Emergence of Zaire Ebola Virus Disease in Guinea — Preliminary Report


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 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 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; 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 probes (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 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 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 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 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 (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 Bayesian Markov Chain Monte Carlo method, as implemented in MrBayes 3.1.2 software, 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 under the

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<tr>
<th>Patient Number</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 Number</th>
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<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>
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<tr>
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<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>
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<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>
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<td>Survived</td>
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<td>March 16</td>
<td>No</td>
<td>ND</td>
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<td>Macenta</td>
<td>March 16</td>
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<td>March 19</td>
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<td>ND</td>
<td>No</td>
<td>ND</td>
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<td>30</td>
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<td>M</td>
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<td>March 12</td>
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<td>Died</td>
<td>March 12</td>
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<td>C14</td>
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<td>M</td>
<td>Macenta, Nzérékoré</td>
<td>March 13</td>
<td>Fever, diarrhea, vomiting, hemorrhage</td>
<td>Died</td>
<td>March 16</td>
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<td>ND</td>
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<tr>
<td>C15</td>
<td>28</td>
<td>F</td>
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<td>March 17</td>
<td>Fever, diarrhea, vomiting, hemorrhage</td>
<td>Survived</td>
<td>—</td>
<td>Yes</td>
<td>KJ660346</td>
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* All sampling and recording of patients’ status were performed in 2014. ND denotes not determined.
Meliandou 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
Onset Dec. 25, 2013; died Dec. 29, 2013
(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
(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

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

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

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

Guéckédou Farako District
First onset Feb. 24, 2014
4 Deaths from Feb. 28 to March 25, 2014
(S14) Family members of S14
Mother-in-law of S14
Died March 22, 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

Kissidougou
Contact with S15 and affected family members of S15
Onset March 3, 2014; died March 12, 2014
2 Further deaths in family

C13
C12
C14
C8
C9
C10
C11
C15
C1
C2
C5
C6
C7
C14
C12
C15
C13
C8
C9
C10
C11
C1
C2
C5
C6
C7
the real-time assays (Table 1). EBOV was identi-
positive in the conventional L gene PCR assay and
specific RT-PCR assays.
were positive for Lassa virus on Lassa virus–spe-
culture from 5 patients. None of the samples
fied in the serum of one patient on electron mi-
croscopy (Fig. 2, inset) and was isolated in cell
particles (arrowheads) are shown (scale bar, 100 nm).
GTR+gamma model with 1000 bootstrap repli-
cations.

EPIDEMIOLOGIC INVESTIGATIONS
We gathered data on possible transmission chains
from hospital records and through interviews
with patients in whom EBOV infection was sus-
pected and their contacts, affected families, in-
habitants 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 conven-
tional Filoviridae-specific RT-PCR assays targeting
a conserved region in the L gene to test sam-
pleS obtained from 20 hospitalized patients who
were suspected of being infected with a hemor-
rhagic fever virus.5,6,9 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 in the conventional L gene PCR assay and
the real-time assays (Table 1). EBOV was identi-
fied in the serum of one patient on electron mi-
croscopy (Fig. 2, inset) and was isolated in cell
culture from 5 patients. None of the samples
were positive for Lassa virus on Lassa virus–spe-
cific RT-PCR assays.8,11 Sequencing of the frag-
ments amplified by the L gene RT-PCR assays
revealed EBOV sequences. The partial L gene se-
quences 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 con-
ventional 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, synony-
mous), 2185 (A→G, NP552 glycine→glutamic acid),
2931 (A→G, synonymous), 4340 (C→T, synony-
mous), 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).

CLINICAL AND EPIDEMIOLOGIC ANALYSIS
The prominent clinical features of the EBOV in-
fec tion in the confirmed cases were fever, severe
diarrhea, and vomiting; hemorrhage was less fre-
quent. The case fatality rate in the initial cases
was 86% (12 of 14 patients with a known out-
come died). Confirmed cases originated from
hospitals in Guéckédou, Macenta, Nzérékoré, and
Kissidougou prefectures (Fig. 1). We performed
an epidemiologic look-back investigation of the
transmission chains by reviewing hospital docu-
ments and interviews with affected families, pa-
tients with suspected disease, and inhab-
habitants of villages in which cases occurred.
According to the current state of the epidemi-
ologic investigation, the suspected first case of the
outbreak was a 2-year-old child who died in Mel-
iandou in Guéckédou prefecture on December 6,
2013 (Fig. 2). Patient S14, a health care worker
from Guéckédou with suspected disease, seems
to have triggered the spread of the virus to Ma-
centa, Nzérékoré, and Kissidougou in February
2014. As the virus spread, 13 of the confirmed
cases could be linked to four clusters: the Balad-
dou district of Guéckédou, the Farako district of
Guéckédou, Macenta, and Kissidougou. Eventu-
ally, all clusters were linked with several deaths
in the villages of Meliandou and Dawa between
December 2013 and March 2014.

CURRENT STATUS OF THE ONGOING OUTBREAK
This report is focused on the initial phase and geo-
graphic 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 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; 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. This introduction seems to have happened in early December 2013 or even before. Further epidemiologic investigation is ongoing to iden-
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 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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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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REFERENCES