Prevalence of IgG Antibodies to Ebola Virus in Individuals during an Ebola Outbreak, Democratic Republic of the Congo, 1995

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During the 1995 outbreak of Ebola (EBO) hemorrhagic fever in Kikwit, Democratic Republic of Congo, two surveys using a new ELISA for EBO (subtype Zaire) virus antigen were conducted to assess the prevalence of EBO IgG antibodies among residents of Kikwit and the surrounding area. The first study determined the proportion of antibody-positive individuals who were self-identified forest and city workers from the Kikwit area. Serum samples from 9 (2.2%) of 414 workers had IgG EBO antibodies. The second study determined the proportion of EBO antibody-positive individuals who lived in villages surrounding Kikwit. The prevalence of IgG EBO antibodies in this population was 9.3% (15/161). The difference in the overall prevalence of EBO antibodies may indicate that villagers have a greater chance of exposure to EBO virus compared with those living in and in close proximity to cities.

From January through July 1995, the Democratic Republic of the Congo (DRC) experienced an epidemic of Ebola (EBO) hemorrhagic fever (EHF) resulting in 315 cases and 250 deaths (81% case fatality; the status of 5 people was unknown) [1]. Kikwit, DRC, was at the center of the epidemic, although additional cases were identified in >20 villages in the surrounding subregion of Kwilu. The presumptive index patient was a farmer and charcoal maker who lived in Kikwit but spent most of his time working in the forest surrounding Kikwit, which was his probable exposure site [1]. During the investigation of this outbreak, two studies were conducted, using current diagnostic methods, to determine the prevalence of antibodies to EBO virus in the local population.

Most previous surveys for EBO antibodies in healthy African populations have used the indirect immunofluorescent antibody test (IFAT) [2–9], a procedure suspected to be limited by both false-positive results and an insensitivity to infections occurring in the distant past [10]. Recently, it has been shown that ELISA using a lysate of EBO virus–infected cells and an appropriate negative control antigen for each unknown serum yields more sensitive and specific results than the IFAT when testing sera from humans and monkeys infected with EBO virus [10].

Informed consent was obtained from all participants in this study.

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The purpose of these studies was to improve our understanding of transmission of EBO viruses outside the health care and domiciliary settings. Specifically, we wanted to (1) compare the prevalence of EBO IgG and IgM antibodies among residents of Kikwit who worked in the surrounding forests with those who worked in the city; (2) determine whether forest workers who worked in the same area as the initial patient had a higher prevalence of antibodies to EBO virus; (3) determine the EBO IgG antibody prevalence among villagers in the area surrounding Kikwit where no EHF cases were reported; and (4) assess the risk factors for possible exposure among any individuals with detectable antibodies.

Methods

Study 1: Comparison of EBO Antibody Prevalence among Forest and City Workers, Kikwit

Selection of forest workers. From 28 July to 16 August 1995, study sites were established in four forested locations surrounding the city of Kikwit (figure 1). The primary site was set up within 1 km of the fields and charcoal pit where the index patient worked, near the village of Mwambala on the southeast side of Kikwit. This area is heavily forested and serves as a source for charcoal, wood, and food and as a hunting area for many people who live in Kikwit. Three other forest survey sites were organized in the small villages of Kuwanga, Kakoi, and Carrefour on the edge of the forested areas west of Kikwit. At each site, investigators collected blood from and interviewed volunteers either going to or returning from the forest.

Selection of city workers. From 7–16 August 1995, the primary study site for individuals who worked in the city was the Grand Marché (i.e., market) within the city of Kikwit. A second site was organized at a church in the adjoining area of Kikwit-3 neighborhood on 11 August. Investigators collected blood from

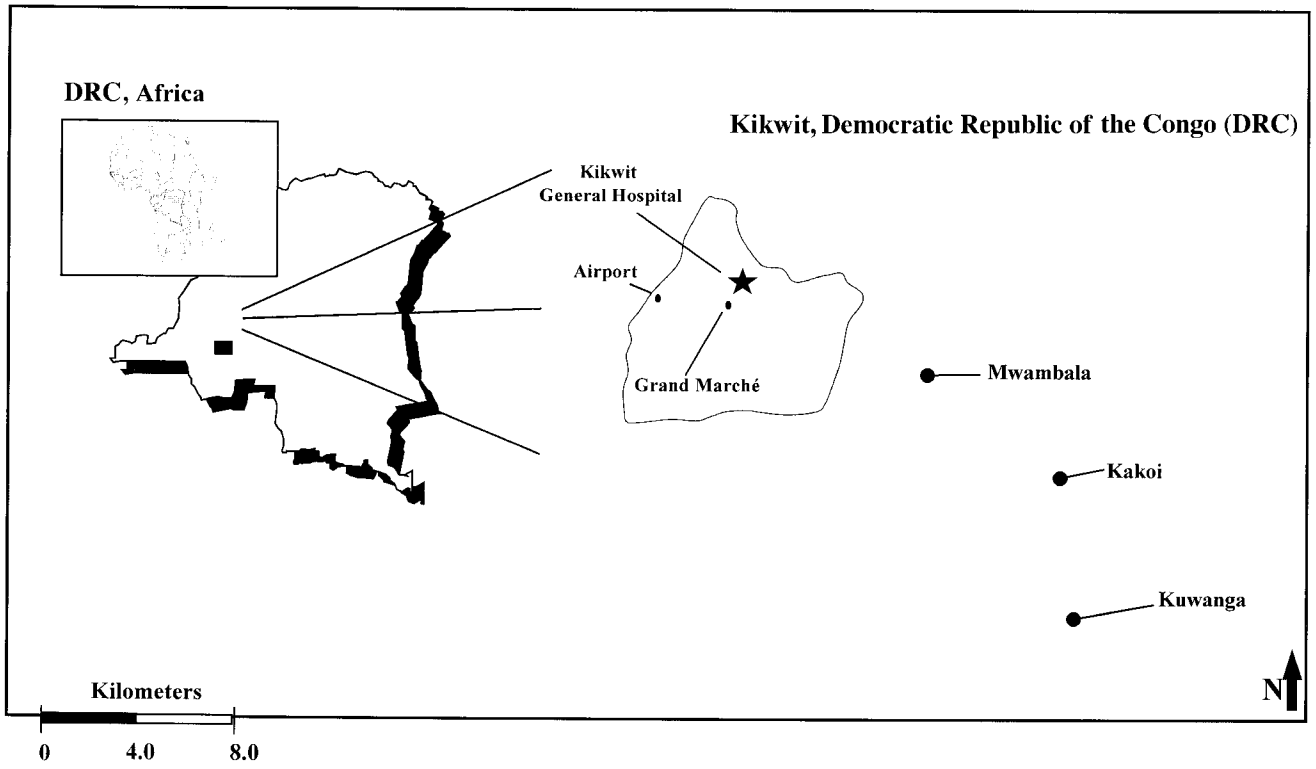

Figure 1. Forest locations in the Democratic Republic of the Congo that were used for the selection of forest workers to participate in a survey to determine the prevalence of Ebola antibodies. The villages of Mwambala, Kakoi, and Kuwanga are shown; Carrefour, which is on the edge of the forested area west of Kikwit, is not shown.

and interviewed volunteers who visited the sites. To facilitate the interviews and collection of blood samples, meetings were held with village chiefs in the collection areas and announcements were made at churches to inform people of the study. Each study participant who answered a one-page questionnaire and donated a blood sample was offered either 22,000 zaires (the equivalent of about US$3) or a can of powdered milk.

Study 2: EBO Antibody Prevalence in Villages in DRC

Selection of villagers. Interview sites were established in the center of five villages in the area surrounding Kikwit. All villages were selected from those that had no reported cases of EHF among residents during the 1995 outbreak and by their proximity to Kikwit. Interviews were conducted for a total of 6 days, between 18 August and 6 September 1995. Residents of the villages were encouraged to participate in the survey through announcements at meetings with the village chief. No individual compensation was given; however, food staples intended for the entire village were donated to the chiefs of the participating villages.

Interviews

All participants were asked to identify their primary and secondary occupations and when they were last employed in that occupation. A person was self-identified as being either a forest worker or a city worker for the first study. In addition, each person was asked how much time each week they worked in the forest during both the wet and dry seasons and where in the forest they worked. Participants were also asked if they ate certain food items or if they had contact with various mammals living in the forest. All participants were asked if they or someone they knew had ever been sick with EHF and, if so, to define their relationship with that individual. Children <14 years of age did not provide blood samples and were not interviewed unless their parents or guardians were present and gave consent. Participants were interviewed either in French or Kikongo, at the discretion of the interviewee.

Blood Collection

For both studies, a 10-mL sample of blood was collected by venipuncture and labeled with a unique identifying study number and date of collection. For children <14 years of age, a 5-mL sample of blood was collected. The unique identifying number was also recorded on the questionnaire to link the serology test results with the interview data.

Laboratory Test

Blood samples from both surveys were centrifuged at Kikwit General Hospital. The sera were separated into aliquots, stored in liquid nitrogen, and shipped frozen to the Special Pathogens Branch, National Center for Infectious Diseases, for testing. The sera collected for both surveys were tested for EBO IgG antibodies

Table 1. Prevalence of Ebola virus IgG antibodies in survey of forest and city workers, by demographics, Democratic Republic of the Congo, 1995.

	All participants		Males		Females	
Characteristic	Total	Positive (%)	Total	Positive (%)	Total	Positive (%)
Age group, years						
0–9	5	0	2	0	3	0
10–19	98	2 (2.0)	63	0	35	2 (5.7)
20–29	91	2 (2.2)	61	2 (3.3)	30	0
30–39	93	3 (3.2)	58	2 (3.4)	35	1 (2.8)
40–49	66	2 (3.0)	47	1 (2.1)	19	1 (5.3)
⩾50	60	0	45	0	15	0
Unknown	1	0	1	0	0	0
Self-identified forest workers	230	5 (2.2)	151	3 (2.0)	79	2 (2.5)
Self-identified city workers	184	4 (2.2)	126	2 (1.6)	58	2 (3.4)
Spent some time in forest	271	6 (2.2)	176	3 (1.7)	95	3 (3.2)
Spent no time in forest	143	3 (2.1)	101	2 (2.0)	42	1 (2.4)
Total	414	9 (2.2)	277	5 (1.8)	137	4 (2.9)

by using an ELISA with EBO (subtype Zaire) virus antigen and correcting the resulting optical density from that obtained by a negative control antigen run for each serum tested [10]. Sera from the survey of forest and city workers were also tested for EBO IgM antibodies [10]. Specimens were considered positive if the antibody titer equaled or exceeded 1:400 and the optical density sum equaled or exceeded 1.25.

Statistical Analysis

Results were analyzed using Mantel-Haenszel χ^2 statistic and Fisher's exact tests to compare proportions. An independent two-sample t test was used to compare means [11].

Results

Study 1: Comparison of EBO Antibody Prevalence among Forest and City Workers

Demographics. Interviews and blood samples were obtained from 414 individuals. Overall participation of forest workers and city workers was difficult to determine since, due to publicity, many participants were waiting to be interviewed and to provide a blood sample when the team arrived. In addition, no accurate census of forest and city occupation groups was available. A summary of participants in the forest workers' survey by age group, sex, type of work, and time spent in the forest is given in table 1. The mean and median ages of all participants were 32 years. Samples were collected from participants who lived in four village areas: 312 (75%) were from Kikwit, 5 (1%) from Kianga, 24 (6%) from Kakoi, and 73 (18%) from Kuwanga.

Self-identified classification. The study participants who were self-identified as forest workers were significantly older (mean, 4.5 years; $P = .001$) than those who were self-identified city workers. The forest workers also tended to spend more time in the forest than city workers (mean, 20 vs. 2.3 h/week; $P < .0001$). Table 2 lists the number of participants and EBO antibody–positive persons in various occupations. All participants with forest occupations worked in the forest during both the dry and wet seasons, with no significant difference in the amount of time spent in the forest in each season.

Nine (2.2%) of the samples were positive for IgG antibodies to EBO virus; none were positive for EBO IgM antibodies. Five of the EBO antibody–positive participants were self-identified forest workers, and 4 were self-identified city workers. The

Table 2. Prevalence of Ebola virus IgG antibodies in survey of forest and city workers, by self-reported occupation, Democratic Republic of the Congo, 1995.

Type of worker	No. in study population	No. seropositive (%)
Forest workers		
Charcoal workers	32	2 (6.2)
Hunters	2	0
Farmers	152	2 (1.3)
Wood gatherers	39	1 (2.6)
Other	3	0
Unknown	2	0
Total	230	5 (2.2)
City workers		
Students	63	2 (3.2)
Military	12	0
Merchants	33	0
Administrators	25	1 (4.0)
Teachers	10	0
Drivers	6	0
Housewives	9	1 (11.1)
Engineers	2	0
Other	24	0
Total	184	4 (2.2)

Table 3. Prevalence of Ebola IgG antibodies in village survey, by demographics, Democratic Republic of the Congo, 1995.

	All participants		Males		Females	
Characteristic	Total	Positive (%)	Total	Positive (%)	Total	Positive (%)
Age group, years						
0–9	11	0	7	0	4	0
10–19	14	1 (7.1)	12	1 (8.3)	2	0
20–29	15	2 (13.3)	6	1 (16.7)	9	1 (11.1)
30–39	35	3 (8.6)	19	2 (10.5)	16	1 (6.3)
40–49	30	4 (13.3)	19	2 (8.9)	11	2 (18.1)
⩾50	56	5 (8.9)	35	3 (8.6)	21	2 (9.5)
Primary occupation						
Farmer	103	11 (10.7)	55	6 (10.9)	48	5 (10.4)
Other	58	4 (6.9)	43	3 (7.0)	15	1 (6.7)
Time spent in forest during wet season						
<20 hours/week	108	10 (9.2)	74	6 (8.1)	34	4 (11.7)
⩾20 hours/week	53	5 (9.4)	24	3 (12.5)	29	2 (6.9)
Time spent in forest during dry season						
<20 hours/week	88	6 (6.8)	68	6 (8.8)	20	0
⩾20 hours/week	73	9 (12.3)	30	3 (10.0)	43	6 (14.0)
Total	161	15 (9.3)	98	9 (9.2)	63	6 (9.5)

youngest EBO antibody–positive individual was 14 years old; the oldest was 43 years old. Charcoal makers had a higher prevalence of antibody than did forest workers with other occupations (6.2% vs. 1.5%; $P = .09$), although the difference was not statistically significant. EBO antibody–positive forest workers spent more time in the forest than EBO antibody–negative forest workers (mean, 27.4 vs. 20 h/week; $P = .18$), although this difference was also not statistically significant. Consumption of certain food items was not significantly associated with EBO antibody status (data not shown). Of all the participants in the forest and city workers' study, 394 (95%), including all 9 of the EBO antibody–positive workers, knew someone who had been ill with EHF. In addition, the proportion of EBO antibody–positive people who had spent any time working in the forest was not statistically significantly different from the proportion who spent no time working in the forest (2.2% vs. 2.1%; $P = .92$).

Study 2: EBO Antibody Prevalence in Villages in DRC

Demographics. For the village survey, a total of 161 individuals participated. Because those who participated in the survey volunteered to be interviewed and were not randomly selected from the village populations, overall participation rates were difficult to determine. The mean age was 40 years, and the median age was 41 years. Classification of participants by age group, sex, primary occupation, and time spent in the forest during the wet and dry seasons is shown in table 3. The number of people from the different villages is listed in table 4.

Table 4. Prevalence of Ebola IgG antibodies in village survey, by village of residence, Democratic Republic of the Congo, 1995.

Village	No. of people	No. seropositive (%)
Buatundu	68	9 (13.2)
Kinganga	6	1 (16.7)
Matende	43	2 (4.7)
Mubungi	8	1 (12.5)
Ngweme	11	2 (18.2)
Other villages	25	0

EBO antibody prevalence. Of the 161 villagers surveyed, 15 (9.3%) had IgG antibodies to EBO virus. The numbers of participants with EBO antibodies are further identified in table 3. The number of EBO antibody–positive participants by village is given in table 4. The youngest EBO antibody–positive person was 15 years old, and the oldest was 75 years old. No EBO antibody–positive persons reported a family member who had been ill with EHF; 5 EBO antibody–positive persons knew a friend or acquaintance that was said to have been ill with EHF. Eleven of the 15 EBO antibody–positive persons reported that farming was their primary occupation. Two of the remaining 4 EBO antibody–positive persons reported they were students. The final 2 EBO antibody–positive individuals reported they were unemployed; their previous occupations were not reported. No statistically significant differences existed for the mean age, the mean time spent in the forest per week, sex, or contact with specific forest animals between the EBO antibody–positive and –negative groups.

Discussion

The results indicate a 2.2% prevalence of IgG antibodies to EBO virus in the population who lived and worked in and around the city of Kikwit. There appeared to be no significantly

greater risk for past infection for self-reported forest workers compared with self-reported city workers or for those who worked in the forest for any amount of time compared with those who never worked in the forest. The EBO antibody survey of persons living in villages unaffected by the 1995 EBO outbreak yielded a higher antibody prevalence (9.3%). Although these studies were not designed to be directly comparative, prevalence was significantly higher in persons living in the outlying villages ($P < .001$). This could indicate that people living in villages had a greater chance of being exposed to the EBO virus than did people living in the city of Kikwit.

The limitations of this study include the nonrandom recruitment of participants into both studies. Because of the intense investigation of the EHF outbreak by multiple international health organizations and publicity of prevention measures to stop further transmission of EBO virus, the sample of participants may not be representative of the population of Kikwit and the surrounding area. In addition, because most of the trained interviewers spoke and conducted interviews in Kikongo, it was difficult to evaluate whether consistent interviewing techniques were being followed. When assessing possible exposures to EBO virus, we may have missed some food items most associated with past infection, since most participants ate all of the foods listed on the questionnaire. Evaluation of the occupational hazard associated with forest or city work is also difficult because we do not know how long people remain antibody positive after infection with EBO virus. However, at least some of the 1967 Marburg virus–infected patients had demonstrable low-titered antibodies against some of the structural proteins >20 years following infection (Feldmann H, unpublished data). The current occupations of the study participants may not be the occupations they had when they were actually exposed and infected. Furthermore, it is possible that no significant differences were found between the different groups because the population surveyed was too small to evaluate potential past exposures.

Previous surveys to determine EBO antibody prevalence found various levels of EBO antibody prevalence, ranging from 1.8% to 21.3% [2–9]. However, all of these studies used the IFAT, which has since been suspected to give results with low specificity [10]. The prevalence of EBO antibody found in this study is lower than the prevalence of EBO antibody in a study using another ELISA technique in the Central African Republic (Gonzalez JP, personal communication). The large differences between the EBO antibody prevalence in native African populations demonstrate the need for additional studies using comparable assays to determine the true prevalence of past infection.

A high prevalence of EBO antibody may indicate that EBO virus causes both a mild or asymptomatic illness and a severe hemorrhagic fever disease. There is also evidence of filoviruses that do not produce disease in humans. In late 1989 and early 1990, four quarantine facility animal handlers developed serologic evidence of acute infection with EBO (subtype Reston) virus after infected monkeys from the Philippines were imported into the United States; none of the handlers had an unexplained febrile illness in the 6 months after exposure to the animals [12]. Two additional employees at quarantine facilities had evidence of past infection, with low antibody titers [13]. In addition, cross-reactivity between antibodies of different EBO subtypes has been documented; therefore, it is possible that another EBO virus or a different virus that is cross-reactive to EBO exists and is inducing an antibody response in some persons [10, 14].

Alternatively, the differences in the prevalence of EBO antibody found in various studies may be real and represent different risks of infection in different populations. Serologic studies of diseases such as EHF with high case fatalities can only roughly estimate the prevalence of previous infection and disease. The majority of those infected will die and no longer be available for enrollment in a survey; thus, the antibody prevalence detected in a population may not reflect the actual incidence of disease in the population. When undertaking surveys to assess the prevalence of antibody to EBO or other filoviruses, investigators must be cautious in interpreting the results until additional validation of the ELISA is obtained.

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