Factors of Risk and Protection for Ebola Exposure in Health Care Workers, Kinshasa Province, Democratic Republic of Congo

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
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ABSTRACT

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Abstract

Health care workers (HCWs) are at great risk of acquiring infections at work given their occupational exposure. However, not all infections present symptomatically leading HCWs to become unknowingly infected. Asymptomatic and pauicsymtpomatic disease in Ebola virus (EBOV) infection is higher than previously thought in areas of previous outbreaks. The rate of asymptomatic and paucisymptomatic disease is unknown in urban parts of the Democratic Republic of the Congo (DRC). Understanding prevalence, risk, and protective factors for asymptomatic and pauicsymptomatic Ebola virus disease (EVD) in HCWs is essential for the security and prosperity of the city. Methods: A total of 424 HCWs were included in a serosurvey from urban and rural areas in the Kinshasa region of the Democratic Republic of Congo (DRC). An ELISA kit from Filovirus Animal Non-Clinical Group (FANG) measured titer levels of human anti-EBOV glycoprotein IgG. Associations between risk factors and seroreactivity were determined through both bivariable and multivariable logistic regression. We then evaluated results using different cutoff values established in different countries around the continent (Mali and Liberia). Results: Twelve (2.9%) participants from our cohort with no previous EVD history were seroreactive for EBOV. Confidence intervals for all observations were wide, crossed 1.00, and within null range, making the observed odds ratios insignificant. Multivariable analysis was not necessary since all findings in bivariable analysis were insignificant but was performed as an

academic exercise. All findings in multivariable analysis were also insignificant. No significant changes to our findings occurred upon further scrutiny in our sensitivity analysis. When compared to cohorts from our previous study site, Boende [4], prevalence for seroreactivity was observed at much lower levels (2.9% vs 22.5%).

Conclusions: Our findings further solidify results from our previous study site and offer additional evidence of asymptomatic and paucisymptomatic EVD, but at significantly lower levels. Further analysis and testing are required for this cohort to determine better quantitative results. Additional investigation is needed to: determine a cutoff value in DRC for the FANG ELISA kit, detect if transmission from minimally symptomatic individuals occurs, and evaluate if seroreactivity equates to immunity for individuals.

Dedication

To all the health care and essential workers who know firsthand what it takes to end outbreaks, thank you.

To the entire INRB and UCLA-DRC team for collecting and sharing data with someone you never met, I hope we meet someday.

To my grandmother for raising my brother and I and getting us to where we are today.

To my husband Kevin, for being my true north.

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Acknowledgements

I would like to acknowledge the incredible team on the ground in the Democratic Republic of Congo including the Institut Nationale pour la Recherche Biomedicale (INRB), the UCLA DRC team, Rimoin Lab and Annie Rimoin. Thank you for being open to sharing your time, expertise, and work with someone who you barely knew. I would like to specifically acknowledge Nicole Hoff and Anna Bratcher for always responding to my never-ending emails and questions, thank you ladies. Thank you to Larry Park for helping me stumble my way through the endless software errors. I would also like to acknowledge and thank Chris Woods whose unwavering mentorship and dedication lead him to answer every call and email, no matter the time and place. Lastly, I would like to acknowledge all of my classmates, without whom I could not made it through this program.

1. Introduction

Background and Study Goals

Ebola is one of the deadliest viruses, with a case-fatality rate of up to 90% in recent epidemics [6]. Healthcare workers have a higher risk of contact with Ebola virus (EBOV) infected individuals [8], and therefore manifest most types of infection, including asymptomatic or paucisymptomatic illness. Ebola virus disease (EVD) was previously thought to always manifest in severe disease, but recent data has suggested the presence minimally symptomatic disease [3]. Our study team has found rates as high as 22.5% for minimally symptomatic EVD in Boende, Democratic Republic of the Congo (DRC) [4].

In order to reduce asymptomatic and undetected spread of EVD in healthcare workers, understanding behavioral and demographic factors that contribute to a healthcare worker's chance of becoming ill is critical. However, risk factors for asymptomatic EVD are not well understood. We can mitigate minimally symptomatic spread of EVD by developing a risk model evaluating factors of risk and protection for healthcare workers. This analysis may inform and shape healthcare worker policy and practices.

The long-term goal of this project is to reduce undetected infection and transmission of EVD among HCWs in the DRC. The principal objectives for this analysis are to: 1) **determine factors of risk and protection** for asymptomatic EVD infection in the Kinshasa province and 2) **compare prevalence, risk, and protection factors** for

asymptomatic EVD between Kinshasa and previously studied Boende. Our comparison of the two sites will clarify if there are patterns of risks and protections that may be used broadly to prevent asymptomatic EVD.

The undertaking of our objective and work products respond to the *current epidemic* declared on 1 June 2020 in the DRC. Conclusions resulting from our objective and work products may influence future healthcare worker training, care giving, policy and intervention initiatives.

Rate of Asymptomatic Infection

Data from a previous serosurvey shows that approximately one in five healthcare workers in Boende, DRC were minimally symptomatic with EVD [8]. Specifically, 21.3% of the sample population of healthcare workers (N=1289) had Ebola antibodies in the absence of previous symptoms or diagnoses [8]. This study will extend the previous analysis by evaluating Kinshasa, DRC--the capital city and *most heavily populated region* of the country--in order to compare significant risk factors of significance with the Boende findings.

A meta-analysis estimated a higher rate of asymptomatic infection of EBOV than the aforementioned serosurvey at 27.1 % [3]. None of the studies used to estimate asymptomatic proportions explicitly stated a rate for subclinical infection but, the authors estimated this value by subtracting the pathogenicity from 1. However, limited quality data were used to provide this estimate which reflects only two of seven studies found. The two studies used were serosurveys conducted in 1999 in Kikwit, DRC and in

2001 in Gabon. Despite the limited amount of usable data, the authors argued that any estimate of the previously unknown asymptomatic infection rate for EBOV was useful [3].

Factors of Risk and Protection

Using nine different studies, the aforementioned meta-analysis concluded transmission and secondary attack rate (SAR) were driven by direct contact with infected individuals, while provision of nursing care was the most significant risk factor. The data used for this meta-analysis came from data collected in DRC (1976, 1995), Sudan (1976, 1979), 3 studies in Uganda (all in 2000), Republic of Congo (2005), and Liberia (2014). A cross-sectional serosurvey reenforced health care work and level of exposure as risk factors for infection [21]. While a third serosurvey concluded that personal protective equipment (PPE) was negatively associated with seroreactivity, making it a protective factor [4].

Hypotheses

We aim to analyze demographic and behavioral factors associated with the presence of EBOV antibodies in asymptomatic healthcare workers surveyed in November and December of 2017 in Kinshasa. We hypothesize that Ebola outbreak involvement, type of healthcare worker, exposure to suspected or confirmed cases, and exposure to the deceased will have the most profound positive associations with seroreactivity.

Additionally, we aim to compare significant risk factors and EBOV antibody prevalence in subjects between Boende and Kinshasa. We hypothesize that significant risk factors for asymptomatic EVD will be similar between the two study sites.

2. Methods

2.1 Setting

A serosurvey was conducted in November and December of 2017 in the Kinshasa province, a region encompassing the capital city and highest population densities of the DRC and the entire continent.

2.2 Participants

In total, 424 participants were recruited and enrolled in this study, representing 15 types of health care workers in the Kinshasa region. Health care worker classification was based on the World Health Organization classification system [22]. We limited the participant pool to those that met the following criteria: 18 years of age or older, healthy (no current illness or underlying health issues), work in a health care facility, and reported no previous known or suspected EBOV infections. Thirteen participants were excluded from analysis due to missing serologic results.

2.3 Procedures

The following procedures took place: ethical review of study proposal and protocols from the Duke Health Institutional Review Board (IRB), Kinshasa School of Public Health IRB and University of California Los Angeles Field School of Public Health IRB. Training of standard operating procedures for personnel, recruitment, consent, and enrollment of participants, participant interviews, participant sample collection, sample storage, sample analysis, and data analysis also took place.

This study underwent ethical reviews from several institutional review boards including: Duke University (Pr00102492), University of Los Angeles Fielding School of Public Health (IRB 16-001346), and the Kinshasa School of Public Health DRC.

Study personnel included staff from the Institute National pour la Recherche Biomedicale (INRB) headquartered in Kinshasa and short-term staff local to the region hired under a subcontract with INRB. All short-term local staff were the same individuals throughout the entire enrollment period.

Participants were recruited from four general hospitals located in three urban health zones and one rural health zone of Kinshasa. Once recruited and enrolled into the study, participants were completed a standardized questionnaire. Upon completion participants contributed blood samples to the study and underwent a physical assessment to capture their vital signs, height, and weight. Participants were then reimbursed for transportation costs to and from the healthcare facility.

Blood samples were collected via venipuncture into BD vacutainer blood collection tubes. Serum was separated from blood samples and stored at -80 degrees Fahrenheit. Sample storage, processing, and analysis of participants' serological specimens was conducted at INRB by INRB personnel. Upon completion of analysis, results of seroreactivity were not provided to participants.

2.4 Measures

Participants were interviewed for demographic information. Serological specimens were evaluated by Enzyme-Linked Immunosorbent Assays (ELISA) from Filovirus Animal Non-Clinical Group (FANG).

2.4.1 Questionnaire

A scripted questionnaire was given in either French or _____ to each participant. Questions posed to participants captured social, demographic, and epidemiologic information. Participant answers were captured via android tablet using a mobile application that allows for offline data collection called Open Data Kit Collect.

2.4.2 Sample Analysis

We assessed for presence of anti-EBOV GP IgG using ELISA (FANG). This study employed the assay to specifically detect human anti-EBOV glycoprotein immunoglobulin G (GP IgG). Detection of anti-EBOV GP IgG is the current standard used to determine previous EBOV infection (seroreactivity) for research use only. Seroreactivity for anti-EBOV GP IgG using the FANG assay has been previously defined as a minimum of 548 ELISA units (EU)/mL in Liberia and 607 EU/mL in Mali. Since there is no currently described cutoff for positivity in DRC, this study used the Liberian established cutoff value of 548 EU/mL to define seropositivity. Efforts to define a cutoff value in DRC are currently being planned.

2.5 Data Analysis

Five phases of data analysis occurred for this study: descriptive statistics for the entire study population, bivariable logistic regression, multivariable logistic regression, sensitivity analysis, and Fisher's Exact Tests. For describing the entire study population, frequencies, medians, and percentages were reported. Bivariable logistic regression was used to determine the association between seroreactivity and selected risk factors assessed in our questionnaire. Unadjusted odds ratios and corresponding confidence interval were reported for bivariable regression. Multivariable logistic regression was used to determine predictors of seroreactivity. Adjusted odds ratios and corresponding confidence intervals were reported for multivariable regression. For our sensitivity analysis, we employed the more conservative Malian cutoff value for seropositivity (607 EU/mL) to determine any significant differences from our original models. Fisher's Exact Tests were conducted because of small sample size found during descriptive analysis. All statistical analysis was completed using RStudio Cloud (version 4.0.3). '

3. Results

3.1 Demographics Characteristics

A total of 424 HCWs were sampled from 4 general hospitals and surrounding clinics in the Kinshasa area, no participants had been previously diagnosed or suspected of EVD, but 13 were excluded from analysis due to missing serologic data. Demographic data from the entire cohort is displayed in Table 1 with some additional details in Table 2. Only 12 participants (2.9%) of the sampled population were seroreactive for anti-EBOV GP IgG using a 2pt dilution with the FANG ELISA kit and a cutoff value of 548 EU/mL. The median age of our participants was 44 (IQR, 34-55). Most participants were female (55%), college or university graduates (55%), and had a partner (60.4%). Nearly all of our participants reported they had not been involved in an EBOV outbreak (96.1%) and were not considered a close contact with anyone who was previously a suspected, probable, or confirmed case of EVD (96.5%). A majority of this cohort worked in general hospitals (67.9%) and nurses were the most represented HCW role (39.4%).

Of the roughly 4% of participants who were not involved with an EBOV outbreak, most were in the 18-39 years old age group, female, single, went to college, and nurses at general hospitals. Only one participant who was not involved in an EBOV outbreak was seroreactive.

Table 1. Sample characteristics and demographics of 424 health care workers from Kinshasa, DRC, November-December 2017

Characteristic	**N = 411**[1]
Age	44 (34, 55)
Unknown	5
Sex	
Male	183 (44.5)
Female	225 (54.7)
Unknown	3
Education Level	
None	1 (0.2)
Started Primary School	6 (1.5)
Finished Primary School	53 (12.9)
Finished Secondary School	118 (28.7)
Apprentice	2 (0.5)
College/University	226 (55)
Graduate School	2 (0.5)
Unknown	3
Civil Status	
Single	112 (27.3)
Married	180 (43.8)
Living Together as Married	70 (17)
Divorced/Separated	22 (5.4)
Widowed	24 (5.8)

Unknown	3
Close Contact of Suspected, Probable, or Confirmed EVD Case	
No	397 (96.6)
Yes	11 (2.7)
Unknown	3
Health Care Worker Type	
Nurse Supervisor	1 (0.2)
Nurse Titulaire	13 (3.2)
Nurse	165 (40.1)
Physician	15 (3.6)
Health Communication Officer	2 (0.5)
Lab Technician	28 (6.8)
Administrator	14 (3.4)
Room Attendant	21 (5.1)
Hygienic Service	18 (4.4)
Medical/Nurse Student	5 (1.2)
Red Cross Worker	4 (1.0)
Midwife	20 (4.9)
Maintenance	5 (1.2)
Other	47 (11.4)
Unknown	53 (12.9)
Reactive ELISA result	
≥ 548 EU/mL[2]	12 (2.9)

≥ 607 EU/mL[3]	9 (2.2)
Unknown[4]	13

[1]Median (IQR); n (%)

[2]Liberia cutoff value

[3]Mali cutoff value

[4]Participants removed from total N and analysis

3.2 Demographics Characteristics by Seroreactivity

Overall, bivariable analysis revealed that being female (odds ratio [OR], 0.80; 95% CI 0.25-2.60), a college or university graduate (odds ratio [OR], 0.57; 95% CI 0.17-1.80), single (odds ratio [OR], 0.51; 95% CI 0.08-1.98), and a nurse (odds ratio [OR], 0.50; 95% CI 0.11-1.82) lowered the odds ratios of seroreactivity. However, none of these findings were statistically significant as demonstrated by the reported confidence intervals. Bivariable analysis also revealed that being over the age of 60 (odds ratio [OR], 1.40; 95% CI 0.31-4.83), divorced or separated (odds ratio [OR], 6.65; 95% CI 1.39-24.46), lab technician (odds ratio [OR], 3.00; 95% CI 0.44-12.68), administrator (odds ratio [OR], 2.88; 95% CI 0.15-17.12), room attendant (odds ratio [OR], 1.83; 95% CI 0.10-10.50, working in a general hospital (odds ratio [OR], 2.41; 95% CI 0.44-44.85), and active involvement in an EBOV outbreak (odds ratio [OR], 2.92; 95% CI 0.15-17.01) increased the odds ratios of seroreactivity. However, none of these findings were statistically significant as demonstrated by the reported confidence intervals.

Taking a closer look at the twelve participants who were seroreactive, we find that: the most represented age group was 40-59 years old (41.7%), there was an even split between men and women (50%, 50%), college and university were the most represented education group (41.7%), half of the group had a domestic partner (50%), all but one did not consider themselves a close contact of an EVD case (91.7%), nurses were the most represented HCW role (25%), most worked at a general hospital (75%), and all but one worked as an HCW during an outbreak (91.7%). When evaluating activities

performed with suspected, probable, or confirmed EVD a majority of the twelve seroreactive participants: entered patients' rooms (66.7%), conversed with patients (75%), participated in funeral rites (75%), and did not use PPE daily (58.3%).

Table 2. Characteristics by seroreactivity (GP > 548 EU/mL) in 412 health care workers from Kinshasa, Democratic Republic of Congo, 2017

Characteristic	N = 411[1,2]	GP ≤ 548 EU/mL N = 399	GP > 548 EU/mL N = 12	Unadjusted Odds Ratio	95% Confidence Interval
Age	44 (34,55)				
18 – 39 y	157 (38.2)	153 (38.3)	4 (33.3)	Reference	-
40 – 59 y	170 (41.4)	165 (41.4)	5 (41.7)	0.99	0.29-3.15
60+ y	79 (19.2)	76 (19)	3 (25)	1.40	0.31-4.83
Unknown	5 (1.2)	5 (1.3)	0 (0.0)	…	…
Sex					
Male	183 (44.5)	177 (44.3)	6 (50)	Reference	-
Female	225 (54.7)	219 (54.9)	6 (50)	0.80	0.25-2.60
Unknown	3 (0.7)	3 (0.8)	0 (0.0)	…	…
Education Level					
None	1 (0.2)	1 (0.3)	(0.0)	…	…
Started Primary School	6 (1.5)	5 (1.3)	1 (8.3)	NA	NA
Finished Primary School	53 (12.9)	51 (12.7)	2 (16.7)	NA	NA
Finished Secondary School	118 (28.7)	114 (28.5)	4 (33.3)	Reference	-
Apprentice	2 (0.5)	2 (0.5)	0 (0.0)	…	…
College/University	226 (55)	221 (55.4)	5 (41.7)	0.57	0.17-1.80
Graduate School	2 (0.5)	2 (0.5)	0 (0.0)	…	…
Unknown	3 (0.7)	3 (0.8)	0 (0.0)	…	…
Civil Status					
Single	112 (27.3)	110 (27.6)	2 (16.7)	0.51	0.08-1.98
Married	180 (43.8)	175 (43.9)	5 (41.7)	0.91	0.27-2.90
Living Together as Married	70 (17)	69 (17.2)	1 (8.3)	Reference	-

Divorced/Separated	22 (5.4)	19 (4.7)	3 (25)	6.65	1.39-24.46
Widowed	24 (5.8)	23 (5.8)	1 (8.3)	1.48	0.08-8.13
Unknown	3 (0.7)	3 (0.8)	0 (0.0)	…	…
Close Contact					
No	397 (96.6)	386 (96.7)	11 (91.7)	Reference	-
Yes	11 (2.7)	10 (2.5)	1 (8.3)	NA	NA
Unknown	3 (0.7)	3 (0.8)	0 (0.0)	…	…
Health Care Worker Type					
Nurse Supervisor	1 (0.2)	1 (0.3)	0 (0.0)	…	…
Nurse Titulaire	13 (3.2)	13 (3.3)	0 (0.0)	…	…
Nurse	165 (40.1)	162 (40.6)	3 (25)	0.50	0.11-1.82
Physician	15 (3.6)	15 (3.7)	0 (0.0)	…	…
Health Communication Officer	2 (0.5)	2 (0.5)	0 (0.0)	…	…
Lab Technician	28 (6.8)	26 (6.5)	2 (16.7)	3.00	0.44-12.68
Administrator	14 (3.4)	13 (3.3)	1 (8.3)	2.88	0.15-17.12
Room Attendant	21 (5.1)	20 (5.0)	1 (8.3)	1.83	0.10-10.50
Hygienic Service	18 (4.4)	18 (4.5)	0 (0.0)	…	…
Medical/Nurse Student	5 (1.2)	5 (1.3)	0 (0.0)	…	…
Red Cross Worker	4 (1.0)	3 (0.8)	1 (8.3)	NA	NA
Midwife	20 (4.9)	20 (5.0)	0 (0.0)	…	…
Maintenance	5 (1.2)	5 (1.3)	0 (0.0)	…	…
Other	47 (11.4)	45 (11.2)	2 (16.7)	1.69	0.25-7.03
Unknown	53 (12.9)	51 (12.7)	2 (16.7)	...	...
Health Facility Type [3]					
General Hospital	284 (69.1)	275(69)	9 (75)	2.41	0.44-44.85
Health Center	68 (16.5)	68 (17)	0 (0.0)	...	...
Other	6 (1.5)	5 (1.3)	1 (8.3)	7.67	0.38- 54.57
Unknown	53 (12.9)	51 (12.7)	2 (16.7)	...	...

Ever involved with an EBOV outbreak					
No	395 (96.1)	384 (96.2)	11 (91.7)	0.37	0.06-7.04
Yes	13 (3.2)	12 (3.0)	1 (8.3)	2.92	0.15-17.01
Unknown	3 (0.7)	3 (0.8)	0 (0.0)	...	...
Activities performed on suspected, probable, or confirmed EVD patient					
Entered patient's room					
No	57 (13.9)	55 (13.8)	2 (16.7)	1.33	0.20-5.49
Yes	301 (73.2)	293 (73.4)	8 (66.7)	0.75	0.18-5.06
Unknown	53 (12.9)	51 (12.8)	2 (16.7)	…	…
Performed patient evaluations (clinical or laboratory)					
No	249 (60.6)	242 (60.7)	7 (58.3)	1.00	0.27-4.72
Yes	107 (26)	104 (26.1)	3 (25)	1.00	0.21-3.66
Unknown	55(13.4)	53 (13.3)	2 (16.7)	…	…
Gave food to patient					
No	258 (62.8)	250 (62.7)	8 (66.7)	1.54	0.38- 10.30
Yes	98 (23.8)	96 (24)	2 (16.7)	0.65	0.10- 2.65
Unknown	55 (13.4)	53 (13.3)	2(16.7)	…	…
Conversed with patient					
No	55 (13.4)	54 (13.5)	1 (8.3)	0.60	0.032- 3.32
Yes	303 (73.7)	294 (73.7)	9 (75)	1.65	0.30- 30.79
Unknown	53 (12.9)	51 (12.8)	2 (16.7)	…	…
Washed patient's clothes					
No	322 (78.3)	313 (78.4)	9 (75)	1.00	0.18- 18.83
Yes	36 (8.8)	35 (8.8)	1 (8.3)	0.99	0.05- 5.52
Unknown	53 (12.9)	51 (12.8)	2 (16.7)	…	…
Exposed to patients' body fluids					
No	187 (45.5)	182 (45.6)	5 (41.7)	NA	NA
Yes	164 (39.9)	159 (39.8)	5 (41.7)	NA	NA

Unknown	53 (12.9)	51 (12.8)	2 (16.7)	…	…
Cleaned patient's room					
No	275 (66.9)	267 (66.9)	8 (66.7)	1.20	0.29- 8.05
Yes	82 (20)	80 (20.1)	2 (16.7)	0.83	0.12- 3.41
Unknown	54 (13.1)	52 (13)	2 (16.7)	…	…
Washed cadaver					
No	332 (80.8)	323 (81)	9 (75)	0.67	0.12-12.59
Yes	25 (6.1)	24 (6)	1 (8.3)	1.50	0.08-8.50
Unknown	54 (13.1)	52 (13)	2 (16.7)	…	…
Participated in funeral rites					
No	124 (30.2)	123 (30.8)	1 (8.3)	0.20	0.01-1.10
Yes	233 (56.7)	224 (56.1)	9 (75)	4.94	0.91-91.67
Unknown	54 (13.1)	52 (13)	2 (16.7)	…	…
Used PPE daily					
No	162 (39.4)	155 (38.8)	7 (58.3)	2.89	0.79- 13.58
Yes	195 (47.4)	192 (48.1)	3 (25)	0.35	0.07- 1.27
Unknown	54 (13.1)	52 (13)	2 (16.7)	…	…

[1] Median (IQR); n (column %)

[2] 13 participants were removed from analysis; missing serologic data

[3] 1 participant did not know what type of health facility they worked in

[4] 1 participant did not know if they actively worked during an outbreak

[5] 7 participants did not know if they were exposed to bodily fluids

3.3 Predictors for Seroreactivity

Multivariable analysis was not necessary since all findings in bivariable analysis were insignificant but was performed as an academic exercise. Multivariable analysis revealed that conversing with patients (odds ratio [aOR], 1.01; 95% CI 0.97-1.07) and participating in funeral rites (odds ratio [aOR], 1.02; 95% CI 0.99-1.05) had odds ratios ever so slightly above 1.00 but were found to be statistically insignificant. All other models were also found to be within null range and statistically insignificant. All exposures were adjusted for age and sex.

Table 3. Adjusted odds ratios of seroreactivity (GP>548 EU/mL) by patient interaction exposures of 412 heath care workers in Kinshasa, Democratic Republic of Congo, 2017

Exposure	Adjusted Odds Ratio	95% Confidence Interval
Activities performed on suspected, probable, or confirmed EVD patient[1]		
Entered patient's room	0.99	0.95-1.04
Performed patient evaluations (clinical or laboratory)	1.00	0.98-1.03
Gave food to patient	1.00	0.98-1.02
Conversed with patient	1.01	0.97-1.07
Washed patient's clothes	1.00	0.95-1.06
Exposed to patient's body fluids	1.00	0.99-1.02
Cleaned patient's room	1.00	0.97-1.03
Washed cadaver	1.00	0.97-1.04
Participated in funeral rites	1.02	0.99-1.05
Used PPE daily[1]		
	0.99	0.96-1.01

[1] Adjusted for age and sex

3.4 Fisher's Exact Tests

Fisher's Exact Tests were performed on variables included during modelling due to our low sample size of seroreactive participants. Table 4 presents results from this phase of analysis for characteristics by seroreactivity. No findings using this mode of analysis were significant.

Table 4. Fisher Exact Tests for characteristics by seroreactivity (GP > 548 EU/mL) in health care workers from Kinshasa, Democratic Republic of Congo, 2017

Characteristic	GP ≤ 548 EU/mL	GP > 548 EU/mL	Row Total	p-value (α = 0.05)
Age				
18 – 39 y	153	4	157	0.77
40+ y	241	8	249	
Column Total	394	12	406	
18 – 59 y	318	9	327	0.71
60+ y	76	3	79	
Column Total	394	12	406	
Sex				0.77
Male	177	6	183	
Female	219	6	225	
Column Total	396	12	408	
Close Contact				0.28
No	386	11	397	
Yes	10	1	11	
Column Total	396	12	408	
Ever involved with an EBOV outbreak				0.33
No	384	11	395	
Yes	12	1	13	
Column Total	396	12	408	
Activities performed on suspected, probable, or confirmed EVD patient				
Entered patient's room				0.66
No	55	2	57	
Yes	293	8	301	
Column Total	348	10	358	

Performed patient evaluations (clinical or laboratory)				1.00
No	242	7	249	
Yes	104	3	105	
Column Total	364	10	354	
Gave food to patient				0.73
No	250	8	258	
Yes	96	2	98	
Column Total	346	10	356	
Conversed with patient				1.00
No	54	1	55	
Yes	294	9	303	
Column Total	348	10	358	
Washed patient's clothes				1.00
No	313	9	322	
Yes	35	1	36	
Column Total	348	10	358	
Exposed to patients' body fluids				1.00
No	182	5	187	
Yes	159	5	164	
Column Total	341	10	351	
Cleaned patient's room				1.00
No	267	8	275	
Yes	80	2	82	
Column Total	347	10	357	
Washed cadaver				0.52
No	323	9	332	
Yes	24	1	25	
Column Total	347	10	357	
Participated in funeral rites				0.17

No	123	1	124	
Yes	224	9	233	
Column Total	347	10	357	
Used PPE daily				0.20
No	155	7	162	
Yes	192	3	195	
Column Total	347	10	357	

3.5 Sensitivity Analysis

For our sensitivity analysis, we increased the cutoff value for seroreactivity from 548 EU/mL to a more conservative 607 EU/mL. These two values were determined from studies in Liberia and Mali, not DRC. After applying the more conservative cutoff value of 607 EU/mL, 3 of the 12 participants who were seroreactive during initial analysis were no longer considered positive for Ebola antibodies. All models and Fisher's Exact Tests were run a second time with the 9 participants who were still considered seroreactive. This did not change the significance of any previous findings from our initial analysis.

4. Discussion

Overall, we can confirm that circulation of asymptomatic or paucisymptomatic EVD in HCWs with no previous diagnosis of EVD has occurred in Kinshasa. The prevalence for asymptomatic EBOV infections in our cohort (2.9%) was far below previous cohorts, particularly in Boende, DRC (22.5%) [4], and below estimates suggested from the previously mentioned meta-analysis (27.1%) as well. Studies have found prevalence of minimally symptomatic EVD to be anywhere from 9-27% [2,3,6,7,9,10,14,16] establishing a well-documented precedent of detectable seroprevalence of anti-EBOV GP IgG amongst individuals with no history of viral hemorrhagic fevers.

It is not surprising that prevalence for this cohort is below previously observed levels since no recent outbreak in the Kinshasa region has occurred. All recorded outbreaks in DRC have been outside the capital, with the most recent outbreaks to the time of our data collection being in the Bas Uélé district and Equateur province in 2017 and 2014, respectively. Higher prevalence in Kinshasa was originally predicted because of the city's significance as the country's capital, lending itself to more travelers passing through from other parts of the country and having a larger HCW workforce that may have been utilized in past containment efforts.

Cross-reactivity for other filoviruses in our serologic analysis is a valid concern for seroreactive individuals in our cohort since the previously mentioned outbreaks

were geographically far from our study site. Further investigation and follow-up is necessary to understand why these 12 participants were seroreactive with no active outbreaks or transmission in the area.

No findings were able to determine any significant predictors for seroreactivity. It is important to note that these HCWs were in Kinshasa, where are exposures are extremely low. This is reflected by the low number of individuals who were seroreactive (12 participants) and contributed to our models.

4.1 Implications for further research

The results from this analysis are preliminary but do suggest the possibility of EVD exposure amongst HCWs with no previous history of EVD in the DRC, as initially shown in our previous study site of Boende [4]. Additionally, further serologic testing is necessary using a 6pt dilution for the FANG ELISA kit to determine better quantitative results. We would also like to do a cross comparison of a subset of samples using ELISA developed by Alpha Diagnostic International (ADI). The 12 individuals who were seroreactive in our cohort need further analysis to determine their source of exposure and what factors lead them to exposure. Investigation is also needed to determine if individuals similar to our cohort are infectious at any point after their initial exposure to EBOV. Future studies could determine if seropositivity of anti-EBOV IgG equates to immunity for EVD. Lastly, a cutoff value for seropositivity using the FANG ELISA kit

needs to be described in the DRC. Plans to determine a cutoff value for the DRC are currently in the making for our study team.

4.2 Study strengths and limitations

There are several limitations to this study. As a cross-sectional study that targeted a specific geographic location, the results may not be generalizable to other populations that vary significantly from our target population and may not be perfectly comparable to our previous study sites. Our study sites differed in choice of ELISA for sample analysis, with Boende using the commercially available ADI kit and Kinshasa using the previously described FANG kit. Additionally, the ELISA kit used to determine seroreactivity is not FDA approved and is permitted for research use only. In addition, our procedure to determine seroreactivity used a 2pt dilution with the FANG kit. A 2pt dilution is enough to determine if a sample is likely to be reactive and seropositive, however a 6pt dilution is preferred for better quantitative results. Furthermore, prevalence of anti-EBOV GP IgG (2.9%) in our study population was low compared to previous study sites, effecting the results of models constructed in analysis.

The data for this study was self-reported and not collected during an active outbreak and may be subject to self-reporting and recall bias. The most recent outbreaks to our data collection were the outbreaks in the Bas Uélé district in early 2017,

approximately 3000 kilometers from Kinshasa, and the 2014 Equateur province outbreak.

We also would like to acknowledge a unique limitation for this study. While the methodology, procedures, and data collection were conducted by a team heavily invested in community based participatory research (CBPR) [12] equitably involving host country nationals, the circumstances of the last year preventing travel have thwarted the full potential and richness of possible outcomes resulting from such a team. Therefore, collaboration restricted to remote work was a novel limitation we faced while conducting analysis of this data.

Additionally, there are many strengths to this study, most notably the aforementioned team who made this study possible. The quality of data and relationships with local partners is a reflection of the nearly 20-year history of the UCLA- DRC research program.

Our choice of the FANG ELISA kit was an additional strength. FANG has been shown to "produce a wider range of relative antibody concentrations, higher assay precision, larger relative accuracy range, lower regional background" and requires only one-third of participants to power a study relative to the competitive ADI ELISA kit [9].

5. Conclusion

Altogether, the findings for this study are preliminary and illustrate some encouraging trends that may overlap with our previous study sites if further recruitment and analysis is conducted. However, confirmation of minimally symptomatic EVD outside a known outbreak region is interesting and prompts further investigation.

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