
Yes	47	23.9	11	2.9
No	150	76.1	364	97.1

a. 1 missing from vaccinated group
b. 55 missing: 13 from unvaccinated, 42 from vaccinated group

Table 3.2 displays the characteristics of those who were vaccinated between the baseline and the 2.5 year follow up visit ("Newly vaccinated") compared to those who were vaccinated at baseline ("Vaccinated at baseline") and those who remained unvaccinated at follow up ("Unvaccinated"). No clear patterns emerged; there were various similarities and differences across groups for each characteristic.

Table 3.2 Characteristics of healthcare workers by vaccination group at the 2.5 year follow up from Mbandaka, Democratic Republic of the Congo, 2020.

	Vaccinated at Baseline n=118		**Newly Vaccinated n=144**		**Unvaccinated n=116**	
	Count (n)	Percent (%)	Count (n)	Percent (%)	Count (n)	Percent (%)
Sex						
Male	75	63.6	63	43.8	50	43.1
Female	43	36.4	81	56.3	66	56.9
Age						
18-24	2	1.7	2	1.4	3	2.6
25-34	18	15.3	35	24.3	20	17.2
35-44	49	41.5	49	34.0	35	30.2
45-54	37	31.4	39	27.1	30	25.9
55-64	8	6.8	15	10.4	24	20.7
65-85	4	3.4	4	2.8	4	3.4
Education[a]						
None or some primary school	2	1.7	5	3.5	6	5.2
Finished primary school or apprenticeship	5	4.2	17	11.8	23	19.8
Finished secondary school	25	21.2	22	15.3	28	24.1
College/University or Graduate school	85	72.0	100	69.4	59	50.9
Marital status[a]						
Single	20	16.9	28	19.4	12	10.3
Married or living together as married	93	78.8	96	66.7	89	76.7
Divorced, separated, or widowed	4	3.4	20	13.9	15	12.9
Type of Healthcare worker						
Nurse	56	47.5	100	69.4	66	56.9
Physician	16	13.6	1	0.7	0	
Lab technician	11	9.3	9	6.3	3	2.6
Administrator	7	5.9	6	4.2	5	4.3
Room Attendant	4	3.4	10	6.9	14	12.1
Hygienic Service	1	0.8	11	7.6	10	8.6
Traditional Healer or Pastor	1	0.8	0		1	0.9
Red Cross Worker	4	3.4	0		0	
Midwife	3	2.5	0		3	2.6
Maintenance	0		1	0.7	2	1.7
Community Volunteer	1	0.8	2	1.4	6	5.2
Other	14	11.9	4	2.8	6	5.2
Contact with patients						
Direct	80	67.8	102	70.8	72	62.1
Indirect	20	16.9	33	22.9	35	30.2
No contact	7	5.9	9	6.3	7	6
Has been in close contact with a suspected, probable, or confirmed Ebola case? [a]						
Yes	29	24.6	7	4.9	2	1.7
No	89	75.4	137	95.1	114	98.3

a. Gathered at baseline

At baseline and the 6 month follow up, there were differences in the proportion of vaccinated versus unvaccinated individuals who participated in occupational EVD transmission behaviors (Table 3.3). At baseline, the most significant differences were that vaccinated individuals were less likely to enter patients' rooms, converse with a patient, and clean patients' room routinely at work. However, they were more likely to participate in funeral rites or rituals. At 6 months of follow-up, vaccinated individuals were less likely to have entered patients' rooms, washed patients' clothes, and cleaned patients' rooms routinely at work in the past 6 months. There were no significant differences at 2.5 years of follow-up. For differences in PPE use (Table 3.4), vaccinated participants were very significantly more likely use face masks, lab coats, and respirators at 6 months of follow-up compared to non-vaccinated participants.

Table 3.3 Percent of sample that participated in occupational EVD transmission behaviors prior to and following the 2018 EVD outbreak in Mbandaka, Democratic Republic of Congo

	Baseline					**6 month follow up**					**2.5 year follow up**				
	Vaccinated (n=197)		Unvaccinated (n=375)			Vaccinated (n=103)		Unvaccinated (n=234)			Vaccinated (n=118)		Unvaccinated (n=116)		
	n	%	n	%		n	%	n	%		n	%	n	%	
Routine work activities															
Been in the patient's room	98	49.7	284	75.7	***	73	70.9	190	81.2	*	67	56.8	71	61.2	
Performed Examinations	63	32.0	85	22.7	*	38	36.9	87	37.2		33	28.0	26	22.4	
Performed Surgery	24	12.2	61	16.3		30	29.1	85	36.3		19	16.1	22	19.0	
Given food to a patient	26	13.2	50	13.3		19	18.4	52	22.2		19	16.1	17	14.7	
Conversed with a patient	110	55.8	258	68.8	**	75	72.8	167	71.4		63	53.4	55	47.4	
Washed the patient's clothes	6	3.0	32	8.5	*	3	2.9	31	13.2	**	6	5.1	13	11.2	
Had contact with patient's bodily fluids	48	24.4	98	26.1		16	15.5	55	23.5		19	16.1	18	15.5	
Processed patient specimens in a lab	21	10.7	26	6.9		10	9.7	34	14.5		8	6.8	8	6.9	
Washed a cadaver	11	5.6	12	3.2		3	2.9	5	2.1		4	3.4	7	6.0	
Cleaned patient's room	14	7.1	80	21.3	***	10	9.7	63	26.9	**	12	10.2	22	19.0	
Participated in funeral rites/rituals	56	28.4	63	16.8	**	73	70.9	190	81.2		19	16.1	10	8.6	
Personal Injury															
Pricked by a contaminated needle	39	19.8	108	28.8	*	10	9.7	34	14.5		9	7.6	12	10.3	
Contact with contaminated sharps	27	13.7	48	12.8		8	7.8	13	5.6		6	5.1	12	10.3	
Contact with biological specimens	49	24.9	101	26.9		16	15.5	31	13.2		12	10.2	10	8.6	
Contact with blood when you had an open cut or wound	22	11.2	58	15.5		6	5.8	13	5.6		8	6.8	8	6.9	
Contact with harmful chemicals while on the job	37	18.8	72	19.2		9	8.7	15	6.4		12	10.2	19	16.4	
Harmed/bitten by a patient	3	1.5	17	4.5		6	5.8	6	2.6		2	1.7	6	5.2	
Threatened/harmed by a patient's family	26	13.2	31	8.3		5	4.9	19	8.1		7	5.9	7	6.0	
Gotten sick from a known patient exposure	2	1.0	16	4.3	*	5	4.9	9	3.8		3	2.5	4	3.4	
Sustained a blunt physical injury at work	18	9.1	41	10.9		7	6.8	8	3.4		6	5.1	8	6.9	
Daily PPE access[a]	110	55.8	273	72.8	-	85	82.5	204	87.2	-	96	81.4	76	65.5	-

a. no p-values gathered for comparisons due to inestimable correlated error by healthcare facility

* p<0.05; ** p<0.01; ***p <0.0001; - p not appropriate

Table 3.4 PPE use among those with daily PPE access prior to and following the 2018 EVD outbreak in Mbandaka, Democratic Republic of Congo

	Baseline					**6 month follow up**					**2.5 year follow up**				
	Vaccinated (n=9) [a]		Unvaccinated (n=273)			Vaccinated (n=85)		Unvaccinated (n=204)			Vaccinated (n=96)		Unvaccinated (n=76)		
	n	%	n	%		n	%	n	%		n	%	n	%	
What type of PPE was used															
Face mask	5	55.6	172	63.0	-	66	77.6	129	63.2	*	88	91.7	70	92.1	
Lab coat	7	77.8	171	62.6	-	68	80.0	130	63.7	**	77	80.2	53	69.7	
Gown	8	88.9	214	78.4	-	69	81.2	170	83.3		78	81.3	58	76.3	
Gloves	9	100.0	262	96.0	-	84	98.8	196	96.1		93	96.9	74	97.4	
Respirator	5	55.6	112	41.0	-	32	37.6	50	24.5	*	16	16.7	9	11.8	

a. small sample size due to mistake on survey logic, no p-values gathered for comparisons including this group

* p<0.05; ** p<0.01; ***p <0.0001; - p not appropriate

In multivariable generalized linear mixed modeling of transmission behaviors (Table 3.5) and PPE use (Table 3.6), there was little evidence of differences between vaccinated and unvaccinated individuals. However, vaccinated individuals were 2.44 times as likely to have participated in funeral rites or rituals than unvaccinated individuals at 2.5 years of follow up (95% CI: 1.02, 5.80). Vaccinated individuals were also more likely to use lab coats at 6 months of follow-up (OR 2.14, 95% CI 1.06, 4.31).

Table 3.5 Adjusted odds ratios for occupational transmission behaviors among vaccinated individuals compared to unvaccinated individuals in the 6 months following an EVD outbreak in Mbandaka, Democratic Republic of Congo 2018-2019

	6 month follow up n=330		2.5 year follow up n=365	
	Odds Ratio*	95% Confidence Interval	Odds Ratio*	95% Confidence Interval
Routine work activities				
Been in the patient's room	0.53	0.26, 1.07	0.79	0.39, 1.59
Performed Examinations (clinical or laboratory)	0.60	0.33, 1.09	0.89	0.44, 1.82
Performed Surgery	-		0.43	0.15, 1.23
Given food to a patient	0.57	0.27, 1.21	1.09	0.46, 2.58
Conversed with a patient	0.77	0.40, 1.48	0.90	0.47, 1.72
Washed the patient's clothes	0.15	0.004, 4.90	1.80	0.06, 55.30
Had contact with patient's bodily fluids	0.53	0.26, 1.08	1.09	0.50, 2.38
Processed patient specimens in a lab	0.14	0.002, 8.95	0.03	0.0001, 4.93
Washed a cadaver	4.08	0.05, 325.08	0.14	0.002, 8.72
Cleaned patient's room	0.12	0.01, 2.36	6.16	0.26, 146.14
Participated in funeral rites/rituals	1.39	0.75, 2.58	2.44	1.02, 5.80
Personal Injury				
Pricked by a contaminated needle	0.39	0.04, 3.90	0.27	0.02, 2.95
Contact with contaminated sharps	2.17	0.09, 51.32	0.10	0.01, 2.26
Contact with biological specimens (droplets)	1.10	0.15, 8.27	0.24	0.01, 4.51
Contact with blood when you had an open cut or wound	0.35	0.02, 6.50	0.62	0.04, 9.62
Contact with harmful chemicals while on the job	1.58	0.14, 17.26	0.20	0.02, 1.76
Harmed/bitten by a patient	6.43	0.08, 505.27	0.001	<0.0001, 0.34
Threatened/harmed by a patient's family	0.35	0.02, 6.41	0.46	0.02, 10.46
Gotten sick from a known patient exposure	1.89	0.05, 76.79	0.09	0.0004, 18.27
Sustained a blunt physical injury at work	5.86	0.28, 121.64	0.28	0.01, 5.61

*Controlled for age, sex, marital status, patient contact, education, EVD exposure, and baseline transmission behavior of interest.

Table 3.6 Adjusted odds ratios for PPE use among vaccinated individuals compared to unvaccinated individuals with daily PPE access following the 2018 EVD outbreak in Mbandaka, Democratic Republic of Congo 2018-2019

	6 month follow up n=330		2.5 year follow up n=330	
	Odds Ratio*	95% Confidence Interval	Odds Ratio*	95% Confidence Interval
What type of PPE was used				
Face mask	1.63	0.83, 3.18	0.62	0.17, 2.20
Lab coat	2.14	1.06, 4.31	1.42	0.64, 3.14
Gown	0.79	0.33, 1.89	1.24	0.46, 3.33
Gloves[a]	-		-	
Respirator	1.25	0.67, 2.34	0.95	0.36, 2.52

a. Estimate not available due to sparse data

* Controlled for age, sex, marital status, patient contact, EVD exposure, and education.

3.5 Discussion

When comparing risk behaviors between vaccinated and unvaccinated HCWs, we found that vaccinated HCWs were more likely to participate in funeral rites or rituals at two and a half years of follow up. As funeral rites carry a large EVD risk due to contact with a deceased body which may be infectious, this finding may demonstrate risk management in the community. Having vaccinated HCWs perform this high-risk activity may be an act of harm reduction as such individuals have more protection than unvaccinated HCWs. If so, this behavior would have been most relevant at the time point we observed it, when there had been an active EVD outbreak during the period for which data was collected. Given the evidence that Ebola vaccination carries significant protection[9–11], this behavior could be replicated at funerals in other locations, along with proper PPE and other infection control, to further decrease overall risk of EVD transmission as a result of funeral rites and rituals.

For the 6 month follow up in which there was no EVD outbreak, vaccinated HCWs were more likely to use lab coats than unvaccinated HCWs. This effect is most likely due to residual

confounding by HCW type. Though patient contact was controlled for, the specific type may have been influential regarding this specific variable. The high likelihood that physicians are both vaccinated and regularly use a lab coat may have produced this effect.

In addition to bias-controlled estimates, we observed evidence of univariate 6-month behavior differences. Interestingly, vaccinated individuals were more likely to use face masks, lab coats, and respirators, ignoring confounders. These findings suggest that unvaccinated individuals may need additional PPE training or motivation to use PPE. This recommendation is particularly pertinent in areas that have experienced a prior EVD outbreak, as they may be at higher risk for resurgent outbreaks due to relapsing infections in EVD survivors.

At baseline, we saw differences between routine activities of vaccinated and unvaccinated HCWs. Activities that involved going into patients' rooms or interacting with a patient tended to show the starkest differences. Unvaccinated individuals were more likely to routinely go into patients' rooms, converse with patients, wash patients' clothes, clean patients' rooms, and get sick from patient exposure. This pattern of findings is concerning. These patient contact activities represent increase EVD risk, and therefore should lead to a higher likelihood of vaccination. Currently, only being a HCW in an EVD affected area qualifies an individual for vaccination.[12] Given this broad criteria, it is unclear if there is a disparity of vaccine access, varying prevalence of vaccine hesitancy, or some other causal mechanism that has led to our observations. More research must be done to fully describe the observed phenomenon. However, regardless of etiology, we recommend that future vaccination campaigns make concentrated efforts to distribute vaccines to healthcare workers who have regular contact with patients, and therefore high occupational EVD risk.

This study is subject to a number of limitations. Vaccinated HCWs were recruited through convenience sampling based on where EVD cases were identified based on the ring vaccination strategy, which may have led to appreciable differences between those who participated in the study and the wider HCW population in the area affected by the 2018 Mbandaka outbreak. This may have affected the generalizability of our findings. Furthermore, loss to follow up could have resulted in biased estimates. Despite rigorous confounding control for the estimates shown in Table 3.5 and 3.6, it is possible there these measures are subject to residual confounding. Additionally, our data was collected through self-report and therefore may have been subject to limitations of recall and translation errors. However, local staff were hired to administer questionnaires and translate instruments from local languages to English and back to address concerns. Additionally, participants who received vaccination during follow up were dropped from the analysis. Future research should consider vaccination as a time-varying exposure to understand the full landscape of EVD transmission behaviors and risk in areas where there have been multiple Ebola vaccination campaigns.

Despite these limitations, this study provides a look at how both vaccinated and unvaccinated HCWs participate in occupational EVD transmission behaviors prior to and following EVD outbreaks. This analysis identified different behavioral patterns among vaccinated and unvaccinated HCWs at baseline and 6 months of follow up. However, few effects were observed when confounding control was employed. In multivariable analysis, vaccinated individuals were more likely to participate in funeral rites or rituals at 2.5 years of follow up and wear a lab coat at 6 months of follow up. Further research must fully describe the risk of transmission behaviors among vaccinated individuals, particularly for those who were vaccinated longer in the past in the case of waning immunity. These results should be taken into

consideration when working with both vaccinated and unvaccinated HCWs affected by EVD outbreaks, particularly in education and communication campaigns that seek to reduce workplace EVD transmission risk.

3.6 References

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Chapter 4: Ebola risk group and rVSVΔG-ZEBOV-GP vaccination response during the 2018 North Kivu Ebola outbreak in the Democratic Republic of the Congo

4.1 Abstract

Background: The newly licensed VSVΔG-ZEBOV-GP vaccine has shown notable efficacy and safety, but there is evidence of heterogeneous serological vaccine responses. Currently, research regarding the causes of varying antibody titers post-Ebola vaccination is sparse. Of particular interest is how baseline antibody titer and prior exposure to Ebola affect vaccine immunogenicity.

Methods: A cohort of vaccinated individuals from the eastern region of DRC, including North Kivu and Ituri provinces, were enrolled and followed prospectively for 6 months. We use linear mixed modeling to examine if baseline immune state, Ebola exposure history, or their joint effect impact serological vaccine response to VSVΔG-ZEBOV-GP vaccination. Further, G-computation was used to assess the causal, marginal effect of Ebola exposure history as mediated by baseline antibody titer on follow-up antibody titers.

Results: In our general linear mixed models, healthcare workers had significantly lower antibody titers at the 21-day visit compared to non-healthcare workers (Antibody titer ratio 0.62, 95% CI 0.44, 0.88), controlling for gender, age, education, and marital status. As baseline antibody titer increased, so did 21-day follow up antibody titers in all models. There was evidence of a positive joint effect of baseline antibody titer and being a contact of a contact of an Ebola case. In our g-computation analysis, we did not find evidence that Ebola exposure history impacted vaccine immunogenicity through mediation by baseline antibody titer.

Conclusion: Short-term vaccine responses are likely impacted by an individual's baseline antibody titer, with an increased baseline titer leading to a stronger vaccine response. Additionally, follow up titers are associated with healthcare worker status, though not mediated though baseline titer.

4.2 Introduction

The newly licensed VSVΔG-ZEBOV-GP vaccine has demonstrated notable efficacy and safety[1–5], yet growing evidence shows that not all individuals are completely protected against Ebola Virus Disease (EVD) post-vaccination.[4,6,7] During the 2018-2020 EVD outbreak in the Eastern Region of the Democratic Republic of the Congo (DRC), there were reports of individuals developing EVD symptoms more than 10 days after vaccination.[4,6] Beyond these breakthrough cases, there has been research which shows heterogeneity in serological response to Ebola vaccination[1,2,4,5,8–23], which is possibly tied to vaccine breakthrough. This heterogeneity can occur due to varying initial immunogenicity in the days to month following vaccination but could also be possibly tied to waning immunity post-vaccination on a scale of months to years.[5] While heterogeneity in vaccine response is well established, there is little research into the causes of varying antibody titers post-Ebola vaccination.

One possible cause of heterogeneous vaccine response is baseline immune state.[24] While currently unexplored for EVD, an impact of baseline immune state on post-vaccination outcomes has been reported for SARS-CoV-2, influenza virus, yellow fever virus, hepatitis virus, and malaria vaccination.[25–31] Furthermore, an increase in vaccine response dependent on baseline antibody titer would be especially relevant to vaccine success in the DRC, as many previous studies have shown varying levels of anti-filovirus antibody seroprevalence across the region regardless of EVD outbreak history, possibly due to undocumented EBOV infection or infection with a antigenically cross-reactive virus.[17,32,41,33–40] This presence of previously developed antibodies may interact with Ebola vaccination to alter vaccine response among Congolese individuals.

Baseline immune state may also serve as a mediator between EVD exposure history and vaccine response. Across risk groups included in the ring vaccination strategy used with VSVΔG-ZEBOV-GP vaccine deployment, it is likely there are variations in pre-vaccination immune state; family members of EVD cases, close contacts, contacts of contacts, and healthcare/frontline workers (HCW/FLWs) may meaningfully differ in baseline immune state due to intensity and duration of EVD exposure.[42,43] Additionally, these groups may experience different vaccine responses through other causal paths. These groups may experience variations in EVD exposure between baseline and follow-up, which may or may not interact with baseline antibody titer to impact follow up antibody titers.[44] Of particular concern is vaccine immunogenicity among healthcare workers, who accounted for 7 out of 15 vaccine failures described in a preliminary report on VSVΔG-ZEBOV-GP vaccine efficacy when used in outbreak response.[4]

In this study, we examine how baseline immune state, EVD exposure history, and their joint effect impact serological response to VSVΔG-ZEBOV-GP vaccination, (hereafter referenced as "Ebola vaccination"). This analysis will include univariate, multivariable regression, and assumed causal level statistics to both describe and analyze post-Ebola vaccination serology. Associations identified will be used to understand the landscape of EVD exposure history and post-vaccination serology. Effects identified will be used to understand the intersection between EVD exposure history, baseline immune state, and serological response to Ebola vaccination in EVD outbreak settings within the DRC.

4.3 Methods

Study population

Cohort enrollment occurred from August 15-29, 2018, in the province of North Kivu in the DRC. At the time of enrollment, the study site was experiencing an EVD outbreak that was declared on August 1st, 2018 and continued to June 25, 2020.[45]

Individuals were targeted for participation if they had received the Merck & Co VSVΔG-ZEBOV-GP Ebola vaccine through WHO/Expanded Programme for Immunizations (EPI) vaccination teams. Additionally, participants were eligible for the study if they were healthy, defined as having no fever (<38□C) or other self-reported acute illness at the time of enrollment. Women who reported being pregnant were not eligible for enrollment. Once enrolled, a survey was administered and blood samples were collected from consenting participants at each study visit for a total of five visits: baseline (day of vaccination), 21-day follow up, 6-month follow up, 1.5-year follow up, and 2.5-year follow up. Serological data was only available for the first three visits at the time of this analysis.

Ultimately, 620 individuals were enrolled in the cohort. Of these, 98.7% (n=612) had both survey and biological data available for at least one visit and were included in this analysis. Eighty-nine percent (n=550) of the sample completed a 21-day follow up, while 71% (n=436) completed a sixth month visit. Any individual who had baseline data was eligible for the 6-month follow up visit, regardless of completion of a 21-day follow up.

Surveys were conducted at each time point by trained interviewers in the participant's preferred local language (French, Lingala, or Swahili), and collected data on demographics and potential exposures to Ebola virus. Serologic testing was completed using the Filovirus Animal Nonclinical Group (FANG) assay to measure IgG antibody levels against the Ebola surface glycoprotein (anti-GP) in the serum. The FANG serologic test is a quantitative immunoassay developed by the National Institute of Allergy and Infectious Disease and approved by the U.S.

Food and Drug Administration for immunogenicity studies of Ebola vaccines.[46] More details can be found elsewhere.[3] Anti-GP IgG antibody titers in arbitrary ELISA units per milliliter (EU/mL) were recorded at 0 days (day of vaccination and baseline visit), 21 days, and 6 months post-vaccination.

Variables

Exposure

At baseline, EVD exposure history was gathered through two methods. First, EVD risk group was gathered through a self-reported list of attributes, including being a healthcare worker in an EVD affected area, having had a family member with EVD, having had a close contact with EVD, and having had a contact of a contact with EVD. Second, our questionnaire asked if the participant had been in contact with any known, suspected, or probable EVD cases.

Anti-GP IgG antibody titers on the day of vaccination (day 0) as measured through the FANG assay was used as the second exposure in regression models and the mediator in the g-computation analysis detailed below. For purposes of calculating geometric means and log of antibody titers, antibody titers of 0 were artificially changed to 1 so that estimates could be calculated.

Outcome

Anti-GP IgG antibody titers at 21-days and 6-months post-vaccination were used as outcomes.

Covariates

Self-reported gender, age, education level, marital status, and healthcare worker status were used for confounding control in multivariable regression. Additionally, g-computation analysis marginalized over these factors.

For gender, participants selected between “Male” and “Female” options.

Age was self-reported by the participant through several possible options. If the participant knew their birth date, age was calculated from this date. If they did not know their birthdate, year of birth was used to estimate age. If the participant had not knowledge of their births date or year, they were asked to estimate their age.

Education level was self-reported as “None”, “Some primary school”, “Finished primary school”, “Apprenticeship”, “Finished secondary school”, “College/University”, or “Graduate school”. Due to sparse data in some categories, some categories were collapsed by expected equivalence into “None or some primary school”, “Finished primary school or apprenticeship”, “Finished secondary school”, or “College/University or Graduate school”.

Marital status was self-reported as “Single”, “Married”, “Living together as marries”, “Divorced or separated”, or “Widowed”. Participants were able to decline to answer. Again, sparse data necessitated collapse into expected equivalent categories as following: “Never marries”, “Married or living together as married”, “Divorced, separated, or widowed”, or “Refused”.

Healthcare worker status as gathered for exposure was used for confounding control in models where healthcare worker status was not the exposure of interest.

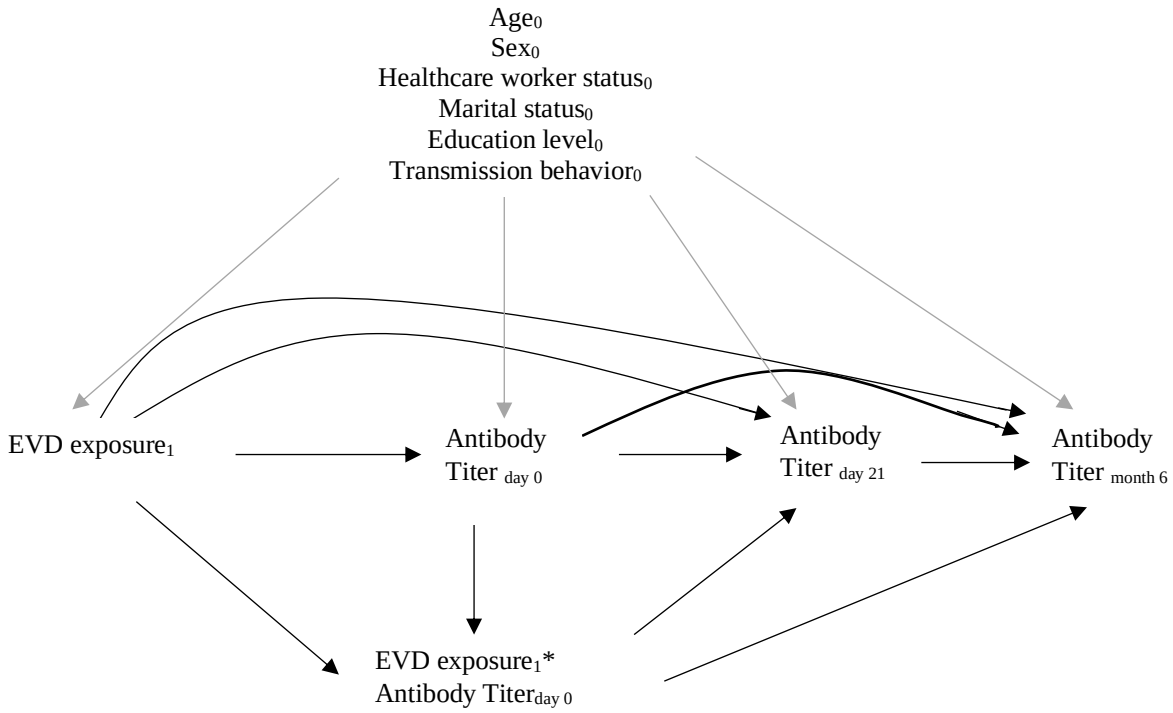
Statistical Analysis

Geometric means of antibody titers were obtained by Ebola exposure at baseline, 21 days, and 6 months of follow up to examine crude relationships. Antibody titer ratios and their

95% confidence intervals were obtained for univariate comparison of exposed versus unexposed individuals. Exposure was considered through several different categorizations: self-reported exposure to an EVD case, exposure to a family member with EVD, being a contact of an EVD case, being a contact of a contact of an EVD case, and being potentially exposed to EVD as a healthcare worker.

Following univariate analysis, linear mixed models were used to assess the longitudinal effects of EVD exposure history, baseline antibody titer, and their joint effect on the log of post-vaccination antibody titers. One model was run for each EVD exposure history, all of which also included a term for log on baseline titer, and an interaction term between these two variables. Participant was treated as the only random effect and an unstructured covariance matrix was used. Confounding control was employed for gender, age, education level, marital status, and healthcare worker status based on the hypothesized underlying causal structure depicted in Figure 4.1.

Figure 4.1 Directed Acyclic Graph (DAG) depicting hypothesized underlying causal structure for Ebola Virus Disease (EVD) exposure and antibody titer among VSVΔG-ZEBOV-GP vaccinated individuals in the Democratic Republic of the Congo



Subscripts denote time points at which variable occurred. X_0 denotes previous to outbreak or variables considered as non-time varying, X_1 denotes between outbreak start and day 0 of study participation

We used the g-computation algorithm, a generalization of the standardization method used commonly for time-varying covariates to further explore the underlying causal structure., G-computation was used to decompose and estimate the natural effects of various Ebola exposure types as mediated by baseline antibody titer on antibody titers 21 days and 6 months post-vaccination. This effect was decomposed into three components that had biologically meaningful interpretations. For the following displayed equations, Y refers to the outcome of

follow up antibody titer (continuous), E refers to EVD exposure history (binary for each classification of risk group, where 1 signifies belonging to the risk group of interest and 0 signifies not belonging to the risk group of interest), and M refers to the mediator—baseline antibody titer. Sublevels of M are m and m*, referring to reference baseline titer (average log baseline titer) for the former and baseline titer of interest for the latter.

Three decompositions were calculated using g-computation. First, we calculated the reference controlled direct effect (CDE_{ref}) or E [$Y_{E=1, M=m} - Y_{E=0, M=m}$]. The CDE_{ref} was calculated to estimate the effect of EVD exposure history on post-vaccination antibody titers if everyone's baseline titer were to be fixed at the geometric mean. This decomposition seeks to understand the marginal effect of EVD exposure history on post-vaccination in the absence of complexities introduced by baseline titer. Next, we obtained the the pure indirect effect (PIE) or E [$Y_{E=0, ME=1} - Y_{E=0, ME=0}$]. The PIE was calculated to estimate the effect of EVD exposure history on post-vaccination antibody titers only through baseline antibody titer. This decomposition attempts to understand if EVD exposure to pre-vaccination antibody titer to post-vaccination antibody titer functions as a sequential cascade of causal effects. Finally, we assessed the proportion attributable to interaction (PAI) or E [$Y_{E=1, ME=1} - Y_{E=0, ME=1}$] - E [$Y_{E=1, M=m*} - Y_{E=0, M=m*}$]. The PAI was calculated to estimate the effect of EVD exposure history on post-vaccination antibody titers due to its interaction with M, regardless of if X causes M. This decomposition attempts to understand if EVD exposure interacts with baseline antibody titer to cause post-vaccinations titers through some other mechanism than a sequential cascade. For example, this measure could elucidate if healthcare workers benefit from higher baseline titers more than non-healthcare workers, even if being a healthcare worker does not cause a higher baseline titer. This measure is particularly important if EVD exposure history is associated with continuing EVD exposure in

the follow-up period. Further discussion of g-computation for mediation analysis, and the interpretations of each decomposition can be found elsewhere.[47]

All provided confidence intervals were obtained using bootstrapping. A 95% CI that did not cross the null value of 1.00 was considered to be evidence of an association. All statistical analyses were carried out using R software, version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

4.4 Results

Our analysis included 612 vaccinated individuals (Table 4.1) with a median baseline antibody titer of 7 EU/mL (IQR=4, 21). It should be noted that this range falls completely below the lower limit of quantification (LLOQ = 66.96 EU/mL) for the FANG assay and should be interpretated with caution. Additionally, our sample had a median age of 33 years (IQR= 25, 43) at baseline. Most participants were men (64%) and married (57%). Our sample population reported various levels of highest education, with 32% having any primary school or apprenticeship, 26% having finished secondary school, and 29% having college, university, or graduate education. The remainder of participants (13%) reported having no formal education.

Table 4.1 Baseline sample characteristics of 612 VSVΔG-ZEBOV-GP vaccine recipients from North Kivu and Ituri provinces in the Democratic Republic of the Congo, August 2018.

	Median	IQR
Age	33	25, 43
Antibody Titer, *day 0*	7	4, 21

	Frequency (n)	Percent (%)
Sex		
Male	389	63.6
Female	223	36.4
Age		
0-17	21	3.4
18-24	114	18.6
25-34	204	33.3
35-44	144	23.5
45-54	71	11.6
55-64	32	5.2
65-82	26	4.2
Education		
None or some primary school	80	13.1
Finished primary school or apprenticeship	195	31.9
Finished secondary school	160	26.1
College/University or Graduate school	177	28.9
Marital status[a]		
Never married	247	40.4
Married or living together as married	347	56.7
Divorced, separated, or widowed	15	2.5
Healthcare worker	428	69.9
Has ever had contact with a confirmed, probable, or suspected EVD case[b]	176	32.1
Family member of an EVD case	106	17.3
Close contact of an EVD case	199	32.5
Contact of a contact of an EVD case	225	36.8
Elevated antibody titer (>607 EU/mL), *day 0*	9	1.5

a. 3 missing
b. 63 missing

Participants consisted of individuals who were targeted for Ebola vaccination by WHO/EPI teams, and therefore included individuals who were exposed or potentially exposed to EVD, belonging to the following risk groups: being a family member of an EVD case, a close contact, a contact of a contact, or a healthcare worker in an outbreak affected area. Thirty-two percent of the sample reported having contact with a confirmed, probable, or suspected EVD case, while 68% reported not having such contact. For specific EVD risk groups, 106 (17%) participants reported being a family member of an EVD case, 199 (32%) had contact with an EVD case, and 428 (70%) had contact with a contact of an EVD case. Thirty-seven percent of participants noted they had been possibly exposed to EVD as a healthcare worker.

In univariate analysis, healthcare workers had significantly lower antibody titers at the 21-day visit, compared to non-healthcare workers (Antibody titer ratio 0.83, 95% CI 0.69, 0.99) (Table 4.2). No other exposure classification showed a univariate association with baseline or follow up antibody titer.

Table 4.2 Unadjusted associations between antibody titer and Ebola exposure history in 612 VSVΔG-ZEBOV-GP vaccine recipients from North Kivu and Ituri provinces in the Democratic Republic of the Congo, August 2018-February 2019.

	Day 0 (n=612)			**Day 21** (n=550)			**Month 6** (n=436)		
	Geometric mean of antibody titer	Mean titer ratio	95% CI	Geometric mean of antibody titer	Mean titer ratio	95% CI	Geometric mean of antibody titer	Mean titer ratio	95% CI
Healthcare worker									
Yes	10	1.14	0.91, 1.44	818	0.83	0.69, 0.99	1233	1.00	0.86, 1.16
No	9			988			1230		
Known EVD contact history									
Yes	9	1.05	0.82, 1.36	844	0.89	0.73, 1.08	1187	0.97	0.65, 1.13
No	9			949			1227		
Family member with EVD									
Yes	10	1.17	0.89, 1.54	891	0.96	0.74, 1.24	1389	1.15	0.71, 1.05
No	8			927			1203		
Close contact of an EVD case									
Yes	10	1.14	0.89, 1.45	829	0.86	0.71, 1.04	1244	1.02	0.84, 1.15
No	9			967			1226		
Contact of a contact of an EVD case									
Yes	10	1.14	0.89, 1.45	939	1.07	0.76, 1.15	1233	1.01	0.83, 1.18
No	9			878			1226		

In our longitudinal regression models, healthcare workers again showed evidence of lower antibody titers at 21-days compared to non-healthcare workers (Antibody titer ratio 0.62, 95% CI 0.44, 0.88). Additionally, there was evidence that baseline antibody titer impacted follow up titers. As baseline antibody titer increased, so did 21-day follow up antibody titer in all models (Table 4.3). We also found evidence of a joint effect where contacts of EVD case contacts with an 172% increase in baseline titer (equivalent to an additional 1 log EU/mL), have 21-day antibody titers that are 52% larger (95% CI 7%, 115%) than non-contacts of EVD case contacts with a baseline titer that is 1 log (EU/mL) lower. However, the interaction term for contacts of EVD case contacts times baseline titer was not significant (p= 0.2261). Therefore, we did not detect interaction on the multiplicative scale for titer, equivalent to no interaction on the additive scale for the log of antibody titer.

Table 4.3 EVD exposure history, baseline antibody titer, and their joint effect on follow up antibody titer for 612 VSVΔG-ZEBOV-GP vaccine recipients from North Kivu and Ituri provinces in the Democratic Republic of the Congo, August 2018-Februrary 2019.

	21 day follow up		6 month follow up	
	Antibody Titer Ratio	95% CI	Antibody Titer Ratio	95% CI
Healthcare worker				
Effect of EVD exposure history	0.62	0.44, 0.88	0.97	0.72, 1.30
Effect of baseline titer	1.12	1.04, 1.22	0.97	0.90, 1.04
Joint Effect	0.81	0.61, 1.08	0.96	0.75, 1.22
Known EVD contact history				
Effect of EVD exposure history	0.86	0.60, 1.22	0.85	0.63, 1.16
Effect of baseline titer	1.18	1.08, 1.29	0.96	0.90, 1.03
Joint Effect	1.02	0.76, 1.37	0.86	0.67, 1.10
Family member				
Effect of EVD exposure history	1.02	0.64, 1.65	1.08	0.71, 1.64
Effect of baseline titer	1.19	1.11, 1.27	0.97	0.92, 1.03
Joint Effect	1.18	0.83, 1.67	1.07	0.79, 1.44
Close contact				
Effect of EVD exposure history	0.84	0.59, 1.20	0.94	0.69, 1.27
Effect of baseline titer	1.17	1.08, 1.27	0.96	0.90, 1.03
Joint Effect	1.01	0.75, 1.35	0.94	0.73, 1.20
Contact of contact				
Effect of EVD exposure history	1.32	0.91, 1.92	0.83	0.60, 1.16
Effect of baseline titer	1.25	1.12, 1.41	0.94	0.84, 1.04
Joint Effect	1.52	1.07, 2.15	0.82	0.60, 1.11

"Effect of baseline titer" refers to a 172% increase in baseline titer (+1 log of baseline titer)
All estimates controlled for gender, age, education level, marital status, and healthcare worker status

None of the decompositions for EVD exposure history's effect on follow up antibody titers showed evidence of an effect (Table 4.4).

Table 4.4 Decomposition of the effect of EVD exposure history and baseline antibody titer on follow up antibody titer using G-computation for 612 VSVΔG-ZEBOV-GP vaccine recipients from North Kivu and Ituri provinces in the Democratic Republic of the Congo, August 2018-Februrary 2019.

	21 day follow up		6 month follow up	
	Antibody Titer Ratio	95% CI	Antibody Titer Ratio	95% CI
Healthcare worker				
Reference Controlled Direct Effect	0.85	0.66, 1.11	1.00	0.82, 1.23
Pure Indirect Effect (PIE)	1.00	0.84, 1.18	1.00	0.88, 1.14
Proportion Attributable to Interaction (PAI)	1.35	0.92, 2.05	0.99	0.13, 8.93
Known EVD contact history				
Reference Controlled Direct Effect	0.91	0.72, 1.18	0.93	0.74, 1.15
Pure Indirect Effect (PIE)	1.00	0.84, 1.21	1.00	0.88, 1.15
Proportion Attributable to Interaction (PAI)	1.01	0.65, 1.55	0.85	0.05, 10.62
Family member				
Reference Controlled Direct Effect	0.92	0.65, 1.30	1.13	0.84, 1.48
Pure Indirect Effect (PIE)	0.98	0.77, 1.25	1.01	0.84, 1.19
Proportion Attributable to Interaction (PAI)	0.98	0.58, 1.69	0.22	0.01, 3.79
Close contact				
Reference Controlled Direct Effect	0.91	0.71, 1.21	0.96	0.78, 1.17
Pure Indirect Effect (PIE)	0.99	0.82, 1.19	1.00	0.88, 1.16
Proportion Attributable to Interaction (PAI)	1.06	0.68, 1.57	0.15	0.01, 1.16
Contact of contact				
Reference Controlled Direct Effect	1.11	0.86,1.43	0.93	0.74, 1.15
Pure Indirect Effect (PIE)	0.97	0.80, 1.17	1.00	0.85, 1.17
Proportion Attributable to Interaction (PAI)	0.84	0.56, 1.28	0.76	0.05, 10.32

Reference Controlled Direct Effect (CDE_{ref}) refers to the direct effect of EVD exposure only
Pure Indirect Effect (PIE) refers to the effect of EVD exposure through elevated baseline titer only
Proportion Attributable to Interaction (PAI) refers to effect attributable to interaction between exposure type and baseline antibody titer
All estimates marginalized over gender, age, education level, marital status, and healthcare worker status

4.5 Discussion

In this analysis of Ebola vaccination immunogenicity, we observed a positive association between baseline antibody titer and antibody titers at the 21 day follow up, but no association at 6 months follow up. This finding adds to the growing body of evidence that baseline immune state contributes to vaccine response heterogeneity.[24–31] Future research must determine correlates of protection for EVD, particularly correlation with EBOV-GP antibody titer. Once correlates are identified, this observed impact on post-vaccination serology can inform what vaccine coverage is needed for effective outbreak and control.

Healthcare workers in our analysis had lower antibody titers 21 days after vaccination compared to non-healthcare workers in both univariate and multivariable analyses. This observation underlines the importance of understanding serological vaccine response among healthcare workers in the DRC, particularly if serological response to this vaccine can be tied to vaccine efficacy. One previous report showed that healthcare workers account for a large proportion of vaccine failures; this preliminary report of vaccine efficacy in the 2018-2020 Beni outbreak showed that out of 15 individuals who developed EVD symptoms 10 or more days after vaccination, 7 of them where healthcare workers.[4] If healthcare workers are indeed at high risk of poor serological response to the vaccine and perhaps reduced vaccine efficacy, it may be beneficial to continue intense EVD prevention precautions in this group at least through 21 days post-vaccination.

Despite these relationships, we did not find effects between healthcare worker status and baseline titer on follow up antibody titers in our g-computation mediation analysis. We did not identify any controlled direct effect, pure indirect effect, or proportion attributable to interaction. These results may suggest that while healthcare workers may have lower short-term post

vaccination titers, this is not a causal link, even through baseline antibody titer. Furthermore, none of the additional types of EVD risk group decompositions showed effects on follow up antibody titers.

This study was subject to a number of limitations. Though retention in the study was high when considering that the area is a highly mobile population and the outbreak was on-going, loss to follow up could have introduced selection bias into our estimates. Additionally, the collection of a convenience sample could have limited our generalizability if those enrolled differed meaningfully from the overall population of individuals vaccinated for Ebola in DRC. Furthermore, limited sample size could have limited the power of this analysis to detect relationships, particularly within the interaction analysis portion. Participants were not recruited pre-vaccination to ensure vaccination remained a primary response activity, thus our enrollment occurred post vaccination, which included a 30-minute observation period post at which point serological samples were obtained. Based on the brevity of this short period post vaccination, this was not expected to lead to any serological differences if the samples had been collected pre-vaccination. Laboratory testing employed duplicate procedures with multiple quality checks to reduce measurement error. Though seropositive cut offs have been used previously, our sample did not have enough non-seroreactive samples to analyze antibody titer as a binary variable.[3,48] Therefore this analysis considered antibody titer as a continuous variable. In the absence of fully defined correlates of protection, any results cannot be extended to definitively discuss protection from EVD. Additionally, our g-computation analysis was done to support causal conclusions but requires that the following assumptions were met: consistency, conditional exchangeability, correct model specification, positivity, and absence of biases, including measurement error and selection bias.

4.6 Conclusion

This analysis suggests that antibody titers in the weeks and months following Ebola vaccination are not uniform. Short-term titers are likely impacted by an individual's baseline antibody titer, with an increase in baseline titer leading to a stronger vaccine response. Additionally, short-term follow up titers are associated with healthcare worker status, though most likely not through a path mediated by baseline antibody titer. Future research much identify modifiable factors that explain variations in serological vaccine response, particularly among healthcare workers. Additionally, more research must examine how these results continue over the long-term to understand the role of EVD exposure history and baseline immune status in the years following Ebola vaccination.

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Chapter 5: Discussion

5.1 Main Findings

In conclusion, this dissertation provides new insights into real-world relationships between Ebola vaccination, transmission behaviors, and EVD risk groups in an outbreak setting. Aim 1 evaluated associations between various EVD risk groups and subsequent behavior that may contribute to outbreak growth. While a short-term reduction in transmission behavior was observed, there was no apparent reduction in EVD transmission behavior during our long-term post-vaccination follow-up period. Additionally, this analysis demonstrated that HCW/FLWs have particularly complex behavior profiles post-vaccination. Given these observations, future investigations should seek to fully define risk of EVD vaccination failure and its connection with post-vaccination transmission behaviors in EVD outbreaks, particularly among HCW/FLWs.

Aim 2 provided a look at how both vaccinated and unvaccinated HCWs participate in occupational EVD transmission behaviors prior to and following EVD outbreaks. This analysis identified different behavioral patterns among vaccinated and unvaccinated HCWs at baseline and 6 months of follow up. However, few effects were observed when confounding control was employed. In multivariable analysis, vaccinated individuals were more likely to participate in funeral rites or rituals at 2.5 years of follow up and wear a lab coat at 6 months of follow up. Further research must fully describe the risk of transmission behaviors among vaccinated individuals, particularly for those who were vaccinated longer in the past in the case of waning immunity. These results should be taken into consideration when working with both vaccinated and unvaccinated HCWs affected by EVD outbreaks, particularly in education and communication campaigns that seek to reduce workplace EVD transmission risk.

Aim 3 examined serological response to vaccination as a result of the interplay between EVD risk group and baseline antibody titer. This analysis suggested that antibody titers following Ebola vaccination are not uniform. Short-term titers are likely impacted by an individual's baseline antibody titer, with an increase in baseline titer leading to a stronger vaccine response. Additionally, 21 day follow up titers were lower in HCW/FLWs, though most likely not through a causal relationship. Future research much identify modifiable factors that explain variations in serological vaccine response, particularly among HCW/FLWs.

These findings together contribute valuable understanding to the use of Ebola vaccination as an outbreak control measure. Ultimately, all three aims highlighted the importance of healthcare workers in any EVD outbreak response that uses vaccination. As individuals with complex behavior profiles and poorer vaccination response, HCWs/FLWs should receive extra attention in Ebola vaccination campaigns. Efforts should be made to support these individuals using supplemental education and infection prevention resources. These additional resources could take multiple forms, such as sufficient PPE available in the workplace or supplemental pay to ease financial burdens that prevent adequate social distancing and therefore exacerbate community transmission.

5.2 Strengths

The primary strength of this dissertation is the inclusion of individuals exposed to Ebola. Since Ebola has not historically occurred in regular, large outbreaks, it has been difficult to obtain data on individuals who are directly affected by the disease. This challenge is exacerbated by safety concerns for scientists that wish to study the disease *in vivo*. Therefore, having such a data set on individuals who experienced a direct Ebola exposure or were otherwise at high risk

for developing EVD is a valuable resource. Here, we were able to examine behavior changes in a non-hypothetical setting, allowing us to observe real world behavior as opposed to collecting what an individual believes they would do after hypothetically exposed to Ebola. Additionally, we were able to study direct effects of EVD exposure on serology.

Another strength of this dissertation is its use of longitudinal data. Using follow up data, we were able to attribute temporality to our associations of interest. Therefore, our follow up data allows us to examine our causal hypotheses in more depth than a cross sectional study would. This data will allow us to make claims not hindered by reverse causality.

One additional strength of this study was the strong local study staff and on the ground knowledge of working with mobile populations, as well as strong community knowledge of the study activities. Much effort was expended to include local communities in study activities so that meaningful insights could be generated from this data.

5.3 Limitations

While there are the above strengths to this dissertation, a number of limitations do exist. Some of these limitations are products of performing epidemiologic research on Ebola affected populations. Most notably, data was collected during an active outbreak period, which may have been a more stressful period. This stress may have impacted those who participated in the study and who was lost to follow up visits. Another effect of performing research in such an area was that we did not have the ability to recruit a control group of non-vaccinated individuals in our cohort in the Eastern DRC provinces of North Kivu and Ituri. The violence and instability in this region limited our access to the community in a way where data collection was only feasible for individuals who had already been gathered for vaccination. This failure to gather a control group limits our conclusions for Aims 1 and 3 to only vaccinated individuals. We cannot comfortably

extend our findings to those who have not been vaccinated for Ebola. However, given our that our aims speak specifically about Ebola exposure, the population that has a known exposure in the absence of vaccination should be limited in the post-vaccination landscape.

Beyond limitations stemming from Ebola-specific challenges, our data is limited by virtue of being an observational study abroad. Data was collected through self-report, which may be subject to bias due to limitations of recall and translation errors. While there was little to be done about limitations of recall, much effort was undertaken to reduce information bias due to translation errors. Local staff were hired to administer questionnaires to conserve information in each translation from local languages to English and vice versa.

5.3 Conclusion

This dissertation expands our knowledge of how various types of Ebola exposure and vaccination can impact transmission behavior and serological response to vaccination. This dissertation provides vital knowledge that healthcare workers are at highest risk for post-vaccination Ebola transmission, both through risk behavior and post-vaccination serology. Thus, the findings from this research inform our use of the Ebola vaccine. This research identifies that vaccination of healthcare workers, particularly those who work in close contact with patients, must be supplemented with education and other infection control practices to enhance transmission prevention. For example, HCW/FLWs that are prone higher Ebola risk after vaccination must be counseled that vaccination does not provide immediate protection, but that they must conduct themselves as susceptible for at least 21 days, ideally indefinitely. This knowledge will ultimately aid in the control of Ebola outbreaks where vaccination is used as an outbreak control measure.