Ebola Virus Disease in the Democratic Republic of Congo


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BACKGROUND

The seventh reported outbreak of Ebola virus disease (EVD) in the equatorial African country of the Democratic Republic of Congo (DRC) began on July 26, 2014, as another large EVD epidemic continued to spread in West Africa. Simultaneous reports of EVD in equatorial and West Africa raised the question of whether the two outbreaks were linked.

METHODS

We obtained data from patients in the DRC, using the standard World Health Organization clinical-investigation form for viral hemorrhagic fevers. Patients were classified as having suspected, probable, or confirmed EVD or a non-EVD illness. Blood samples were obtained for polymerase-chain-reaction–based diagnosis, viral isolation, sequencing, and phylogenetic analysis.

RESULTS

The outbreak began in Inkanamongo village in the vicinity of Boende town in Équateur province and has been confined to that province. A total of 69 suspected, probable, or confirmed cases were reported between July 26 and October 7, 2014, including 8 cases among health care workers, with 49 deaths. As of October 7, there have been approximately six generations of cases of EVD since the outbreak began. The reported weekly case incidence peaked in the weeks of August 17 and 24 and has since fallen sharply. Genome sequencing revealed Ebola virus (EBOV, Zaire species) as the cause of this outbreak. A coding-complete genome sequence of EBOV that was isolated during this outbreak showed 99.2% identity with the most closely related variant from the 1995 outbreak in Kikwit in the DRC and 96.8% identity to EBOV variants that are currently circulating in West Africa.

CONCLUSIONS

The current EVD outbreak in the DRC has clinical and epidemiologic characteristics that are similar to those of previous EVD outbreaks in equatorial Africa. The causal agent is a local EBOV variant, and this outbreak has a zoonotic origin different from that in the 2014 epidemic in West Africa. (Funded by the Centre International de Recherches Médicales de Franceville and others.)
O

N AUGUST 24, 2014, WHEN THE EYES OF
the world were on the spreading West
African outbreak of Ebola virus disease
(EVD), the World Health Organization
(WHO) was notified of another EVD outbreak in the vicinity
of Boende town, Equateur province, in western
Democratic Republic of Congo (DRC). Boende
town lies 700 km northeast of the capital city,
Kinshasa, and 300 km east of Mbandaka, the
capital of Équateur province (Fig. 1). The affected
area is situated in humid tropical forest and de-
limited by two large rivers, which are the main
channels for moving people and goods to and
from Mbandaka. The roads in the area of Boende
town are in poor condition, as are the bridges
that cross a multitude of smaller rivers. There are
few commercial flights in and out of Mbandaka
(two to three each week) and none out of Boende.

This is the seventh Ebola outbreak in the DRC
since the first was reported in 1976. The index
patient was a pregnant woman living in Inkana-
mongolo village (Watsi Kengo Health Area) who
butchered a monkey (of an unknown arboreal
species) that had been found dead by her husband.
She became ill on July 26 and died on August 11.
A local doctor and three health workers who
undertook a postmortem cesarean section (to
separate the fetus from the mother before burial,
according to the local culture) also became in-
fected and died. These health workers were evi-
dently the source of further cases in this outbreak.

We report the cases and deaths that have
been identified as of October 7, on the geo-
graphic distribution and rate of spread of infec-
tion, and on the identity of the agent causing
this EVD outbreak, a novel Ebola virus (EBOV,
Zaire species) variant, in comparison with vari-
ants isolated from West Africa.

METHODS

CASE DEFINITIONS

Epidemiologic and clinical data were recorded on
the standard WHO clinical-investigation form
for viral hemorrhagic fevers2 with the use of
standard case definitions, which are provided in
the Supplementary Appendix (available with the
full text of this article at NEJM.org; also see vid-
eo, available at NEJM.org). A suspected case (ei-
ther before or after death) was defined as a sud-
den onset of high fever in a patient who had
contact with a person with suspected, probable,
or confirmed EVD, with a dead or sick animal, or
with any person with a sudden onset of high fever
and at least three of the following symptoms or
clinical signs: headache, vomiting, anorexia, diar-
hea, lethargy, stomach pain, aching muscles or
joints, difficulty swallowing or breathing, or hic-
cup; unexplained bleeding; or any sudden, unex-
plained death. A probable case was defined as
any suspected case evaluated by a clinician or any
person who died from suspected EVD and had an
epidemiologic link to a confirmed case but was
not tested and did not have laboratory confirma-
tion of the disease. A probable or suspected case
was classified as confirmed when a sample from
the affected person tested positive for EBOV in
the laboratory. In this study, the final classifica-
tion of patients remained as suspected or proba-
ble when a definitive diagnosis could not be made
(usually because no blood sample was obtained).
We attempted to identify the source of infection
in each patient with EVD by tracing contacts,
mainly retrospectively.

BLOOD SAMPLES

The first samples were collected from eight
symptomatic patients with suspected EVD who
visited the Lokolia health center in Équateur prov-
ince. As in previous EVD outbreaks, blood sam-
ples were collected, with oral consent, either at
the homes of patients or in hospital isolation
wards, by a team that included staff members of
the Ministry of Health in the DRC and the WHO.3
Samples were placed in dry tubes and immedi-
ately transported to Institut National de Recherche
Biomédicale (INRB), Kinshasa, DRC, for labora-
tory testing and storage. Blood samples were also
sent to the WHO reference center for the diagno-
sis of viral hemorrhagic fever, the Centre Interna-
tional de Recherches Médicales de Franceville
(CIRMF), Gabon, for confirmation. At all stages,
essential biosecurity measures were taken to avoid
contamination of personnel and the environment.

DIAGNOSTIC ASSAYS

Total RNA was extracted from 100 μl of serum
with the use of BioRobot EZ1 and the EZ1 Virus
Mini Kit, version 2.0 (both from Qiagen), accord-
ing to the manufacturer’s instructions. RNA was
first converted to complementary DNA (cDNA)
with the use of a commercial kit (High Capacity
cDNA Reverse Transcription Kit, Applied Biosys-
tems), and specific real-time reverse-transcriptase–
polymerase-chain-reaction (RT-PCR) assays tar-
geting the nucleoprotein gene of EBOV4 were
performed on the 7500 Fast Real-Time PCR System (Applied Biosystems). The amplification cycle involved 2 minutes at 55°C and an initial denaturation at 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds and at 58°C for 1 minute.

For samples with positive results on RT-PCR, a PCR assay targeting a fragment of the filovirus polymerase (L) gene was performed with the use of the SuperScript III One-Step RT-PCR Kit (Invitrogen). The reaction was carried out in a final volume of 25 μl, containing 12.5 μl of 2X buffer, 1.5 μl of each primer (10 μM) (Filo A and Filo B), bovine serum albumin (1 μg per microliter), 1 μl of SuperScript III Platinum Taq Mix, RNase-free water, and 5 μl of RNA. Amplification involved a step of reverse transcription for 30 minutes at 45°C, which was followed by denaturation at 95°C for 2 minutes and then by 45 cycles of 15 seconds at 94°C, 30 seconds at 55°C, and 45 seconds at 68°C, with a final elongation step of 5 minutes at 68°C.

**VIRAL SEQUENCING**

The PCR fragment of 346 bp was purified by ultrafiltration before sequencing (Millipore). Sequencing was performed with the use of the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and purified by means of ethanol precipitation. Sequence chromatograms from both strands were obtained on an automated sequence analyzer (ABI 3730XL, Applied Biosystems) with the PCR primers. For coding-complete genomic characterization, viral RNA was directly extracted from serum samples with the EZ1 Viral RNA Kit (Qiagen), according to the manufacturer’s instructions. Extracted total RNA was retrotranscribed into cDNA with Phi29 polymerase, as described previously. Amplified DNA was quantified with the use of the Quant-iT DNA Assay Kit (Life Technologies), and a di-
solution was performed in order to obtain 0.2 ng of amplified DNA per microliter in the final solution. The library was performed with the use of the Nextera XT DNA Sample Preparation Kit (Illumina), according to the manufacturer’s instructions.

DNA was initially sequenced with the MiSeq sequencer (Illumina), following by a second run with the Illumina NextSeq sequencer. A total of $5.6 \times 10^6$ reads were obtained for each sample. All reads were filtered according to quality, and those corresponding to human genomes were removed with mapping software with the use of the *Homo sapiens* hg19 sequence as reference. The viral reads corresponding to the EBOV genome were selected by means of a similarity approach with the use of the BLASTN search tool, referring to the complete sequence of a 1976 Yambuku isolate of EBOV available in GenBank (accession number, KC242801). For each selected read, only the region that matched the viral genome was considered. All reads were assembled with SPAdes (St. Petersburg genome assembler) software in order to obtain the coding-complete viral genome.

**PHylogenetic Analysis**

We aligned 29 complete genome sequences of EBOV that are available in GenBank with the new DRC (abbreviated COD) sequence of EBOV (accession number, KM519951), using the ClustalW algorithm and MEGA 5 software package. We used the Bayesian Markov chain Monte Carlo method in MrBayes, version 3.2, software to infer the phylogenetic trees, using two runs of four chains with 1 million generations, with a burn-in rate of 25% and the GTR+G+I nucleotide substitution model.

**Viral Isolation**

Tissue cultures were performed in a biosafety level 4 laboratory. Monolayers of Vero cells in six-well plates were incubated for 1 hour at 37°C with 50 μl of serum from each sample at 1:10 dilution in Dulbecco’s modified Eagle’s medium. Medium containing 2.5% fetal-calf serum and antibiotic mix was added (5 ml), and cells were incubated at 37°C in 5% carbon dioxide for 7 days. Supernatant was harvested and stored at −80°C until it was used for genomic characterization. Viral growth in the cell lines was confirmed with the use of a specific real-time RT-PCR assay, as described above.
It is possible, by plotting the daily case incidence, to discern several generations of cases, as indicated by six peaks in incidence, including the initial index case (Fig. 2C). These covered approximately five serial intervals (average, 16.1 days), generating a total of 69 cases in 70 days (Fig 2D).

The rise in case incidence during August was apparently driven by multiple infections acquired from the index case. Of the 29 patients in whom EVD was diagnosed during first 24 days of the outbreak, 21 were reported to be direct contacts of the index case (i.e., they had physical contact or contact with bodily fluids). If all these secondary cases did indeed acquire infection from a single source, this represents a basic case reproduction number ($R_0$) of 21 for this outbreak.

The number of secondary cases arising from each primary case during this outbreak was highly variable. Among other patients with EVD who infected named contacts, 1 patient generated 3 secondary cases, 2 patients generated a further 2 cases each, 30 patients generated a single extra case, and 11 patients generated no further cases. Counting all secondary cases arising among named contacts, including the index case, the average case reproduction number ($R$) for the whole outbreak was 1.29 (95% confidence interval [CI], −4.71 to 7.29). However, after the exclusion of the 21 cases generated by the index case, the average case reproduction number during the outbreak was 0.84 (95% CI, −0.38 to 2.06), which is below the threshold value for persistent transmission ($R > 1$). This explains the

Figure 2. Suspected, Probable, and Confirmed Cases of Ebola Virus Disease (EVD) in the Democratic Republic of Congo.

Shown is the distribution of suspected, probable, and confirmed cases of EVD according to age (Panel A) and weekly incidence (Panel B) in Équateur province. Also shown are the numbers of EVD cases reported daily since the onset of symptoms in the index patient on July 26 (Panel C) and the cumulative number of cases (Panel D). The case incidence shown in Panel C is presented as a 3-day running mean to highlight six peaks in incidence (as numbered), including the index case.
observed decline in the EVD case incidence after mid-August. The last reported patient with EVD became ill on October 4, and no further cases were reported as of October 7.

A total of 1121 contacts of the patients were registered for follow-up. By October 7, a total of 830 had been followed for at least 21 days, which is considered to be the maximum incubation period. The index patient and her contacts had no history of travel to the EVD-affected countries in West Africa (Guinea, Liberia, Nigeria, Senegal, and Sierra Leone) and no history of contact with residents of the affected areas.

**Identification and Characterization of the Virus**

To identify the causative agent of the Boende EVD outbreak, investigators at Institut National de Recherche Biomédicale in Kinshasa and at Centre International de Recherches Médicales de Franceville in Gabon analyzed eight samples from patients who were suspected of having EVD. On the basis of both a conventional filoviridae-specific RT-PCR assay targeting a conserved region in the L gene and a real-time RT-PCR assay targeting the nucleoprotein gene in EBOV, six of the eight samples tested positive for EBOV. Sequencing of the 346-bp fragment amplified from the L gene in four of the samples revealed the viral sequences. These four sequences were identical and closely related to the EBOV variant that caused an EVD outbreak around Kikwit, Zaire (now part of the DRC), in 1995, indicating that the current outbreak in the DRC was unrelated to the ongoing outbreak in West Africa (Fig. 3). We obtained a coding-complete sequence of the virus from the sample having the highest RNA load and obtained a sequence of 18,953 nucleotides in length, which was called EBOV/H.sap/COD/14/Boe-Lok according to the recently estab-

<table>
<thead>
<tr>
<th>Sign or Symptom</th>
<th>Non-EVD Cases (N = 60)</th>
<th>Probable EVD Cases (N = 28)</th>
<th>Odds Ratio of Probable EVD (95% CI)</th>
<th>Confirmed EVD Cases (N = 38)</th>
<th>Odds Ratio of Confirmed EVD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. of patients (%)</td>
<td>no. of patients (%)</td>
<td>no. of patients (%)</td>
<td>no. of patients (%)</td>
<td>no. of patients (%)</td>
</tr>
<tr>
<td>Fever</td>
<td>41 (68)</td>
<td>28 (100)</td>
<td>NA</td>
<td>35 (92)</td>
<td>5.4 (1.7 to 9.1)</td>
</tr>
<tr>
<td>Headache</td>
<td>7 (12)</td>
<td>7 (25)</td>
<td>2.5 (-0.7 to 5.7)</td>
<td>17 (45)</td>
<td>6.1 (3.4 to 8.9)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>22 (37)</td>
<td>23 (82)</td>
<td>7.9 (4.7 to 11.1)</td>
<td>26 (68)</td>
<td>3.7 (1.4 to 8.9)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>12 (20)</td>
<td>7 (25)</td>
<td>1.3 (-1.9 to -4.5)</td>
<td>18 (47)</td>
<td>3.6 (1.1 to 6.1)</td>
</tr>
<tr>
<td>Vomiting or nausea</td>
<td>18 (30)</td>
<td>22 (79)</td>
<td>8.6 (5.4 to 11.8)</td>
<td>26 (68)</td>
<td>5.1 (2.6 to 7.5)</td>
</tr>
<tr>
<td>Intense fatigue or general weakness</td>
<td>11 (18)</td>
<td>11 (39)</td>
<td>2.9 (-0.3 to 6.1)</td>
<td>27 (71)</td>
<td>10.9 (8.3 to 13.5)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>9 (15)</td>
<td>11 (39)</td>
<td>3.7 (0.5 to 6.9)</td>
<td>15 (39)</td>
<td>3.7 (1.1 to 6.3)</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>3 (5)</td>
<td>7 (25)</td>
<td>6.3 (3.1 to 9.5)</td>
<td>17 (45)</td>
<td>15.4 (11.6 to 19.1)</td>
</tr>
<tr>
<td>Difficulty swallowing</td>
<td>3 (5)</td>
<td>6 (21)</td>
<td>5.2 (2.0 to 8.4)</td>
<td>10 (26)</td>
<td>6.8 (2.9 to 10.7)</td>
</tr>
<tr>
<td>Difficulty breathing</td>
<td>5 (8)</td>
<td>6 (21)</td>
<td>3.0 (-0.2 to 6.2)</td>
<td>6 (16)</td>
<td>2.1 (-1.5 to 5.6)</td>
</tr>
<tr>
<td>Cough</td>
<td>5 (8)</td>
<td>5 (18)</td>
<td>2.4 (-0.8 to 5.6)</td>
<td>2 (5)</td>
<td>0.6 (-4.8 to 6.0)</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (2)</td>
<td>2 (7)</td>
<td>4.5 (1.3 to 7.7)</td>
<td>3 (8)</td>
<td>5.1 (-4.9 to 15.0)</td>
</tr>
<tr>
<td>Bleeding from injection site</td>
<td>0</td>
<td>2 (7)</td>
<td>NA</td>
<td>3 (8)</td>
<td>NA</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>1 (2)</td>
<td>3 (11)</td>
<td>7.1 (3.9 to 10.3)</td>
<td>3 (8)</td>
<td>5.1 (-4.9 to 15.0)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>1 (2)</td>
<td>4 (14)</td>
<td>9.8 (6.6 to 13.0)</td>
<td>6 (16)</td>
<td>11.0 (2.4 to 19.7)</td>
</tr>
<tr>
<td>Bloody or black stools</td>
<td>4 (7)</td>
<td>12 (43)</td>
<td>10.5 (7.3 to 13.7)</td>
<td>8 (21)</td>
<td>3.7 (0.1 to 7.3)</td>
</tr>
<tr>
<td>Vomiting blood</td>
<td>3 (5)</td>
<td>10 (36)</td>
<td>10.6 (7.4 to 13.8)</td>
<td>6 (16)</td>
<td>3.6 (-0.7 to 7.8)</td>
</tr>
<tr>
<td>Bleeding from nose</td>
<td>0</td>
<td>4 (14)</td>
<td>NA</td>
<td>4 (11)</td>
<td>NA</td>
</tr>
<tr>
<td>Bleeding from vagina</td>
<td>0</td>
<td>2 (7)</td>
<td>NA</td>
<td>4 (11)</td>
<td>NA</td>
</tr>
</tbody>
</table>

* EVD denotes Ebola virus disease, and NA not applicable.
† Odds ratios were calculated for patients with probable or confirmed EVD as compared with those who had negative test results for EVD. CI denotes confidence interval.
lished filovirus variant nomenclature (GenBank accession number, KM519951; see also GenBank numbers KM517570.1 and KM517571.1, which were obtained from initial RT-PCR analysis). The sequence showed 99.2% identity (0.8% difference, 145 mutations) with the most closely related variant that was isolated during the 1995 Kikwit outbreak (EBOV/H.sap/COD/95/Kikwit-13709). Among the 145 mutations, 22 were nonsynonymous, but none were expected to induce any important change in viral protein sequences. In particular, we observed 5 nonsynonymous mutations in the NP gene, 1 in the VP35 gene, 1 in the VP40 gene, 8 in the GP gene, none in the VP30 gene, 2 in the VP24 gene, and 5 in the L gene.

We then performed high-throughput sequenc-
ing on two additional positive samples after random amplification of extracted total RNA from serum samples. Given the moderate viral load in the serum, we could not obtain the complete sequences but only 2270 bp from one sample and 12,501 bp from the other sample. Analysis of these partial sequences showed an identity of 100% with the complete sequence obtained previously, further suggesting that the current outbreak in the DRC is due to a single introduction of this novel EBOV variant into the human population.

In contrast to the similarity between the Boende variant and other equatorial African variants (especially EBOV/H.sap/COD/95/Kikwit-13709), the gene sequence of the Lokolia isolate showed only 96.8% identity (3.2% difference) with the West African variants, with 601 mutations as compared with a Guinean variant (KJ660347) and 602 mutations as compared with a Sierra Leonean variant (KM233116).

**DISCUSSION**

EVD first emerged in human populations in 1976, causing nearly simultaneous, but unrelated, outbreaks in Zaire (now DRC) and Sudan (now South Sudan). The EBOV strain that is causing the present outbreak in the DRC is most closely related to another EBOV variant, which was isolated from a patient in Kikwit (then in Zaire) in 1995. On the basis of the genetic characterization of the virus, together with the geographic location of the outbreak, it is clear that the current outbreak in the DRC is an independent event that has no epidemiologic or virologic connection with the continuing epidemic in West Africa.9

The rise and fall of EVD cases in the Boende area between July and October 2014 suggests that the present EVD outbreak in the DRC will probably be typical of other EVD outbreaks in equatorial Africa — that is, characterized by a comparatively low incidence of spillover (probably originating in a local animal reservoir) and chains of human-to-human transmission that are brought under control within 2 to 3 months. Nearly one third of the cases (21 of 69) in this outbreak appear to have arisen from direct contact with the index case. In subsequent chains of transmission, each patient generated relatively few secondary cases, and the average case reproduction number ($R_s=0.84$) was below the threshold for persistent transmission (i.e., $R_s<1$).

There are at least five possible reasons why previous EVD outbreaks in equatorial Africa, and the present outbreak in the DRC, have been smaller than the current epidemic in West Africa. The first is that cultural practices associated with EVD differ between equatorial Africa and West Africa, so behaviors and customs in equatorial Africa carry a lower risk of infection among potential contacts. However, customs in the two regions of Africa appear to have much in common — in the way relatives and friends attend the sick and in funeral rites and burial practices, all of which involve bodily contact. In the two regions, there are examples of the use of nonmedical diagnostics and therapies (by herbalists, diviners, healers, and pastors), inadequate availability of disinfectants and protective equipment, and lack of knowledge of microbiologic hygiene by health care workers, along with local resistance to public health measures proposed by national authorities.

The second possible reason why EVD outbreaks have been smaller in equatorial Africa is that infection and illness caused by EBOV variants that are characteristic of equatorial Africa, including the variant that emerged in the Boende district, take a different clinical course from those in West Africa, with different epidemiologic consequences. We have found that equatorial and West African variants of EBOV are genetically distinct, as did Baize et al.9 In addition, the genetic diversity among equatorial African isolates appears to be low, as compared with the extensive variation, including many nonsynonymous mutations, that has been identified among West African EBOV isolates, even though the equatorial African variants have been collected over a period of nearly four decades.10 Against this background, there is currently no evidence that variants from the two regions are associated with such factors as differences in the infectious period, case fatality rate, or frequency of hemorrhagic disease.

The third possible explanation for the smaller outbreaks in equatorial Africa, such as the one in the Boende area, is that they have largely occurred in remote forested areas, where the number of human contacts is limited in small populations living at low density, with infrequent or slow connections by road, river, or air. This is in contrast to Guinea, Liberia, and Sierra Leone, where villages, towns, and capital cities are connected by an extensive network of footpaths, dirt roads, and paved highways. The West African
road network is international: in the areas where Guinea, Liberia, and Sierra Leone have common borders, single ethnic groups inhabit more than one country, and cross-border travel is frequent, for purposes of commerce and to maintain family ties. Unlike the outbreak in the Boende area, the West African epidemic has been multinational almost from the start, with the transportation of both patients with EVD and bodies of deceased patients across borders, complicating the organization of public health measures.

The fourth possible explanation for the smaller outbreaks in equatorial Africa is that the response to Ebola outbreaks has been faster and more effective in the DRC and neighboring countries. With the experience of six previous EVD epidemics, the DRC is now well prepared. The time that it takes to respond to news of an outbreak has been shortened over the years. There is also substantial national expertise in managing EVD outbreaks, including skills in epidemiology, laboratory analysis, and patient care, with readily available international support. During the present outbreak, action was quickly taken to inform the affected communities and to assign responsibilities for control measures to village chiefs, religious and social leaders, traditional healers, and medical staff members. These actions were coupled with the introduction of basic infection-control measures — for example, on the basis of the slogan “no family without detergent,” hand sanitizers were distributed to all affected communities to help stop transmission.

Finally, EVD may have spread more widely in West Africa because the human population is less resistant to infection than in equatorial Africa, either because there has been no previous exposure to EBOV or for some other reason. This possibility has not yet been investigated because, for example, we currently have little information about the risk that EVD will develop in a person after exposure to EBOV.

Although none of these possibilities can be ruled out, the third and fourth possibilities appear to be the most plausible on the basis of the current evidence. The coming days and weeks will reveal whether the new EBOV variant that was isolated in the Boende area is on the point of being eliminated from the human population, as in previous EVD outbreaks in the DRC. Regardless of the clinical and epidemiologic characteristics of this outbreak that are associated with the virus or the animal and human host populations, a continuing, comprehensive public health response will be critical for success.

The views expressed in this article are those of the authors and do not necessarily reflect the views of the national governments or the WHO.

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**REFERENCES**


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