Emergence of Zaire Ebola Virus Disease in Guinea — Preliminary Report


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SUMMARY

In March 2014, the World Health Organization was notified of an outbreak of a communicable disease characterized by fever, severe diarrhea, vomiting, and a high fatality rate in Guinea. Virologic investigation identified Zaire ebolavirus (EBOV) as the causative agent. Full-length genome sequencing and phylogenetic analysis showed that EBOV from Guinea forms a separate clade in relationship to the known EBOV strains from the Democratic Republic of Congo and Gabon. Epidemiologic investigation linked the laboratory-confirmed cases with the presumed first fatality of the outbreak in December 2013. This study demonstrates the emergence of a new EBOV strain in Guinea.

OUTBREAKS CAUSED BY VIRUSES OF THE GENERA EBOLAVIRUS AND MARBURGVIRUS represent a major public health issue in sub-Saharan Africa. Ebola virus disease is associated with a case fatality rate of 30 to 90%, depending on the virus species. Specific conditions in hospitals and communities in Africa facilitate the spread of the disease from human to human. Three ebolavirus species have caused large outbreaks in sub-Saharan Africa: EBOV, Sudan ebolavirus, and the recently described Bundibugyo ebolavirus.1,2 Epidemics have occurred in the Democratic Republic of Congo, Sudan, Gabon, Republic of Congo, and Uganda. Reston ebolavirus circulates in the Philippines. It has caused disease in nonhuman primates but not in humans.3 The fifth species, Tai Forest ebolavirus, was documented in a single human infection caused by contact with an infected chimpanzee from the Tai Forest in Ivory Coast.4 Although this event indicated the presence of Tai Forest ebolavirus in West Africa, this subregion was not considered to be an area in which EBOV was endemic.

On March 10, 2014, hospitals and public health services in Guéckédou and Macenta alerted the Ministry of Health of Guinea and — 2 days later — Médecins sans Frontières in Guinea about clusters of a mysterious disease characterized by fever, severe diarrhea, vomiting, and an apparent high fatality rate. (Médecins sans
Frontières had been working on a malaria project in Guéckédou since 2010. In Guéckédou, eight patients were hospitalized; three of them died, and additional deaths were reported among the families of the patients. Several deaths were reported in Macenta, including among hospital staff members. A team sent by the health ministry reached the outbreak region on March 14 (Fig. 1). Médecins sans Frontières in Europe was notified and sent a team, which arrived in Guéckédou on March 18. Epidemiologic investigation was initiated, and blood samples were collected and sent to the biosafety level 4 laboratories in Lyon, France, and Hamburg, Germany, for virologic analysis.

**Methods**

**Patients**

Blood samples were obtained from 20 patients who were hospitalized in Guéckédou, Macenta, and Kissidougou with fever, diarrhea, vomiting, or hemorrhage. Demographic and clinical data for the patients were provided on the laboratory request forms. Clinical data were not collected in a systematic fashion. This work was performed as part of the public health response to contain the outbreak in Guinea.

**Diagnostic Assays**

Viral RNA was extracted from 50 to 100 mm$^3$ of undiluted plasma and 1:10 diluted plasma with the use of the QIAmp viral RNA kit (Qiagen). The following assays were performed (Table XX in the Supplementary Appendix, available with the full text of this article at NEJM.org).

**VirAl Sequencing**

Fragments amplified by filovirus L gene-specific primers were sequenced with the use of polymerase-chain-reaction (PCR) primers. Complete EBOV genomes were sequenced directly with the use of RNA extracted from serum obtained from three patients with high levels of viral RNA, as measured on real-time reverse-transcriptase–PCR (RT-PCR) analysis. The genome was amplified in overlapping fragments with the use of EBOV-specific primers. The fragments were sequenced from both ends with the use of conventional Sanger techniques. The sequence of the contig was verified by means of visual inspection of the electropherograms.

**VIRAl Isolation**

About 100 mm$^3$ of all serum samples were used to inoculate Vero E6 cells maintained in 25-cm$^2$ flasks in Dulbecco’s Modified Eagle’s Medium containing 2 to 5% fetal-calf serum and penicillin–streptomycin. Cells and supernatant were passaged several times. Virus growth in the cells was verified on immunofluorescence with the use of polyclonal mouse anti-EBOV–specific antibodies or an increase in viral levels in the cell-culture supernatant over several orders of magnitude, as measured on real-time RT-PCR.

**Electron Microscopy**

Specimens from two patients were prepared for electron microscopy with the use of a conventional negative-staining procedure. Briefly, a drop of 1:10 diluted serum was adsorbed to a glow-discharged carbon-coated copper grid and stained with freshly prepared 1% phosphotungstic acid (Agar Scientific). Images were taken at room temperature with the use of a Tecnai Spirit electron microscope (FEI) equipped with an LaB6 filament and operated at an acceleration voltage of 80 kV.

**Phylogenetic Analysis**

We obtained all 48 complete genome sequences of filoviruses that are currently available from
GenBank and aligned them with the new EBOV Guinea sequences (18,959 nucleotides). We used software designed to perform statistical selection of best-fit models of nucleotide substitution ([Q10] jModelTest) to identify the general time-reversible model of sequence evolution with gamma-distributed rate variation among sites (GTR+gamma) as the model that best describes the phylogenetic data. We used the ([Q11] Bayesian Markov Chain Monte Carlo method, as implemented in MrBayes 3.1.2 software, [Q21] to infer the composition of one phylogenetic tree, using two runs of four chains with 1,000,000 steps with a burn-in rate of 25% and the GTR+gamma model. A second tree was inferred for the same alignment with a maximum-likelihood method implemented in PhyML software [Q14] under the GTR+gamma model with 1000 bootstrap replications.

Table 1. Demographic, Clinical, and Virologic Characteristics of 15 Patients with Confirmed Ebola Virus Disease during the 2014 Outbreak in Guinea.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age</th>
<th>Sex</th>
<th>Hospital</th>
<th>Date of Sampling</th>
<th>Symptoms</th>
<th>Outcome</th>
<th>Date of Death</th>
<th>Virus Isolation [Q21]</th>
<th>GenBank Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>20</td>
<td>F</td>
<td>Guéckédou</td>
<td>March 12</td>
<td>Fever, diarrhea, vomiting</td>
<td>Died</td>
<td>March 18</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C2</td>
<td>25</td>
<td>F</td>
<td>Guéckédou</td>
<td>March 13</td>
<td>Fever, diarrhea, vomiting</td>
<td>Died</td>
<td>March 25</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C3</td>
<td>35</td>
<td>M</td>
<td>Guéckédou</td>
<td>March 13</td>
<td>Fever, vomiting</td>
<td>Died</td>
<td>March 17</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C4</td>
<td>25</td>
<td>M</td>
<td>Guéckédou</td>
<td>March 18</td>
<td>Fever, diarrhea, vomiting, hemorrhage</td>
<td>Died</td>
<td>March 18</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C5</td>
<td>16</td>
<td>F</td>
<td>Guéckédou</td>
<td>March 19</td>
<td>Spontaneous abortion</td>
<td>Survived</td>
<td>NA</td>
<td>Yes</td>
<td>KJ660348</td>
</tr>
<tr>
<td>C6</td>
<td>27</td>
<td>F</td>
<td>Guéckédou</td>
<td>March 20</td>
<td>Fever, diarrhea, vomiting</td>
<td>Died</td>
<td>ND</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C7</td>
<td>47</td>
<td>F</td>
<td>Guéckédou</td>
<td>March 20</td>
<td>Fever, diarrhea, vomiting</td>
<td>Died</td>
<td>March 22</td>
<td>Yes</td>
<td>KJ660347</td>
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<tr>
<td>C8</td>
<td>29</td>
<td>M</td>
<td>Macenta</td>
<td>March 16</td>
<td>Fever, hemorrhage</td>
<td>Died</td>
<td>March 16</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C9</td>
<td>55</td>
<td>F</td>
<td>Macenta</td>
<td>March 16</td>
<td>Fever, diarrhea, vomiting</td>
<td>Died</td>
<td>March 19</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C10</td>
<td>17</td>
<td>M</td>
<td>Macenta</td>
<td>March 16</td>
<td>Fever, diarrhea, vomiting</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C11</td>
<td>7</td>
<td>M</td>
<td>Macenta</td>
<td>ND</td>
<td>Fever, diarrhea, vomiting</td>
<td>Died</td>
<td>March 26</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C12</td>
<td>30</td>
<td>M</td>
<td>Macenta, Nzérékoré</td>
<td>February 28</td>
<td>Fever, vomiting</td>
<td>Died</td>
<td>February 28</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>C13</td>
<td>50</td>
<td>M</td>
<td>Macenta</td>
<td>March 12</td>
<td>Fever, diarrhea, vomiting</td>
<td>Died</td>
<td>March 12</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>C14</td>
<td>41</td>
<td>M</td>
<td>Macenta, Nzérékoré</td>
<td>March 13</td>
<td>Fever, diarrhea, vomiting, hemorrhage</td>
<td>Died</td>
<td>March 16</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>C15</td>
<td>28</td>
<td>F</td>
<td>Kissidougou</td>
<td>March 17</td>
<td>Fever, diarrhea, vomiting, hemorrhage</td>
<td>Survived</td>
<td>NA</td>
<td>Yes</td>
<td>KJ660346</td>
</tr>
</tbody>
</table>

* All sampling and recording of patients’ status were performed in 2014. F denotes female, M male, NA not applicable, and ND not available.
Village Meliandou
9 Deaths from Dec. 2, 2013, to Feb. 8, 2014
2 Deaths on March 26, 2014
First recorded cases of the outbreak
(S1) Child, 2 yr of age
Fever, black stool, vomiting
Onset Dec. 2, 2013; died Dec. 6, 2013
(S2) Sister of S1, 3 yr of age
Fever, black diarrhea, vomiting
Onset Dec. 25, 2013; died Dec. 29, 2013
(S3) Grandmother of S1 and S2
Fever, diarrhea, vomiting
Died Jan. 1, 2014
(S4) Mother of S1 and S2
Bleeding
Died Dec. 13, 2013
(S5) Nurse
Fever, diarrhea, vomiting
Onset Jan. 29, 2014; died Feb. 2, 2014
(S6) Village midwife
Fever
Hospitalized in Guéckédou Jan. 25, 2014; died Feb. 2, 2014

Village Dandou Pombo
6 Deaths from Feb. 11 to March 31, 2014
(S13) Family member of S6, took care of S6
Fever, hemorrhage
Onset Feb. 4, 2014; died Feb. 11, 2014

Village Dawa
8 Deaths from Jan. 26 to March 27, 2014
(S7) Sister of S3, attended funeral of S3
Fever, diarrhea, vomiting, hemorrhage
(S8) Attended funeral of S3
Fever, bleeding
Onset Jan. 25, 2014; died Jan. 30, 2014
(S9–S12) Onset Feb. 2–16, 2014; died Feb. 11–March 5, 2014

Village Gbandou
3 Deaths from March 9 to March 12, 2014

Guéckédou

Guéckédou Baladou District
First onset Feb. 23, 2014
14 Deaths from March 1 to March 31, 2014
C1 C2 C5

Guéckédou Farako District
First onset Feb. 24, 2014
4 Deaths from Feb. 28 to March 25, 2014
C6 C7

Kissidougou
5 Deaths from March 7 to March 26, 2014
(S16) Brother of S15
Fever, vomiting
Onset Feb. 24, 2014; died March 7, 2014
(S17) Brother of S15
Fever, vomiting, hiccups
Onset Feb. 24, 2014; transferred from Guéckédou hospital to Kissidougou; died March 8, 2014
C15

Macenta
15 Deaths from Feb. 10 to March 29, 2014
(S15) Doctor at Macenta hospital; took care of S14
Vomiting, bleeding, hiccups
Funeral in Kissidougou

S14 Health care worker at Guéckédou hospital
Fever, diarrhea, vomiting
Onset Feb. 5, 2014
Went to Macenta hospital; died Feb. 10, 2014

C10 C11
C14

C8 C9
C12
C13
Contact with S15 and affected family members of S15
Onset March 3, 2014; died March 12, 2014
2 Further deaths in family
Family member of S15 and contact of S14
Died Feb. 28, 2014, in Nzérékoré
Contact with C12 in Macenta
Hospitalized in Macenta March 6, 2014, in Nzérékoré

(S1) Child, 2 yr of age
Fever, black stool, vomiting
Onset Dec. 2, 2013; died Dec. 6, 2013

(S2) Sister of S1, 3 yr of age
Fever, black diarrhea, vomiting
Onset Dec. 25, 2013; died Dec. 29, 2013

(S3) Grandmother of S1 and S2
Fever, diarrhea, vomiting
Died Jan. 1, 2014
(S4) Mother of S1 and S2
Bleeding
Died Dec. 13, 2013
(S5) Nurse
Fever, diarrhea, vomiting
Onset Jan. 29, 2014; died Feb. 2, 2014
(S6) Village midwife
Fever
Hospitalized in Guéckédou Jan. 25, 2014; died Feb. 2, 2014

First onset Feb. 23, 2014
14 Deaths from March 1 to March 31, 2014
C1 C2 C5

First onset Feb. 24, 2014
4 Deaths from Feb. 28 to March 25, 2014
C6 C7

C10 C11
C14

Contact with S15 and affected family members of S15
Onset March 3, 2014; died March 12, 2014
2 Further deaths in family
Family member of S15 and contact of S14
Died Feb. 28, 2014, in Nzérékoré
Contact with C12 in Macenta
Hospitalized in Macenta March 6, 2014, in Nzérékoré

(S1) Child, 2 yr of age
Fever, black stool, vomiting
Onset Dec. 2, 2013; died Dec. 6, 2013

(S2) Sister of S1, 3 yr of age
Fever, black diarrhea, vomiting
Onset Dec. 25, 2013; died Dec. 29, 2013

(S3) Grandmother of S1 and S2
Fever, diarrhea, vomiting
Died Jan. 1, 2014
(S4) Mother of S1 and S2
Bleeding
Died Dec. 13, 2013
(S5) Nurse
Fever, diarrhea, vomiting
Onset Jan. 29, 2014; died Feb. 2, 2014
(S6) Village midwife
Fever
Hospitalized in Guéckédou Jan. 25, 2014; died Feb. 2, 2014

First onset Feb. 23, 2014
14 Deaths from March 1 to March 31, 2014
C1 C2 C5

First onset Feb. 24, 2014
4 Deaths from Feb. 28 to March 25, 2014
C6 C7

C10 C11
C14

Contact with S15 and affected family members of S15
Onset March 3, 2014; died March 12, 2014
2 Further deaths in family
Family member of S15 and contact of S14
Died Feb. 28, 2014, in Nzérékoré
Contact with C12 in Macenta
Hospitalized in Macenta March 6, 2014, in Nzérékoré
We gathered data on possible transmission chains from hospital records and through interviews with patients in whom EVOL infection was suspected and their contacts, affected families, inhabitants of villages in which deaths occurred, attendants of funerals, public health authorities, and hospital staff members.

**RESULTS**

**IDENTIFICATION OF THE EBOV STRAIN**

To detect the causative agent, we used conventional Filoviridae-specific RT-PCR assays targeting a conserved region in the L gene to test samples obtained from 20 hospitalized patients who were suspected of being infected with a hemorrhagic fever virus. In addition, we performed EBOV-specific real-time RT-PCR assays targeting the GP or NP gene. Samples from 15 of 20 patients tested positive in the conventional L gene RT-PCR assay and the real-time assays (Table 1). EBOV was identified in the serum of one patient, and two degraded particles (arrowheads) are shown (scale bar, 100 nm).

**SEQUENCING OF SAMPLES FROM PATIENTS**

The EBOV in samples obtained from 3 patients was completely sequenced with the use of conventional Sanger techniques (GenBank accession numbers, KJ660346, KJ660347, and KJ660348). The three sequences, each 18,959 nucleotides in length, were identical with the exception of a few polymorphisms at positions 2124 (G→A, synonymous), 2185 (A→G, NP552 glycine→glutamic acid), 2931 (A→G, synonymous), 4340 (C→T, synonymous), 6909 (A→T, sGP291 arginine→tryptophan), and 9923 (T→C, synonymous). The Guinean EBOV strain showed 97% identity to EBOV strains from the Democratic Republic of Congo and Gabon. Phylogenetic analysis of the full-length sequences by means of Bayesian and maximum-likelihood methods revealed a separate, basal position of the Guinean EBOV within the EBOV clade (Fig. 3).

The prominent clinical features of the EBOV infection in the confirmed cases were fever, severe diarrhea, and vomiting; hemorrhage was less frequent. The case fatality rate in the initial cases was 86% (in 12 of 14 patients with a known outcome).
clinically suspected cases with 79 deaths (71% case fatality rate on the basis of clinical suspicion) had been recorded in the prefectures of Guéckédou, Macenta, and Kissidougou. According to the timeline of the transmission chains (Fig. 2), the outbreak of confirmed disease started in the prefecture Guéckédou and then spread to Macenta and Kissidougou (Fig. 4). The male-to-female ratio among patients who died was 41:59, with a median age of 35 years (interquartile range, 25 to 51).

Figure 3. Phylogenetic Analysis of the Ebolavirus Genus, Including the EBOV Strains from Guinea.

The phylogenetic tree was inferred with the use of the Bayesian Markov Chain Monte Carlo method. A second tree that was inferred for the same set of sequences with a maximum-likelihood method confirmed the Bayesian tree (data not shown). Bayesian posterior probabilities and bootstrap percentages (1000 replicates of the maximum-likelihood tree) are shown on the branches. For clarity of presentation, the branches for the non-EBOV species were shortened and condensed (dashed branches). The GenBank accession number, strain designation, country of origin, and year of isolation are indicated on the EBOV branches. The EBOV Guinea strain is available from the European Virus Archive (www.european-virus-archive.com).

Discussion

This study demonstrates the emergence of EBOV in Guinea. The high degree of similarity among the 15 partial L gene sequences, along with the three full-length sequences and the epidemiologic links between the cases, suggest a single introduction of the virus into the human population. This introduction seems to have happened in early December 2013 or even before. Further epidemiologic investigation is ongoing to iden-
tify the presumed animal source of the outbreak. It is suspected that the virus was transmitted for months before the outbreak became apparent because of clusters of cases in the hospitals of Guékédou and Macenta. This length of exposure appears to have allowed many transmission chains and thus increased the number of cases of Ebola virus disease.

The clinical picture of the initial cases was predominantly fever, vomiting, and severe diarrhea. Hemorrhage was not documented for most of the patients with confirmed disease at the time of sampling but may have developed during the later course of the disease. The term Ebola virus disease (rather than the earlier term Ebola hemorrhagic fever) takes into account that hemorrhage is not seen in all patients and may help clinicians and public health officials in the early recognition of the disease. The case fatality rate was 86% among the early confirmed cases and 71% among clinically suspected cases, which is consistent with the case fatality rates observed in previous EBOV outbreaks.

Phylogenetic analysis of the full-length sequences established a separate clade for the Guinean EBOV strain in sister relationship with other known EBOV strains. This suggests that the EBOV strain from Guinea has evolved in parallel with the strains from the Democratic Republic of Congo and Gabon from a recent ancestor and has not been introduced from the latter countries into Guinea. Potential reservoirs of EBOV, fruit bats of the species Hypsignathus monstrosus, Epomops franqueti, and Myonycteris torquata, are present in large parts of West Africa. It is possible that EBOV has circulated undetected in this region for some time. The emergence of the virus in Guinea highlights the risk of EBOV outbreaks in the whole West African subregion.

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Figure 4. Number of Suspected Cases of Ebola Virus Disease, According to Prefecture and Week.

The cases in Guékédou, Macenta, and Kissidougou prefectures were recorded by the local public health authorities in collaboration with the World Health Organization and Médecins sans Frontières.
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**REFERENCES**


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