Human Infection Due to Ebola Virus, Subtype Côte d'Ivoire: Clinical and Biologic Presentation

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In November 1994 after 15 years of epidemiologic silence, Ebola virus reemerged in Africa and, for the first time, in West Africa. In Côte d'Ivoire, a 34-year-old female ethologist was infected while conducting a necropsy on a wild chimpanzee. Eight days later, the patient developed a syndrome that did not respond to antimalarial drugs and was characterized by high fever, headache, chills, myalgia, and cough. The patient had abdominal pain, diarrhea, vomiting, and a macular rash, and was repatriated to Switzerland. The patient suffered from prostration and weight loss but recovered without sequelae. Laboratory findings included aspartate aminotransferase and alanine aminotransferase activity highly elevated, thrombocytopenia, lymphopenia, and, subsequently, neutrophilia. A new subtype of Ebola was isolated from the patient's blood on days 4 and 8. No serologic conversion was detected among contact persons in Côte d'Ivoire ($n = 22$) or Switzerland ($n = 52$), suggesting that infection-control precautions were satisfactory.

In 1976, two outbreaks of hemorrhagic fever in the Democratic Republic of the Congo (Yambuku area) and Sudan [1, 2] were associated with a new etiologic agent, the Ebola (EBO) virus [3]. More than 600 cases were recorded, but only 15 documented observations were made, and few biologic investigations were done [4, 5]. Clinical data from other cases were obtained by retrospective studies [6, 7]. The only human case rigorously studied was a laboratory infection in Great Britain [8].

During infectious outbreaks of the Reston subtype of EBO (EBO-R) among monkeys imported from the Philippines in 1989, 1990 [9], and 1996, some animal handlers were found to be EBO seropositive, but all were asymptomatic. EBO-R appears to be less pathogenic than the African EBO subtypes for humans.

Although anti-EBO antibodies have been found repeatedly in African populations [10], there was no confirmed case in Africa for 15 years. In November 1994 in Côte d'Ivoire, a new subtype of EBO was isolated from a febrile patient [11]. We report here the clinical and pathologic findings and the management of this case.

Case Report

In the Taï National Park, Côte d'Ivoire, the behavior of a community of free-living chimpanzees has been studied since 1979 [12]. In early November 1994, several decomposed corpses of chimpanzees were found. On 16 November 1994,

3 research workers dissected the body of a chimpanzee that had died <12 h earlier: They found signs of hemorrhage and non-clotting blood. Eight days later, 1 of the researchers, a 34-year-old woman, became ill.

Clinical Course

On 24 November 1994 (day 1), around 6 PM, the patient started shivering with fever (figure 1). She took a curative dose of halofantrine for suspected malaria. On day 3, as there was no notable improvement, the patient was transported by car to Abidjan (600 km) and admitted to a clinic. Despite persistent chills, headache, and myalgia, her general condition was satisfactory. Physical examination of the abdomen, heart, lung, throat, and tongue was normal.

On day 4, she was treated with intravenous quinine (1.6 g daily) for suspected malaria. Her temperature remained around 40°C, and quinine was discontinued due to progressive deafness.

On day 5, the patient developed diarrhea (seven stools/day) without blood traces, and then nausea, vomiting, and anorexia. A non-itching rash developed on her left shoulder, spread to her back, and finally became generalized. She also suffered from central nervous system disorders, such as temporary loss of memory, anxiety, confusion, and irritability. Urinary output stopped from day 5 to day 7. Repeated blood examinations did not reveal parasites, and blood culture results remained negative. She was rehydrated with lactated Ringer's solution, and antibiotics were given (initially pefloxacin by mouth, then amoxicillin-clavulanate intravenously). A chest radiograph was normal, and a diagnosis of gram-negative sepsis was evoked.

On day 6, there was no improvement in the patient's condition; therefore, she was repatriated to Switzerland on day 7 in

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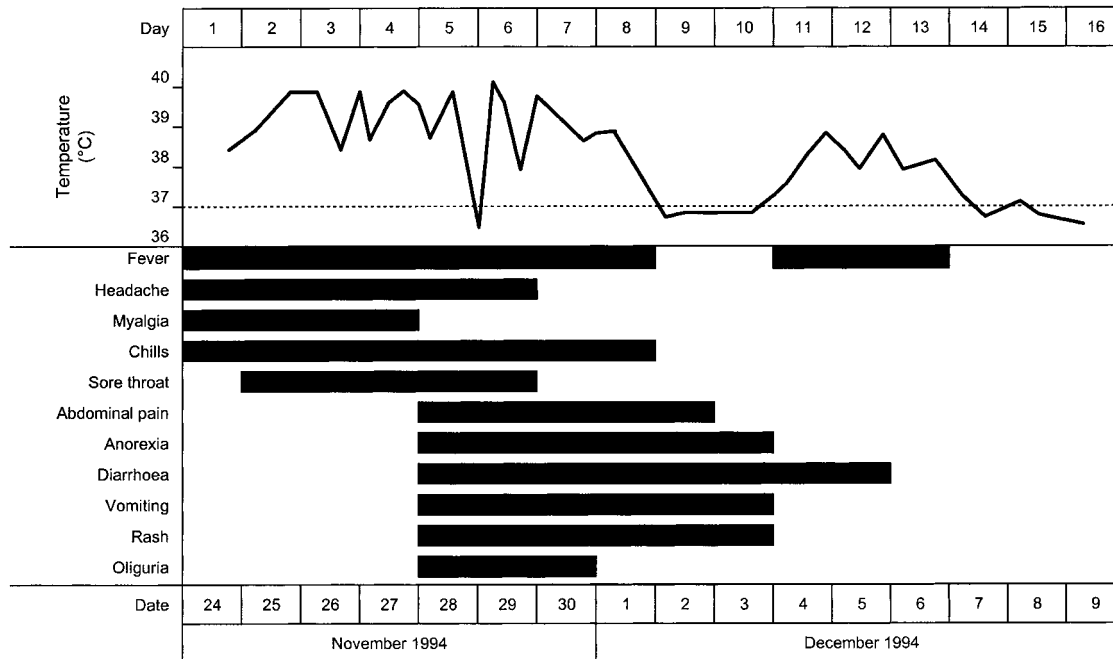
Figure 1. Clinical course of EBO-CI disease in patient presumably infected during necropsy of infected chimpanzee.

a Swiss Air Ambulance jet. During the flight, the patient wore a mask, and the physician and nurse wore surgical masks, gloves, and gowns. Due to the geographic closeness of Taï National Park to Liberia, it was considered that the patient might have Lassa fever.

On day 8, the patient was admitted to the University Hospital of Basel. She was transferred to a single, double-door isolation room with negative pressure. All health care workers wore high-quality gloves, gowns, and dust-and-mist masks (3M Pharmaceuticals, St. Paul, MN). Because her illness started a week after the necropsy of a nonhuman primate, infection-control practice did comply with guidelines of the Centers for Disease Control and Prevention [13]. On admission, she was tired but awake. Physical examination revealed tender spleen and liver on palpation. An ultrasound scan of the abdomen was normal. The differential diagnosis included a viral or bacterial infection of unknown origin, dengue fever, rickettsial disease, hantavirus infection, leptospirosis, typhoid fever, and malaria. A form of hemorrhagic fever (Lassa fever or EBO virus) was considered as unlikely. The patient was treated intravenously with ciprofloxacin and doxycycline for suspected gram-negative sepsis, leptospirosis, or rickettsial disease.

On day 9, the patient became afebrile for 2 days. Therefore, clothes were disposed of as usual, using the double-bag technique, and used dishes were returned to the kitchen. On day 11, strict barrier isolation was reduced to body substance isolation practice. The same day, fever recurred. Diarrhea changed to constipation, and the patient started to eat normally.

On day 15, she was discharged from the hospital, having lost 6 kg (10% of her initial weight). The patient fully recovered after 6 weeks. A month after onset, her hair became dry, lost its elasticity, and began falling out in large quantities. Hair loss lasted ~3 months.

Laboratory Investigation (Tables 1, 2)

Persistent thrombocytopenia and an early lymphopenia followed by neutrophilic hyperleukocytosis were observed. The coagulation profile and platelet counts evoked an intravascular coagulation. The most notable biochemical finding was highly elevated aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) activity. γ-glutamyl transferase was normal to slightly elevated, and alkaline phosphatase was normal to highly elevated. Creatinine kinase was slightly above normal on day 8 but normal thereafter. Lactate dehydrogenase was 20 times normal on day 7, and amylase levels increased from day 11 to 14. As of day 15, all values progressively stabilized.

Results for tests for parasites in blood and stool, blood cultures, and cultures of urine were negative. Urine samples were examined on days 5, 10, and 12: Hematuria was observed on day 5 and proteinuria on day 10. Electrolyte levels (sodium, potassium, calcium, phosphate) and lipid levels (triglycerides and cholesterol), which were investigated from day 7 to 14, were normal.

While the patient was in the Swiss hospital, serologic tests for dengue fever, hantavirus, hepatitis B, C, and E, leptospirosis, *Rickettsia mooseri, Rickettsia conorii,* and brucellosis were negative. She was IgG positive but IgM negative for Epstein-Barr and cytomegalovirus. Due to her lack of bleeding,

Table 1. Hematologic values on various days after onset of illness for a female ethologist who became infected with EBO-CI, presumably while doing a necropsy on an infected chimpanzee.

	November 1994			December 1994							January 1995	Reference standard	Unit
	26	28	30	2	3	4	5	6	8	17	5		
Red blood cell count	4.45	4.11	4.78	4.45	4.13	4.93	4.39	3.99	3.9	4.11	4.49	4.2–5.4	10^{12}/L
Hemoglobin	13.9	12.2	14.9	13.4	11.8	15.1	13.1	11.9	11.3	11.8	13.2	12–16	g/dL
Hematocrit	41.4	40.1	42.4	39.1	36.9	43.0	37.4	35.0	36.1			36–46	%
MCV	92.5	97.5	88.6	87.8	89.3	87.3	85.1	87.7	92.5			79–95	μ^3
MCH	31.0	29.8	31.2	30.2	28.6	30.6	29.8	29.8	29.0			27–31	pg
MCHC	33.5	30.5	35.2	34.4	32.0	35.1	35.1	34.0	31.3			32–36	%
Platelet count	150	83	114	117	105	126	141	141	227			150–450	$\times10^9$/L
White blood cell count	4.1	3.6	6.6	7.83	11.54	14.76	12.8	10.76	11.89	7.3	4.7	3.5–10	$\times10^9$/L
Neutrophils	79	87	82	80	78	77	63	49		55	65	40–74	%
Neutrophil band*			52	41	27.5			13				5–15	%
Lymphocytes	15	12	9	10	8	9	19	28		31	27	19–48	%
Eosinophils	0	0	1	1	1	5	4	4		1	2	0–7	%
Monocytes	6	1	4	5	10	5	5	14		8	4	3–9	%
Basophils	0	0	1	1	0	1	2	2		4	1	0–2	%
CRP		42	127					15				<10	mg/L
aPTT					30	24	26	26				25–34	/s
Thrombin time					19	15	13	15				13–18	/s
FDP					>40	>40						<10	μg/mL
Fibrinogen					0.8	1.4	2.3	2.6				1.7–4.0	g/L
Factor II					55							70–120	%
Factor V					87	>100	>100	>100				70–120	%
Factor VII/X					40							70–120	%

NOTE. Day 1 of illness = 24 November 1994. MCV = mean cell volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration, CRP = C-reactive protein, aPTT = activated partial thromboplastin time, FDP = fibrin degradation product.

* Toxic granulations were seen on days 7–9 and 12.

tests for hemorrhagic fever (Lassa fever or EBO) had not been required.

Virologic Investigation

An epidemiologic investigation was done during the first days of December 1994 to determine the cause of deaths among the community of chimpanzees being studied by the ethologist who became ill (see [14], in this supplement). The survey indicated a highly lethal epidemic with hemorrhagic syndrome. Etiology was unknown, and differential diagnosis included a viral infection of unknown origin, anthrax, dengue, and African hemorrhagic fevers. A serologic survey was done among the research workers, and 8 samples were collected. Thinking that the case-patient could have been contaminated during the necropsy, we asked the Ivoirian clinic for a serum sample from the patient. Nine samples, including 1 from the case-patient, were sent to Institut Pasteur on 14 December. We asked for differential diagnostics, notably, anthrax, dengue, Crimean-Congo hemorrhagic fever (CCHF), Rift Valley fever, yellow fever, chikungunya, Lassa fever, EBO virus, Marburg (MBG) virus, and hantaviruses. Virus isolation was attempted on a patient serum sample obtained during the febrile phase of the illness (26 November, day 3 of illness).

A new EBO virus (subtype Côte d'Ivoire; EBO-CI) was isolated [11]. Antibody and antigen titers in serum samples obtained 3, 7, 22, 41, 81, 211, and 466 days after onset of illness were determined by IFA and ELISA (table 3). The IFA test was done using EBO-infected Vero E6 cells as described [15]. The IgG ELISA was done using Vero cell lysates as antigens as described [16]. For the IgM capture assay, plates were coated with anti-IgM sera overnight, and then diluted serum was added, followed by specific and control antigen. Antigen fixation was revealed with anti-EBO rabbit serum and peroxidase-labeled anti-rabbit serum. For the antigen detection test, we used the reagents and technique described by Ksiazek et al. [17].

The other research workers were negative for EBO, including 2 who participated in the necropsy in which the case-patient presumably was infected. All researchers (including the case-patient) had observed the chimpanzees, using noninvasive methods and avoiding all physical contact with them and keeping a minimum of 3–4 m away from the animals.

Table 2. Biochemical values at various times after infection for a female ethologist who became infected with EBO-CI, presumably while doing a necropsy on an infected chimpanzee.

	November 1994			December 1994						Reference standard	Units
	26	28	30	1	2	3	4	5	7		
AST	19		1380	854	543	636	125	61	39	11–36	U/L
ALT	9		510	398	306	486	272	176	100	10–37	U/L
AST:ALT ratio	2.1		2.7	2.1	1.8	1.3	0.5	0.3	0.4		
γ-glutamyl transferase			48	50	53	87	104	82	60	8–66	U/L
Alkaline phosphatase			219	279	226	191	130	105	74	31–108	U/L
Creatinine kinase				199	125		46	36	41	38–157	U/L
Lactate dehydrogenase			8690							232–437	U/L
Amylase				289	355		913	792	793	20–220	U/L
Creatinine			108	96	81	84	63	56	49	45–93	μmol/L
Bilirubin			7	8	8	9	6	8	8	5–18	μmol/L
Albumin		38		39	29		32	30	33	38–52	g/L
Globulin		23		28	26		26	25	29	18–34	g/L
Albumin:globulin ratio		1.7		1.4	1.1		1.2	1.2	1.1	<1.0	

NOTE.   Day 1 of illness = 24 November 1994. AST = aspartate aminotransferase, ALT = alanine aminotransferase.

Mode of Contamination and Contact Persons

The chimpanzee organs from the implicated necropsy were shown by immunohistochemistry to be infected with EBO [11]. During the necropsy, all 3 researchers had worn gloves but not masks or gowns. No wounds or punctures had been noted. Two researchers had worn latex examination gloves, but the case-patient had worn household gloves. Therefore, it is highly probable that she became contaminated during the necropsy by direct contact with chimpanzee blood on her hand or by projection of droplets onto her face. This would indicate an 8-day incubation period.

Contact persons were defined as those having had direct face-to-face contact with the patient either 2 days before onset of illness or during illness [18]. In Côte d'Ivoire, sera were sampled from 18 contact persons and 4 laboratory technicians who handled the chimpanzee's organs. In Switzerland, 52 contacts were interviewed and serologically investigated. These contacts included 8 family members and friends, the 4 crew members of the air-rescue service, and the hospital and laboratory staff. No antibodies against EBO were found by ELISA or IFA in any of the 74 sera tested.

Discussion

This is the first case of EBO hemorrhagic fever (EHF) reported in West Africa and the first documented human infection associated with naturally infected nonhuman primates in Africa. Although several EHF cases have been briefly described [6], only the English case investigated by Emond et al. [8] is well documented. Our patient is thus the second fully investigated case of EHF.

The clinical course, the length of the disease, and signs during the patient's convalescence are consistent with the non-lethal EHF described among Yambuku's survivors and with the English patient (in [8]). Loss of memory, central nervous system disorders, and loss of hair during the convalescent phase were also reported in persons with EHF or other hemorrhagic fevers (MBG virus, Lassa fever, and Argentinean hemorrhagic fever). Loss of weight is a constant feature of EHF, due to asthenia and anorexia and probably to a direct action of the virus on tumor necrosis factor production.

Raised levels of transaminases but normal levels of γ-glutamyl transferase and bilirubin indicate that hepatic damage was not major. These findings suggest extrahepatic targets of infection. Higher AST than ALT activity has previously been described during MBG hemorrhagic fever (MHF) [19, 20] and in experimentally inoculated monkeys [21, 22]. The higher activity may indicate myocardial involvement during the first week of the illness, which would correlate with the increased lactate dehydrogenase and initial above-normal values of creatinine kinase. Damage to the myocardium was reported in mon-

Table 3. Serum antigen and antibody titers against EBO-CI in a female ethologist who presumably became infected while doing a necropsy on an infected chimpanzee.

Date, day after onset	IFA	IgG ELISA	IgM-capture ELISA	Antigen detection ELISA
27 November 1994, 3	0	0	1250	512
1 December 1994, 7	0	0	15,000	8192
16 December 1994, 22	2048	6000	10,000	0
4 January 1995, 41	4096	10,000	3000	0
13 February 1995, 81	2048	5500	200	0
23 June 1995, 211	1024	5000	0	0
4 March 1996, 466	128	3200	0	0

keys with natural MHF or EHF and in experimentally inoculated monkeys. Slightly elevated creatinine levels suggest dehydration rather than major impairment of the renal function. Despite the lack of obvious bleeding, the patient presented with a hematologic profile consistent with an intravascular coagulation.

The patient's mild form of EBO disease may have been due to the virus subtype, the mode of contamination, or the biologic response of the patient (or a combination of all three factors). But regarding the high mortality rate (25%) among chimpanzees [14], EBO-CI has to be considered as potentially highly pathogenic in humans.

Although containment measures were not always tight and there were no specific precautions taken during laboratory tests (blood specimens were not inactivated prior to analysis), no secondary cases appeared among the contacts. Contacts included a man who ate from the same plate as the patient on day 1 and a woman who comforted her in Côte d'Ivoire. Transmission of EHF requires physical contact with a patient or contact with blood or secretions, as described in 1976 [1, 2] and confirmed during the Kikwit outbreak [23]. Universal precautions and barrier nursing methods are effective in preventing cross-infection. Our hypothesis is that the patient contaminated herself by simple contact with infected chimpanzee blood, but the exact route of transmission in this case is unknown.

This episode emphasizes the difficulty of diagnosing new tropical diseases. In the tropics, many patients present malaria-like syndromes, and most illnesses in such cases are caused by malaria. The nonmalarial cases can be bacterial (i.e., typhoid fever, typhus, leptospirosis) or viral infections. If malaria tests are negative and antibiotic treatment and blood cultures unsuccessful, an arboviral or hemorrhagic fever infection has to be considered. More than 100 arboviruses can cause human infections, but the number of tests required can be reduced according to the geographic origin of the infection and the clinical signs.

Furthermore, few viruses present a risk of mortality and the need for isolation measures: Those that do present such a risk include Lassa fever, EBO and MBG viruses, Rift Valley fever, and CCHF in Africa. IgM-specific ELISAs are available for these viruses in World Health Organization (WHO) reference centers. The tests show positive results 4–10 days after the beginning of the disease. A more rapid answer may be obtained by antigen-capture assay. Antigen detection tests have been developed for EBO and Lassa fever but not for CCHF or Rift Valley fever. Unfortunately, reagents for the antigen-detection test are not always available in WHO and Office International des Epizooties centers for hemorrhagic fevers. For an unknown virus or a new subtype, such as EBO-CI, the best approach is to isolate the virus from a sample collected during the febrile phase of the disease.

Infections by hemorrhagic fevers are not always associated with hemorrhages. The absence of bleeding is insufficient to reject this etiology. Fewer than 40% of EBO and Lassa fever infections present with gum bleeding, petechia, hematemesis, or melena. Beside nonspecific but constant symptoms, high fever, myalgia, headache, and nausea, the most predictive signs for EBO and MBG infections is abdominal pain, which is often accompanied by diarrhea and, for Lassa fever infection, chest pain and sore throat [24]. The hemorrhagic form of Rift Valley fever infections represents <10% of the cases and begins with jaundice.

The case presented herein highlights the threat of importation of a disease by a sporadic case: Air travel allows viruses to travel from continent to continent within hours. The Kikwit, Democratic Republic of the Congo, epidemic (recognized in May 1995) showed that EBO and other deadly viruses may kill people for months before an outbreak and its agent are identified. Thus, as recommended by WHO, there is an urgent need to improve both national surveillance efforts and the capacity of laboratories around the world to diagnose these emerging diseases [25].

During this investigation, cooperation between researchers with different specialties (i.e., physicians, biologists, veterinarians, and microbiologists) was essential for determining the case-patient's diagnosis. Further collaboration between these disciplines will be necessary to improve surveillance and control of emerging infectious diseases.

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