## Title

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MAJOR ARTICLE

# Identifying Paucisymptomatic or Asymptomatic and Unrecognized Ebola Virus Disease Among Close Contacts Based on Exposure Risk Assessments and Screening Algorithms

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***Background.*** There is limited evidence to evaluate screening algorithms with rapid antigen testing and exposure assessments as identification strategies for paucisymptomatic or asymptomatic Ebola virus (EBOV) infection and unrecognized EBOV disease (EVD).

***Methods.*** We used serostatus and self-reported postexposure symptoms from a cohort study to classify contact-participants as having no infection, paucisymptomatic or asymptomatic infection, or unrecognized EVD. Exposure risk was categorized as low, intermediate, or high. We created hypothetical scenarios to evaluate the World Health Organization (WHO) case definition with or without rapid diagnostic testing (RDT) or exposure assessments.

***Results.*** This analysis included 990 EVD survivors and 1909 contacts, of whom 115 (6%) had paucisymptomatic or asymptomatic EBOV infection, 107 (6%) had unrecognized EVD, and 1687 (88%) were uninfected. High-risk exposures were drivers of unrecognized EVD (adjusted odds ratio, 3.5 [95% confidence interval, 2.4–4.9]). To identify contacts with unrecognized EVD who test negative by the WHO case definition, the sensitivity was 96% with RDT (95% confidence interval, 91%–99%), 87% with high-risk exposure (82%–92%), and 97% with intermediate- to high-risk exposures (93%–99%). The proportion of false-positives was 2% with RDT and 53%–93% with intermediate- and/or high-risk exposures.

***Conclusion.*** We demonstrated the utility and trade-offs of sequential screening algorithms with RDT or exposure risk assessments as identification strategies for contacts with unrecognized EVD.

**Keywords.** Ebola virus disease; Filovirus; epidemiology; screening tests; infection control; Liberia.

Although Ebola virus (EBOV) disease (EVD) is widely known to result in severe illness and death [1–4], milder clinical manifestations can occur and be characterized in contacts of EVD cases as paucisymptomatic or asymptomatic EBOV infection and unrecognized EVD [3, 5–7]. During the 2013–2016 EVD outbreak in West Africa, a significant proportion of these contacts were later identified by serological testing [8–10], as part of natural history studies, including the US National Institutes of Health and Liberia Ministry of Health Partnership for Research on Ebola Virus in Liberia (PREVAIL) study [11, 12].

Evidence in recent years suggests that the distinct populations of paucisymptomatic or asymptomatic EBOV infection and unrecognized EVD exert differing impacts on public health and may require targeted clinical care strategies. Contacts with paucisymptomatic or asymptomatic EBOV infection do not contribute significantly to onward transmission [2, 3, 13], nor do they experience clinical sequelae [14]. In contrast, contacts with unrecognized EVD can transmit EBOV and may experience significant clinical sequelae, such as joint pain and memory loss [14].

Exposure risk assessments can identify both individuals with unrecognized EVD and those with paucisymptomatic or asymptomatic EBOV infection. Although several studies report that lower-risk exposures are associated with less severe disease [1, 2, 15, 16], the precision of their measurements was limited

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by small sample sizes [2, 17], and they did not confirm whether there is a dose-response relationship between the intensity of exposure to EVD and transmission. Larger, confirmatory studies are needed to evaluate specific sets of exposure questions and improve the efficiency of overstretched surveillance teams.

Historic use of the World Health Organization (WHO) case definition to screen for suspected EBOV has suffered from known poor sensitivity and specificity [18]. Newer screening algorithms that incorporate rapid diagnostic testing (RDT) [19] along with the WHO case definition for suspected cases of EBOV hold promise for overcoming this limitation. Given the cost and logistics of distributing rapid tests in the field, a screening algorithm that sequentially used the WHO case definition followed by RDT depending on case definition results could substantially increase identification of individuals who would otherwise have unrecognized EVD. However, to our knowledge, no study to date has evaluated the hypothetical test performance of this strategy.

To test the hypotheses that a dose-dependent relationship occurs between EVD exposure risk and severity of illness and that the sequential addition of RDT to the WHO case definition would improve the performance of screening algorithms, we constructed 2 study populations from household contacts of EVD survivors in the PREVAIL cohort (see below) with known serostatus: contacts reporting paucisymptomatic or asymptomatic infection and contacts with unrecognized EVD. We then assessed them for exposure risk and characterized their disease status using hypothetical screening algorithms both with and without RDT. The aim was to validate the use of exposure risk and RDT as tools that improve acute detection of paucisymptomatic or asymptomatic EBOV infection and unrecognized EVD.

## METHODS

### Study Design and Participants

The cross-sectional study reported here used data from the PREVAIL Ebola natural history Study, an observational, longitudinal cohort study that enrolled EVD survivors and their close contacts (PREVAIL III; ClinicalTrials.gov NCT02431923). Its procedures and methods have been described elsewhere [12].

In brief, individuals of all ages listed in the Liberian Ministry of Health Registry with an EVD diagnosis were recruited from 7 of the 15 counties in Liberia through local outreach and via the media. PREVAIL III enrolled eligible participants at 3 research sites (John F. Kennedy Medical Center and Duport Road Clinic in Monrovia and C. H. Rennie Hospital in Kakata) from June 2015 through June 2017. Survivors (case patients) were typically enrolled a median of 362 days after symptom onset, at which time they were asked to refer up to 5 contacts for enrollment. Contacts were either living in the same house during the case patient's acute illness with EVD or were someone with whom the survivor had sexual contact after discharge from an Ebola treatment unit. Contacts with a previous EVD diagnosis were ineligible. Contacts who were exposed to the survivor only after he or she was discharged from the treatment unit were not asked about health symptoms or relationship to a survivor and were therefore excluded from this analysis.

### Ethics Statement

The study protocol was approved by the National Research Ethics Board of Liberia and the National Institute of Allergy and Infectious Diseases Institutional Review Board at the US National Institutes of Health. All participants provided written informed consent. Children aged 7 to <18 years old provided informed assent; all children regardless of age were required to have consent from ≥1 parent or guardian.

### Measurements

Survivor-participants and contact-participants completed an interviewer-administered questionnaire on sociodemographics and medical history (prior diagnosis of hypertension, stroke, ischemic heart disease, diabetes mellitus, cancer, tuberculosis, malaria, syphilis or other sexually transmitted infection, human immunodeficiency virus (HIV)/AIDS, hepatitis B, hepatitis C, or typhoid fever). For all participants aged <18 years, questions were answered by a parent or guardian. Survivors were asked whether they had experienced any of the following 16 EVD-related symptoms or signs during their acute illness: fever, loss of appetite, nausea, vomiting, diarrhea, headache, abdominal pain, unexplained bleeding, myalgia, arthralgia, difficulty breathing, shortness of breath, hiccups, red eyes, fatigue, or sore throat. Contacts were also asked to report whether they had experienced any of these symptoms or signs within 21 days of the survivor's Ebola event. Contacts reported the type of physical contact they had with the survivor and/or contaminated environment during acute illness, categorized according to the hierarchy of exposure risk [20]: low risk (no physical contact), intermediate risk (slept or ate in the same room, contact with clothing, direct physical contact), or high risk (contact with body fluid). The exposure categories were mutually exclusive; contact-participants were included in the category with the highest level of contact. The relationship of a contact to a survivor was described as spouse or other sexual contact, nonspousal relative (parent, child, sibling, other relative), or nonrelative. All participants underwent a brief physical examination that included height and weight measurements to calculate body mass index [21].

At enrollment, venous blood samples were collected from both survivors and contacts for serum testing of immunoglobulin G antibody levels against EBOV glycoprotein using the Filovirus Animal Nonclinical Group (FANG) assay [22]. We used a positive cutoff value of 548 enzyme immunoassay units (EU) or 2.74 $\log_{10}$ EU/mL [23], below which participants were characterized as seronegative. This cutoff determined seropositivity with 94.4% sensitivity and 96.7% specificity. All antibody

testing was performed at the Liberian Institute for Biomedical Research. HIV rapid testing was performed on site using the HIV/Syphilis Duo SD Bioline as first choice for testing. Confirmatory testing for HIV was done using the Unigold Recombigen HIV1/2 test. Those with HIV diagnosed during the study screening protocol were referred for care.

### Disease Classification

We used serostatus and self-reported postexposure symptoms of each contact-participant and survivor-participant to determine disease classification. As in other published studies [13, 14], we defined groups and severity of acute illness based on the average number of symptoms reported by groups (from least to most severe), as follows: uninfected contacts (seronegative), contacts with paucisymptomatic or asymptomatic EBOV infection (seropositive but denied having any of the 16 EVD-related symptoms), contacts with unrecognized EVD (seropositive and endorsed ≥1 symptom), and EVD survivors listed on the Liberian Ministry of Health EVD Registry (seropositive) (Figure 1). Our definitions of these groups made assumptions related to potential recall error of symptoms and probable extent of severity of illness without presentation for diagnosis. Although polymerase chain reaction testing was recommended for contacts with symptoms during the post-EVD exposure period, those with unrecognized EVD were able to remain at home during their acute illness because they reported fewer symptoms on average than reported EVD survivors [12].

### Statistical Analysis

We described baseline characteristics by disease classification. Among the groups of contact-participants, we assessed the association of exposure risk with infection (seropositivity) and severity of acute illness (3-level categorical ordinal outcome). The 3 categories of the severity outcome were as follows: lowest severity, uninfected contacts; medium severity, paucisymptomatic or asymptomatic contacts; and highest severity, unrecognized EVD. We fit logistic regression models with generalized estimating equations, adjusting for age, sex, site, body mass index, HIV status, and other medical illness, and accounting for clustering among contacts. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were estimated.

We evaluated the performance of the WHO case definition by constructing true-positive and true-negative groups. Because self-report of symptoms is requisite to use of the WHO case definition, we defined 2 true-positive groups: EVD survivors and contacts with unrecognized EVD. We defined our true-negative group as uninfected contacts who reported symptoms (seronegative and reported ≥1 of the 16 EVD-related symptoms). We then applied the WHO suspected case definition used during the 2013–2016 EVD outbreak to recategorize each of the true-positive and true-negative groups in our cohort as to how they would have been categorized had the case definition been used to screen them during the postexposure period (Figure 1). We estimated sensitivity for each true-positive group (EVD survivors and contacts with unrecognized

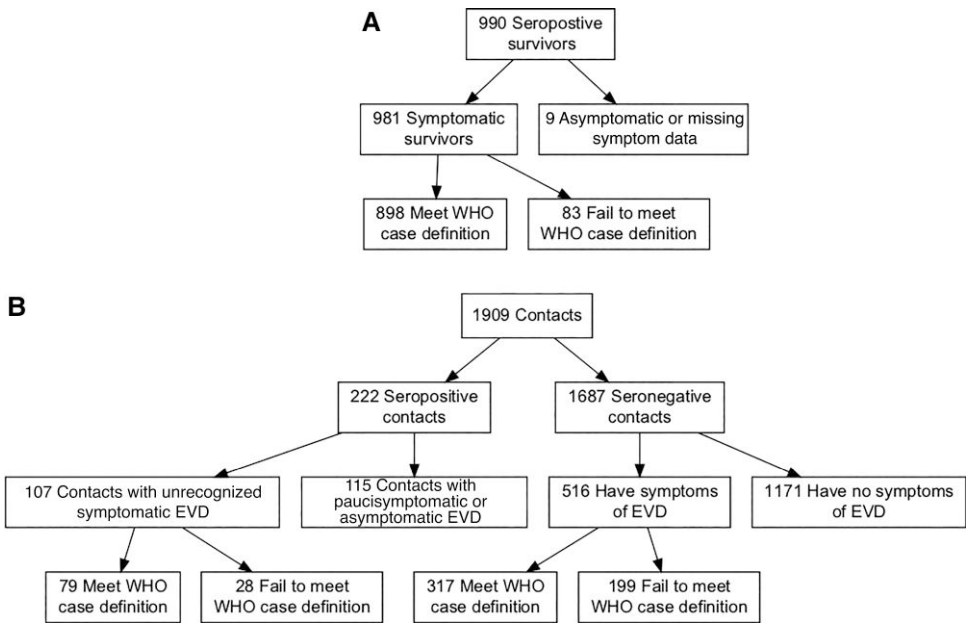


**Figure 1.** Flow diagrams of study populations for exposure assessments and hypothetical scenarios. *A*, Ebola virus disease (EVD) survivors (n = 990) who were symptomatic (n = 981), with results according to the World Health Organization (WHO) case definition. *B*, Contacts (n = 1909) who were seropositive with unrecognized symptomatic EVD (n = 107) or who were seronegative and symptomatic (n = 516), with WHO case definition results for the symptomatic individuals.

EVD) and specificity for the true-negative group (uninfected, symptomatic contacts).

We created a hypothetical scenario to evaluate screening algorithms: for those categorized as negative according to the WHO case definition, we assumed that they were subsequently tested with RDT or an exposure assessment. Given that the WHO case definition has been applied to symptomatic individuals, we did not include paucisymptomatic or asymptomatic EBOV-infected individuals in this hypothetical screening algorithm. For the purpose of the hypothetical, we applied the test characteristics of the WHO, US Food and Drug Administration, and European Union–approved Oraquick rapid test (sensitivity of 84.0% [95% CI, 63.9%–95.5%] and specificity of 98.0% [89.4%–99.9%] in this population) [24] because it has been widely used in several EVD outbreaks (OraSure Technologies) [25]. Applying RDT with this test to our groups who tested negative according to the WHO case definition, we identified participants who were RDT positive, reclassified them to the combined test-positive group, and reestimated sensitivities and specificity. When applying the exposure assessment to our groups who tested negative by WHO case definition, we used data from this cohort and identified participants with positive test results as those with high-risk exposure only or high- and intermediate-risk exposures. We reclassified them to the combined test-positive group and reestimated sensitivities and specificity.

## RESULTS

Our analysis cohort included 990 EVD survivors who were confirmed with anti-EBOV antibodies (Figure 1*A*) and 1909 contacts who were present in the survivors' households while the survivor was acutely ill (mean number of household contacts per survivor, 1.7). Among the 1909 contacts, 1687 (88%) were uninfected and 222 (12%) were infected; 115 (6%) had paucisymptomatic or asymptomatic EBOV infection and 107 (6%) had unrecognized EVD (Figure 1*B*).

Demographic characteristics of survivors and contacts and their relationship status, comorbid conditions and mean anti-EBOV antibody titers are shown in Table 1. EVD survivors had an older mean age (standard deviation [SD]) than contacts with paucisymptomatic or asymptomatic infection or unrecognized EVD (survivors, 29.7 [14.8] years; contacts, 26.4 [15.7] years; $P < 0.01$). EVD survivors also had a higher mean number of EVD-related symptoms than contacts with unrecognized EVD (survivors, 10.8 [SD. 3.3]; contacts: 7.0 [4.8]; $P < 0.001$). EVD survivors had higher mean antibody levels than other groups. Among the infected contacts, mean (SD) antibody titers were higher among contacts with unrecognized EVD (4.0 [0.7] EU) than among those with paucisymptomatic or asymptomatic infection (3.5 [0.6] EU) ($P < .001$) (Figure 2).

Table 2 presents the maximal exposure risks to EVD cases reported by contact groups. A majority of contacts with unrecognized EVD reported high-risk exposures (60% had contact with bodily fluids), in contrast to contacts with paucisymptomatic or asymptomatic infection (46%) or uninfected contacts (46%). Uninfected contacts had a similar distribution of exposure risks as contacts with paucisymptomatic or asymptomatic infection; low-risk exposures (living in the same house but without direct contact) were reported in 14%–15% of each group. In contrast, contacts with unrecognized EVD reported about 50% less of the low-risk and 20% less of the intermediate-risk exposures reported in other contact groups.

Table 3 shows the results of adjusted analyses performed to examine the associations of EVD exposure risk with infection and severity of illness among household contacts. Contacts with high-risk exposures had higher adjusted odds of infection (aOR, 2.1 [95% CI, 1.2–3.6]) compared with those with low-risk exposures; the odds of infection were also higher in the intermediate-risk exposure group than in to the low-risk exposure group, but this difference was not statistically significant.

When we considered the 3 levels of outcome describing the severity of illness, we found that higher-risk exposures were associated with greater adjusted odds of a more severe disease. This trend was observed based on the finding that contacts with high-risk exposures had the largest magnitude, which was 3.5 times the adjusted odds of more severe disease compared with contacts with low-risk exposures (95% CI, 2.4–4.9). In this analysis, we also found that intermediate-risk exposures were associated with a higher level of disease severity (aOR, 2.0 [95% CI, 1.4–2.8]).

Table 4 shows our evaluation of screening algorithms using the WHO case definition, with or without RDT or exposure assessments. The WHO case definition identified EVD survivors with a sensitivity of 92%, contacts with unrecognized EVD with a sensitivity of 74%, and uninfected symptomatic contacts with a specificity of 39%. When we applied the RDT performance characteristics to the group characterized as test negative according to the WHO case definition, we identified 70 additional EVD survivors (95% CI, 53–79), 24 additional contacts with unrecognized EVD (18–27), and 4 additional uninfected symptomatic contacts (0.1–21). Using the sequential screening algorithm of the WHO case definition followed by RDT, the performance of the combined algorithm was as follows: sensitivity of 99% for EVD survivors (95% CI, 97%–99.6%), sensitivity of 96% for contacts with unrecognized EVD (91%–99%), and specificity of 38% for uninfected symptomatic contacts (35%–39%).

We applied a second screening algorithm using the WHO case definition with and without exposure assessments. In the identification of contacts with unrecognized EVD who test negative by the WHO case definition, sensitivity was 87% with high-risk exposure (95% CI, 82%–92%) and 97% with intermediate- to high-risk exposures (93%–99%). Although we found increasing sensitivity of the combined algorithm as

**Table 1. Characteristics of Study Population by Disease Classification**

| Characteristic | EVD Survivors (n = 990) | | Contacts (n = 1909) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Uninfected (n = 1687) | | Infected Contacts | | | | | |
| | | | | | All (n = 222) | | Paucisymptomatic or Asymptomatic Infection (n = 115) | | Unrecognized EVD (n = 107) | |
| | No. | % | No. | % | No. | % | No. | % | No. | % |
| Age, y | | | | | | | | | | |
| ≤10 | 101 | 10.2 | 309 | 18.3 | 35 | 15.8 | 21 | 18.3 | 14 | 13.1 |
| 11–20 | 186 | 18.8 | 495 | 29.3 | 57 | 25.7 | 32 | 27.8 | 25 | 23.4 |
| 21–20 | 260 | 26.3 | 376 | 22.3 | 49 | 22.1 | 22 | 19.1 | 27 | 25.2 |
| 31–40 | 225 | 22.7 | 235 | 13.9 | 41 | 18.5 | 20 | 17.4 | 20 | 19.6 |
| ≥41 | 218 | 22.0 | 272 | 16.1 | 40 | 18.0 | 20 | 17.4 | 21 | 18.7 |
| Female sex | 545 | 55.1 | 934 | 55.4 | 129 | 58.1 | 63 | 54.8 | 66 | 61.7 |
| Level of formal education | | | | | | | | | | |
| None | 218 | 22.0 | 451 | 26.7 | 63 | 28.4 | 29 | 25.2 | 34 | 31.8 |
| Less than high school | 413 | 41.7 | 811 | 48.1 | 111 | 50.0 | 61 | 53.0 | 50 | 46.7 |
| High school or above | 359 | 36.3 | 425 | 25.2 | 48 | 21.6 | 25 | 21.7 | 23 | 21.5 |
| Relationship to survivor | | | | | | | | | | |
| Spouse or sexual partner | … | … | 222 | 13.2 | 29 | 13.1 | 18 | 15.7 | 11 | 10.3 |
| Relative | … | … | 1345 | 79.7 | 172 | 77.5 | 91 | 79.1 | 81 | 75.7 |
| Nonrelative | … | … | 120 | 7.1 | 21 | 9.5 | 6 | 5.2 | 15 | 14.0 |
| Enrollment site in Liberia | | | | | | | | | | |
| JFK | 618 | 62.4 | 802 | 47.5 | 99 | 44.6 | 51 | 44.3 | 48 | 44.9 |
| Rennie | 160 | 16.2 | 365 | 21.6 | 62 | 27.9 | 34 | 29.6 | 28 | 26.2 |
| Duport Rd | 212 | 21.4 | 520 | 30.8 | 61 | 27.5 | 30 | 26.1 | 31 | 29.0 |
| HIV seropositive | 12 | 1.4 | 37 | 2.8 | 5 | 2.8 | 3 | 3.3 | 2 | 2.3 |
| History of other illness | 400 | 40.4 | 614 | 36.4 | 94 | 42.3 | 43 | 37.4 | 51 | 47.7 |
| EVD-related symptoms, mean (SD), no. | 10.8 (3.3) | | 1.2 (2.6) | | 3.4 (4.8) | | … | | 7.0 (4.8) | |
| Antibody titer, mean (SD), $\log_{10}$ EU | 4.3 (0.3) | | 1.9 (0.4) | | 3.7 (0.7) | | 3.5 (0.6) | | 4.0 (0.7) | |

Abbreviations: Duport Rd, Duport Road Clinic, Monrovia; EU, enzyme immunoassay units; EVD, Ebola virus disease; HIV, human immunodeficiency virus; JFK, John F. Kennedy Medical Center, Monrovia; Rennie, C. H. Rennie Hospital, Kakata; SD, standard deviation.

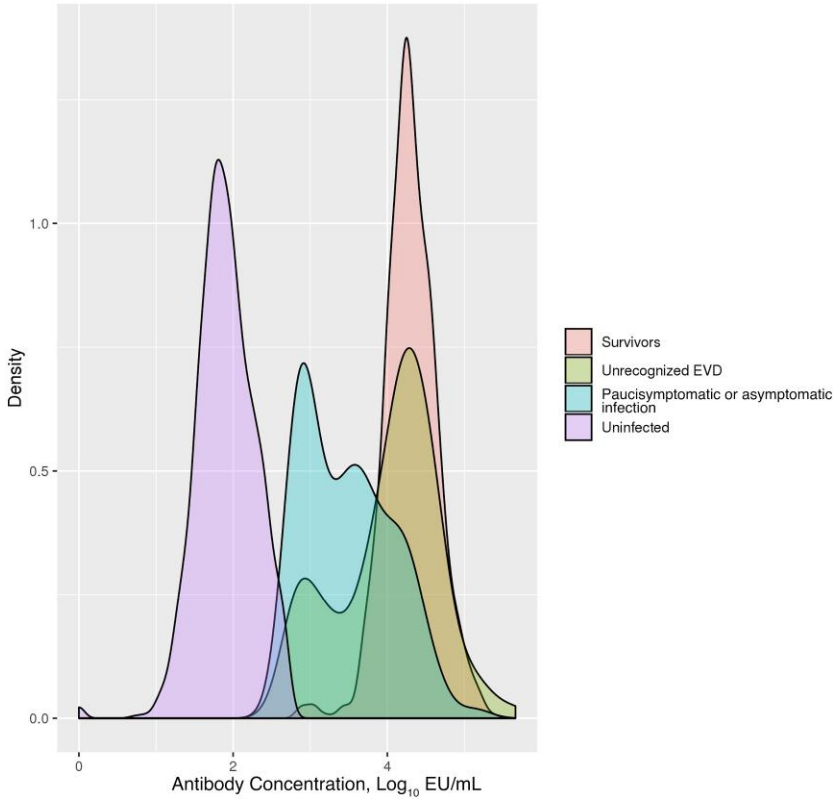


**Figure 2.** Kernal density plot of anti–Ebola virus antibody concentrations by group (Ebola virus disease [EVD] survivors, contacts with unrecognized EVD, contacts with paucisymptomatic or asymptomatic infection, and uninfected contacts). Abbreviation: EU, enzyme immunoassay units.

we broadened use of exposure levels from high risk only to both intermediate and high risk, the proportion of false-positives was 2% with RDT and 53%–93% with intermediate- and/or high-risk exposures.

## DISCUSSION

In this large cohort of EVD survivors and contacts, we demonstrated the utility of sequential screening algorithms as identification strategies for contacts with unrecognized EVD, starting with the WHO case definition, and followed by RDT or exposure risk assessments. Compared with RDT, however, exposure assessments were a less favorable option because of the high proportion of false-positives (uninfected symptomatic contacts) identified. Our findings highlight the importance of incorporating RDT into screening algorithms for triaging individuals suspected of having EVD. By identifying contacts who would have had unrecognized EVD, outbreak surveillance teams will break hidden cycles of transmission and link these missed cases into acute and postacute care.

When the WHO case definition and RDT are independently applied to these EVD populations, test performance has been reported as suboptimal [18, 26]; when they are applied in sequence, however, we found acceptable test performance characteristics (sensitivity of >95%) for screening symptomatic contacts whose EVD would otherwise have been unrecognized. Optimizing surveillance teams' strategies for screening symptomatic contacts can add public health and clinical care value to response work while improving efficiency, with fewer exposure questions to ask and fewer contacts to monitor. EVD outbreaks can be explosive in limited-resource settings, resulting in overworked responders and overstretched diagnostic testing capacity [27], even in Congolese outbreaks that used decentralized GeneXpert testing [28]. During EVD outbreaks in West Africa and the Democratic Republic of the Congo, validation studies of RDT created an arsenal of new screening tools that have been increasingly deployed in subsequent outbreaks for various reasons, including burial and clinic-level surveillance [25, 29–31]. These RDT implementation efforts were independent of WHO case definition. Our findings support the implementation of a cost-effective, time-efficient, point-of-care screening algorithm by applying the WHO case definition first and then performing antigen testing on individuals who are highly likely to have false-negative results. We found that almost all false-negative individuals missed by the WHO case definition were identified by antigen testing, which should reassure policy makers that this sequential screening algorithm could optimize EVD outbreak response activities and goals.

**Table 2. Exposure Risk to Ebola Virus Disease (EVD) Cases Among Contacts With Paucisymptomatic or Asymptomatic Infection and Those With Unrecognized EVD Compared With Uninfected Contacts**

| Level of Exposure Risk[a] | All Contacts (n = 1909) No. | Uninfected Contacts (n = 1687) No. | Uninfected Contacts (n = 1687) % | Infected Contacts: Paucisymptomatic or Asymptomatic Infection (n = 115) No. | Infected Contacts: Paucisymptomatic or Asymptomatic Infection (n = 115) % | Infected Contacts: Unrecognized EVD (n = 107) No. | Infected Contacts: Unrecognized EVD (n = 107) % |
|---|---|---|---|---|---|---|---|
| Low | 275 | 251 | 14.9 | 16 | 13.9 | 8 | 7.4 |
| Intermediate | 746 | 665 | 39.4 | 46 | 40.0 | 35 | 32.7 |
| High | 888 | 771 | 45.7 | 53 | 46.1 | 64 | 59.8 |

Abbreviation: EVD, Ebola virus disease.

[a]Low-risk exposure was defined as living in the same house but with no physical contact; intermediate-risk exposure, sleeping or eating in the same room, contact with clothing, or other physical contact; and high-risk exposure, contact with bodily fluids.

For scenarios where RDT is not available, we considered exposure assessments in sequential screening algorithms among WHO case definition–negative individuals. We observed a trade-off in terms of performance of the combined algorithm, such that the specificity was too low among contacts reporting high- or intermediate-risk exposure for both those exposure levels to be used. Using high-risk exposures in a sequential screening algorithm, however, may serve as an acceptable alternative for scenarios without RDT. High-risk exposures have also been associated with more severe illness in small studies [2, 13, 17]. In our study, we confirm findings suggesting that virus inoculum, presumably higher from high-risk exposure, has an impact on disease severity. Furthermore, we extend this literature with a more granular and definitive assessment of exposure risk levels by groups.

Thinking more broadly about the value of exposure assessments for contacts with paucisymptomatic or asymptomatic infection or unrecognized EVD, we found that uninfected contacts presented a similar distribution of exposure risk as contacts with paucisymptomatic or asymptomatic infection; this suggests that exposure risk questions would not assist surveillance teams to definitively categorize these 2 groups. In contrast, there was a difference in the distribution of exposure risk between uninfected contacts and contacts with unrecognized EVD. A higher proportion of contacts with unrecognized EVD had high-risk exposures (contact with body fluid). Although low-risk exposures (living in the same house but with no physical contact) were rare events among contacts with unrecognized EVD, intermediate-risk exposure (sleeping or eating in the same room, contact with clothing, other physical contact) still occurred in a significant yet lower proportion than in uninfected contacts. During the 2013–2016 EVD outbreak, households were quarantined regardless of exposure risk assessments [3, 32], yet our findings suggest that quarantine could be targeted to only household contacts with direct exposures to EVD case patients (physical contact related) [33].

**Table 3. Association Between Risk of Exposure to an Ebola Virus Disease Case and Ebola Virus Infection or More Level of Disease Severity Among Household Contacts (n = 1909)**

| Degree of Exposure | aOR[a] | 95% CI | *P* Value |
|---|---|---|---|
| Association with infection | | | |
| Low | Reference | … | … |
| Medium | 1.57 | 0.91–2.70 | .11 |
| High | 2.10 | 1.23–3.59 | .006 |
| Association with severity of illness[b] | | | |
| Low | Reference | … | … |
| Medium | 1.95 | 1.38–2.76 | <.001 |
| High | 3.45 | 2.43–4.91 | <.001 |

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval.

[a]All models were adjusted for age, sex, site, body mass index, human immunodeficiency virus status, and other medical illness, and all accounted for clustering.

[b]Severity of illness was characterized by a 3-level categorical outcome (no infection, paucisymptomatic or asymptomatic infection, and unrecognized Ebola virus disease).

Our study has several limitations. First, baseline interviews at enrollment in the PREVAIL study were conducted on average 1 year after the survivors were discharged from an Ebola treatment unit. Self-reported symptoms were subject to recall error, but because contacts were not aware of their serostatus at the time of the interview, any misclassification would have been nondifferential in nature and biased to the null. Second, our questions about exposure risk did not include types of contact shown elsewhere to be associated with infection, such as burial preparation; however, any resulting imprecision from misclassification error of the exposure was overcome by the large sample size. Third, our disease classification used serostatus, which cannot be used as a diagnostic tool for infection because of cross-reactivity and measurement error. This serological measurement has highly accurate test performance over time and has been used in several other studies [11–13]. Fourth, the measurement error associated with disease classifications of unrecognized EVD and no infection likely led to some amount of imprecision in sensitivity and specificity estimates in the hypothetical screening algorithms. Furthermore, the reported RDT sensitivity of the OraQuick test was not conditioned on a

**Table 4. Sensitivity and Specificity of World Health Organization Case Definition Among True-Positive (Symptomatic Ebola Virus Disease [EVD] Survivors and Contacts with Unrecognized, Symptomatic EVD) and True-Negative (Uninfected Symptomatic Contacts) Participants[a]**

| Screening Algorithm | Symptomatic EVD Survivors (n = 981)[b] | Contacts with Unrecognized EVD (n = 107) | Uninfected Symptomatic Contacts (n = 516) |
|---|---|---|---|
| WHO case definition | | | |
| Positive test result | 898 | 79 | 317 |
| Negative test result | 83 | 28 | 199 |
| Test performance | Sensitivity, 91.5% | Sensitivity, 73.8% | Specificity, 38.6% |
| WHO case definition followed by RDT | | | |
| Expected no. of additional individuals identified by RDT if WHO case definition were negative | 69.7 (53.1–79.2)[c] | 23.5 (17.9–26.7) | 4 (0.1–20.8) |
| Performance of combined algorithm | Sensitivity, 98.6% (97%–99.6%)[c] | Sensitivity, 95.8% (90.6%–98.8%) | Specificity, 37.8% (34.5%–38.5%) |
| WHO case definition followed by exposure assessment | | | |
| No. of additional individuals identified by exposure assessment if WHO case definition were negative | High-risk exposure | 14 | 106 |
| | Intermediate- or high-risk exposure | 25 | 186 |
| Performance of combined algorithm | High-risk exposure | Sensitivity, 86.9% (81.8%–92.0%) | Specificity, 18% (15.3%–20.8%) |
| | Intermediate- or high-risk exposure | Sensitivity, 97.2% (92.6%–99.4%) | Specificity, 2.5% (1.4%–4.2%) |

Abbreviations: EVD, Ebola virus disease; RDT, rapid diagnostic testing; WHO, World Health Organization.

[a]Individuals who would have tested negative by the WHO case definition were hypothetically tested with RDT to demonstrate the expected change in sensitivity and specificity of a sequential screening algorithm of the WHO case definition followed by RDT. A second screening algorithm was assessed for individuals who would have tested negative by WHO case definition. They were sorted by exposure level to demonstrate the expected change in sensitivity and specificity of a sequential screening algorithm of the WHO case definition followed by exposure analysis.

[b]Nine EVD survivors reported no symptoms.

[c]All ranges within Table 4 represent 95% confidence intervals.

negative WHO case definition, so test performance in our study population could be lower than published estimates. Fifth, our study was retrospective, and contacts may have been reluctant to disclose their symptoms to surveillance teams. The study should be replicated using a prospective study design to ensure external validity in future outbreak settings. Finally, inclusion of contacts exposed only to survivors and the limit of 5 referred contacts were both factors that may have induced some degree of selection bias. Despite these limitations, however, our study represents the largest cohort of individuals with paucisymptomatic or asymptomatic EBOV infection or unrecognized EVD studied during an EVD outbreak.

In our cohort, a significant proportion (approximately 20%) of symptomatic contacts were identified as having had unrecognized EVD; these are individuals who may be able to transmit EBOV and experience clinical sequelae [6, 14]. While meaningful and effective interventions to increase acceptance of EVD treatment and control efforts must consider the sociopolitical determinants of health in the region [34, 35], exposure risk assessments and sequential screening protocols can be viable strategies for identifying contacts with unrecognized EVD. Additional prospective research is needed to determine the public health and clinical significance of identifying contacts with paucisymptomatic or asymptomatic infection.

Sequential screening strategies still require confirmatory polymerase chain reaction testing, but use of rapid antigen testing or exposure assessments after a symptomatic individual tests negative by the WHO case definition can prevent EVD case patients from returning to communities (false-negatives) and sparking new transmission chains. Exposure risk and other variables that emerge as acute determinants of unrecognized EVD can be used by surveillance teams to prevent transmission, and they can be collectively used to build prediction models that identify individuals who have a high probability of unrecognized EVD and experiencing postacute sequelae. Because there are important public health and clinical implications of unrecognized EVD, we need an arsenal of evidence-based strategies beyond exposure risk assessments and sequential screening algorithms that can identify this group during future EVD outbreaks.

## Notes

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the manuscript, and wrote the final version. D. G. D., M. P. F., C. D., M. B., J. S. M., T. F., K. J., M. C. S., G. W. R., C. R., C. P. L., and J. D. K. revised the manuscript. All authors approved the final version.

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