SUPPLEMENT ARTICLE

Assessment of the Risk of Ebola Virus Transmission from Bodily Fluids and Fomites

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Although Ebola virus (EBOV) is transmitted by unprotected physical contact with infected persons, few data exist on which specific bodily fluids are infected or on the risk of fomite transmission. Therefore, we tested various clinical specimens from 26 laboratory-confirmed cases of Ebola hemorrhagic fever, as well as environmental specimens collected from an isolation ward, for the presence of EBOV. Virus was detected by culture and/or reverse-transcription polymerase chain reaction in 16 of 54 clinical specimens (including saliva, stool, semen, breast milk, tears, nasal blood, and a skin swab) and in 2 of 33 environmental specimens. We conclude that EBOV is shed in a wide variety of bodily fluids during the acute period of illness but that the risk of transmission from fomites in an isolation ward and from convalescent patients is low when currently recommended infection control guidelines for the viral hemorrhagic fevers are followed.

Ebola virus (EBOV; family *Filoviridae*, genus *Ebolavirus*, type species *Zaire ebolavirus*) circulates in sub-Saharan Africa, where it occasionally causes large outbreaks of severe hemorrhagic fever with high case fatality rates [1]. The natural reservoir remains unknown, although bats are suspected [2]. Because no effective vaccine or specific antiviral therapy is available for Ebola hemorrhagic fever (EHF), the primary control strategy relies on aggressive contact tracing and isolation of patients with suspected cases in specialized isolation wards [3].

Large outbreaks of EHF are usually driven by person-to-person transmission, with caregivers both at home and in hospitals being at particular risk [4]. Although direct contact with bodily fluids is considered to be the major risk factor [5–7], other than confirmation of EBOV in blood during acute illness, few data exist on which specific bodily fluids pose a risk and at what stages of infection. Furthermore, although extreme caution is recommended to prevent environmental contamination and exposure in isolation wards and detailed safety guidelines and protocols for decontamination have been developed [3], the role of fomites in the transmission of EBOV has not been explored. To better understand the precise modes of transmission, we sampled various clinical specimens from patients as well as from environmental surfaces in an isolation ward for EHF and analyzed them for the presence of EBOV.

METHODS

Clinical specimens. The study was conducted in the isolation ward at Gulu Regional Hospital during an outbreak of EHF (Sudan EBOV) in Gulu, Uganda, in 2000 [8]. The ward was divided into patients with "suspected EHF" and patients with "probable EHF" on the basis of the clinician's judgment with subsequent use of the laboratory data when available. The daily cleaning/decontamination procedure in the ward consisted of spraying a 0.5% bleach solution on the floors each

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

The study is dedicated to Matthew Lukwiya, who died in service to his patients during the Ebola hemorrhagic fever outbreak in Gulu, and to his family.

[a] Deceased.

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morning and a 0.05% solution on other visibly contaminated surfaces as needed [3].

Informed consent was obtained from the patient or guardian. A convenience sample of various clinical specimens, primarily bodily fluids, was obtained from patients with laboratory-confirmed EHF (table 1). Samples of urine, stool, vomit, and sputum were taken from plastic receptacles used by the patients at their bedsides, by use of a transfer pipette. Skin surface samples, tears, and nasal blood from a patient with epistaxis were collected with a Dacron swab that was then placed into a cryovial containing sterile PBS. Saliva, breast milk, and semen (obtained from 1 convalescent patient) were obtained by providing patients with a sterile container and asking them to produce the specimen. A body louse *(Pediculus humanus corporis)* found on a patient's clothing by the treating physician was collected with a forceps. The color and absence or presence of blood was noted for each sample. All specimens were placed into sterile cryovials and stored at ambient temperature (~25°C–30°C) in the isolation ward for the rest of the day (typically ≤6 h) before being stored in liquid nitrogen at the field laboratory established for the outbreak.

Environmental specimens. Environmental surfaces in the isolation ward that were considered to have varying probabilities of EBOV contamination were selected by researchers and clinicians familiar with EHF (table 2). All environmental samples were collected over a 2-h period in the mid-afternoon, ~6 h after the last routine cleaning. A Dacron swab was rubbed over the surface in question for ~10 s and then placed immediately into a sterile cryovial containing 1 mL of PBS. An opened and closed tube and unused swab were taken as negative

Table 1. Virus culture and reverse-transcription polymerase chain reaction (RT-PCR) results from 54 clinical samples collected from 26 patients with laboratory-confirmed Ebola hemorrhagic fever.

Sample type, phase of illness	Patients, no.	Samples, no.	Day after disease onset that sample was collected, range (mean)	Virus culture positive, no. (% sample type tested)	RT-PCR positive, no./total tested (%)	Latest day positive after disease onset
Saliva						
Acute	10	12	4–14 (6)	1 (8)	8/12 (67)	8
Convalescent	4	4	12–23 (16)	0 (0)	0/4 (0)	…
Skin[a]						
Acute	7	8	4–10 (7)	0 (0)	1/8 (13)	6
Convalescent	3	3	7–15 (12)	0 (0)	0/3 (0)	…
Urine						
Acute	5	7	5–22 (14)	0 (0)	0/7 (0)	…
Convalescent	4	4	8–40 (28)	0 (0)	0/4	…
Vomit						
Acute	1	1	NA (9)	0 (0)	0/1 (0)	…
Convalescent	1	1	NA (20)	0 (0)	0/1 (0)	…
Sputum						
Acute	1	1	NA (8)	0 (0)	0/1 (0)	…
Convalescent	1	1	NA (16)	0 (0)	0/1 (0)	…
Breast milk						
Acute	1	1	NA (7)	1 (100)	1/1 (100)	7
Convalescent	1	1	NA (15)	1 (100)	1/1 (100)	15
Stool,[b] acute	4	4	4–12 (8)	0 (0)	2/4 (50)	12
Sweat,[b] acute	1	1	NA (9)	0 (0)	0/1 (0)	…
Tears,[b] acute	1	1	NA (6)	0 (0)	1/1 (100)	6
Nasal blood,[b] acute	1	1	NA (10)	0 (0)	1/1 (100)	10
Body louse,[b] acute	1	1	NA (9)	0 (0)	0/1 (0)	…
Semen,[c] convalescent	1	2	40–45 (43)	1 (50)	1/2 (50)	40
Subtotal acute	23	38	4–22 (9)	2 (5)	14 (37)	12
Subtotal convalescent	8	16	7–45 (21)	2 (13)	2 (13)	40
Total	26[d]	54	4–45 (12)	4 (7)	16 (30)	…

NOTE. Samples are classified as either acute phase (serum ELISA antigen positive and/or RT-PCR positive) or convalescent phase (previously serum ELISA antigen positive or RT-PCR positive but now reverted to negative, often with the appearance of ELISA IgG antibody). Clinical samples were classified as acute or convalescent phase on the basis of the results of the most closely matched serum sample by date, which was a mean difference of 1.2 days (range, 0–13 days) and 7.3 days (range, 0–29 days) for acute- and convalescent-phase samples, respectively. NA, not applicable.

[a] Samples were swabbed from the hand (10) or forehead (1). The sole positive sample was from a hand.
[b] No convalescent-phase samples were available for this sample type.
[c] No acute-phase sample was available for this sample type.
[d] Both acute- and convalescent-phase samples were collected from some patients.

Table 2. Virus culture and reverse-transcription polymerase chain reaction (RT-PCR) results from 33 environmental samples.

Sample	Color	Virus culture result	RT-PCR result
Outside of ward			
Changing room wall	Clear	–	–
Changing room desk	Clear	–	–
Exterior surface of door of isolation ward	Clear	–	–
Inside ward, suspected side			
Nurse's newly placed glove	Clear	–	–
Bed frame	Clear	–	–
Instrument tray for ward rounds	Clear	–	–
Inside ward, probable side			
Air (tube opened and capped, negative control 1)	Clear	–	–
Sterile swab (negative control 2)	Clear	–	–
Intravenous fluid support pole	Clear	–	–
Light switch	Clear	–	–
Floor	Clear	–	–
Handle of 0.05% bleach solution dispenser	Clear	–	–
Nurse's clean apron	Clear	–	–
Nurse's clean glove	Clear	–	–
Clean stethoscope	Clear	–	–
Stethoscope after use	Clear	–	–
Stethoscope after use and rinsing with 0.05% bleach solution	Clear	–	–
Bed frame	Clear	–	–
Bedside chair (2 different samples)	Clear	–	–
Food bowl	Clear	–	–
Spit bowl	Clear	–	–
Skin (hand) of patient attendants (3 different samples)	Clear	–	–
Clean glove of patient attendant	Clear	–	–
Corpse decontaminated with 0.5% bleach solution	Clear	–	–
Body bag decontaminated with 0.5% bleach solution (2 different samples)	Clear	–	–
Clean mattress	Clear	–	–
Intravenous tubing	Clear	–	–
Doctor's blood-stained glove (positive control 1)	Pink	–	+
Bloody intravenous insertion site (positive control 2)	Red	–	+
Total (% of all samples)	…	0 (0)	2 (7)

controls, and swabs of samples considered to be highly probable to contain EBOV (a doctor's bloody glove and a bloody intravenous site) were collected as positive controls. The cryovials were immediately transported to the field laboratory and stored in liquid nitrogen.

Laboratory testing. A field laboratory for the diagnosis of EHF was set up at the beginning of the outbreak at St. Mary's Hospital Lacor in Gulu. Serum samples were tested by ELISA for EBOV-specific antigen and IgG antibody, as described elsewhere [9, 10]. IgM antibody testing was not possible in the field because of technical difficulties. Most serum samples were also tested at the field laboratory by conventional reverse-transcription polymerase chain reaction (RT-PCR) [11]. A patient with a laboratory-confirmed case of EHF was considered to be any person who was ELISA antigen positive or RT-PCR positive. Patients considered to be convalescent were those who previously had confirmed cases but whose ELISA antigen and RT-PCR results had reverted to negative. Most convalescent patients were ELISA IgG antibody positive.

The bodily fluids and environmental specimens collected for this study were stored in liquid nitrogen containers at the field laboratory. However, because of limited space, the specimens were periodically transported to the Uganda Virus Research Institute for temporary storage in mechanical freezers at −80°C. At the end of the outbreak, all samples were transported on dry ice in International Airline Transport Association–compliant safety shippers to the biosafety level 4 laboratory at the Centers for Disease Control and Prevention in Atlanta, Georgia, where they were catalogued and stored in liquid nitrogen until testing.

Clinical and environmental samples were tested in duplicate for virus by culture [9] and by real-time RT-PCR [11], as described elsewhere. For virus culture, 100 μL of each specimen was inoculated onto Vero E6 cell monolayers. Stool samples were first passed through a 0.22-μm filter to remove bacteria. The body louse was homogenized in a small amount of sterile PBS.

Data collection and analysis. Data were analyzed using SPSS (version 12.0; SPSS). Fisher's exact test was used when appropriate.

RESULTS

Clinical specimens. Fifty-four specimens from 26 patients, 12 (46%) of whom died, were collected (table 1). Sixteen clinical specimens from 12 patients were positive by virus culture (4 specimens) and/or RT-PCR (16 specimens), including saliva (8 of 16), skin swab (1 of 11), stool (2 of 4), semen (1 of 2), breast milk (2 of 2), tears (1 of 1), and nasal blood (1 of 1). No virus was found in urine (0 of 11), vomit (0 of 2), sputum (0 of 2), sweat (0 of 1), or the body louse (0 of 1). Three of the 16 positive specimens (2 saliva and 1 nasal blood) visibly contained blood.

As indicated by RT-PCR and ELISA antigen results from blood (data not shown), the shedding of EBOV in saliva corresponded almost exactly to the period of viremia, with the last positive saliva specimen noted at day 8 after disease onset. In contrast, specimens of breast milk and semen were found to be culture positive and RT-PCR positive at days 15 and 40 after disease onset, respectively, when EBOV was already cleared from the blood. The same patient's semen was negative when retested at day 45. Despite the fact that 7 of the 11 skin swabs were collected from patients during a period of high antigenemia (reciprocal antigen titer, $\geqslant$256) and/or RT-PCR positivity in the blood, only 1 was RT-PCR positive. All 11 urine specimens were negative by both culture and RT-PCR, even though 2 of the specimens were collected during periods of high antigenemia. Overall, mortality was not significantly different for patients with positive versus negative clinical samples, although the small sample size for most sample types calls for caution in interpretation of this finding. Mortality was significantly higher among patients with RT-PCR–positive saliva than among those who were RT-PCR negative (4 of 6 versus 0 of 7, respectively; $P = .02$).

Environmental specimens. Thirty-three environmental specimens were collected (table 2). None were culture positive, but 2 specimens (1 bloody glove and 1 bloody intravenous insertion site sampled as positive controls) were positive by RT-PCR. Both specimens were visibly colored by blood (i.e., red or pink), whereas all 31 of the negative samples were clear.

DISCUSSION

We found EBOV to be shed in a wide variety of bodily fluids during the acute phase of illness, including saliva, breast milk, stool, and tears. In most cases, the infected bodily fluid was not visibly contaminated by blood. Of particular concern is the frequent presence of EBOV in saliva early during the course of disease, where it could be transmitted to others through intimate contact and from sharing food, especially given the custom, in many parts of Africa, of eating with the hands from a common plate. However, the isolation of EBOV from only 1 saliva specimen, in contrast to the 8 that were RT-PCR positive, could suggest that the virus is rapidly inactivated by salivary enzymes or other factors in the oral cavity that are unfavorable to virus persistence and replication. EBOV has been previously documented in saliva by RT-PCR, but no attempt was made to culture virus or to explore the temporal dynamics of virus shedding in that study [12]. Marburg virus, the other member of the *Filoviridae* family, has been isolated as well as detected by RT-PCR in saliva from a patient with a fatal case of Marburg hemorrhagic fever in the Democratic Republic of the Congo (authors' unpublished data). The higher mortality among patients with RT-PCR–positive saliva likely reflects increased virus shedding in patients with high viremia, which has been previously noted to be an indicator of a poor prognosis [9, 11].

The finding of EBOV in breast milk raises the possibility of direct mother-to-child transmission. In fact, breastfed children of both of the mothers whose milk was later tested in this study died of laboratory-confirmed EHF during early stages of the outbreak. The isolation of virus from breast milk in one case even after clearance from the blood suggests that transmission may occur even during convalescence. It is possible that the mammary gland, like the gonads [5] and chambers of the eye [13, 14], is an immunologically protected site in which clearance of virus is delayed. However, we cannot rule out that the finding simply represents residual EBOV secreted into the milk during the period of viremia but not expressed until some days later, since the patient was not actively breastfeeding during admission in the isolation ward, nor can we determine whether the detected EBOV was actually a component of the milk or, rather, was contained in accompanying macrophages. At any rate, it seems prudent to advise breastfeeding mothers who survive EHF to avoid breastfeeding for at least some weeks after recovery and to provide them with alternative means of feeding their infants.

The isolation of EBOV from semen 40 days after the onset of illness underscores the risk of sexual transmission of the filoviruses during convalescence. Zaire EBOV has been detected in the semen of convalescent patients by virus isolation (82 days) and RT-PCR (91 days) after disease onset [5, 14]. Marburg virus has also been isolated from the semen and linked

conclusively to sexual transmission 13 weeks into convalescence [15].

The absence of EBOV infection in multiple tested urine specimens suggests that the virus may not be efficiently filtered in the kidney. Consequently, exposure to urine appears to be of low risk during both acute illness and convalescence. The absence of EBOV in the urine, low prevalence on the skin, and rapid clearance from the saliva in surviving patients provides some reassurance that the risk of secondary transmission from casual contacts, fomites, or the sharing of toilet facilities in the home after discharge from the hospital is minimal. This conclusion is supported by previous empirical observations [5, 6].

Abstinence from sex or the use of condoms during sex, as well as avoidance of breastfeeding and contact with the mucous membranes of the eye for at least 3 months after recovery, are still recommended to avoid possible exposure to EBOV in the aforementioned immunologically protected sites.

Other than in samples grossly contaminated with blood, EBOV was not found by any method on environmental surfaces and by RT-PCR on the skin of only 1 patient. These results suggest that environmental contamination and fomites are not frequent modes of transmission, at least in an isolation ward. However, the infectious dose of EBOV is thought to be low, and neither cell culture nor the RT-PCR assay used for EBOV in this study have not been extensively validated for use in environmental detection. Hence, the sensitivity and specificity are unknown. It is possible that EBOV was present in the environment below the threshold of detection or that environmental surfaces in the isolation ward were, at times, initially contaminated by EBOV but then decontaminated through the daily cleaning routine. However, many of the inanimate objects tested, such as bed frames and bedside chairs, would not routinely be specifically decontaminated with bleach solutions under existing guidelines unless they happened to be visibly contaminated [3], suggesting that environmental contamination did not occur. Taken together with empirical epidemiological observations during outbreaks, our results suggest that current recommendations for the decontamination of filoviruses in isolation wards [3] are effective. The risk from environmental contamination and fomites might vary in the household or other settings where decontamination would be less frequent and thorough, especially if linens or other household materials were to become visibly soiled by blood.

There was a significant discrepancy between the results of virus culture and RT-PCR testing in our study, with many more frequent positive results from RT-PCR. Possible explanations for this finding include virus degradation from breaks in the cold chain during sample collection, storage, and shipping; the greater sensitivity of RT-PCR relative to culture; and, in the case of the saliva specimens, possible virus inactivation by salivary enzymes. The less-than-ideal storage conditions of the specimens in the isolation ward immediately after acquisition and the fact that even the nasal blood from 1 patient was culture negative suggest that some virus degradation indeed occurred. Nevertheless, we cannot exclude the possibility of a true absence of viable virus in the original samples. We hope to be able to repeat this study in the future with better maintenance of the cold chain to resolve this question.

Taken together, our results support the conventional assumptions and field observations that most EBOV transmission comes from direct contact with blood or bodily fluids of an infected patient during the acute phase of illness. The risk of casual contacts with the skin, such as shaking hands, is likely to be low. Environmental contamination and fomites do not appear to pose a significant risk when currently recommended infection control guidelines for the viral hemorrhagic fevers are followed. Prospective studies with the collection of a greater number of clinical samples from patients at different stages of EHF, as well as environmental samples analyzed with an assay validated for EBOV detection in such samples, should be performed to confirm our results.

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