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Surveillance for Ebola hemorrhagic fever was conducted in the Democratic Republic of the Congo from 1981 to 1985 to estimate the incidence of human infection. Persons who met the criteria of one of three different case definitions were clinically evaluated, and blood was obtained for antibody confirmation by IFA. Contacts of each case and 4 age- and sex-matched controls were also clinically examined and tested for immunofluorescent antibody. Twenty-one cases of Ebola infection (persons with an antibody titer of $\geq 1:64$, or lower if they fit the clinical case definition) were identified, with a maximum 1-year incidence of 9 and a case fatality rate of 43%. Cases occurred throughout the year, but most (48%) occurred early in the rainy season. Fifteen percent of contacts had antibody titers $\geq 1:64$ to Ebola virus, compared with 1% of controls ($P < .0001$). Results suggest that Ebola virus periodically emerges from nature to infect humans, that person-to-person transmission is relatively limited, and that amplification to large epidemics is unusual.

The Ebola (EBO) virus was first identified in simultaneous outbreaks of hemorrhagic fever in the Democratic Republic of the Congo (DRC) and Sudan in 1976, where case fatality rates were 88% and 49%, respectively, and 602 persons were known to have been infected [1, 2]. The 2 viruses causing these outbreaks were later shown to be biologically and genetically related but distinct; the difference in virus subtypes may account, in part, for the difference between case fatality rates in the two countries [3]. A second appearance of EBO virus in DRC was detected in 1977 when the virus was isolated from a 12-year-old girl in Tandala Hospital, a rural facility in the Sud-Ubangi subregion of northwestern DRC, ~500 km from Yambuku, which was the site of the first outbreak in DRC [4]. The girl was admitted to the hospital with clinical signs and symptoms of hemorrhagic fever and died shortly after admission. In this instance, strict isolation and barrier nursing methods were implemented early, and there was no further hospital transmission from this isolated case; however, investigation led to the identification of a probable related infection in a surviving sister of this patient. A previous symptomatic infection in a member of the hospital staff, which appears to have occurred in 1972, suggested that infection with EBO was perhaps common in that area even before the first identified outbreaks in 1976.

Three years after the isolated infection was reported from Tandala, an intensified 5-year surveillance system for hemorrhagic fever was implemented in the same geographic region (figure 1). Herein, we describe this surveillance system and the results obtained from it.

Methods

Surveillance site. The Sud-Ubangi subregion, which is situated in the Zaire River basin, is covered by second-growth tropical rain forest. More than 85% of the inhabitants live in small villages with populations of <1,500. These villages, which are in clearings, are surrounded by fields used for traditional farming. The inhabitants are farmers and hunters, who are regularly in contact with domestic and peridomestic animals that are raised as a food source in close proximity to households. Inhabitants are also frequently in contact with wild animals, which they hunt in the forest and prepare for eating or for storage in their villages. The economy is thus based on agriculture, livestock (goats, sheep, and poultry), and hunting, with the main agricultural crops being maize, manioc, ground nuts, cotton, coffee, and palm for oil. Diseases endemic to the area include malaria, diarrhea, intestinal parasites, measles, tuberculosis, onchocerciasis, malnutrition, goiter, and cretinism.

Surveillance system. Twenty-eight health facilities (11 government and/or missionary hospitals, 10 dispensaries, and 7 peripheral health units) participated in the EBO hemorrhagic fever (EHF) surveillance activities in the Sud-Ubangi subregion from 1981 to 1985. The chief medical officer in charge of each participating health facility identified a surveillance agent for that facility and supervised surveillance activities, using the following case definitions for identifying possible, clinical, and probable cases: possible case: no contact specified and presence of sudden-onset fever, a single bleeding episode, and any three EBO-defining symptoms (headache, myalgia, arthralgia, nausea or vomiting, diarrhea, and lumbar, chest, or abdominal pain); clinical case: no contact specified, presence of sudden-onset fever, three bleeding episodes, and headache, myalgia or arthralgia (or both), nausea or vomiting, diarrhea, and lumbar, chest, or abdominal pain (or all three); and probable case: contact with known clinical case, no bleeding, and presence of sudden-onset fever and any three EBO-defining symp-
Figure 1. Tandala Hospital, Sud-Ubangi subregion, Equateur Region, Democratic Republic of the Congo. Hosp. = hospital; Disp. = dispensary; IRS = Institut de Recherche Scientifique; PLZ = Plantation Lever au Zaire.

toms (headache, myalgia, arthralgia [or both], nausea or vomiting, diarrhea, and lumbar, chest, or abdominal pain [or all three]).

The surveillance agent regularly contacted peripheral health and social workers, including traditional healers and village leaders, who were requested to immediately report any patient who met the criteria of one of the three case definitions to a health worker at the health facility. Once a report was received by the health facility, an epidemiologic investigation was conducted. Patients were examined, a clinical history was taken, and a 10-cm³ venous blood specimen was obtained from all those who fit the case definition and from their family members. (Later, blood specimens were obtained from other patient contacts and age- and sex-matched village controls.)

For purposes of the epidemiologic investigation, a contact was defined as a person who lived in the same house as the person who was reported to the surveillance system or as a non-household member who had visited or cared for the case. A village control was defined as a person who lived in the same village and was of the same age and sex as but not a contact of the reported case. Four village controls were identified for each case.

When possible, surveillance agents ensured that patients who fit one of the case definitions were transported to a hospital, where they were placed under isolation. When this was not possible, patients were isolated in their homes, and palliative medication, protective material, and disinfectants were provided to the caretaker. At the same time, travel to and from the village was decreased to a minimum, and intensive information campaigns on the hemorrhagic fevers and their control were conducted throughout the village and in neighboring villages. Surveillance agents made follow-up visits every 3–5 days to ensure that appropriate treatment was being provided and that isolation procedures were adequately maintained. These visits continued until 21 days after the recovery or death of the last person fitting the case definition, during which time patient contacts other than family and village controls were identified and interviewed and blood specimens were collected.

A mobile surveillance team of 1 physician and at least 1 nurse and 1 health inspector regularly visited participating health facilities to provide technical and material support to the surveillance agent, to review the report form for completeness, and if necessary, to further assist in the epidemiologic investigation. A senior surveillance officer was based in one of the health facilities (the Tandala Protestant Hospital) during the period of the surveillance to serve as coordinator and ensure that the system was implemented as conceived.

Laboratory confirmation. Blood specimens collected from patients, contacts, and village controls were held at ambient temperature until clot retraction, after which the serum was decanted and
stored at −20°C until analysis. Laboratory testing of sera was done by the WHO Collaborating Center for Hemorrhagic Fevers (Special Pathogens Branch, Viral Diseases Division, Centers for Disease Control and Prevention, Atlanta). Specimens were transported to Atlanta on wet ice in standard specimen transport containers.

An IFA was used to detect antibodies to filoviruses in the serum specimens, using acetone-fixed virus-infected cells inactivated by gamma radiation [5, 6]. Combined antigen slides called “CRE2LM” (Crimean-Congo hemorrhagic fever virus, Rift Valley virus, EBO [subtypes Sudan and Zaire] viruses, Lassa fever virus, Marburg virus) were used for the screening [7]. Those sera with a positive reaction on filovirus screening were titrated to end point, using EBO-specific virus-infected cells (end point was defined as the highest dilution giving weak but specific staining). A titer ≥1:64 was taken as evidence of EBO virus-specific antibodies, and virus isolation using Vero cell tissue culture or in suckling mice was planned but not done because of bacterial contamination of specimens.

Results

A total of 98 persons from 46 different villages were reported to the surveillance system. Epidemiologic investigation of these cases determined that 30 (12 male, 6 female) of the 98 actually had symptoms that met one of the three case definitions. Detailed analysis was done on information obtained from these 30 persons, all of whom had provided at least 1 blood specimen, and on information from their contacts and village controls, who had also provided blood samples.

Of the 30 persons, 18 (60%) had EBO antibody titers ≥1:64; these persons were from 14 different localities (figure 2). An additional 3 persons who fit the clinical case definition had antibody titers <1:64 but died before a second blood specimen could be obtained. The year the illness occurred and the case definition of all 21 persons are shown in table 1. Of these 21 persons, 3 (14%) fit the definition of a possible case, 11 (52%) fit the definition of a clinical case, and 7 (34%) fit the definition of a probable case. Three (14%) of the 21 were ≤14 years old, 5 (24%) were between 15 and 19 years old, and the remaining 13 (62%) were ≥20 years old. Nine of the 21 died, giving a case fatality rate of 43%. During the 5-year period, cases occurred during each month except February and December, with a maximum of 10 cases (48%) occurring during the months of August and September (the start of the rainy season).

During the epidemiologic investigation, 188 contacts of the 30 persons were identified who had symptoms that met one of the three case definitions. Among the 188 contacts, 28 had antibody levels ≥1:64; all were contacts of either the 18 persons with antibody titers ≥1:64 or the 3 persons with low-titer antibody who had died before the second blood specimen could be obtained, giving a maximum overall secondary attack rate of 15% (table 2). Four (14%) of the 28 contacts with antibody titers ≥1:64 were or had been sick with an illness with symptoms that met the definition of a possible case, and 24 (86%)

Figure 2. Fourteen localities in Sud-Ubangi subregion where 18 persons were reported with Ebola antibody titers ≥1:64 and 3, who fit clinical case definition but died before second blood sample could be obtained, were reported with low-titer antibody (<1:64).
were or had been sick with an illness fitting the definition of a clinical case.

A total of 137 villagers were selected during the epidemiologic investigation as controls for the 30 persons who had symptoms that met one of the three case definitions. Among these controls, 2 (1%) had antibody titers \( \geq 1:64 \) (table 2). The difference in frequency of antibody to EBO virus between contacts and village controls was statistically significant \( (P < .0001, \chi^2) \).

A total of 51 contacts had cared for a case patient who was detected through the surveillance system. Among these contacts, 9 (18%) had antibody \( \geq 1:64 \), as did 4 (7%) of the 58 who stated they had only visited a case.

### Discussion

The results obtained from the EHF surveillance that was conducted from 1981 to 1985 in northwestern DRC suggest that EBO virus caused sporadic infections that led to low levels of secondary transmission. During the epidemiologic investigations that followed each reported case, information about EHF and about its means of spread was disseminated. This may have prevented further transmission and thus led to the low levels of secondary virus transmission. The study design did not permit an evaluation of the impact of this information on secondary transmission. Information from investigations of large-scale outbreaks demonstrates, however, that amplification of transmission can occur when sporadic cases enter a hospital with poor public health practices, such as occurred in Yambuku in 1976 and Kikwit in 1995 [1, 8].

The maximum overall secondary attack rate of 15% among contacts is similar to that of 16% found in villages surveyed during the Yambuku outbreak in 1976. It is also similar to the 13% secondary attack rate in the simultaneous epidemic in Sudan [1, 2] and in the 1979 outbreak in Sudan [9]. The 43% case fatality rate was similar to that reported from Sudan in 1976 (49%) and 1979 (60%) but much lower than that in DRC in 1976 (88%).

Some individuals without a history of clinical illness compatible with EHF had low-titer reactions \( (< 1:64) \) to the IFA that was used in the 1980s. Nevertheless, it seems reasonable to conclude that those persons who met one of the surveillance case definitions for EHF and who had antibody titers \( \geq 1:64 \) were truly infected. That 30% of those who were judged to fit one of the three case definitions did not have antibody \( \geq 1:64 \) suggests that other diseases can confound surveillance, and they demonstrate the importance of laboratory diagnosis in any hemorrhagic fever surveillance system. Because of its dependence on blood-sampling equipment and supplies and on the cold chain, which must be respected to preserve sera until it is tested in the laboratory, such a system may not be feasible over the long term. The collection, storage, and safe transportation of specimens was a major problem during the 1981–1985 surveillance; inadequate storage facilities and electricity failures often resulted in thawing and refreezing of stored sera. This contributed to the heavy bacterial contamination of specimens and, thus, to the interference of virus isolation procedures in the laboratory.

Whole blood that has been dried on filter paper and then eluted for testing has been shown to be feasible for use in IFAs; however, the procedure has not been fully evaluated. If shown to be useful, filter-paper specimens would still require storage in cold and dry conditions, and while this procedure could decrease some of the problems of maintaining a cold chain under difficult field conditions, it would not completely eliminate the need for reasonably rapid transport to a cold storage facility. To our knowledge, dried whole blood has not

### Table 1. Case definition of 18 persons with Ebola antibody titers \( \geq 1:64 \) and of 3 with low-titer antibody who fit the clinical case definition but died before a second blood sample could be obtained, Ebola hemorrhagic fever surveillance, Democratic Republic of the Congo, 1981–1985.

<table>
<thead>
<tr>
<th>Case definition</th>
<th>1981 (n = 0)</th>
<th>1982 (n = 4)</th>
<th>1983 (n = 36)</th>
<th>1984 (n = 27)</th>
<th>1985 (n = 31)</th>
<th>1981–1985 (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Clinical</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Probable</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>21</td>
</tr>
</tbody>
</table>

**NOTE.** \( n = \) no. of surveillance reports investigated.

### Table 2. Ebola antibody titers \( \geq 1:64 \) among contacts and age- and sex-matched controls of patients identified during an Ebola hemorrhagic fever surveillance in the Democratic Republic of the Congo, 1981–1985.

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>No. of contacts tested/no. with titers ( \geq 1:64 ) (%)</th>
<th>No. of controls tested/no. with titers ( \geq 1:64 ) (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–14</td>
<td>61/4 (7)</td>
<td>43/2 (5)</td>
<td></td>
</tr>
<tr>
<td>15–19</td>
<td>13/2 (15)</td>
<td>10/0</td>
<td></td>
</tr>
<tr>
<td>( \geq 20 )</td>
<td>114/22 (19)</td>
<td>84/0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>188/28 (15)</td>
<td>137/2 (&lt;1)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
been evaluated using the more reliable antibody ELISA, which has now replaced the IFA.

A recently developed testing system that uses immunohistochemical staining to test for the presence of antigen in skin snips has been tested in persons who died of EHF. The test can be read by use of a light microscope and provides some promise for more feasible surveillance [10]. Because this procedure can be done on formalin-preserved specimens, cold storage is not required; however, a major disadvantage is that the procedure can be used only when virus titers are extremely high, such as in persons who are acutely ill or have died from infection. Thus, the testing system has potential, but its sensitivity among living cases varies, and it is not a feasible diagnostic tool for persons who may have recovered from EHF.

In summary, information gained during the 1981–1985 surveillance for EHF in DRC suggests that EBO virus periodically emerges from nature to infect humans and that in so doing, it usually causes self-limited outbreaks, which do not regularly amplify into large epidemics. Newer and more reliable tests for EBO, ranging from improved serologic tests to immunohistochemical staining techniques, which can be done on skin necropsy specimens, must continue to be evaluated for their utility and feasibility in surveillance so that the most refined and useful surveillance methodology can be identified and implemented.

References