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Ebola haemorrhagic fever in Zaire, 1976

Report of an International Commission[1]

Between 1 September and 24 October 1976, 318 cases of acute viral haemorrhagic fever occurred in northern Zaire. The outbreak was centred in the Bumba Zone of the Equateur Region and most of the cases were recorded within a radius of 70 km of Yambuku, although a few patients sought medical attention in Bumba, Abumombazi, and the capital city of Kinshasa, where individual secondary and tertiary cases occurred. There were 280 deaths, and only 38 serologically confirmed survivors.

The index case in this outbreak had onset of symptoms on 1 September 1976, five days after receiving an injection of chloroquine for presumptive malaria at the outpatient clinic at Yambuku Mission Hospital (YMH). He had a clinical remission of his malaria symptoms. Within one week several other persons who had received injections at YMH also suffered from Ebola haemorrhagic fever, and almost all subsequent cases had either received injections at the hospital or had had close contact with another case. Most of these occurred during the first four weeks of the epidemic, after which time the hospital was closed, 11 of the 17 staff members having died of the disease. All ages and both sexes were affected, but women 15–29 years of age had the highest incidence of disease, a phenomenon strongly related to attendance at prenatal and outpatient clinics at the hospital where they received injections. The overall secondary attack rate was about 5%, although it ranged to 20% among close relatives such as spouses, parent or child, and brother or sister.

Active surveillance disclosed that cases occurred in 55 of some 550 villages which were examined house-by-house. The disease was hitherto unknown to the people of the affected region. Intensive search for cases in the area of north-eastern Zaire between the Bumba Zone and the Sudan frontier near Nzara and Maridi failed to detect definite evidence of a link between an epidemic of the disease in that country and the outbreak near Bumba. Nevertheless it was established that people can and do make the trip between Nzara and Bumba in not more than four days: thus it was regarded as quite possible that an infected person had travelled from Sudan to Yambuku and transferred the virus to a needle of the hospital while receiving an injection at the outpatient clinic.

Both the incubation period, and the duration of the clinical disease averaged about one week. After 3–4 days of non-specific symptoms and signs, patients typically experienced progressively severe sore throat, developed a maculopapular rash, had intractable abdominal pain, and began to bleed from multiple sites, principally the gastrointestinal tract. Although laboratory determinations were limited and not conclusive, it was concluded that pathogenesis of the disease included non-icteric hepatitis and possibly acute pancreatitis as well as disseminated intravascular coagulation.

This syndrome was caused by a virus morphologically similar to Marburg virus, but immunologically distinct. It was named Ebola virus. The agent was isolated from the blood of 8 of 10 suspected cases using Vero cell cultures. Titrations of serial specimens obtained from one patient disclosed persistent viraemia of $10^{6.5}$–$10^{4.5}$ infectious units from the third day of illness until death on the eighth day. Ebola virus particles were found in formalin-

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fixed liver specimens from three cases. Survivors of infection were found to have indirect fluorescent antibodies to Ebola virus in titres of 1 : 64–1 : 256 within three weeks after onset of disease and these serum titres persisted with only slight decrease for a period of 4 months.

A total of 201 units (200–300 ml each) of plasma containing Ebola virus antibodies in titres of at least 1 : 64 were obtained and frozen. Two of these units were used to treat a laboratory worker infected with Ebola virus. This person recovered, which suggests that the antibodies may have helped therapeutically.

Virus transmission was interrupted by stopping injections and by isolation of patients in their villages. Use of protective clothing and respirators, strict isolation of patients, and careful disposal of potentially contaminated excreta and fomites will almost certainly prevent future major outbreaks. The virus is probably rarely transmitted by infectious aerosols, although infection via large droplets remains a possibility.

*Only limited ecological investigations were made, since the epidemiology of the outbreak strongly suggested that the virus had been imported into Bumba Zone. Ebola virus was not recovered from representative samples of bedbugs or of rodents (*Rattus rattus *and* Mastomys *spp.) having more or less close contact with humans. Ebola virus antibodies were found, however, in five persons who were not ill and had not had contact with the " infected " villages or the Yambuku hospital during the epidemic. If these findings can be confirmed by an independent method of testing, they would suggest that the virus is in fact endemic to the region and should lead to further effort to uncover a viral reservoir in Zaire.*

This paper describes the work of an International Commission formed in Kinshasa, Zaire, on 18 October 1976 and directed by the Minister of Health of that country, Dr Ngueté Kikhela. Its mission was to investigate the cause, the clinical manifestations, and the epidemiology of a newly recognized disease, termed Ebola haemorrhagic fever, and to advise and assist the Ministry with measures for controlling it.

THE OUTBREAK

Chronology of events

An understanding of many of the things that were done or not done in these investigations is best acquired by reference to the chronology of salient events (Table 1) and to the following summary of the major logistic problems encountered by the Commission.

The first visit of a Commission subgroup to the epidemic area in and near Yambuku was on 19 October 1976. It was found there was no effective communication between Yambuku and Kinshasa. The commercial air service to Bumba had ceased because of the quarantine regulations. The mission had very little electricity, no diesel fuel, no functional laboratory, and no protective clothing for the staff. When the advance team returned on 27 October with these facts and a report that active cases had been seen or had died in at least 20 villages in the Bumba Zone in the previous ten days, the Commission mobilized all available resources, national and international, to cope with a possible major threat to public health in Zaire and elsewhere.

Following an assessment of the situation in the epidemic area and in Kinshasa, the priorities of the Commission in late October were: (1) to stop transmission of the disease in Kinshasa; (2) to search out and control the epidemic in the Bumba Zone; (3) to establish national disease surveillance and to ensure that no unreported cases were occurring between the Bumba Zone and Sudan; (4) to document the clinical features and epidemiology of the disease; (5) to obtain a large amount of plasma from convalescent patients for therapeutic and prophylactic use; and (6) to search for the natural reservoirs and possible vectors of the disease. Transportation, communications, and supplies had to be organized, and in many cases improvised. Literally hundreds of persons were involved and more than one million dollars were spent. The following sections summarize the work carried out under circumstances which at times seemed to us those of a small war.

Table 1. Chronology of events.

Date	Event
1976	
1 September	Onset of symptoms in first recognized case, Yambuku.
16 September	Dr Ngoy of Bumba reviewed 17 cases. Concluded it was an unknown disease.
21 September	First message received in Kinshasa about the epidemic.
23 September	Visit by Professor Muyembe (Université Nationale du Zaïre) and Dr Omombo (Service d'Hygiene) from Kinshasa. Typhoid fever suspected. They returned on 25 September.
25 September	Belgian nursing sister transferred from Yambuku to hospital in Kinshasa.
30 September	Belgian nursing sister died of haemorrhagic fever in Kinshasa.
30 September	Yambuku hospital closed. 11 of 17 staff members dead.
2 October	Visit by Dr Krubwa (UNAZA), Dr Raffier (Mission Médicale Française) and Dr Ruppol (Fonds Médical Tropical) from Kinshasa. Specimens collected. They returned on 6 October.
3 October	Bumba Zone quarantined by Minister of Health upon recommendation of second medical mission.
8 October	2nd Belgian nursing sister developed the disease in Kinshasa.
12 October	Zairian nurse at Kinshasa hospital developed the disease.
13 October	Virus morphologically similar to Marburg agent isolated in three laboratories overseas.
14 October	New agent shown to be immunologically distinct from Marburg virus. 2nd nursing sister died.
16 October	Zairian nurse given Marburg convalescent plasma. Strict patient isolation and quarantine of exposed hospital staff instituted.
18 October	International Commission formed.
19 October	Survey team sent to Bumba Zone.
20 October	Zairian nurse died of the disease in Kinshasa.
27 October	Survey team reported active cases in at least 8 villages.
30 October	Two mobile teams airlifted to Isiro to search for disease between Sudan and the Bumba Zone. Advance group to Yambuku to search for cases and convalescent patients and to make preliminary ecological survey.
2 November	First units of convalescent plasma obtained in Kinshasa.
4 November	Recruitment and training of surveillance teams began in the Bumba Zone.
5 November	Last case of the disease in the region died in village of Bongulu II.
9 November	Widespread village surveillance initiated.
16 November	Clinical, virological, and plasmapheresis teams with equipment arrived in Yambuku. Radio communication Yambuku–Ebonda–Kinshasa established.
13 December	Main teams returned to Kinshasa.
16 December	The emergency officially ended.
1977	
28 January	Plasmapheresis programme terminated.

Description of the epidemic area

The Republic of Zaire has a population of about 26 million and an area of almost 2 million km^2, making it the second largest country of Africa. Kinshasa, the capital city with a population of 2 million, is on the lower reaches of the Zaire River. The river separates the northern sectors from the remainder of the country. The main epidemic area was in north-west Zaire; in the Bumba Zone of the Equateur Region (Fig. 1). This zone has a population of about 275 000 persons, half of whom are less than 15 years of age. More than three-quarters of the people live in villages with a population of less than 5000 and most in localities with fewer than 500 persons. The zone is part of the middle Zaire river basin and is predominantly tropical rain-forest. Cash crops include palm oil, rice, coffee, cocoa, and rubber. The people are avid hunters and come in contact with a variety of wild animals. Staple foods are transported from the Equateur Region to other parts of Zaire, to Sudan, and to the Central African Empire in exchange for cloth, utensils, transistor radios, etc. The major ethnic group is the Budza. Dysentery, malaria, filariasis, measles, amoebiasis, pneumonia, tuberculosis, and goitre are some of the common endemic diseases.

The Yambuku Catholic Mission was established by Belgian missionaries in 1935 in the Yandongi collectivity (county), one of seven in the Bumba Zone. It is located about 100 km north of Bumba and serves as the principal source of health care for, perhaps, 60 000 people in Yandongi and adjacent collectivities. Because it maintained a good supply of medicines, people passing through Bumba Zone frequently travelled long distances to attend clinics there. In 1976, the hospital had 120 beds, and a medical staff of 17 including a Zairian medical assistant, and three Belgian nurse/midwives, who were nuns. There was an active prenatal and obstetrical service and an outpatient clinic that treated 6000–12 000 persons monthly.

Five syringes and needles were issued to the nursing staff each morning for use at the outpatient department, the prenatal clinic, and the impatient wards. These syringes and needles were apparently not sterilized between their use on different patients but rinsed in a pan of warm water. At the end of the day they were sometimes boiled. The surgical theatre had its own ample supply of instruments, syringes, and needles, which were kept separately and autoclaved after use.

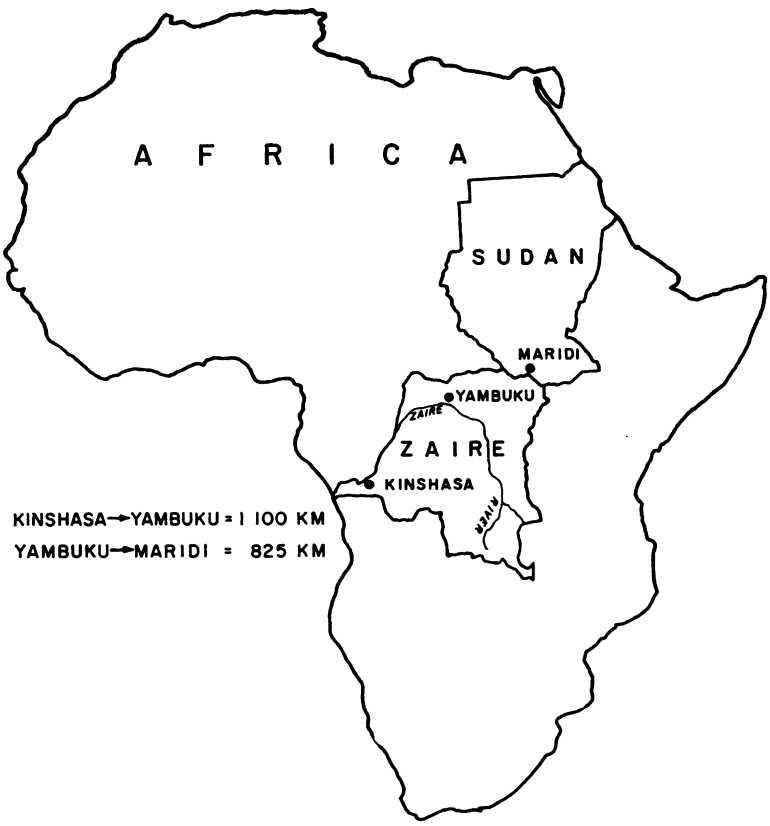

Fig. 1. Locations of the principal centres of the outbreaks of Ebola haemorrhagic fever, Sudan-Zaire, 1976.

SURVEILLANCE AND CONTROL ACTIVITIES

METHODS

Definitions

A *probable case* of Ebola haemorrhagic fever was a person living in the epidemic area who died after one or more days with two or more of the following symptoms and signs: headache, fever, abdominal pain, nausea and/or vomiting, and bleeding. The patient must have, within the three preceding weeks, received an injection or had contact with a probable or a proven case, the illness not having been otherwise diagnosed on clinical grounds. A *proven case* was a person from whom Ebola virus was isolated or demonstrated by electron microscopy or who had an indirect fluorescent antibody (IFA) titre of at least 1: 64 to Ebola virus within three weeks after onset of symptoms. An *Ebola virus infection* was deemed to have occurred in persons who had a similar IFA antibody titre, but had not been ill during the period 30 August to 15 November 1976.

A *possible case* was a person with headache and/or fever for at least 24 hours, with or without other signs and symptoms, who had contact with a *probable* or a *proven* case within the previous three weeks. These patients were treated with antimalarial drugs, antibiotics, and antipyretics to exclude other diseases common to the area. Persons reporting such symptoms retrospectively were bled and their sera were tested for Ebola virus antibodies. Also any case of fever with bleeding reported to the Ministry of Health from any part of Zaire, whatever the clinical outcome, was regarded as a *possible* case, and every effort was made to establish a diagnosis by virological or histopathological means.

Infants born to patients who were probable cases of Ebola haemorrhagic fever were called *neonatal* cases if they died within 28 days of birth.

A *primary contact* was any person having direct face-to-face contact with a probable or a proven case (sleeping in same room, sharing meals, caring for patients, preparing a cadaver for burial, touching the body at a funeral, etc.) in the period between two days prior to onset of symptoms and the death or clinical recovery of the patient. The surveillance interval for primary contacts was 21 days from the last such contact. *Secondary contacts* were persons having face-to-face contact with a primary contact.

ORGANIZATION OF SURVEILLANCE

National

Two teams led by physicians, with Land Rovers, were airlifted to north-east Zaire to seek a link between the epidemics in Sudan and Zaire. These teams searched for recent and active cases and tried to identify commercial trade routes and human travel patterns between the Bumba Zone and southern Sudan.

Medical personnel throughout Zaire were informed of the epidemic by distribution of a technical note containing basic information on the disease. Practical advice for protection of health workers was given, along with instructions for notification of the disease and collection and shipment of specimens if a possible active case occurred. This information was disseminated to health units and administrative authorities throughout the country by official government channels, through the radio networks of the Catholic and Protestant missions, and by informal methods. Requests were made for immediate telegraphic reporting of possible cases and for weekly radio reports from peripheral health units to the Ministry of Health in Kinshasa. A national surveillance/investigation team was established in Kinshasa and was responsible for the immediate investigation of possible cases in the capital and elsewhere in the country. Liaison was established with the Zaire Air Force and Air Zaïre for transporting the team anywhere into the interior. The Pathology Laboratory at Mama Yemo Hospital, Kinshasa, was equipped to fix and stain liver sections and to make the histological diagnosis of different types of hepatitis. Sera were tested for Ebola virus antibodies at Yambuku, and were sent to Atlanta, Georgia, USA, for attempts to isolate a virus.

Kinshasa

The entire medical staff of one ward at Ngaliema Hospital, Kinshasa, where three proven cases were treated, was quarantined in the ward, which was an isolated pavilion; they were considered as primary contacts. Others from outside the hospital who were primary contacts of one of the cases were quarantined in groups of 1–8 persons in another pavilion of the hospital, which was set aside for that purpose. Temperatures of all these people were taken daily during a 21-day period.

Secondary contacts were identified and requested to report daily to a physician or clinic in the capital city for temperature recording and a general health check. This surveillance programme was abandoned when investigations in the epidemic area disclosed that secondary contacts were not at risk of contracting the disease.

EPIDEMIC AREA

The objectives of surveillance teams were to find past and active cases of Ebola haemorrhagic fever, to detect possible convalescent cases, to educate the public as to the nature of and means of preventing the disease, and to establish beyond question the termination of the outbreak. Ten special active surveillance teams were recruited and trained. Each consisted of four persons; a team leader (physician or nurse), two nurses, and a chauffeur. The subjects covered during training were the differential diagnosis of Ebola haemorrhagic fever, its epidemiology (including possible modes of transmission), the means of protecting personnel, and methods for obtaining family census data and recording probable and possible cases. The teams were provided with standard forms, a written schedule, and detailed maps showing the villages they were to cover during a two-week period. Each team was assigned a four-wheel-drive vehicle, some of which had radios, and was provided with food, water, gowns, caps, gloves, boots, respirators, and equipment for obtaining blood samples. Chloroquine, tetracycline, aspirin, and a drug against intestinal parasites were all supplied in tablet form. A physician supervised five teams by frequent field visits and administrative reviews.

The teams were based either at Yambuku or Ebonda, a small town on the Zaire river 12 km west of Bumba. They were assigned 10 different routes as shown schematically in Fig. 2. These routes extended up to 200 km from the epicentre at Yambuku. The

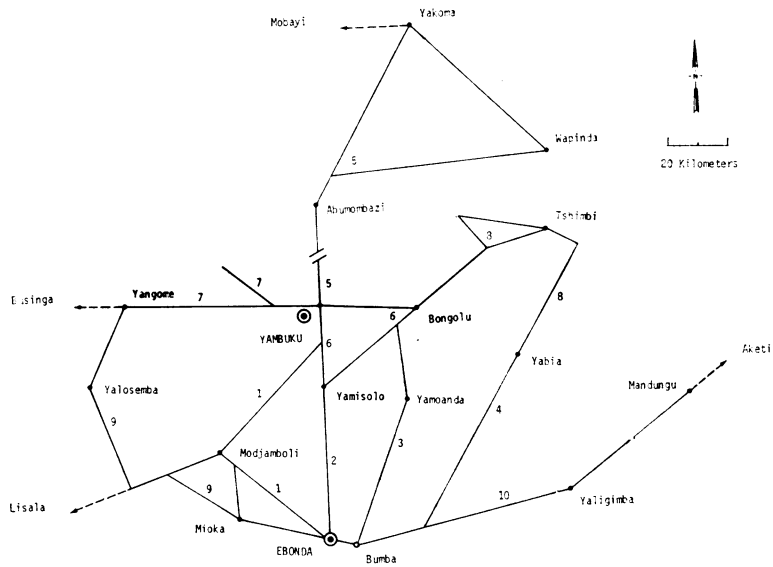

Fig. 2. Routes followed by the surveillance teams. The numbers identify the teams.

teams contacted the village chief and enlisted support for a house-to-house survey. The name of the family head and the number of persons in each family were recorded. Data on past cases were collected. Suspect cases were not closely examined, but medicines were given to them and arrangements were made for their isolation in the village. Barriers were erected along roads and paths to restrict entry to, and egress from, such villages. Work reviews were held daily at Yambuku and Ebonda, and physicians were sent to follow up suspect cases and to bleed candidate convalescent cases.

After an interval of 7–14 days, the teams travelled their respective circuits a second time using these methods, and a third rapid survey was made in which village chiefs were asked whether any new suspect cases had occurred.

METHODS USED TO PREVENT TRANSMISSION

Isolation of patients, safe disposal of potentially contaminated articles and disposable clothing, plus high-efficiency respirators and goggles were the principal means employed to prevent contact infection. Sodium hypochlorite (2%), boiling, or burning were the methods used to decontaminate or dispose of potentially infectious excreta, utensils, and clothing; cadavers were wrapped in shrouds soaked in formalin or phenol and buried deeply.

RESULTS

National surveillance

Fifteen possible cases of haemorrhagic fever occurring outside the main epidemic area were investigated from Kinshasa. Ebola haemorrhagic fever was ruled out in each instance on clinical, virological, or pathological grounds. Final diagnoses included typhoid fever, viral hepatitis, amoebiasis, acute pulmonary oedema, and carbon monoxide poisoning. Two surveillance teams travelled a total of more than 5000 km in northern Zaire, between the Sudanese frontier near Nzara and Yambuku. They found no evidence of any outbreak resembling Ebola haemorrhagic fever, although they were obliged to track down many rumours and they investigated several instances of acutely fatal infection with either jaundice or respiratory symptoms and signs. It was established, however, that trucks carrying people made frequent trips from south-western Sudan to Bumba, a journey which could be completed in as little as four days. In contrast, there was little evidence of human travel from Sudan through the extreme northern part of Zaire to either Yambuku or towns north of it along the border with the Central African Empire.

Surveillance and disease control in Kinshasa

A nursing sister exposed to patients with Ebola

haemorrhagic fever in Yambuku was admitted to Ngaliema hospital on 25 September 1976, the third day after onset of symptoms. Her primary care was given by a colleague who had accompanied her from Yambuku. This patient died on 30 September. The second nursing sister, who had not used protective clothing, became ill on 8 October and died 6 days later. Thus the incubation period was estimated to be 8–14 days. A third nurse had helped with the first case from 27 September, but not the second. She was on leave in Kinshasa during the second week of October and had onset of headache and low grade fever on 13 October; so the incubation period in her case was 13–16 days. Prior to her admission on 15 October, she had exposed personnel at the Ministry of Foreign Affairs, where she made arrangements for an overseas study visit, at two other hospital emergency rooms in Kinshasa, and various members of her family. This patient, who died on 20 October, was cared for by her sister who was a nurse. Although careful barrier nursing had been practised and emergency arrangements made for disposal of all potentially contaminated articles, the possibility that further cases would occur was considered highly likely and it was deemed imperative to stop the chain of transmission in Kinshasa. To this end a portable negative-pressure bed isolator was obtained from Canada and installed in a room in the pavilion where the three cases were managed. Quarantined medical staff were trained in operation of this equipment. Fortunately, no further cases occurred. In addition to about 14 staff members, 37 primary contacts of the third case were quarantined for three weeks. Serum specimens obtained from 25 persons at the end of quarantine were tested for Ebola IFA antibodies and were all negative.

Surveillance in the epidemic area

The first special surveillance teams began work on 9 November 1976 and by 16 November all teams were functioning. Although an average of about 5–10 possible cases were seen per week, none had Ebola haemorrhagic fever. At the end of November it was clear that the last probable case had died on 5 November in the village of Bongulu II, some 30 km east of Yambuku. During their work the surveillance teams visited 550 villages, interviewed more than 34 000 families with an average of about 7 members, that is, approximately 238 000 persons. A total of 231 probable cases were identified during active surveillance, but this figure did not include persons from the village of Yambuku itself. Also, many possible convalescent persons were identified and bled. The results of this work are given in the following section.

The teams completed second and third visits to villages as planned, and teams 1, 5, 6, and 7 made a fourth village check in late December. Based on these data the International Commission advised lifting quarantine in Bumba Zone on 16 December 1976, which was six weeks after the last death from Ebola haemorrhagic fever.

EPIDEMIOLOGICAL INVESTIGATIONS

METHODS

Information collected on the occurrence of Ebola haemorrhagic fever in Bumba Zone was largely retrospective. Two independent assessments were made. One member of the International Commission searched 21 villages from 1 to 9 November 1976 in the course of a concerted effort to identify and obtain serum specimens from possible convalescent patients. He recorded 136 fatal cases. Six teams led by physicians with nurse translators, working independently of the 10 active surveillance teams, subsequently visited all villages reporting probable cases to the surveillance teams and gathered detailed information on individual cases, using a standard form. The findings in these two surveys agreed remarkably well: the same cases were found in 91% of instances. Moreover, there was complete agreement in sex recording; age (within five years) and death date (within seven days) corresponded in 87% and 92% of instances, respectively. Definitions of cases and infection were the same as those described above. Family members provided information on fatal cases. A control was a person from the same village as a probable case. If possible, a control was matched by age and sex with a probable case in the same family.

Serum specimens were collected from persons in villages in the epidemic area if they had acute febrile illness during the epidemic period and were in contact with probable cases, and from all volunteers in 8 villages, each of which had 5 or more probable cases.

Mosquitos were caught directly with glass tubes

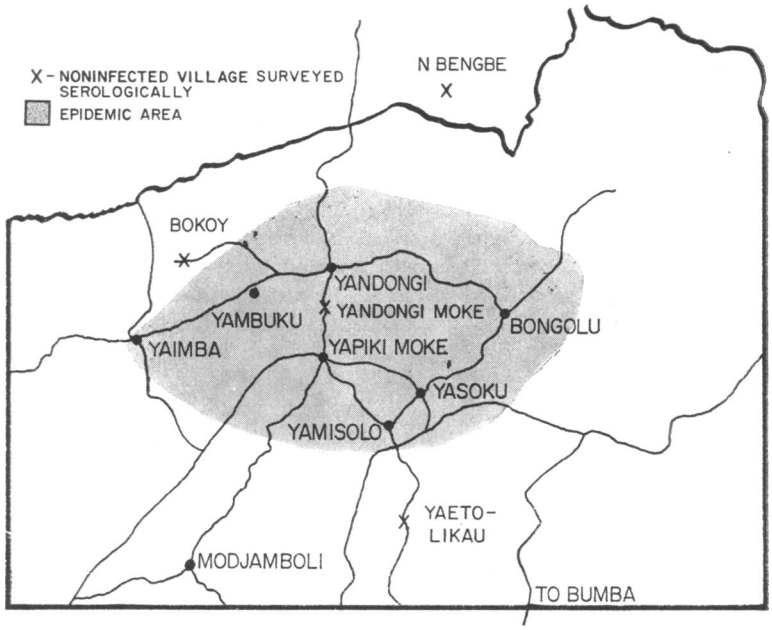

Fig. 3. Location of epidemic area and the noninfected villages surveyed serologically.

from resting sites in homes. Cimicid bugs were picked from bamboo beds and clothing. Wild rodents were hand caught by village youths and a few other mammals were shot by local hunters. Mist nets were employed to capture bats emerging from houses at dusk.

RESULTS

Origin and course of the epidemic

The first known case, a 44-year-old male instructor at the Mission School, presented himself to the outpatient clinic at Yambuku Mission Hospital (YMH) on 26 August 1976 with a febrile illness thought to be malaria. This man had toured the Mobaye-Bongo zone in the northern Equateur Region by automobile from 10 to 22 August with 6 other Mission workers. The group visited some of the larger towns (Abumombazi, Yakoma, Katokoli, Wapinda) along the road from Yambuku to Badolité, but never arrived at that village because a bridge had been washed away a few kilometres east of the town. On 22 August, fresh and smoked antelope and monkey meat were purchased on the road about 50 km north of Yambuku. The patient and his family ate stewed antelope on his return, but not the monkey meat. He was given chloroquine by parenteral injection on 26 August. His fever resolved rapidly and he was afebrile until 1 September when he again had fever to 39.2°C. Other symptoms and signs ensued and he was admitted to YMH on 5 September with gastrointestinal bleeding. He died on 8 September.

At least 9 other cases occurred during the first week of September, all among persons who had received treatment for other diseases at the outpatient clinic at YMH. Names of persons treated at the outpatient clinic and specific diagnoses were not recorded. Thus, it was impossible to determine whether persons with fever had visited YMH in late August. It was of interest, however, that a man about 30 years of age had been admitted to the medical ward on 28 August suffering from " dysentery and epistaxis ", a diagnosis not otherwise listed in the preceding eight months. This man, listed as resident in Yandongi, the capital village of the collectivity some 7 km from Yambuku, was taken from the hospital two days later. He turned out to be a person completely unknown to the residents and authorities of Yandongi.

Case histories quickly suggested that YMH was a major source of dissemination of Ebola haemorrhagic fever. It was learned that parenteral injection

Table 2. Distribution of numbers of cases in villages

Number of cases	Number of villages	% of villages	Cumulative %
1	17	30.9	30.9
2–5	18	32.7	63.6
6–9	12	21.8	85.4
10–14	4	7.3	92.7
15–19	1	1.8	94.5
20–29	1	1.8	96.3
⩾30	2	3.7	100.0
Total	55		

was the principal mode of administration of nearly all medicines.

Between 1 September and 24 October, 318 probable and confirmed cases/infections of Ebola haemorrhagic fever occurred with 280 deaths, an infection fatality rate of 88%. The epidemic reached a peak during the fourth week, at which time the YMH was closed, then it receded over the next four weeks.

Fifty-five of about 250 villages in the epidemic area recorded cases. All but one of these were in Bumba Zone, the exceptions being to the north. All affected villages were within 120 km of Yambuku, and the majority were along roads running east and west of the mission (Fig. 3). These roads had more villages per kilometre than roads running north and south of the epicentre. Forty-three of 73 villages in Yandongi collectivity had cases and the attack rate for this area was 8.0 per 1000 persons. None of the other 6 collectivities in the epidemic region had attack rates of greater than 2 per 1000. Sixty-four percent of cases occurred in villages having 1–5 cases and only 3 villages had more than 20 cases (Table 2).

The epidemic spread relatively slowly. During the first two weeks all cases were restricted to a radius of 30 km from Yambuku. Nearly two more weeks passed before a patient was evacuated to Kinshasa. Cases were introduced in early October into Abumombazi and Bumba, the two large towns limiting the north–south extension of the outbreak. The mean duration of the epidemic in the villages was 25.6 days.

Thirteen of 17 staff members at YMH acquired the disease and 11 of these died, with the result that the hospital closed on 3 October. Although all staff members had contact with patients, their families, and instruments used in treatment, one male nurse and three female midwives escaped infection.

Attack rates, incubation period, and mode of transmission

All ages and both sexes were affected (Table 3) but females slightly predominated. Age/sex attack rates, using the Yandongi collectivity as the population denominator, showed that adult females had the highest attack rate. Much of this excess illness was associated with receipt of parenteral injections at YMH or one of its clinics. The distribution of disease by age group and type of transmission was essentially equal for both sexes except for injection-associated illness among persons 15–29 years old. Females comprised 22 of 24 such cases in the 21-village study.

The single common risk factor in comparison with matched family and village controls for 85 of 288 cases where the means of transmission was deter-

Table 3. Age and sex distribution of cases

Age (years)	Male		Female		Total	
	No.	%	No.	%	No.	%
Newborn & Infants	10	3.1	14	4.4	24	7.5
1–14	18	5.7	22	6.9	40	12.6
15–29	31	9.7	60	18.9	91	28.6
30–49	57	17.9	52	16.4	109	34.3
⩾50	23	7.2	26	8.2	49	15.4
Unknown	2	0.6	3	0.9	5	1.6
Total	141	44.2	177	56.0	318	100

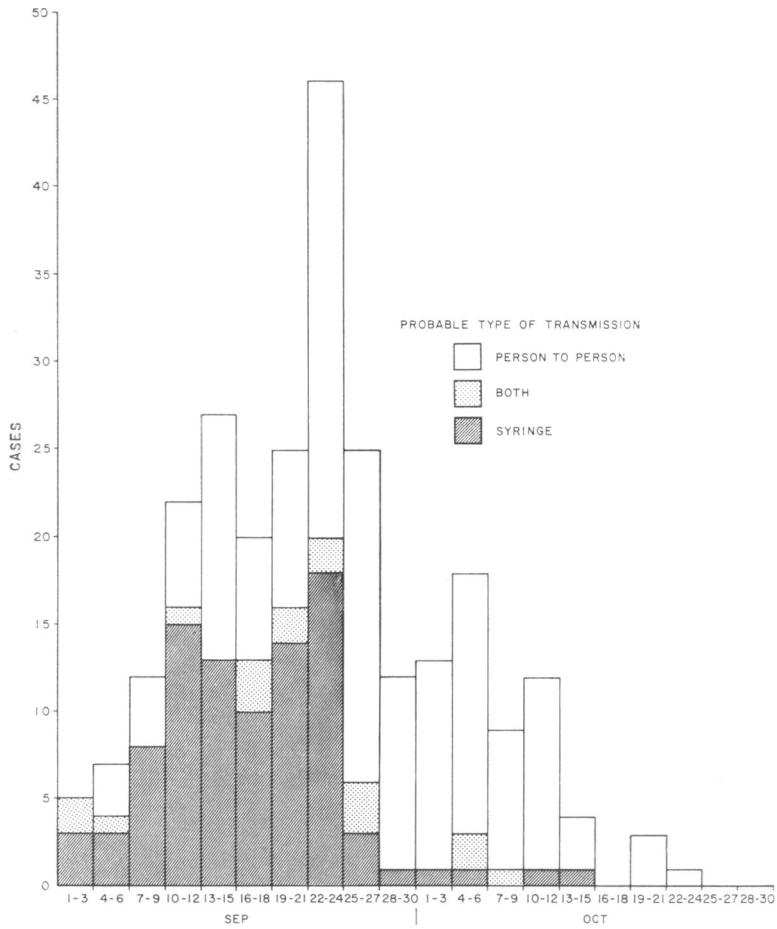

Fig. 4. Number of cases of Ebola haemorrhagic fever in the Equateur Region, by day of onset and by probable type of transmission.

mined, was receipt of one or more injections at YMH. Injections received away from YMH were very unusual. Other factors such as previous case-contact, exposure to food, water, hospital buildings, domestic and wild animals, or travel within three months prior to onset, were not associated with this type of transmission. An additional 149 persons acquired the disease following contact with patients, usually in their home villages, and 43 cases had a history of both patient contact and receipt of injection within three weeks prior to onset of illness. Seventeen persons who lived outside Yambuku had contact at YMH and may have received injections there without reporting this fact to their family.

Most of the cases related to injection occurred during the first 4 weeks of the epidemic (Fig. 4). Indeed, it seems likely that closure of YMH was the single event of greatest importance in the eventual termination of the outbreak.

Several parameters were compared for persons acquiring infection by contact and injection, respectively. Although no statistically significant differences were found in terms of duration of symptoms and signs of illness (Table 4), no person whose contact was exclusively parenteral injection survived the disease. All 20 survivors with symptoms and signs had documented contact with at least one other patient, although in 4 instances the survivor had also

Table 4. Mean duration (in days) of symptoms and signs presenting in patients [a] by type of transmission

	Fatal Cases				Convalescents	
	Injection		Person-to-person			
	Duration	± SE	Duration	± SE	Duration	± SE
Symptoms						
Fever	7.3	(0.53)	7.1	(0.43)	6.4	(1.63)
Headache	7.2	(0.52)	7.4	(0.46)	4.8	(0.86)
Sore throat	6.3	(0.49)	6.5	(0.49)	10.7	(2.57)
Abdominal pain	5.5	(0.53)	6.2	(0.46)	8.3	(2.28)
Myalgia	7.3	(0.55)	6.9	(0.43)	5.7	(1.01)
Nausea	5.0	(0.61)	5.4	(0.63)	4.8	(2.37)
Arthritis	6.3	(0.48)	7.0	(0.47)	9.9	(2.08)
Other [b]	7.2	(2.10)	3.3	(0.61)	4.4	(0.76)
Signs						
Bleeding	3.1	(0.30)	4.0	(0.33)	9.3	(2.95)
Diarrhoea	4.8	(0.47)	4.9	(0.36)	7.5	(1.75)
Oral/throat lesions	5.1	(0.60)	5.7	(0.50)	5.3	(0.64)
Vomiting	3.7	(0.40)	4.3	(0.44)	3.9	(1.17)
Conjunctivitis	4.8	(0.78)	5.1	(0.60)	6.2	(1.80)
Cough	7.5	(1.08)	6.9	(0.87)	10.0	(3.98)
Abortion	2.3	(0.98)	1.0	(0)	7.0	(0)
Oedema	2.5	(1.48)	2.0	(0)	0	–
Jaundice	2.0	(0)	9.7	(3.70)	0	–
Other [c]	0	–	3.0	(0)	3.0	(0)

[a] Persons ⩾ 1 year of age.
[b] Anorexia, chest pain/pleuritis, chills, tinnitis, vertigo.
[c] Amenorrhoea, ataxia, dark urine, dysarthria, hiccoughs, hyperhidrosis, lymphadenitis, paralysis, polyuria, rash.

received an injection. The mean incubation period for cases acquired by injection was 6.3 days. In 17 cases where only one or two days of patient contact occurred, the mean incubation period was also 6.3 days.

Persons acquiring the disease by contact had a variety of close associations with cases as indicated in Table 5. No statistically significant differences occurred among cases or family or village control groups, except that cases were more likely than controls to have aided at childbirth of another patient. Contact cases had a mean of 9.5 days of contact with active cases before becoming ill (range 1 to 21 days). Several persons had contact with more than one patient before becoming ill, particularly medical staff at YMH. However, in one case of the disease, the only possible source of infection was contact with a probable case 48 hours before the latter developed symptoms.

Five consecutive generations of transmission of Ebola haemorrhagic fever were documented in one instance. No sporadic, apparently spontaneous, probable cases were recorded. When " family " was defined as all persons living in contiguous housing and sharing common eating facilities, secondary attack rates never exceeded 8% (Table 6). However, when 92 families affected in the 21 villages surveyed along an east–west axis close to Yambuku were

Table 5. Factors associated with person-to-person spread for cases, family controls, and village controls

Risk factor	Person-to-person cases		Family controls		Village controls	
	No.	% Yes	No.	% Yes	No.	% Yes
Cared for case	119	70.6	84	71.4	22	68.2
Touched case	126	85.7	91	83.5	22	68.2
Slept in same room	116	69.0	86	66.3	22	22.7
Aided in delivery of child of sick patient	104	18.3	74	9.5	22	4.5
Prepared cadaver	116	58.6	87	57.5	22	54.5
Attended funeral	126	85.7	98	85.7	22	95.5

examined, contact attack rates were 16.7, 3.6, and 9.0% in three successive generations of transmission. Moreover, there were marked differences in secondary contact transmissions related both to sex of the primary case and to blood and marital relationships within households. The secondary attack rate was 27.3% among spouses, brothers, sisters, parents, and children but only 8.0% among all other relatives. Among the high-risk relatives, 10 of 60 contacts became ill when the primary case was male, and 27 of 75 when the primary case was female. Although the precise factors involved were impossible to determine, direct care of cases and intimate family contact, including sexual intercourse, were possibly the important variables.

Epidemic in the village of Yamolembia I

This village, located 5 km from Yambuku, was chosen for detailed analysis of disease transmission. The town was mapped and a door-to-door census revealed that 415 persons were resident in 71 households prior to the epidemic. Between 4 September and 18 October 1976, 24 persons became probable or confirmed cases of the disease. The first case was a man 27 years old who received an injection at the YMH outpatient clinic on 29 August. Within 6 days, 4 more persons with a history of injection at YMH became ill. During this same period 2 nurses, a medical assistant and a catechist contracted the disease. These persons had all been in frequent contact with patients at the hospital, but had not received injections. By mid-October, 15 additional villagers had become ill, 12 of them following close contact with patients in this or other villages. Seven of these contacts occurred in the homes of neighbours, and 3 were contacts with sick relatives in other villages. Information on 3 cases was lacking.

Table 6. Attack rate in family contacts by generation of transmission

Generation	No. of families with cases	No. of family exposures	No. of subsequent cases	Attack rate (%)
1	61	498	38	7.6
2	62	459	20	4.4
3	18	117	3	2.6
4	5	29	1	3.4
Total	146	1103	62	5.6

Ten cases were among males, 14 among females. Adults of 15–45 years of age were most commonly affected. Only 2 persons survived, both contact infections. Cases occurred in 15 households with 98 members; four households had secondary cases, and one other had more than one case but it could not be documented sufficiently to arrive at a conclusion as to the mode of transmission. There were 6 secondary and 3 tertiary cases, giving transmission rates of 7.2 and 4.0%, respectively. Households with cases were scattered through the village and no pattern of transmission other than very close patient contact was established.

In December 1976 and January 1977, sera were solicited from as many people as possible; a total of 236 were obtained. Three persons, 2 of them in clinically noninfected households, who had not had symptoms during or since the epidemic, were found to have Ebola virus IFA titres of at least 1 : 64. All

Table 7. Ebola virus IFA antibodies among residents of the epidemic area by age group, sex, and exposure status

Category	Sex	Age group (years)						Unknown	Total
		1–9	10–19	20–29	30–39	40–49	⩾ 50		
Ill	M	2	5	15 (2)	16 (2)	7	10 (1)		55 (5)
	F	2 (2) [a]	11 (2)	14 (3)	16 (6)	11 (2)	12		66 (15)
Contact, not ill	M	19	19	55 (1)	40	35	64 (2)	11	243 (3)
	F	31 (1)	25 (1)	22 (1)	30	29 (1)	35 (3)		172 (7)
Not contact, no illness	M	49	57	35	36	27 (1)	44 (2)		248 (3)
	F	43	48	33	24 (1)	21	31		200 (1)
Total		146 (3)	165 (3)	174 (7)	162 (9)	130 (4)	196 (8)	11	984 (38) [b]

[a] Numbers in parentheses indicate the number of persons with Ebola virus IFA titres of at least 1 : 64.
[b] Includes the 4 positive titres with unknown history.

3 had experienced contact with fatal cases. Extrapolating to the entire population, 2 more silent infections might be expected. Thus it appeared that 29 people (7%) in the village had been infected, clinical illness ensued in 83% with an infection-mortality rate of 76%.

Serological and ecological studies

Serum specimens were obtained in November and December 1976 and January 1977 from 984 persons resident in 48 of the 55 towns and villages reporting probable cases of the disease. These individuals were classed as clinically ill, not ill but in contact with a case, or neither ill nor in contact. More than half of the subjects were resident in 8 villages, each having more than 5 probable cases. These persons were bled during rapid survey excursions, taking the entire family as the unit of study. The composition of these groups by age, sex, and epidemiological characteristic is given in Table 7 together with the number and category of persons having Ebola virus IFA titres of at least 1 : 64. Data from Yamolembia I are included. Thirty-eight positives were found. Twenty (16.5%) of 121 ill persons were confirmed as having had Ebola haemorrhagic fever, and 10 (2.5%) of 404 persons in contact with cases also had such antibodies. There were 4 antibody-positive persons who admitted neither illness nor contact with patients. These people were questioned a second time and bled again, and confirmed to have Ebola IFA antibodies. Antibodies were found also in sera of 4 people whose history was not clear and who could not be found a second time for confirmatory study.

In a further effort to document either concurrent asymptomatic infection or possible past infection with Ebola virus, 442 persons were bled in 4 neighbouring villages that had had no fatal cases of the disease. Sera from 5 persons 8–48 years old contained IFA antibodies in titres of 1 : 64. None of these people were sick, had had contact with persons in other villages, or had visited YMH during the epidemic.

Sera from 58 persons in various exposure categories had anti-Ebola IFA titres of 1 : 4–1 : 32. The specificity of these reactions was doubted when it was found that samples from 4 of 200 San Blas Indians from Panama also had such " antibodies " for Ebola but not Marburg virus. Final interpretation of these data awaits development of another method for measurement of type-specific antibodies to these agents.

Suspensions of 818 bedbugs, *Cimex hemipterus* F., formed into 21 pools, were inoculated into Vero cell cultures. No virus was recovered. Negative results also were obtained from the following: 8 *Culex cinereus* Theo., 2 *Culex pipiens fatigans* Wied. and 5 *Mansonia africana* Theo. mosquitos; sera from 10 domestic pigs and one cow; viscera (usually spleen, liver, kidney, and heart) from 7 unidentified bats, 123 rodents including 69 *Mastomys* (= *Praomys*) (among which *P. tullbergi minor* (Hatt.) predominated) and 30 *Rattus rattus*, 8 squirrels, 6 *Cercopithecus* monkeys (not *aethiops*) and 2 small duikers (*Cephalophus monticola* Thünberg). *Rattus rattus*, bats, bedbugs and mosquitos were captured in infected villages; other wild species were captured or

shot in the nearby forest. *Rattus rattus* were found in village dwellings but *Mastomys* were not.

The mosquito man-biting-rate was low in villages, as were the *Aedes aegypti* indices (Yandongi village, 5 November 1976, Breteau index 3.6; container index 2.3).

CLINICAL MANIFESTATIONS

METHODS

Well documented serial observations were made on 3 patients at the Ngaliema Hospital in Kinshasa. Several others were seen briefly in villages by physicians in the survey teams. Information on other cases was obtained exclusively in villages of the affected region by six teams led by physicians working with nurse interpreters. Data were obtained as part of a retrospective epidemiological study using standardized forms. Family members provided information on fatal cases. Case and infection criteria have been described above under " Surveillance and control ". A control was a person from the same village as a probable case. Controls were matched as far as possible with cases by sex and age, and a member of the same family was chosen if available.

To measure IFA antibodies, a small field laboratory was established in Yambuku. A Leitz microscope with indirect halogen lamp illumination was used. A KP filter was fitted to give ultraviolet light of 490 nm. Ebola virus-infected Vero cells, which were inactivated by exposure to ultraviolet light (1200 $\mu W/cm^2$ for 10 min) were sent from Atlanta on dry ice and stored frozen until needed. The procedure used has been described (*1*). A 1 : 40 dilution of fluorescein isothiocyanate-conjugated anti-human immunoglobulin prepared in goats (Burroughs-Wellcome, MF-03) containing a 1 : 2000 concentration of Evan's blue dye as counterstain was employed. Test slides were prepared in a portable negative-pressure plastic isolator fitted with high-efficiency, particulate air (HEPA) filters, after safety tests at Atlanta had shown that the slides contained a small amount of Ebola virus after ultraviolet light treatment. All tests done in Zaire were subsequently repeated in Atlanta with excellent agreement in results.

RESULTS

Hospitalized patients

Early symptoms and signs in all 3 patients at Ngaliema Hospital included fever, headache, anorexia, and vomiting. A morbilliform rash appeared on the anterior trunk in each of these patients on day 5 or 6, spread to the back and limbs, then faded within 48 hours. Haemorrhage and severe sore throat began between the fourth and seventh days of illness.

One patient had oral and conjunctival petechiae beginning on day 4, haematemesis and melaena from day 4, gingival bleeding on day 7, and bleeding from injection sites on day 8. Another had melaena only, beginning on the sixth day of illness. The third patient had a single episode of haematemesis on day 7 followed by melaena and ecchymosis on the next day. Progressive glossitis and pharyngitis beginning on day 3 were noted in one patient who developed severe erythema and oedema of the soft palate and pharynx leading to pronounced dysphagia. All three patients were febrile throughout the course of illness, with temperatures frequently above 39°C. Two patients had terminal tachycardia. One patient died on day 7 and two on the eighth day of illness.

Clinical laboratory tests were done on the first patient, but only a few measurements were carried out on the other two cases to avoid undue exposure of hospital laboratory staff to the virus. Leucocyte counts on the first patient were 7600 and 8900/mm^3 on days 5 and 7, respectively. Platelets on days 4, 6, and 7 were 162 000, 150 000 and 150 000/mm^3; these were days when frank haemorrhage occurred. During this time, serum SGOT rose from 90 to greater than 200 units/ml and the SGPT increased from 40 to more than 200 units/ml.[a] Serum bilirubin rose from 25.6 μmol/l on day 5 to 59.8 μmol/l on day 7. Partial thromboplastin time (PTT) was 47 seconds on the fifth day. This patient produced only 200 ml of urine on the seventh day and none during the next day when she died. The second patient, on whom no laboratory tests were done, became anuric during the last 2 days of life.

The third patient had white blood cell counts of 9400 and 12 300/mm^3 on days 7 and 8, respectively. Platelet counts on these days were 253 000 and 205 000/mm^3, while PTT values were 45 and 50

[a] SGOT = aspartate aminotransferase (EC 2.6.1.1). SGPT = alamine aminotransferase (EC 2.6.1.2).

seconds, respectively. Fibrin degradation products, measured with a commercial kit (Burroughs-Wellcome) were recorded as 1+ and 2+ on days 7 and 8.

The first case was treated with aspirin, antibiotics, corticosteroids, blood transfusion, and intravenous fluids. The second patient received aspirin, hydrocortisone, immunoglobulin, intravenous fluids, and an experimental drug, moroxydine. Enterovioform was given to control diarrhoea but without success. The third patient was treated for unconfirmed malaria during the first 2 days of illness. When the etiological agent of the epidemic was shown to be a Marburg-like virus, she was given, on day 4, 500 ml of Marburg human plasma obtained from a recovered patient in South Africa. This plasma had an IFA titre of 1 : 32. In anticipation of disseminated intravascular coagulation (DIC), she was given 16 000 units of heparin on day 6 and 30 000 units daily thereafter. Although anticoagulation was unsatisfactory, as shown by the normal PTT on days 7 and 8, she had less clinical bleeding than the other two patients. On the day prior to death she complained of substernal chest pain and had a tachycardia of 136 with a gallop rhythm. Digitalization slowed this rate only slightly. Marked oedema of the face and upper limbs was present.

Although no autopsies were performed, it appeared clinically that these patients died of hypovolaemic shock. Evidence for DIC was fragmentary, but this syndrome may well have precipitated the bleeding and shock in all cases. Postmortem liver biopsy in the first case revealed marked focal hepatic-cell necrosis with large intracytoplasmic eosinophilic inclusions. Marburg virus-like particles were visualized with an electron microscope (*2*).

Ebola virus was recovered on day 6 from blood specimens from patient 1 and on days 3 and 6 from patient 2. Quantitative virus assays on blood from the third patient are shown in Table 8. No IFA antibodies against Ebola or Marburg viruses were present.

Table 8. Virological findings in a patient treated at Ngaliema Hospital, Kinshasa, October 1976

Day of disease	Viraemia (log_{10}/ml)	IFA antibodies to:	
		Ebola virus	Marburg virus
3	5.5		
4[a]	5.5	< 2	< 2
5	5.5	< 2	< 2
	5.5	< 2	< 2
	4.5		
6	4.5		
	5.0		
7	6.5		
	4.5		
8[b]	4.5		

[a] Patient received 500 ml of anti-Marburg plasma.
[b] Patient died.

Retrospective field studies

Questionnaire forms were completed on 231 probable cases 1 year of age or older, 34 individuals who were found to have Ebola virus IFA antibodies, and 198 controls. The numbers of responses obtained for each symptom and the percentages responding positively in these groups are shown in Table 9. Fever and headache were almost invariably present. The headache often radiated to the cervical spine and was associated with low-back pain radiating into the legs. Sore throat was often reported in association with a sensation of a " ball " in the throat. Chest pain and pleuritis were uncommon. Of persons with antibodies, 59% had one or more symptoms, the most prominent being fever, headache, abdominal pain, and arthralgia. Many more persons who had been in contact with fatal cases reported symptoms but had no Ebola virus antibodies. Illness in antibody-positive individuals was, in general, marked by profound prostration, weight loss, and a convalescent period of 1–3 weeks.

The signs observed among the three study groups are also depicted in Table 9. Diarrhoea (3 or more liquid stools for 1 or more days), bleeding, and oral/throat lesions were more common in fatal cases than among survivors. History of symptoms and signs for the control group covered the interval from 1 September to 15 November 1976 and were much less frequent.

The gastrointestinal tract was the most common site of bleeding, and haemorrhage occurred more often among fatal than nonfatal infections (Table 10). Bleeding varied from melaena and slow oozing from gums to brisk haemorrhage from multiple sites in fulminating cases. Oral lesions, which were observed in a few patients, were typically herpetic, although a greyish patchy exudate was noted on the soft palate and oropharynx in one

Table 9. Percentage of symptoms and signs in fatal cases of Ebola haemorrhagic fever,[a] in persons with IFA antibodies, and in controls

	Fatal cases (Probable cases) [b]		Positive IFA [b]		Controls [b]	
	No.	%	No.	%	No.	%
Symptoms						
Fever	231	98	34	59	198	11
Headache	210	96	34	59	196	14
Abdominal pain	201	81	34	50	186	9
Sore throat	207	79	34	32	192	2
Myalgia	206	79	34	47	195	7
Nausea	178	66	30	33	187	2
Arthritis	193	53	34	38	188	8
Other [c]	42	5	23	26	115	5
Signs						
Diarrhoea	228	79	34	44	194	6
Bleeding	223	78	34	18	196	1
Oral/throat lesions	208	74	34	27	195	2
Vomiting	225	65	34	35	193	2
Conjunctivitis	208	58	34	35	195	4
Cough	208	36	34	18	192	4
Abortion	73	25	9	11	56	0
Jaundice	191	5	34	0	190	0
Oedema	193	4	34	0	194	0
Lymphadenitis	141	4	33	3	192	1
Other [d]	166	2	32	3	183	0

[a] Persons ⩾ 1 year of age.

[b] No. = total no. of cases, etc., for whom there was a response to this questions; % = percentage of cases for whom that symptom or sign was reported.

[c] Anorexia, chest pain/pleuritis, chills, tinnitis, vertigo. For each of these conditions the percentage of fatal cases was equal to or less than the figure shown.

[d] Amenorrhoea, ataxia, dark urine, dysarthria, hiccoughs, hyperhidrosis, paralysis, polyuria, rash. For each of these conditions the percentage of fatal cases was equal to or less than the figure shown.

instance. Conjunctivitis was nonpurulent, and cough appeared to be associated with oral/throat lesions rather than with lower respiratory tract pathology. One patient observed in a village on two successive days was leaning forward, restless, and complained of severe epigastric pain radiating to the back. He vomited frequently and had intractable singultus. Abortion occurred among 25% of 73 pregnant women who died. One of 9 pregnant survivors also aborted.

Eleven live infants were born to mothers who died of haemorrhagic fever. All of these children died in turn within 19 days. These cases of possible neonatal Ebola haemorrhagic fever had few signs and symptoms. Seven were said to have had fever but bleeding was infrequent. In the absence of virological and pathological data it was not possible to decide whether these deaths represented actual cases of neonatal infection or resulted from the many other causes of high infant mortality in this area.

The duration of symptoms and signs among persons with and without haemorrhagic fever is given in Table 11. Data for the " convalescent " group were by far the most reliable. Nonspecific symptoms

among the small number of controls reporting illness lasted as long as in haemorrhagic fever patients. Serum samples were obtained from 63 of the controls and none was positive for Ebola virus antibodies. Illness among fatal cases ranged from 1 to 15 days with a strong unimodal peak at 6–8 days.

The only clinical laboratory test done on patients admitted to Yambuku Hospital was urinary protein. This was reported as uniformly positive and was used as a major diagnostic criterion by the nursing sisters early in the epidemic.

Virological studies were limited. Ebola virus was isolated in African green monkey kidney cells (Vero) from blood specimens in 8 of 10 cases attempted. These specimens were taken 2–13 days after onset of symptoms. Of interest was the simultaneous detection of virus and IFA Ebola antibodies to a titre of 1 : 32 in one patient. This man was in the 13th and penultimate day of his illness. Ebola virus particles were also visualized in 3 of 4 postmortem liver qiopsies obtained from clinically suspect cases.

Table 10. Bleeding manifestations in patients [a] with Ebola haemorrhagic fever

Type of bleeding	Fatal cases (probable cases) [b]		Cases with positive IFA [b]	
	No.	%	No.	%
Melaena	210	66	33	15
Haematemesis	222	43	33	6
Mouth/gingival	215	26	33	0
Vaginal	108	20	24	4
Epistaxis	216	17	33	0
Injection sites/ scarification	197	7	33	3

[a] Persons ⩾ 1 year of age.

[b] No. = no. of cases for whom there was a response to this question; % = percentage of cases for whom this type of bleeding was reported.

Table 11. Mean duration (days) of symptoms and signs in patient [a] who died probably from Ebola haemorrahgic fever, convalescents with positive IFA titres, and controls

Symptoms and signs	Fatal cases (probable cases)		Convalescents		Controls	
	Duration	± SE	Duration	± SE	Duration	± SE
Fever	7.2	(0.28)	6.8	(1.48)	7.7	(1.87)
Headache	7.3	(0.29)	5.5	(1.04)	7.3	(1.43)
Sore throat	6.5	(0.31)	10.7	(2.57)	9.5	(4.53)
Abdominal pain	5.9	(0.30)	7.9	(2.11)	5.6	(1.16)
Myalgia	7.1	(0.30)	5.7	(1.01)	7.7	(1.27)
Arthralgia	6.5	(0.32)	9.9	(2.08)	10.8	(2.13)
Bleeding	3.5	(0.20)	9.3	(2.95)	2.0	(0)
Diarrhoea	4.9	(0.24)	7.5	(1.75)	3.5	(0.66)
Oral/throat lesions	5.5	(0.32)	5.3	(0.64)	3.7	(0.87)
Vomiting	4.0	(0.25)	3.9	(1.17)	6.8	(4.45)
Conjunctivitis	5.1	(0.40)	6.3	(1.51)	5.6	(0.98)
Cough	6.9	(0.57)	10.0	(3.98)	5.4	(1.70)
Abortion	1.7	(0.54)	7.0	(0)	0	
Oedema	2.3	(0.87)	0		0	
Jaundice	7.8	(3.25)	0		0	
Others if < 5 %	4.5	(1.03)	4.0	(0.73)	5.0	(0.98)

[a] Persons ⩾ 1 year of age.

PLASMAPHERESIS OF CONVALESCENTS

METHODS

One of the early priorities of the Commission was to obtain plasma from Ebola-virus positive individuals for treatment of patients and of any member of the investigating teams who might suffer an overt accident while working with the sick, or specimens obtained from them. The Mama Yemo Hospital in Kinshasa provided a refrigerated Sorvall centrifuge suitable for plasma separation. The original survey team brought two probable convalescent persons to Kinshasa on 27 October and another, by then proven, convalescent person arrived on 7 November. The entire operation was moved to Yambuku on 16 November and was functional four days later.

Donors were drawn from persons 16–50 years of age who had Ebola virus antibody titres of 1 : 64 or greater. Standard clinical and laboratory examinations were done. Lower limits for haematocrit were set at 35% for males and 30% for females. First and last units of plasma obtained from each donor were tested for hepatitis B virus antigen by radioimmunoassay. All donors were placed on malaria prophylaxis and given multivitamins, iron tablets, and supplementary food, and were paid a small stipend. Two units of plasma (200–300 ml) were obtained weekly in most instances.

RESULTS

A total of 201 units of plasma was obtained between 2 November 1976 and 25 January 1977. The majority were collected from 12 of the 26 persons eventually bled. Only 4 units from three donors had titres of less than 1 : 64. There were no untoward clinical reactions, and none of the donors had malaria or hepatitis B antigen; virtually all had circulating filarial larvae, principally *Loa loa*. The plasma samples were kept frozen at —15°C in Kinshasa, Yambuku, Antwerp, Johannesburg, and Atlanta pending a final decision concerning the best method of processing for long-term storage. Several specimens taken 6 weeks to 4 months after onset of symptoms were tested for Ebola virus in Vero cells with negative results. These comprised serum samples from 14 donors, throat swabbings from 13, urine samples from 11, and faecal and cervical swabbings, and semen specimens from 4, 2, and 2 donors, respectively.

Antibody titres were measured in a single test on plasma samples from 6 donors obtained 1–4 months after onset of their disease. The mean titre at one month was 1 : 256, and it was 1 : 64 thereafter. A single donor experienced a decline to 1 : 16 four months after illness.

So far, 4 units of plasma from a single donor with antibody titre of 1 : 256 have been given to 2 patients. One was a proven case of Ebola haemorrhagic fever; the other was a Commission member who suffered an illness that included fever, headache, myalgia, and rash, which remains undiagnosed but was not due to Ebola virus. Both patients had Ebola virus antibody titres of 1 : 32 one day after receiving plasma. The first case experienced a small decline in titre over the next 2 weeks followed by an increase due to autologous antibody production (*3*). The second patient had a titre of 1 : 16 one week after receipt of plasma, values of 1 : 8 and 1 : 4 at two and three weeks, and no detectable antibody at 35 days.

DISCUSSION

No more dramatic or potentially explosive epidemic of a new acute viral disease has occurred in the world in the past 30 years. The case mortality rate of Ebola haemorrhagic fever in Zaire of 88% is the highest on record except for rabies infection. In the circumstances it was not surprising that much desired information was never obtained. Delays in recognition, notification to international health agencies, and specific diagnosis of the disease contributed greatly to this outcome. No better example comes to mind to illustrate the need for national disease surveillance and the prompt solicitation of international assistance, nor of the need for the development of international resources, comprising personnel, equipment, transport, communication, and finance, that can be made available in a very few days to cope with such emergencies.

Although laboratory data were virtually nonexistent, the clinical picture seen in this outbreak resembled illness produced by the related Marburg virus. If anything, the evolution of Ebola haemorrhagic fever appeared to be more inexorable and less

variable than Marburg virus infection. Though far from proven, we suspect that acute defibrination syndrome and pancreatitis were major features of the syndrome and severe liver disease was evident.

At the time of its formation on 18 October 1976, the International Commission knew that the epidemic was of at least 7 weeks duration, that the Yambuku mission hospital was nonfunctional because many of the personnel had died of the disease and that transmission was occurring in Kinshasa. We suspected, but did not know, that the fatality rate of the disease was very high. There was no information concerning secondary infection rates and it was impossible to foretell that the last case during this epidemic would die within 3 weeks.

In contrast to observations made simultaneously in Sudan, the illness in Zaire had fewer respiratory symptoms, a shorter clinical course, and a higher fatality rate (*4*). Whether this was due to differences in the virulence of the virus *per se* or to host and ecological variables such as climate (relative humidity) is not known. At the present time the agents recovered from Sudan and Zaire are thought to be identical, although definitive neutralization tests have not yet been done.

Viraemia appears to be a constant feature of Ebola virus infection in man. The virus persisted in large amounts in the blood in the single, well studied case. The finding of both virus and antibodies in the blood of another agonal case 13 days after onset of symptoms raises the possibility, regarded as unlikely, that antigen–antibody complexes may contribute to the pathology of infection. This and a number of other important virological questions can only be pursued for the moment through studies using monkeys. One of the most pressing is the need for a way to make rapid diagnosis in suspected cases of the disease by searching for cells containing viral antigen. Retrospective specific diagnosis of fatal cases by electron microscopic examination of formalin-fixed liver biopsies appears quite promising and should be attempted in all cases of acute febrile haemorrhagic disease in Africa.

No evidence was obtained for persistent viral carriage in the Zaire cases of Ebola infection, a phenomenon documented on two occasions for Marburg virus (*5*, *6*). But it should be remembered that the number of appropriate, immunosequestered sites sampled was very small. However, semen from one patient infected with a Zaire strain of Ebola virus in the United Kingdom contained virus for more than 2 months after onset of symptoms (*3*).

The Zaire epidemic had all the attributes of a common source outbreak, together with a fortunately low rate of secondary person-to-person transmission. The means by which the virus was introduced into Yambuku Mission Hospital will probably never be precisely known, but it seems possible that it was brought directly from the Sudan by man. Dissemination of the agent into the villages of the region was principally through contaminated equipment used for parenteral injections. The epidemic waned when the hospital was closed for want of medical staff. That careful disposal of contaminated excreta and fomites, as well as strict barrier nursing using respirators, could break the chain of transmission was demonstrated during the small outbreak in Kinshasa. Still simpler isolation precautions and a change in the cultural customs at funerals appears to have contributed to the dying out of infection in the villages.

Although the data were not always statistically convincing, we had the strong impression that Ebola haemorrhagic fever acquired by injection differed from that due to contact with another case. The mortality was higher. In one study, secondary transmission rates also were higher from index cases that were parenterally induced. It may be that increased virus replication and excretion following parenteral infection accounts for all or most of these differences, but other causes were by no means excluded.

The observed " neonatal " cases of the disease were not definitively elucidated. One wishes to know whether Ebola virus can pass through the placenta and infect the fetus, and whether virus is present in human milk and is infectious if ingested.

Finally, a better method for measuring Ebola virus antibodies is needed in order to interpret the serological findings reported here. That less than 20% of persons gave a history of acute illness following contact with a fatal case was no surprise. Most of these persons had mild, self-limiting diseases, these being highly endemic in the area. But if the IFA data are correct, at least 2.5% of persons in contact with fatal cases experienced subclinical infection. In addition, the finding of antibodies in a few individuals in the absence of any known contact with Ebola virus during the epidemic raises the possibility that the agent is in fact endemic in the Yambuku area and is occasionally transmitted to man. A definitive answer is essential to further ecological exploration of what is now a very mysterious agent. As in the case of Marburg virus, the source of Ebola virus is completely unknown beyond the simple fact that it is African in origin.

RECOMMENDATIONS

The International Commission was disbanded on 29 January 1977. At that time it made the following recommendations to the Government of the Republic of Zaire:

1. Maintain active national surveillance for acute haemorrhagic disease. Require regular positive and negative reporting. Investigate all suspected cases and take appropriate action including collection of diagnostic specimens, the institution of clinical isolation procedures, and the use of protective clothing for medical personnel.

2. Distribute pertinent information to medical and other personnel participating in surveillance and update this material by appropriate documents.

3. Organize a national campaign to inform health personnel of the proper methods for sterilizing syringes and needles in order to ensure that patients are not infected with diseases from other patients as a result of poor technique. Reconsider the need for parenteral injections when patients can take medicines by mouth. Prohibit or strictly regulate the activities of itinerant nurses who treat all diseases by injection.

4. Maintain a list of experienced Zairian personnel so that appropriate action can be taken without delay in the event of a new epidemic.

5. Maintain a stock of basic medical supplies and protective clothing for use in future suspected outbreaks.

6. Keep plasma from immune donors in readiness. Make standardized clinical observations and obtain serial serum specimens following use of plasma in the treatment of suspected cases of Ebola haemorrhagic fever in order to obtain further information concerning the effectiveness of this treatment.

Commission members also participated in a consultation sponsored by WHO in January 1977. Detailed recommendations were made for dealing with future outbreaks wherever they may occur, as well as for continued investigations of the biology of Ebola virus. These suggestions have been published (*7*), and are wholeheartedly endorsed by this Commission.

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The detailed contributions of governments, agencies, and individuals are too lengthy to enumerate here. The government of Zaire made a continuous, concerted effort to provide leadership, personnel, transport, and supplies. Belgium, Canada, France, South Africa, the United Kingdom, the United States of America, and the World Health Organization (WHO) all sent materials and supplies. Critical administrative and logistic roles were assumed in Kinshasa and Bumba Zone by personnel of FOMECO (Fonds Médical de Coordination), FOMETRO (Fonds Médical Tropical, Belgium), Mission Médicale Française, the US Agency for International Development, and WHO. Mr N. Staehling and Mrs E. Duckett of the Center for Disease Control, Atlanta, kindly provided statistical assistance. We wish to thank particularly the following individuals who performed vital roles in maintaining communications and supplies, without which the work of the Commission could not have been performed: Mrs G. Allen, Mr L. Armour, Father L. Claes. Mr. G. Cook, Father G. A. Slegers, Mr J. Jansens, Mr T. Lindland, Mr J. Masters, Father C. Rommel, Father G. Six, and Ms L. Welch.

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RÉSUMÉ

FIÈVRE HÉMORRAGIQUE EBOLA AU ZAÏRE, 1976

Entre le 1er septembre et le 24 octobre 1976, 318 cas de fièvre hémorragique virale aiguë se sont produits dans le Zaïre septentrional. La poussée avait son centre dans la Zone de Bumba, Région de l'Equateur, et la plupart des cas ont été observés dans un rayon de 70 km autour de Yambuku; quelques malades cependant se sont fait soigner à Bumba, à Abumombazi et à Kinshasa, la capitale, où on a enregistré des cas isolés secondaires et tertiaires. Il y a eu 280 décès, et seulement 38 survivants dont l'infection a été confirmée sérologiquement.

Le cas initial avait ressenti les premiers symptômes le 1er septembre 1976, cinq jours après une injection de chloroquine pour paludisme présumé faite au service de consultation externe de l'Hôpital de la Mission de Yambuku et suivie de rémission clinique des symptômes de paludisme. Dans l'espace d'une semaine, plusieurs autres personnes à qui des injections avaient été administrées, au même hôpital, ont été également atteintes de fièvre hémorragique d'Ebola, et presque tous les cas ultérieurement observés ou bien avaient reçu des injections à l'hôpital, ou bien avaient été en contact étroit avec un autre malade. La plupart de ces cas se sont produits au cours des quatre premières semaines de l'épidémie, après quoi l'hôpital a été fermé, 11 des 17 membres de son personnel ayant succombé à la maladie. Des sujets de tout âge et des deux sexes ont été affectés, mais l'incidence la plus élevée a été observée chez les femmes de 15 à 29 ans, et on a de fortes raisons d'associer ce fait à la fréquentation des consultations prénatales et des consultations externes de l'hôpital, où ces femmes avaient reçu des injections. Le taux général d'atteinte secondaire a été d'environ 5‰, mais il s'élevait à 20% parmi les personnes étroitement apparentées au malade, telles que conjoint, parents, enfants, frères ou sœurs.

La surveillance active a permis de constater que des cas s'étaient produits dans 55 villages sur un total d'environ 550 qui ont été examinés maison par maison. La maladie en cause était jusqu'alors inconnue de la population de la région affectée. La recherche intensive des cas dans la région du nord-est du Zaïre située entre la Zone de Bumba et la frontière du Soudan, près de Nzara et de Maridi, n'a pas permis d'établir la preuve certaine d'un lien entre une épidémie de la même maladie dans ce pays et la poussée qui s'est produite autour de Bumba. On a toutefois établi que les gens peuvent faire, et font effectivement, le voyage entre Nzara et Bumba en quatre jours: il paraît donc tout à fait possible qu'une personne infectée se soit rendue du Soudan à Yambuku et qu'elle ait contaminé par le virus une aiguille de seringue lors d'une injection subie à la consultation externe de l'hôpital.

La durée de la période d'incubation comme celle de la maladie clinique ont été en moyenne d'une semaine. Après avoir présenté pendant 3-4 jours des symptômes et signes non spécifiques, les malades ont eu progressivement de fortes douleurs à la gorge, des éruptions maculopapuleuses et de douleurs abdominales irréductibles, et commencé à présenter des saignements à des sièges multiples, principalement dans le tractus gastro-intestinal. Bien que les analyses de laboratoire qui ont été faites aient été limitées, ne permettant pas de conclusion, on a estimé que la pathogenèse de la maladie comprenait une hépatite non ictérique et éventuellement une pancréatite aiguë ainsi qu'une coagulation intravasculaire diffuse.

Le syndrome en question était dû à un virus morphologiquement analogue au virus Marburg, mais immunologiquement distinct de celui-ci, virus qu'on a appelé Ebola. Cet agent a été isolé dans le sang de huit cas, sur un total de dix cas suspects, en cultures de cellules Vero. Les titrages de spécimens successifs prélevés sur un malade ont révélé une virémie persistante de $10^{6,5}$ à $10^{4,5}$ unités infectantes à partir du troisième jour de la maladie et jusqu'au décès, survenu au huitième jour. Des particules de virus Ebola ont été trouvées dans des spécimens hépatiques fixés au formol, provenant de trois cas. On a constaté par l'épreuve de la fluorescence indirecte que les survivants présentaient des titres d'anticorps anti-virus Ebola de 1 : 64 à 1 : 256 dans les trois semaines suivant le début de la maladie, et ces titres persistaient, avec seulement une faible diminution, pendant une période de quatre mois.

Au total on a obtenu et congelé 201 unités (de 200-300 ml chacune) de plasma contenant des anticorps anti-virus Ebola au titre d'au moins 1 : 64. Deux de ces unités ont servi à traiter un travailleur de laboratoire infecté par le virus Ebola. Le sujet a guéri, ce qui fait penser que les anticorps peuvent avoir joué un rôle thérapeutique.

On a pu interrompre la transmission du virus en cessant les injections et en isolant les malades dans leurs villages. L'utilisation de vêtements protecteurs et de masques respiratoires, un strict isolement des malades et une soigneuse élimination des excreta et des objets éventuellement contaminés devraient presque certainement permettre d'éviter de nouvelles grandes poussées de la maladie. Il est probable que le virus est rarement transmis par des aérosols contaminés, mais l'éventualité d'une infection par des gouttelettes de grande taille ne peut pas être écartée.

On n'a fait que des enquêtes écologiques limitées, puisque l'épidémiologie de la poussée fait fortement penser que dans la Zone de Bumba, le virus avait été importé. Le virus Ebola a été mis en évidence dans des échantillons représentatifs de punaises de lit ou de rongeurs (*Rattus rattus* et *Mastomys* spp.) ayant des contacts plus ou moins étroits avec l'homme. Mais on a aussi mis en évidence des anticorps anti-virus Ebola chez cinq

personnes qui n'avaient pas été malades et n'avaient pas eu de contact avec les villages « infectés » ou l'hôpital de Yambuku au cours de l'épidémie. Si elles pouvaient être confirmées par une autre méthode d'épreuve, ces observations pourraient indiquer qu'en fait le virus existe à l'état endémique dans la région, ce qui devrait inciter à entreprendre de nouveaux efforts pour découvrir un réservoir de virus au Zaïre.

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Annex 1

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