# Clinical, Virologic, and Immunologic Follow-Up of Convalescent Ebola Hemorrhagic Fever Patients and Their Household Contacts, Kikwit, Democratic Republic of the Congo

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**A cohort of convalescent Ebola hemorrhagic fever (EHF) patients and their household contacts (HHCs) were studied prospectively to determine if convalescent body fluids contain Ebola virus and if secondary transmission occurs during convalescence. Twenty-nine EHF convalescents and 152 HHCs were monitored for up to 21 months. Blood specimens were obtained and symptom information was collected from convalescents and their HHCs; other body fluid specimens were also obtained from convalescents. Arthralgias and myalgia were reported significantly more often by convalescents than HHCs. Evidence of Ebola virus was detected by reverse transcription–polymerase chain reaction in semen specimens up to 91 days after disease onset; however, these and all other non-blood body fluids tested negative by virus isolation. Among 81 initially antibody negative HHCs, none became antibody positive. Blood specimens of 5 HHCs not identified as EHF patients were initially antibody positive. No direct evidence of convalescent-to-HHC transmission of EHF was found, although the semen of convalescents may be infectious. The existence of initially antibody-positive HHCs suggests that mild cases of Ebola virus infection occurred and that the full extent of the EHF epidemic was probably underestimated.**

From January to June 1995, the city of Kikwit, Democratic Republic of the Congo (DRC), was the focus for an epidemic of Ebola (EBO) hemorrhagic fever (EHF) that involved an additional 20 villages in the Kwilu subregion. There were 315 cases, of whom 244 died [1]. Many of the survivors of the epidemic returned home to their families. Because little was known about their infectiousness, most hospitalized patients were held in an observation ward for 3 weeks after recovering from their acute illness before they were allowed to be discharged.

There is evidence that convalescent EHF patients may be infectious: EBO virus has been isolated from the semen of a convalescent 61 days after disease onset [2]; Marburg virus, a filovirus that is genetically related to EBO virus, has been shown to exist in the semen of a convalescent patient for up to 83 days after disease onset and may have been the source of infection to a contact [3–5]; and Marburg virus has also been isolated from the anterior chamber of the eye of a convalescent patient with uveitis 80 days after disease onset [6].

We conducted a prospective study of patients who recovered from EBO (subtype Zaire; EBO-Z) virus infection to investigate the potential for sexual and nonsexual transmission. The objectives of the study were to describe the clinical course of convalescence following EHF, determine whether body fluids of convalescent EHF patients contain EBO virus and quantify the duration of infectivity, and monitor household contacts (HHCs) for evidence of secondary transmission from the convalescents.

## Methods

*Population and enrollment.* All convalescent EHF patients and their HHCs who lived in or near Kikwit were eligible for enrollment. An EHF convalescent was defined as any person living in the Kwilu subregion of Bandundu region, DRC, from 1 January to 26 August 1995 who had a history of one of the following: (1) unexplained fever plus three or more of any of the following symptoms: headache, nausea, vomiting, anorexia, intense fatigue, abdominal pain, general myalgia, general arthralgias, dysphagia, dyspnea, or hiccups; (2) unexplained fever plus contact with an EHF case; or (3) unexplained acute hemorrhagic symptoms, such as gingival bleeding, conjunctival bleeding, petechiae, purpura, melena, or hematemesis [1]. In addition, for the purposes of this

Informed consent was obtained from the participants or their guardians. The study was approved by the Institutional Review Board of the Centers for Disease Control and Prevention (CDC) US Department of Health and Human Services; and the Ministry of Health of the former Zairian government.

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study, convalescents were defined as persons who had laboratory evidence of EBO infection and had recovered from the acute phase of their illness.

An HHC was any person who resided in the same household as or shared a cooking fire with an EHF convalescent at the time of enrollment. HHCs were considered to have been infected with EBO-Z virus if they had an initial blood specimen that tested negative for IgM and IgG antibodies and EBO antigen and a subsequent specimen that was positive for one or more of these three tests. HHCs were considered to have EHF disease if they met the laboratory diagnostic criteria for EBO infection and if they met the clinical criteria for EHF as described in the paragraph above. An HHC who met the clinical criteria for EHF but who lacked laboratory confirmation of EBO infection was classified as having symptoms ''consistent with EHF.''

The study protocol was approved by the Ministry of Health of the former Zairian government, Bandundu region health authorities, and the Centers for Disease Control and Prevention (CDC; Atlanta) Human Subjects Review Board. Convalescents and their sex partners in the household were counseled regarding the possibility of sexual transmission of EHF, and condoms were provided. All HHCs were educated about EHF, care of the convalescent person, and avoidance of the convalescent's body fluids. Latex gloves were provided in case contact with a convalescent's body fluids could not be avoided. The convalescents who participated were given food packages at each visit as an incentive; convalescents and their families who participated in the 21-month follow-up visit were also given US$100. All convalescents who could be contacted were given a 1-month supply of iron and folate supplements, regardless of whether they chose to participate.

We used a prospective cohort design, with the beginning of the follow-up period defined as the date of discharge of the convalescent from the EBO isolation ward of Kikwit General Hospital. For the 2 convalescents who were never hospitalized, follow-up began on the day of symptom onset. As soon as the study protocol was approved, we began contacting potential convalescents for enrollment. Some potential convalescents had already been discharged from the hospital, while others were held for observation in an open ward of the hospital. Potential convalescents who were enrolled were later dropped from the study if they lacked laboratory evidence of EBO infection.

*Data and specimen collection.* After enrollment, participants were visited by the study team up to six times over the first 6 months and once at 21 months. On the initial visit, the EHF convalescent and his or her HHCs were interviewed regarding any symptoms experienced since the convalescent's release from the hospital. Convalescents were examined by a physician on the study team. Because of anecdotal reports of hearing loss during acute EHF, audiometric testing (model 92680; Welch Allyn, Skaneateles Falls, NY) for hearing loss of four frequencies (500, 1000, 2000, and 4000 Hz) at a 25-decibel level was performed on convalescents and 2 household controls closest in age to the convalescent. In addition, HHCs were asked about their exposure to the convalescent, including whether they had touched, embraced, or kissed a convalescent; had slept in the same bed or had sexual intercourse with a convalescent; or had other exposures to the body fluids of a convalescent. If an HHC reported having sexual intercourse with a convalescent, he or she was asked whether a condom was used always, sometimes, or never. A blood specimen was obtained from all convalescents and their HHCs. The following additional body fluid specimens were obtained from the convalescents: urine, feces (from a rectal swab), tears, sweat, saliva, semen (ejaculate was collected in a latex condom), and vaginal secretions (from a vaginal swab). None of the convalescents was lactating, so no breast milk was collected.

On subsequent visits during the first 6 months of follow-up, a standardized questionnaire was administered to participants about intercurrent illnesses and contact with the convalescent. Audiometric testing was also performed, and a full set of body fluid specimens was obtained from the convalescents. During the first 3 months of follow-up, we attempted to obtain 3 specimens of each body fluid from the convalescents.

To determine whether HHCs had become EBO antibody positive, a follow-up blood specimen was collected from HHCs on the last visit made during the initial 6 months of the study. However, because of a mishap that occurred either during specimen storage or transport, the labels on the follow-up specimen tubes were illegible, and individual specimens could not be identified; these specimens were discarded. Therefore, one additional visit was made in April 1997, 21 months after follow-up began, to obtain blood specimens again. For some HHCs, only 1 specimen was obtained, and it was obtained after exposure to the convalescent had begun. When such a specimen tested negative for anti-EBO IgG antibody, we assumed that the HHC had been antibody negative since the beginning of follow-up.

During the 21-month follow-up visit, in addition to the blood specimen that was obtained from all participants, a semen specimen was obtained from all male convalescents. A standardized in-depth symptom questionnaire was administered to each convalescent and up to 2 household controls. We attempted to select 2 controls of the same sex as and close in age to the convalescent. During the in-depth symptom interview, we first asked a series of close-ended questions about symptoms that occurred in the past month; then in an open-ended format, we asked the interviewee to describe the major health problems that he or she had experienced in the past year. Because of the civil unrest that affected Bandundu region, the field work to complete the 21-month follow-up visits was prematurely terminated. All interviews were conducted by a single team of Congolese health workers in either French or Kikongo, the native language of the participants.

In the course of other epidemiologic studies and serologic surveillance activities that were conducted in Kikwit during the EHF epidemic, a small number of additional blood specimens were obtained from convalescents and their HHCs who participated in this study. These specimens were tested by the same laboratory that analyzed the specimens collected for this study, and results from these additional specimens were included in this analysis.

*Laboratory.* All specimens were tested by the Special Pathogens Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, CDC. Virus isolation was attempted on all body fluid specimens, and they were also tested with an ELISA to detect EBO virus protein antigens [7, 8]. Blood specimens were tested by ELISA for the presence of EBO antigen and IgM and IgG antibodies [7, 8]. Semen specimens were also tested for the presence of viral RNA, using reverse transcription–polymerase chain reaction (RT-PCR) [9].

*Statistical analysis.* For visits completed during the first 6 months of follow-up, we calculated the proportion of interviews in which a symptom was reported. Proportions were calculated separately for convalescents and HHCs. Odds ratios (ORs) for the

comparison of symptoms between convalescents and HHCs were estimated by use of the SAS GENMOD procedure [10]. This software package performs logistic regression, using generalized estimating equations, a method that accounts for the correlation of responses given by the same participant over time. An exchangeable working correlation matrix was chosen because an inspection of the data revealed no obvious temporal pattern in the symptoms reported. This structure models the correlation in the symptoms reported in a way that does not depend on the order in which the responses were given. For each symptom, we created a model containing case status (convalescent or HHC), sex, age group (using 10-year intervals), and a set of dummy variables coded for the number of months since illness onset of the convalescent of the participant's household. For all comparisons, ORs and 95% confidence intervals (CI) were estimated, and statistical significance was defined by a 95% CI, which did not contain the null value of one.

For visits completed at 21 months of follow-up, participants were only visited once; therefore, there was no need to analyze these data with a method for correlated data. Instead, comparisons of symptom frequency were made using a logistic regression method, which added 0.5 to zero cells of stratum-specific tables without a zero marginal [11]. ORs were adjusted for sex and age group using age terciles.

The analysis of symptoms revealed that most convalescents had arthralgias. To investigate the association between arthralgias and antibody levels, a comparison of the median summed optical densities of the ELISA test for IgG antibody for convalescents with and without arthralgias was performed using an exact method of the Wilcoxon rank sum test. In testing for the association between summed optical densities of the ELISA test and reported arthralgias, we used the ELISA test result from the blood specimen that was obtained at 21 months of follow-up and arthralgias reported at 21 months of follow-up; we excluded convalescents who had arthralgias predating the onset of EHF.

## Results

*Enrollment, follow-up, and epidemiologic characteristics of the study population.* From 20 June to 24 July 1995, we identified 58 patients from the epidemic surveillance database who met the clinical criteria of the convalescent EHF case definition; 49 of them lived in or near Kikwit. Of these 49 potential convalescents, 32 agreed to participate, 14 refused, 2 could not be located, and 1 was found to be antibody negative during the enrollment period and was dropped from the study. Of the 32 potential convalescents who agreed to participate, 29 were included in the study because they were antibody positive and therefore met the complete case definition for an EHF convalescent. These 29 convalescents lived in 27 households: 25 households each contained 1 convalescent, and 2 households each contained 2 convalescents. Table 1 shows the epidemiologic characteristics of the serologically confirmed EHF convalescents who were included in the study.

All 152 HHCs of the 29 participating convalescents were enrolled. The median age of the HHCs was 15 years (range: 3 months to 58 years), and 74 (48.7%) were female.

**Table 1.** Epidemiologic characteristics of Ebola hemorrhagic fever (EHF) convalescents, Bandundu region, Democratic Republic of the Congo, 1995.

| Characteristic | Convalescents ($n$ = 29) |
|---|---|
| Age (years) | |
| Mean ± SD | 30.9 ± 11.6 |
| Median (range) | 27 (8–56) |
| Sex (% female) | 69.0 |
| Range of EHF onset dates | 14 April to 20 June |
| First case in household* (%) | 67.9 |
| Health care worker (%) | 44.8 |
| Delay between EHF onset and seeking care at a hospital (days)† | |
| Mean ± SD | 4.0 ± 3.3 |
| Median (range) | 3.0 (0–14) |

* Missing data: $n$ = 28.
† Missing data: $n$ = 24.

During the initial 6 months of follow-up, convalescents gave a median of 5 interviews per person (range: 1–6); the median follow-up time per convalescent was 18.9 weeks (range: 2–27.3). A median of 5 interviews was completed per HHC (range: 1–6), and the median follow-up time per HHC was 18.8 weeks (range: 2–27.3).

At 21 months of follow-up, in-depth symptom interviews were completed, and blood specimens were obtained from 20 convalescents. In-depth symptom interviews were completed for 28 HHCs, and blood specimens were obtained from 20 HHCs.

*Serologic results for convalescents.* In all, we tested 373 blood specimens from the 29 convalescents enrolled in the study: 353 specimens were collected during the first 6 months of follow-up and 20 specimens were collected at the 21-month follow-up visit. A median of 10 (range: 2–31) specimens was obtained from each convalescent.

As required by the case definition, all convalescents had at least 1 blood specimen that tested positive for antigen or IgM or IgG antibody. The earliest specimen that tested positive for antigen was obtained 3 days after symptoms began; however only 1 specimen was obtained earlier in a convalescent's illness (figure 1). All specimens obtained 3–6 days after symptoms began tested positive for antigen, and antigen positivity disappeared 7–16 days after symptoms began. All convalescents tested were antigen negative after 16 days. Results from tests for IgM antibody showed that IgM appears between 2 and 9 days after symptom onset, and disappears between 30 and 168 days after onset. Since the convalescent who tested positive for IgM 168 days after symptom onset had no subsequent specimens tested for IgM, we were unable to determine whether antibody persists >168 days. IgG antibody appears between days 6 and 18 after symptom onset and persists. All 20 of the specimens drawn at the 21-month follow-up visit (661–749 days after symptom onset) were IgG positive.

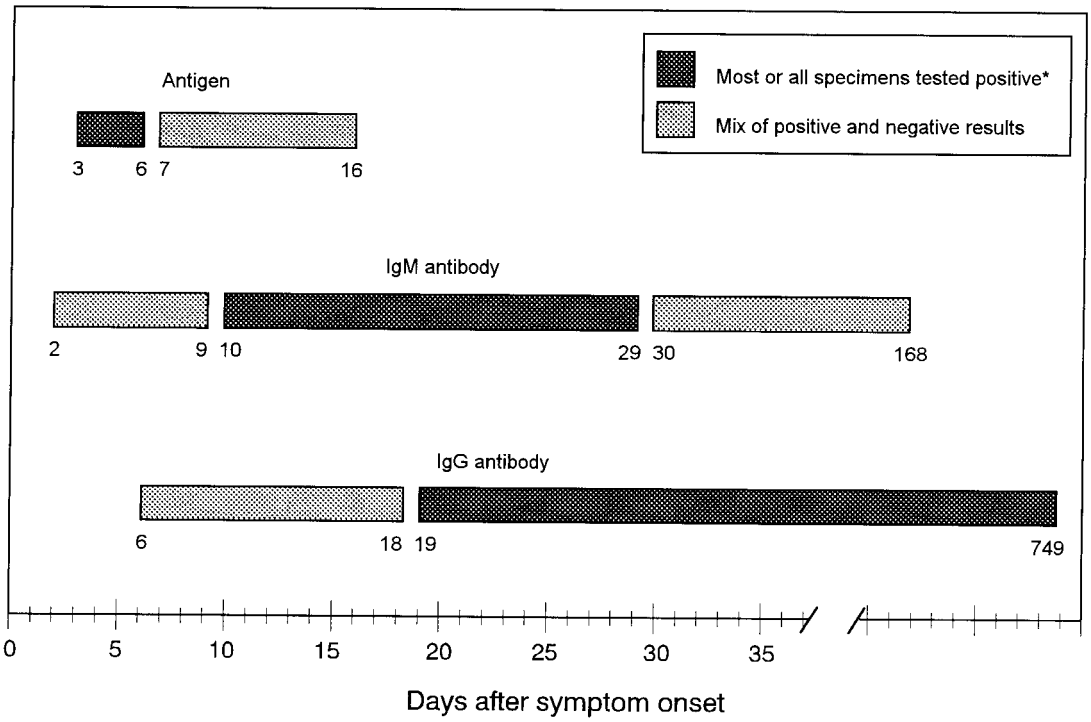


**Figure 1.** Antigen and antibody response among Ebola hemorrhagic fever convalescents, 1995–1997. Nos. under gray bars denote days after symptom onset. * For IgG antibody testing, 260/271 (96%) of specimens obtained 19–749 days after symptom onset tested positive. All other specimens shown in black bars tested positive.

Eleven specimens were negative for all tests (7 were tested for antigen and IgM and IgG antibody, and 4 were tested for only IgM and IgG). Of the latter group, 2 of the 4 specimens were obtained in the first week after symptoms began and thus may have been antigen positive. If these 2 early specimens were ignored, then 9 (2.4%) of 371 specimens tested negative for antigen, IgM, and IgG. However, all patients with a negative specimen had ⩾3 other positive specimens.

*Infectivity of convalescent body fluids.* During the first 3 months of follow-up, body fluid specimens were obtained from 28 of the 29 convalescents: Specimens included 85 of tears, 84 of sweat, 79 of feces, 95 of urine, 86 of saliva, 8 of semen, and 44 of vaginal secretions. These specimens were obtained between 12 and 157 days after symptom onset. During the 21-month follow-up visit, 3 additional semen specimens were obtained. All specimens were negative for virus isolation, and all were negative for antigen by ELISA. For semen, 4 of the 5 convalescents tested had at least 1 specimen with EBO RNA detected by RT-PCR (table 2). Of the 6 RT-PCR–positive specimens, the earliest was obtained 47 days after the onset of illness, and the latest was obtained 91 days after the onset of illness. Of the 5 RT-PCR–negative specimens, the earliest was obtained 62 days after the onset of illness and the latest was obtained 701 days after the onset of illness [9].

*Clinical symptoms of convalescents.* During the first 6 months of follow-up, convalescents reported arthralgias, myalgia, abdominal pain, extreme fatigue, and anorexia more frequently than did HHCs (table 3). The odds of a convalescent reporting fever, headache, diarrhea, dysphagia, hiccups, or signs of hemorrhage (conjunctival or gingival bleeding, petechiae, melena, and hematemesis) were not significantly differ-

**Table 2.** Results of semen specimens obtained from Ebola hemorrhagic fever convalescents, Kikwit, Democratic Republic of the Congo, 1995–1997.

| Identification no. of convalescent, age (years) | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| 2060, 27 | | | |
| Days after illness onset | 52 | 82 | 697 |
| RT-PCR | + | + | − |
| 2032, 25 | | | |
| Days after illness onset | 47 | 91 | ND |
| RT-PCR | + | + | |
| 96, 29 | | | |
| Days after illness onset | 63 | 97 | 698 |
| RT-PCR | + | − | − |
| 11, 33 | | | |
| Days after illness onset | 63 | 701 | ND |
| RT-PCR | + | − | |
| 2110, 32 | | | |
| Days after illness onset | 62 | ND | ND |
| RT-PCR | − | | |

NOTE. RT-PCR = reverse transcription–polymerase chain reaction; ND = not done; + = positive; − = negative.

**Table 3.** The number and percentage of interviews in which symptoms were reported by Ebola hemorrhagic fever convalescents and their household contacts during the first 6 months of study follow-up, Kikwit, Democratic Republic of the Congo, 1995.

| | Convalescents (123 interviews) | | Household contacts (626 interviews) | | |
|---|---|---|---|---|---|
| Symptom | *n* | (%) | *n* | (%) | OR (95% CI) |
| Arthralgias | 59 | (48.0) | 17 | (2.7) | 23.40 (11.5–47.7) |
| Myalgia* | 29 | (23.8) | 18 | (2.9) | 6.39 (2.9–14.1) |
| Abdominal pain | 15 | (12.2) | 29 | (4.6) | 2.91 (1.3–6.4) |
| Extreme fatigue | 10 | (8.1) | 17 | (2.7) | 3.20 (1.2–8.8) |
| Anorexia | 9 | (7.3) | 18 | (2.9) | 3.00 (1.2–7.6) |

NOTE. OR = odds ratio; CI = confidence interval.

* Because of missing values, responses were analyzed for 122 convalescent interviews and 625 household contact interviews.

ent from those of HHCs. Arthralgias and myalgia were the most commonly reported symptoms among convalescents: The proportions of convalescents reporting these symptoms at least once during the first 6 months of follow-up were 23 (79.3%) and 16 (55.2%), respectively. Physical examinations of involved joints did not reveal redness, swelling, or reduced range of motion with passive movement.

Fifteen months later, at the 21-month follow-up visit, convalescents reported both general and specific health problems. Compared with their health before the EBO epidemic, convalescents reported more often than controls that their general health was a little or much worse (70.0% vs. 17.9%; OR: 8.1; 95% CI: 1.7–37.7) and that their capacity to work was worse (70.0% vs. 7.1%; OR: 13.6; 95% CI: 2.7–69.1). Regarding specific symptoms, only arthralgias and myalgia were reported significantly more often by convalescents than controls. Excluding those who had myalgia before the EBO epidemic, 9 (47.4%) of 19 convalescents and 1 (3.7%) of 27 controls reported having myalgia at least once a week during the month preceding the interview (OR: 8.8; 95% CI: 1.9–42.0); and 5 (26.3%) of 19 convalescents and none of 27 controls described myalgia as a major health problem affecting them in the past year (OR: 5.3; 95% CI: 0.9–31.2).

Excluding those who had arthralgias before the EBO epidemic, 8 (61.5%) of 13 convalescents and 1 (3.8%) of 26 controls reported having arthralgias at least once a week during the month preceding the interview (OR: 16.6; 95% CI: 2.5–107.6), and 9 (64.3%) of 14 convalescents and none of 26 controls described arthralgias as a major health problem affecting them in the past year (OR: 18.0; 95% CI: 2.5–128.7). The median summed optical density (SOD) of the ELISA test for IgG antibody was significantly greater for convalescents with arthralgias (median SOD = 3.90) than for convalescents without arthralgias (median SOD = 2.13; $P = .045$).

Most convalescents who reported arthralgias at 21 months also reported muscle or joint pain during the acute phase of EHF. Arthralgias usually were worse in the morning and after working, and convalescents rarely reported that painful joints were red or swollen. Almost all joint involvement was symmetric, and 3–5 pairs of joints were typically involved. From most to least commonly affected, the joints reportedly involved were the knees, back, hips, fingers, wrists, neck, shoulders, ankles, and elbows.

Serial audiometric testing was performed on 28 of the 29 convalescents during follow-up visits (median of 3 tests per convalescent; range: 1–4). Among these 28 convalescents, 11 (39.3%) could not hear at least one of four frequencies at some point during the first 6 months of follow-up. Among 8 convalescents who had follow-up at 21 months and who had denied having hearing problems before their EBO infections, 7 still could not hear at least one frequency; however, only 4 had self-reported hearing loss. After adjusting for age and sex, we failed to show a significant association between being a convalescent and hearing loss, defined either audiometrically (OR: 1.4; 95% CI: 0.4–5.0) or subjectively (OR: 3.5; 95% CI: 0.3–39.6).

*Serologic results and symptoms of HHCs of convalescents.* We tested 160 blood specimens from HHCs enrolled in the study. A median of 1 specimen was collected (range: 1–6) and ⩾2 blood specimens were obtained from 49 HHCs (32.2%). Of 152 HHCs, 5 had serologic evidence of EBO virus infection on their initial specimen, 81 were antibody negative on their initial specimen and were serologically monitored prospectively, 16 had an antibody-negative specimen obtained through surveillance efforts before enrollment began but had no subsequent specimens and thus had no serologic follow-up; no blood specimens were obtained from 50 HHCs.

Five HHCs (HHCs A–E) had serologic evidence of EBO virus infection on their initial specimen. HHC A had a single IgG-positive specimen obtained 6 days before she had exposure to a convalescent. The timing of her serologic results excluded the convalescent as the source of the infection. She had a spontaneous abortion 3 days before her specimen was obtained, but otherwise she had no symptoms of EHF during the study period.

HHCs B and C each had a single IgG-positive specimen (antigen- and IgM-negative) and no symptoms consistent with

EHF during the study period. Assuming a minimum incubation period of 2 days for EHF [12] and a minimum of 30 days between symptom onset and IgG-positivity (figure 1), we estimated that these HHCs were infected at least 32 days before the blood specimen was obtained. Assuming the reported contact dates are accurate, since the exposure of HHCs B and C to convalescents began 18 and 19 days, respectively, before the blood specimens were obtained, it seems unlikely that the source of EBO virus for these HHCs was the convalescent.

One specimen was obtained from HHC D 3 days after her exposure to the convalescent: The specimen was positive for both IgM and IgG antibodies. During the following 3 months, 3 more specimens were obtained from HHC D, and they were positive for IgG only. A fifth specimen obtained 20 months after her exposure was negative for IgG. She had no symptoms consistent with EHF during the study period. Using the same argument as above and making the additional assumption that a minimum of 6 days elapses between symptom onset and combined IgM and IgG positivity (figure 1), we estimated that the EBO virus infection of HHC D occurred at least 8 days before the blood specimen was obtained (i.e., 5 days before she was exposed to the convalescent in her household). If the reported contact dates are accurate, it would seem unlikely that the source of the EBO virus was the convalescent.

HHC E was a 20-year-old woman who had no symptoms consistent with EHF during the study period. Her initial specimen was weakly positive for IgM, and a follow-up specimen 21 months later was IgG negative. Since the IgM result from the first specimen could have been a false-positive or the IgG from the second specimen could have been a false-negative, it is difficult to determine whether this HHC was infected with the EBO virus. If she had been infected with EBO, it is possible that her infection was sexually transmitted. The IgM-positive specimen was obtained 52 days after her exposure to the convalescent had begun. The semen of the convalescent, with whom HHC E reported having sexual intercourse, was positive for EBO virus RNA as determined by RT-PCR, and condoms were not always used.

In summary, of the 5 initially antibody-positive HHCs, there is indirect evidence that 1 may have been infected by a convalescent and that the infection may have been sexually transmitted via semen. Of the remaining 4 HHCs, only 1 reported a symptom consistent with EHF, and all had histories of exposure to convalescents that began after the time we estimated they were infected with the EBO virus.

Among the 81 HHCs who were antibody negative on their initial specimen and were serologically monitored, none seroconverted. However, during serologic follow-up, 15 of these 81 HHCs experienced 18 episodes of symptoms that were consistent with EHF. After serologic follow-up ended, prospective monitoring of symptoms continued, and 7 additional episodes of symptoms consistent with EHF were identified among 6 HHCs. Five episodes occurred during the first 4 months of follow-up and two at the 21-month follow-up visit. Among the 66 HHCs with no serologic follow-up (16 with an initial antibody-negative result and 50 from whom no blood specimens were obtained), 3 experienced 3 episodes of symptoms consistent with EHF. We identified 2 episodes during the first 4 months of follow-up and 1 at the 21-month follow-up visit.

During the follow-up period, we identified 2 deaths among HHCs. The first was a 9-year-old boy whose cause of death was reported as poisoning, and no symptoms were reported. A second death occurred in a 2-week-old infant; the child was born prematurely, and because he only lived 2 weeks, he was never enrolled in the study.

*Other serologic results.* Among 22 household members of 3 suspected EHF cases who ultimately were found to be antibody negative, 2 had laboratory evidence of EBO virus infection (HHCs F and G). HHC F had no reported symptoms and a single blood specimen. The specimen was divided into two portions: One portion was weakly EBO IgM positive, while the other was IgM negative. Neither portion was positive for antigen or IgG. HHC G was asymptomatic until the 21-month follow-up visit, at which time she reported fever, headache, abdominal pain, and back pain during the 15 months since the previous study visit. Her only specimen, which was obtained at the 21-month follow-up visit, was positive for EBO IgG.

## Discussion

The primary public health issue addressed by this study was to assess whether convalescent EHF patients who had returned home posed any danger of transmitting EBO virus to their HHCs. On the basis of clinical and serologic criteria of a cohort followed for up to 21 months, we found no direct evidence of convalescent-to-HHC transmission of EBO-Z virus. However, for 4 of the 5 male convalescents from whom semen specimens were obtained, we demonstrated the presence of EBO virus RNA in the semen up to 91 days after illness onset. Furthermore, we have indirect evidence that 1 HHC may have been infected by exposure to semen. These findings are consistent with those in previous reports of EBO virus and Marburg virus being isolated from the semen of convalescents [2–4] and Marburg virus being implicated in a potentially sexually transmitted case [5].

In the 6 months following their recovery from the acute phase of EHF, convalescents experienced arthralgias, myalgia, abdominal pain, extreme fatigue, and anorexia; however, many of these symptoms seemed to resolve by the 21-month follow-up visit. A striking finding was that almost two-thirds of the survivors continued to suffer from arthralgias or myalgia 21 months after their acute illness, and many described their symptoms as major health problems. Symmetric polyarthropathies have been associated with other virus infections, such as hepatitis B, rubella, and parvovirus [13]. These arthropathies result from either viral replication or the deposition of immune complexes in joint tissue. In this study, antibody levels were greater among convalescents with arthralgias, suggesting a role of persistent immune activation in the pathogenesis. If the clinical course of EBO-associated arthralgias is similar to that of other

viral pathogens, then we might expect the symptoms to resolve completely with no permanent joint damage. Similarly, we might expect the arthralgias to respond to conservative management, such as bed rest and treatment with nonsteroidal anti-inflammatory drugs.

Throughout the study, we discovered HHCs had episodes of symptoms that were consistent with the outbreak definition of EHF (fever and three accompanying symptoms or unexplained bleeding). All the episodes that occurred among HHCs for whom we had a blood specimen were demonstrated not to be EHF because the HHCs were antibody negative. In central Africa, there are many possible causes for fevers (e.g., malaria, yellow fever, dysentery, and hepatitis), constitutional symptoms, and bleeding episodes. That antibody negative HHCs frequently met the clinical criteria for EHF underscores the point that the outbreak case definition should not be used for endemic disease detection, and it points to the necessity of etiologic diagnosis.

This study provides evidence for asymptomatic or mild cases of EBO virus infection. We identified at least 4 HHCs who had laboratory evidence of IgG antibodies without symptoms when they were initially interviewed, suggesting disease concurrent or prior to that of the family member first enrolled as a convalescent in the study. One of these asymptomatic individuals was among the contacts of a suspected EHF case who was subsequently excluded from our study because she did not have laboratory evidence of disease. In addition, 3 children of an EHF patient who was reported to the epidemic surveillance system but not enrolled in this study because of death, were IgM-positive for EBO virus infection, suggesting infection but asymptomatic disease (data not shown). Therefore, it is valid to test for asymptomatic infection among contacts of convalescents. These findings also suggest that the full extent of the Kikwit epidemic was underestimated.

One antibody-positive HHC, who we suspected may have been infected through contact with a convalescent, had IgM antibodies on her initial specimen but no IgG. She reported no symptoms and did not develop IgG antibodies by day 687. A second HHC had IgM initially and IgG antibodies through day 97, but she had no IgG on day 629. These findings suggest that IgG may not develop or may be transient in mild cases. Alternatively, misclassification error could explain these anomalous findings.

There are several important limitations to this study. First, there was considerable loss to follow-up. Although we will never know the serologic status of almost half of the HHCs enrolled after having exposure to convalescents, at least we can say that during the first 6 months of follow-up, there were no cases of serious, unexplained illness or death that appeared to be due to EHF. This suggests that the consequences most feared by the HHC of EHF convalescents were not occurring. Loss to follow-up also may have affected our analysis of symptoms at the 21-month follow-up visit, since the small number of subjects we had to study decreased the power of the statistical comparisons. Second, non-semen specimens were tested only with ELISA and virus culture. It is possible that other techniques, especially RT-PCR, may have detected EBO virus in these body fluids, although the significance of positive findings in relationship to their infectiousness is unknown. Third, there was the potential for misclassification of serologic results (false-positives and false-negatives). This problem became evident when a few specimens that were retested several times yielded conflicting results. Fourth, there was the potential for misclassification of symptoms. The clinical criteria of the outbreak definition of EHF are nonspecific, and as already mentioned, have numerous other causes. While serologic results were usually sufficient to rule out EHF, in instances where no results were available, we found it difficult to interpret the symptoms that were reported. This problem underscores the importance of serologic confirmation. Finally, responses to the in-depth questionnaire that we used to characterize the long-term clinical sequelae of EHF were subject to recall error, and comparisons were subject to recall bias, particularly with regard to the timing of symptoms that may have occurred months or years in the past.

## Conclusions

On the basis of the results of this study, we make the following recommendations. First, since we found no direct evidence of convalescent-to-HHC transmission of EBO-Z virus and we were unable to detect virus by culture from any of our study participants, EHF convalescents should be allowed to return home after resolution of their acute illness. However, since EBO virus RNA was detected by RT-PCR in samples collected up to 91 days after illness onset, further study is warranted. EHF convalescents and their contacts should be followed in a research setting that includes screening of body fluids, especially semen; educating contacts to avoid body fluids, especially semen; and providing latex gloves when contact with body fluids is unavoidable. Furthermore, condoms should be provided for 3 months to prevent potential sexual transmission of EBO. A key question to be addressed by such a study is whether semen is infectious, and if so, for how long. Second, we recommend that in a future EHF outbreak, surveys should be conducted to characterize the full spectrum of the illness and to prevent transmission that might otherwise have occurred unnoticed from mild cases. Surveys should not be delayed, since people with mild or asymptomatic infections may have a transient antibody response. Third, convalescents should be screened for arthralgias and myalgia and treated appropriately. Finally, work should continue to improve rapid diagnostic assays because in nonepidemic situations, the clinical case definition is too nonspecific to be useful.

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