Emergence of Zaire Ebola Virus Disease in Guinea


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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 EBOVIRUS 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 deaths 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 because of 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; informed consent was not obtained.

**DIAGNOSTIC ASSAYS**

Viral RNA was extracted from 50 to 100 μl of undiluted plasma and 1:10 diluted plasma with the use of the QIAmp viral RNA kit (Qiagen). Nucleic acid amplification tests for detection of filoviruses and arenaviruses were performed with the use of commercially available kits and published primers and probes5-11 (Table S1 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 from both ends with the use of conventional Sanger techniques. The sequence of the contigs was verified by visual inspection of the electropherograms.

**VIRAL ISOLATION**

About 100 μl of all serum samples was used to inoculate Vero E6 cells maintained in 25-cm² 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 in the serum of mice challenged with EBOV or on the basis of 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. In brief, 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 a 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 (jModelTest\(^1^2\)) 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 Bayesian Markov Chain Monte Carlo method, as implemented in MrBayes 3.1.2 software,\(^1^3\) to infer the composition of one phylogenetic tree, using two runs of four chains with 1 million 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\(^1^4\) under the GTR+gamma model with 1000 bootstrap replications. A reconstruction of the EBOV phylogenetic tree with the use of molecular clock

### 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 No.</th>
<th>Age (yr)</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</th>
<th>GenBank Accession No.</th>
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</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>—</td>
<td>Yes</td>
<td>KJ660348</td>
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<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>
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<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>
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<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>
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<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>
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<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>
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<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>
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<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>
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<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>
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<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>—</td>
<td>Yes</td>
<td>KJ660346</td>
</tr>
</tbody>
</table>

* All sampling and recording of patients’ status were performed in 2014. ND denotes not determined.
models is provided in Figure S1 in the Supplementary Appendix.

**Epidemiologic Investigations**

We gathered data on possible transmission chains from hospital records and through interviews with patients in whom EBOV 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.\(^5\) In addition, we performed EBOV-specific real-time RT-PCR assays targeting the glycoprotein (GP) or nucleoprotein (NP) gene.\(^7,10\)

Samples from 15 of 20 patients tested positive on the conventional L gene PCR assay and the real-time assays (Table 1). EBOV was identified in the serum of one patient on electron microscopy (Fig. 2, inset) and was isolated in cell culture from 5 patients. None of the samples were positive for Lassa virus on Lassa virus–specific RT-PCR assays.\(^8,11\)

Sequencing of the fragments amplified by the L gene RT-PCR assays revealed EBOV sequences. The partial L gene sequences were identical for all confirmed cases, except for a synonymous T-to-C polymorphism at position 13560, which was found in Patients C12 and C14.

**Sequencing of Samples from Patients**

The EBOV in samples obtained from three patients was completely sequenced with the use of conventional Sanger techniques (GenBank accession numbers, KJ660346, KJ660347, and KJ660348; the sequences in the preliminary report have been updated). The three sequences, each 18,959 nucleotides in length, were identical, with the exception of a few polymorphisms at positions 2124 (G→A, NP552 glycine→glutamic acid), 2185 (A→G, synonymous), 6909 (A→T, sGP291 arginine→tryptophan), 9923 (T→C, synonymous), 13856 (A→G, L759 aspartic acid→glycine), and 15660 (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).

**Clinical and Epidemiologic Analysis**

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% (12 of 14 patients with a known outcome died). Confirmed cases originated from hospitals in Guékédou, Macenta, Nzérékoré, and Kissidougou prefectures (Fig. 1). We performed an epidemiologic look-back investigation of the transmission chains by reviewing hospital documentation and interviews with affected families, patients with suspected disease, and inhabitants of villages in which cases occurred.

According to the initial epidemiologic investigation, the suspected first case of the outbreak was a 2-year-old child who died in Meliandou in Guékédou prefecture on December 6, 2013 (Fig. 2). A second investigation confirmed the origin of the outbreak in Meliandou but revealed a somewhat different timing of the early events (including the death of Patient S1 at the end of December and the deaths of Patients S2, S3, and S4 in January). Patient S14, a health care worker from Guékédou with suspected disease, seems to have triggered the spread of the virus to Macenta, Nzérékoré, and Kissidougou in February 2014. As the virus spread, 13 of the confirmed cases could be linked to four clusters: the Baladou district of Guékédou, the Farako district of Guékédou, Macenta, and Kissidougou. Eventually, all clusters were linked with several deaths in the villages of Meliandou and Dawa between December 2013 and March 2014.

**Initial Phase of the Outbreak**

This report is focused on the initial phase and geographic origin of the EBOV outbreak. Before the end of March 2014 (week 13), a total of 111 clinically suspected cases with 79 deaths (71% case fatality rate on the basis of clinical suspicion) had been recorded in the prefectures of Guékédou, Macenta, and Kissidougou. According to the timeline of the transmission chains (Fig. 2), the outbreak of confirmed disease start-
Méliandou Village, Guéckédou
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
(S3) Mother of S1 and S2
Bleeding
Died Dec. 13, 2013
(S4) Grandmother of S1 and S2
Fever, diarrhea, vomiting
Died Jan. 1, 2014
(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

Dandou Pombo Village, Guéckédou
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

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

Guéckédou Baladou District
First onset Feb. 23, 2014
14 Deaths from March 1 to March 31, 2014
(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
(S3) Mother of S1 and S2
Bleeding
Died Dec. 13, 2013
(S4) Grandmother of S1 and S2
Fever, diarrhea, vomiting
Died Jan. 1, 2014
(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

Guéckédou Farako District
First onset Feb. 24, 2014
4 Deaths from Feb. 28 to March 25, 2014
(S7) Sister of S4, attended funeral of S4
Fever, diarrhea, vomiting, hemorrhage
(S8) Attended funeral of S4
Fever, bleeding
Onset Jan. 25, 2014; died Jan. 30, 2014
(S9–S12)
Onset Feb. 2–16, 2014; died Feb. 11–March 5, 2014

Dawa Village, Guéckédou
8 Deaths from Jan. 26 to March 27, 2014
(S7) Sister of S4, attended funeral of S4
Fever, diarrhea, vomiting, hemorrhage
(S8) Attended funeral of S4
Fever, bleeding
Onset Jan. 25, 2014; died Jan. 30, 2014
(S9–S12)
Onset Feb. 2–16, 2014; died Feb. 11–March 5, 2014

Families

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

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
(C13) Contact with S15 and affected family members of S15
Onset March 2, 2014; died March 12, 2014
2 Further deaths in family

(C14) Contact with C12 in Macenta
Hospitalized in Macenta March 6, 2014
Died March 16, 2014, in Nzérékoré

(C12) Family member of S15
Hospitalized in Macenta
Died March 6, 2014, in Nzérékoré
ed 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; the median age was 35 years (interquartile range, 25 to 51).

**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.

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**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).
This introduction seems to have happened in December 2013. Further epidemiologic investigation is ongoing to identify 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 Guéckédou 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 later in the 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 a 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. However, the determination of both the timing of the introduction of the virus into Guinea and its phylogenetic origin also depend on our understanding of the evolutionary rate of EBOV in nature (Fig. S1 in the Supplementary Appendix). A reliable estimate for this rate, such as one derived from archaeological calibration, may be required to answer these questions.

Potential reservoirs of EBOV, fruit bats of the species *Hypsiprymnodon mosstonus*, *Epomops franqueti*, and *Myonycteris torquata*, are present in large parts of West Africa. Therefore, 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 Guéckédou, Macenta, and Kissidougou prefectures were recorded by the local public health authorities in collaboration with the World Health Organization and Médecins sans Frontières.
Office, Brazzaville, Republic of Congo (B.I.).

**REFERENCES**