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Ebola haemorrhagic fever in Sudan, 1976

Report of a WHO/International Study Team [1]

A large outbreak of haemorrhagic fever (subsequently named Ebola haemorrhagic fever) occurred in southern Sudan between June and November 1976. There was a total of 284 cases; 67 in the source town of Nzara, 213 in Maridi, 3 in Tembura, and 1 in Juba. The outbreak in Nzara appears to have originated in the workers of a cotton factory. The disease in Maridi was amplified by transmission in a large, active hospital. Transmission of the disease required close contact with an acute case and was usually associated with the act of nursing a patient. The incubation period was between 7 and 14 days. Although the link was not well established, it appears that Nzara could have been the source of infection for a similar outbreak in the Bumba Zone of Zaire.

In this outbreak Ebola haemorrhagic fever was a unique clinical disease with a high mortality rate (53% overall) and a prolonged recovery period in those who survived. Beginning with an influenza-like syndrome, including fever, headache, and joint and muscle pains, the disease soon caused diarrhoea (81%), vomiting (59%), chest pain (83%), pain and dryness of the throat (63%), and rash (52%). Haemorrhagic manifestations were common (71%), being present in half of the recovered cases and in almost all the fatal cases.

Two post mortems were carried out on patients in November 1976. The histopathological findings resembled those of an acute viral infection and although the features were characteristic they were not exclusively diagnostic. They closely resembled the features described in Marburg virus infection, with focal eosinophilic necrosis in the liver and destruction of lymphocytes and their replacement by plasma cells. One case had evidence of renal tubular necrosis.

Two strains of Ebola virus were isolated from acute phase sera collected from acutely ill patients in Maridi hospital during the investigation in November 1976. Antibodies to Ebola virus were detected by immunofluorescence in 42 of 48 patients in Maridi who had been diagnosed clinically, but in only 6 of 31 patients in Nzara. The possibility of the indirect immunofluorescent test not being sufficiently sensitive is discussed.

Of Maridi case contacts, in hospital and in the local community, 19% had antibodies. Very few of them gave any history of illness, indicating that Ebola virus can cause mild or even subclinical infections. Of the cloth room workers in the Nzara cotton factory, 37% appeared to have been infected, suggesting that the factory may have been the prime source of infection.

In response to a request from the Government of the Democratic Republic of the Sudan, WHO sent a team to investigate an outbreak of haemorrhagic disease in the townships of Nzara and Maridi in the north of Sudan, close to the Zairian border. This team worked in close collaboration with Sudanese colleagues and with staff provided by other governments. This report describes, in separate sections, the outbreak and the investigations undertaken, the epidemiology of the outbreak, the clinical manifestations, the pathological studies, and the virological and serological investigations undertaken in the human population.

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THE OUTBREAK AND INVESTIGATION

In Sudan, the first cases of haemorrhagic fever are thought to have originated in Nzara township in three employees of a cotton factory situated near the town centre. The factory forms part of a large agricultural cooperative with 2000 employees. A staff of 455 is employed in the factory, which produces cotton cloth from raw cotton grown in small holdings throughout the region.

The first identifiable case was YG, a storekeeper in the factory, who became ill on 27 June 1976 with a severe febrile illness, headache, and chest pains. He developed haemorrhagic manifestations on the fifth day of illness with profuse bleeding from the nose and mouth, and bloody diarrhoea. He was admitted to hospital in Nzara on 30 June and died on 6 July. During his illness at home he was nursed by his brother, who in turn became ill on 13 July. The brother's symptoms were similar but he recovered after a two-week illness and was able to give a description of both illnesses. A second storekeeper, BZ, who worked alongside YG, was admitted to Nzara hospital on 12 July and died on 14 July. Soon afterwards his wife also became ill and died at home on 19 July. Both had had severe febrile illnesses complicated by bleeding. The third man from the factory to fall sick was PG, who was employed in the cloth room beside the store where YG and BZ worked. PG became sick on or around 18 July and was admitted to Nzara hospital on 24 July; he died on 27 July. Although all three men were employed in the same section of the factory, their homes were far apart and their life-styles were very different. There appeared to be no social contact between any of them. YG lived 10 km south of Nzara in a remote rural homestead while BZ lived 3 km east of the town. Both men lived quietly with their families and had few friends or contacts within their home environments. The third man, PG, was a bachelor who lived in the centre of Nzara close to a shop belonging to a general merchant, MA. PG was reportedly an ebullient character, known to almost everyone in the area and he was closely associated with the merchant's family and employees. He was particularly friendly with two brothers, Samir S and Sallah S, who were staying in the merchant's household. The three young men helped in the shop and organized parties and dances in the area. During PG's illness he was visited and comforted by many people including two women, HW and CB, who nursed him before he was admitted to hospital.

Samir S became ill on 26 July, and after a few days in Nzara he travelled with his brother, Sallah, to Maridi on 6 August. There he became so ill that he was admitted to Maridi hospital on 7 August where he died on 17 August. Sallah helped to care for Samir in Maridi hospital and then returned to Nzara on 18 August, when he too had begun to feel ill. Meanwhile in Nzara four close contacts of PG—the two women HW and CB, together with SU, another cotton factory employee and close friend of PG, and RJ a nurse at Nzara hospital—had become ill and died from the disease. They in turn infected several others who had nursed them during their illnesses at home. Sallah S arrived in Nzara on 18 August and was so ill the following day that he was visited by a hospital nurse, AI, who administered chloroquine and antibiotic injections. Sallah and one of the merchant MA's sons died later in the same house from the disease. The nurse, AI, fell ill on 24 August and was eventually taken to Maridi hospital where he died on 3 September. The merchant, MA, became ill on 21 August and went for treatment to Omdurman, travelling by road to Juba, and thence by plane to Khartoum. He died in Omdurman hospital on 30 August. Shortly after he left Nzara, several of his family and employees also contracted the same illness. They, in turn, sequentially infected several others and small pockets of infection were set up around Nzara.

It was possible to relate 48 cases and 27 deaths in Nzara to the original infection in PG, all acquired by direct close contact, usually involving nursing and care of an infected individual. However, in July, September, and October, further unrelated cases continued to occur in cotton factory employees for which no direct contact with previously sick persons could be established. They, in turn, infected members of their own families but, as each family lived in a relatively remote homestead, few contacts outside their families were involved, and these pockets of infection were self-limiting. The onset of the last recorded infection in Nzara was reported to have been on 27 October 1976.

The disease was introduced to Maridi, 128 km

away, by Samir S when he was admitted to Maridi hospital on 7 August 1976. A close friend, a hospital nurse, a hospital cleaner, and a hospital messenger, all of whom had had close contact with Samir during his illness, all developed the same disease and were admitted to various wards in the hospital. Hospital contacts of these patients, including visitors to the hospital who often helped to care for patients, seeded the infection around Maridi township. A further focus of infection was introduced to Maridi hospital on 29 August when AI, the nurse from Nzara, was admitted with the same disease. The hospital served as an efficient amplifier from which the virus was disseminated throughout the town. The number of cases gradually increased until mid-September and at the end of the month there was a large number of cases, particularly in hospital staff. The number of cases declined in early October, possibly as a result of the use of protective clothing. A considerable increase in the number of cases was observed in late October and early November, which may have been partly due to lack of protective clothing when supplies ran out in mid-October. These later cases were more frequent in individuals who were not employed in the hospital, but who had been in contact with patients either in hospital or in their homes.

THE INVESTIGATION

Members of the WHO team arrived in Maridi on 29 October 1976. At the time of their arrival the principle objectives were:

1. To determine as quickly as possible the method of spread of the infection within the human population; particular emphasis to be directed towards interrupting the chain of infection.

2. Any active focus of infection to be investigated immediately and control measures instituted.

3. Houses where known and possible cases occurred to be identified and accurately located on a map of the area; all contacts and possible contacts to be identified and their movements recorded.

4. As no specific therapy was available, all convalescent patients to be identified and, if possible, bled to provide stocks of immune plasma.

5. All possible contacts to be bled for antibody studies, and a random sample of the population also to be bled for similar investigations.

6. Extra-human reservoirs of the virus to be actively sought.

7. Protective clothing to be provided for the investigating teams and all hospital staff caring for patients.

Immediate contact was made with Sudanese colleagues. It was quickly evident that the deteriorating situation in Maridi needed immediate attention, whereas Nzara, where only sporadic cases remained, did not present such an acute problem. Maridi hospital was a cause of grave concern, with a worsening situation resulting from the fear and alarm caused by the disease among nursing staff and the local people. There were almost no patients left in the hospital, since most of them had either absconded or been forcibly removed by their relatives. Very few nurses were reporting for duty and there was obvious discontent among those present. The alarm and near panic among hospital staff could be readily understood when it was found that the doctor-in-charge and 61 members of the 154 nursing staff had developed the disease, and 33 had died. Eight further deaths had occurred in hospital cleaning and ancillary staff. There was certainly justification for the feeling among the local community that the hospital was the prime source of the outbreak in the town.

The WHO and Sudanese investigators quickly agreed that the first priority was to understand the epidemiology of the disease. Much useful information about the spread of the illness was obtained from nurses who were themselves recovering from the disease in their own homes. In Maridi the disease was spread by direct contact from person to person, and it affected particularly those in very close contact with patients with active disease. The most urgent task was to find all the cases of active infection in the community and isolate them as quickly as possible.

Adequate isolation premises had been completed just before the WHO team's arrival. Situated in the hospital grounds, some distance from existing buildings, they consisted of two separate wards made of mud and wattle surrounded by a high fence. Access to the isolation wards was strictly limited to authorized staff. Nursing staff who had had the infection and had now fully recovered were persuaded to return to work and undertake the care of the patients in isolation. Ample supplies of disposable gowns, gloves, masks, and caps (donated by CDC, Atlanta; the Microbiological Research Establishment, Porton;

and the Government of the Federal Republic of Germany) were distributed to the hospital staff. Full-face biological respirators, goggles, and naso-oral respirators were also issued. The staff were carefully instructed about the use of protective clothing and its subsequent decontamination.

The next task was to find all cases of the disease. A large surveillance team of 30 people, including schoolteachers and senior schoolboys, was quickly recruited. The team was divided into groups of six, each under the care of a Public Health Officer or Public Health Inspector. Each group was issued with protective clothing and dispatched at once to visit every homestead in Maridi to seek out cases. They were specifically instructed *not* to enter any house, so as to minimize the chance of any of the team becoming infected. A large number of cases of active infection were soon discovered; each was reported to the Sudanese officials and an ambulance accompanied by a Public Health Officer was sent to the house. Patients were persuaded to enter the isolation wards at the hospital. Some refused, and in these cases the relatives were warned of the grave risks, and advised to restrict close contact with the patient, and to limit it to only one close relative or friend. Protective clothing was offered but usually refused.

At the same time, an early meeting of all the local chiefs and subchiefs was called in the District Inspector's Office in Maridi. The nature of the disease and its method of spread was fully explained. The urgent necessity to isolate all patients in order to prevent further spread was pointed out and accepted by the chiefs, who promised all possible assistance. The response from local administrative officials greatly facilitated the surveillance teams, which were soon able to expand their investigations to areas outside Maridi. By 17 November all areas within a 30-mile radius of the town had been covered.

In addition to tracing all active cases of infection, the surveillance teams directed their attention to finding all past cases of infection and ascertaining their contacts. All recovered cases who could be traced were approached regarding the possibility of obtaining immune plasma. A considerable number of convalescent patients volunteered, and within three weeks, with the very considerable assistance of Dr J. Knobloch and Dr Dorothea Lutter, 51 fully recovered patients were bled, using a modified plasmapheresis technique, to obtain aliquots of immune plasma. Also, all known and possible contacts of cases were bled whenever possible for antibody studies. In addition, serum samples were collected from a reportedly unexposed control population in an attempt to build up an overall picture of the disease pattern.

The clinical care of patients in the isolation premises remained the responsibility of Sudanese medical staff, although WHO team members did collect throat swabs, blood samples, and urine from patients in the early stages of illness for virological studies. No specific therapy, such as the use of immune plasma, was given because team members could not be sure that the plasma available did not still contain active virus. Attention was directed particularly towards maintaining fluid and electrolyte balance.

Two post mortems were carried out by WHO team members; specimens of liver, spleen, kidney, heart, lung, brain, and bone marrow were collected for virus isolation, histopathology, and electron microscopy.

As soon as the situation in Maridi appeared to be improving, attention was directed towards Nzara, where epidemiological evidence suggested that the disease had originated. The cotton factory received particular attention. Six cases of infection had occurred in the cloth room and adjacent store, 3 in the weaving section, 1 in the drawing-in section, and 2 among maintenance staff. Only one of these appeared to have had contact with a previous case within the estimated incubation period. One other factory worker developed the disease and he had had direct contact with a previous case. Thus out of 24 employees in the cloth room, 6 (25%) were infected, while only 10 workers (4%) in the weaving section developed the disease, and there were no infections among 184 employees in the spinning section.

The concentration of apparently primary cases suggested that infection was acquired by these workers at their place of employment, either from direct or indirect animal contact. Rodents (particularly *Rattus rattus*) were numerous throughout the factory, and the roof-space housed large populations of the insectivorous bat *Tadarida* (*Mops*) *trevori*. Rodents and bats were collected especially from the area around the cloth room, and blood and tissues removed for virological studies. Members of the factory staff were bled for serological evidence of infection. Careful surveillance was also carried out in Nzara to trace all case-contacts, and, wherever possible, blood was obtained for serological study.

The possibility that the virus might have been transmitted by an arthropod vector was also con-

sidered, and limited mosquito collections were carried out in Nzara and in rural homesteads outside the township. Mosquitos were pooled by species, and frozen in liquid nitrogen for attempts at virological isolation.

No active cases were found in Nzara and attention was focused once more on Maridi. The active surveillance and isolation procedures were extremely successful, and the last known case of infection was admitted to Maridi hospital on 25 November 1976. No further cases of infection have been reported in Sudan since then.

EPIDEMIOLOGY

Epidemiological investigation of the outbreak in Sudan involved finding all of the cases and recording pertinent information. Several tactics were used to find as many cases as possible:

1. examining hospital records,

2. visiting homes of patients and searching for other cases or their contacts,

3. searching house-to-house in the infected areas for additional cases, and

4. contacting local chiefs for information.

A case was defined as anyone:

1. having the symptoms of fever and headache lasting for at least two days with the addition of gastrointestinal symptoms (diarrhoea or vomiting) or chest pain; or

2. diagnosed by a physician in a hospital.

Using this definition, a total of 284 cases occurred in Sudan between June and November 1976 — 67 in Nzara, 213 in Maridi, 3 in Tembura, and 1 in Juba.

DISTRIBUTION OF CASES

Nzara

The origin of the epidemic was Nzara, a small town with clusters of houses scattered in the dense woodlands bordering the African rain forest zone. The total population of the area within 16 km is estimated to be about 20 000, most of whom live in mud-walled, thatched-roofed houses surrounding the town proper. The main employer in Nzara is a large agricultural corporation which has 2000 employees, half of whom work in a large cotton factory in the town. The corporation has excellent records of employee absenteeism and these facilitated the investigation. A small hospital is operative in Nzara, but at the time of the outbreak the facilities were limited and few patients were admitted.

Discussions with local people and a review of the factory records for the previous two years did not reveal any fatal haemorrhagic disease in Nzara until late June or early July, 1976. At that time, one or two factory workers started dying each week of haemorrhagic disease and subsequently their families or friends who cared for them would manifest the same symptoms. By the first week in September, 6 factory workers and 25 of their contacts had developed the same syndrome and 21 had died. Of the 6 factory employees, 5 worked in one particular end of the cotton factory. Extensive discussions with friends and families of these workers did not reveal any possible link between them except the factory. None had cared for any pre-existing cases of the disease, none had had a previous illness for which they might have received an injection with a contaminated needle, and none had had any known contact with monkeys or any other wild animal. Their houses were widely scattered over the area and their social circles very different.

Since their only link was the cotton factory, the investigation for an animal reservoir of infection was concentrated in Nzara and specifically in the cotton factory itself. The results of this investigation will be reported elsewhere.

Tembura

The outbreak in Nzara continued until late October, infecting a total of 67 people, of whom 31 (46%) died. Before the outbreak died out spontaneously, cases were exported to two neighbouring areas. One was Tembura, a small town 160 km to the north where an ill woman went to be nursed by her family. Subsequent to her death, three women who cared for her died of the same haemorrhagic disease. No subsequent cases were discovered in the area, despite active searching.

Maridi, Juba, and Khartoum

The other exportation was to Maridi with a very different outcome. Maridi, a town with an estimated

Fig. 1. Map of Sudan and Zaire showing main centres of infection (Scale, 1 cm = 450 km, approximately).

population of 10 000 with another 5000 people in its environs, is located about 180 km east of Nzara, between Nzara and the regional capital of Juba (Fig. 1). The hospital in Maridi, in contrast to the one in Nzara, is an active hospital with a large staff, and serves as a teaching hospital where student nurses are taught patient care. With its staff of 230, it served as an ideal centre for the large, hospital-associated outbreak that followed the introduction of the disease from Nzara. After Maridi was infected, four cases (one from Nzara and three from Maridi) were transferred to the regional hospital in Juba. In late September, three of these cases were flown to Khartoum (1200 km north), where two died. Fortunately, only one secondary case, in a nurse from Juba, occurred as a result of these exportations.

MORTALITY

A total of 284 cases occurred with 151 deaths (mortality rate, 53%). All but 4 of the cases were in Nzara and Maridi. In these two areas, the clinical disease appeared identical and the mortality rates (46% in Nzara and 54% in Maridi) very similar (Table 1). Moreover, the mortality rate stayed relatively constant through the 5 months of the outbreak, during which approximately 15 generations of person to person transmission occurred. Monthly mortality ranged from 63% in October to 40% in November, with intermediate rates in the previous months. The mortality rate by age and sex showed similar, but insignificant, variations. For children, teenagers, and adults it was 44%, 39%, and 56%, respectively, while the overall mortality rate for males was 56%, and for females 48%.

COMPARISON OF OUTBREAKS AT NZARA AND MARIDI

The similarity of the clinical disease and its mortality in Nzara and Maridi is contrasted with the types of outbreak in the two locations. The Nzara outbreak was centred on factory workers and spread to their families. In Maridi, the hospital served both as the focus and amplifier of the infection. After two

Table 1. Mortality rate by month of onset

	July		August		September		October		November		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Nzara	8[a]	4	21	14	34	12	4	1	0	0	67	31
	—		67%		35%		—		—		46%	
Maridi	0	0	9	3	104	53	80	52	20	8	213	116
	—		—		51%		65%		40%		54%	
Total	8	4	30	17	138	65	84	53	20	8	280	147[b]
	—		57%		47%		63%		40%		53%	

[a] Includes 1 fatal case with onset during the last week of June.
[b] Does not include 4 fatal cases — 3 from Tembura and 1 from Juba.

Table 2. Sources of infection

Location	Total no. of cases	Source of infection [a]		
		Hospitals	Homes	Unknown
Nzara	67	2 (3%)	51 (76%)	14 (21%)
Maridi	203 [b]	93 (46%)	105 (52%)	5 (2%)
Other	4	1	3	0
Total	274	96 (35%)	160 (58%)	18 (7%)

[a] Numbers of cases (percentage of total from same location).
[b] 10 cases not investigated (total no. of cases = 213)

separate importations from Nzara (the first during the last week of July, and another during the first week of September), Maridi hospital workers became ill, and they were in turn hospitalized. Those caring for them were then infected, and the cycle repeated itself. The difference between the Nzara and the Maridi outbreaks is best exemplified by examining the focus where patients most probably became infected. Few patients (26%) were even hospitalized in Nzara, and they seldom stayed more than a few days, but in Maridi, almost three-quarters of the patients were hospitalized, and often for more than two weeks. As a result, Maridi hospital was a common source of infection (46% of cases), whereas the Nzara hospital was not (3% of cases) (Table 2).

The outbreaks reflected the initial seeding of infection in each locality. That in Maridi was larger (213 cases) and accelerated rapidly after the initial importation within the hospital, whereas that in Nzara was smaller (67 cases) and more sustained. The age-specific attack rates in Maridi were higher (overall 14.2 cases per 1000 population compared with 3.4 cases per 1000 in Nzara) and the cases were primarily adult males, which reflected the predominance of male staff in the hospital (75%). In contrast, Nzara had equal attack rates in adults and had a moderate number of teenage male cases. These teenage cases were all associated with a single chain of transmission that links Nzara to Maridi and Juba.

TRANSMISSION

The transmission of Ebola viral haemorrhagic fever at Nzara and Maridi was similar in that it required intimate contact with a previous case. There was seldom any problem in determining the source of infection for a case. Usually the possible source (or sources) was well known to the patient being investigated. This is well documented in Maridi where sources were determined for all but 5 of 203 patients investigated (Table 2). This contrasts with and re-emphasizes the uniqueness of the Nzara outbreak where no contact with a previous case could be established for 14 (21%) of 67 cases. Nine of these were employees of the cotton factory, and possibly represent the initial introduction of the disease into the human population of the area.

Once the infection was in humans, transmission from one person to the next was not rapid. It required close and usually prolonged contact with an acutely ill patient. Transmission did not seem to occur via the airborne route. To better define the pattern of transmission, we took a small sample of 17 highly-infected households and studied the type of contact that resulted in spread of infection. In this group, all secondary cases had slept in the same room, and all secondary cases had touched the primary case during the acute stage of the disease. However, touching and sleeping in the same room were associated with a relatively low attack rate (23%) (Table 3), whereas actually nursing the case greatly increased the chances of becoming infected (81%). This requirement for close contact explains the lack of cases in children, who, although sleeping in the same room, did not become infected. No

Table 3. Attack rate in household contacts sleeping in the same room as an infected case Maridi

Risk factor	No. of contacts at risk	No. of contacts subsequently acquiring Ebola haemorrhagic fever	Attack rate (%)
Touching patient	23	5	23
Nursing patient	48	39	81

casual infections in passers-by or in contacts of primary contacts were observed.

HOSPITAL SPREAD

From these observations, it is evident that nursing a patient was almost a requirement for becoming infected (39 of 48 instances in Table 3). Therefore, a hospital should be, and was, an ideal environment in which to transmit the disease. At least one-third of the staff of Maridi hospital had the disease and 41 staff members died. All of the 6 medical assistants were infected and 41% of the student nurses. Before the disease was recognized, most wards of the hospital had haemorrhaging patients in them, and at the height of the epidemic the hospital was in chaos. Altogether, 93 of Maridi's 213 patients acquired their disease in the hospital. Most of these (72) were hospital staff infected during their duties. At least 6 others were patients who were infected by contact or injection with infectious material from nearby acutely ill patients. Fifteen additional people probably received their infection as visitors of infectious patients in hospital. All of them helped care for an acutely ill patient during their visit to the hospital.

SECONDARY ATTACK RATES IN THE COMMUNITY

Once out of the hospital and into the community, the disease spread in a similar manner, but did not have the large substrate on which to feed that the hospital provided. We studied 36 families with 38 primary cases and listed contacts that resided in the same house. The original 38 cases had 232 contacts, of whom 30 (13%) developed subsequent disease. Similar rates (14% and 9%) were observed in the subsequent generations, giving an overall secondary attack rate of 12%. These results document the relatively low rate of transmission of this disease.

INCUBATION PERIOD

Since most cases had had prolonged contact with their source case, it was difficult to make accurate measurements of incubation periods. However, 11 cases were discovered in whom relatively short exposure times were documented and subsequent onset of disease accurately recalled. From these cases, and the lack of any documented extremely short or long generation times, we concluded that the incubation period was usually between 7 and 14 days.

CONTROL

The control of this outbreak in the Sudan relied on the classic public health principles of identification and isolation. The outbreak in Nzara died out spontaneously. However, the outbreak in Maridi required intervention. Strict barrier nursing was established initially in early October 1976, and reinforced with additional disposable isolation equipment in mid-October. In early November, surveillance teams were established to search house-to-house in Maridi, and any patients discovered were placed in a specially constructed isolation ward. With time, the surveillance was expanded to include most of Western Equatorial Province. The last known case in Sudan occurred on 20 November 1976.

LINK BETWEEN SUDAN AND ZAIRE

An outbreak of Ebola fever also occurred in Zaire during the latter half of 1976 (see the article on pages 271-293 of this issue). The first recognized case was in September. Despite extensive efforts, the exact link between the outbreak of viral haemorrhagic fever in Nzara, Sudan, and Bumba Zone, Zaire, remains undetermined. There is no doubt that there is extensive traffic in commercial goods between the two areas. In fact, we interviewed one truck driver in Nzara who personally escorted an early case in Nzara to hospital on 25 July 1976 with the help of two other friends. The two friends developed disease on 1 and 3 August 1976, respectively, and died 10 days later. The driver left the day after aiding his friend, and arrived in Bumba 4 days later. He stated he had no disease, and that he knew of no one who travelled with him who had become ill. It is very possible that several other people who had had similar close contact with sick patients travelled from Nzara to Zaire during the course of the outbreak in Nzara. One of them may have become ill and set up a secondary chain of infection in Zaire.

DISCUSSION OF EPIDEMIOLOGY

Ebola fever is a newly recognized disease with many similarities to two other haemorrhagic diseases (Lassa and Marburg fevers) that are known to spread from person to person (*1*, *2*, *3*, *4*). These three diseases, although caused by different agents, are clinically similar, have high mortality rates, and are all spread among humans by close contact with infected effluvia from acutely ill patients. The most likely sources of infectious virus responsible for this person-to-person transmission are blood (or blood-containing excreta) and urine. Respiratory spread has been postulated in one outbreak of Lassa fever (*2*), but since large numbers of secondary cases are unusual for all three diseases, the frequency of

spread by droplet infection is probably low. Additional late spread via semen has been documented in Marburg disease (*3*).

Because of the relatively inefficient mode of spread, most outbreaks of these diseases have undergone few generations, and have been short-lived. However, the outbreak in Maridi is an example of the potential of these diseases to proliferate under the right conditions. The obvious means of preventing such a disaster is to recognize cases early, and establish barrier nursing. Considering that 30–71% of patients with these diseases have haemorrhagic manifestations (*3*, *5*), it is not difficult to recognize a cluster of epidemiologically related cases. Once recognized, the diseases are not difficult to contain.

Any hospital in the tropics, or any hospital receiving patients from the tropics, should be on constant alert for haemorrhagic signs in febrile patients. Once cases are recognized, the following procedures should be followed:

1. Collect specimens (blood or tissue) for diagnosis, but handle the specimens with extreme caution. The World Health Organization will arrange for proper shipment of specimens to high-security laboratories.

2. Establish a surveillance system to identify other cases of influenza-like disease with or without haemorrhagic manifestations.

3. Establish barrier nursing with gowns, gloves, and masks (or better, respirators). Disinfect patients' excreta with an effective disinfectant, such as formaldehyde.

4. Identify contacts and perform daily temperature surveillance. If temperature rises, patients should be isolated at once.

5. As soon as the diagnosis is made, convalescent plasma should be dispatched to the area.

With such a simple system, these diseases can be identified, isolated, and patients properly treated.

It is evident that Lassa fever is far more prevalent than previously thought (J. B. McCormack, unpublished report, 1977). The same could be equally true for Marburg and Ebola fevers. With experience, no doubt, more outbreaks of these diseases will be identified. We shall probably find that large outbreaks are rare and can be prevented with simple precautions. However, if appropriate precautions are not taken early, as was the case with the first outbreak of viral haemorrhagic fever in Sudan, these diseases can spread far and wide, and across international borders. The hospital, especially the referral hospital, is the site where such outbreaks can either be recognized and halted, or unrecognized and disseminated. With them rests the responsibility for stopping the spread of these dangerous diseases.

CLINICAL MANIFESTATIONS

The clinical manifestations of viral haemorrhagic fever in the Sudan (Ebola haemorrhagic fever) were uniform in their presentation. Although there was a wide range in severity from mild (and probably even asymptomatic) to rapidly fatal, the presentation of the vast majority of cases was clinically unique and relatively easy to diagnose.

The following is our description from observing the disease in hospital and interviewing patients or their families retrospectively. Interviews were completed for 183 of the 280 cases in Nzara and Maridi and are summarized in Table 4 and Fig. 2.

INITIAL PRESENTATION

The illness always had a sudden onset. In its initial stages the disease presented as a progressively more severe influenza-like illness. By the time the patients reported to hospital (mean, day 5), they exhibited

Table 4. Clinical symptoms in 183 cases investigated in Maridi and Nzara

Symptom	Frequency (%)
Fever	100
Headache	100
Chest pain	83
Diarrhoea	81
Vomiting	59
Dry painful throat	63
Rash or desquamation	52
Cough	49
Bleeding (any)	71
melaena	59
bleeding (recovered cases)	48
bleeding (fatal cases)	91

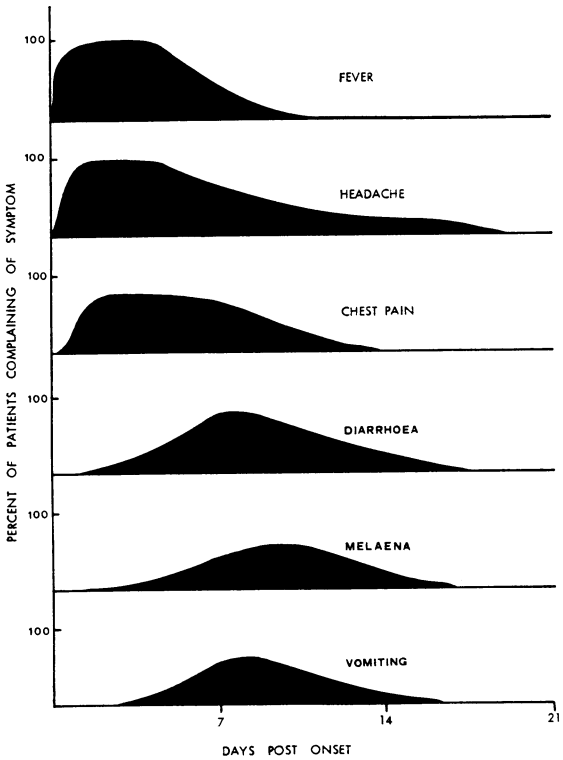

Fig. 2. Frequency and duration of symptoms of Ebola haemorrhagic fever.

the classical appearance of the disease with deep-set eyes, ghost-like, expressionless faces, and extreme lethargy. Severe cases, after increasing toxicity, became moribund and died on the average at day 8.5. Those that recovered did so slowly and painfully. Their appearance remained characteristic with deep-set eyes, drawn faces, a stooped walk, and extreme cachexia. Their condition slowly improved over several weeks, but many complained of pain and weakness for 6–8 weeks after discharge.

The first symptom was usually headache, which invariably began suddenly. Its pain was usually severe and persistent; it was located frontally at first, and soon progressed occipitally. Simultaneously with the development of headache, patients complained of marked weakness, fever, arthralgia of the larger joints, and pain of the cervical and lumbar musculature. After 2 days (range 0–9 days) of increasing headache, weakness, fever (38–39°C) and body pain, gastrointestinal and pulmonary symptoms began.

GASTROINTESTINAL SYMPTOMS

Diarrhoea was the most common (81%) gastrointestinal symptom and was severe in many cases. It began abruptly with frequent watery stools, and it usually lasted for about 7 days. The stools, typically clear at onset, often (59%) became tarry or contained red blood. The diarrhoea, plus probably third space (intracellular space) losses, often resulted in moderate to severe dehydration manifested by poor skin turgor and dry mucous membranes.

Vomiting was also common (59%), usually starting just after the diarrhoea began, and stopping just before the diarrhoea subsided. Abdominal pain commonly coexisted with the gastrointestinal symptoms. It was often cramping in nature and mid-abdominal in location, and it frequently lasted for several weeks. Concurrently with these initial symptoms, patients complained of a complete loss of appetite, which often continued for several weeks despite profound weight loss.

CARDIORESPIRATORY SYMPTOMS

Chest pain was almost universal (83%). It was often associated with a dry cough, and was described either as knife-like at the lower-lateral rib margins (frequently pleuritic) or central and associated with abnormal precordial sensations like " turning " or palpitations.

In many patients (63%), a severe dryness of the throat coexisted with other cardiorespiratory symptoms. Although this discomfort often became painful (" a dry rope in the throat "), it was not typical of pharyngitis at onset, and few patients complained of a " sore throat " when asked. With time, the oral dryness (and the ulcerative enanthem often seen clinically) frequently resulted in fissures and open sores of the lips and tongue.

CUTANEOUS MANIFESTATIONS

Skin rash was difficult to evaluate in this dark-skinned population. Most cases reporting rash noticed it on or about day 5. We believe that most cases did have some rash, since 52% reported either a noticeable rash or desquamation later. When observed, the rash was measles-like, progressing caudally and most marked on the lateral upper arms and upper legs. A subsequent desquamation occurred about 2 weeks later and was most marked on the shoulders, palms, soles, and the pretibial areas.

HAEMORRHAGIC MANIFESTATIONS

Bleeding tendencies, were clinically very common, although seldom tested for during this outbreak. Almost all (91%) of the fatal cases and half of the nonfatal ones had some visible blood loss. Melaena was most common (59% overall) but haematemesis, epistaxis, and oral, vaginal, cutaneous, and subconjunctival bleeding was frequently observed. Fatal cases often bled from multiple sites. Gross haematuria, however, was rarely seen.

CENTRAL NERVOUS SYSTEM SYMPTOMS

Central nervous system symptoms were relatively common. Hospitalized patients were often combative and difficult to nurse. Bizarre behaviour such as stripping off clothes and climbing out of bed was occasionally observed. Sometimes this bizarre behaviour continued for several weeks after recovery.

PHYSICAL EXAMINATION

There were few unique findings on physical examination. The patient's appearance was characteristic, as mentioned above: the face drawn, eyes sunken, and skin showing poor turgor. The conjunctivae were often slightly injected but not icteric. The oral cavity was dry and often showed small aphthous-like ulcers, and the posterior pharynx was commonly slightly injected. In severe cases, the neck was stiff and painful to flex. Examination of the chest commonly revealed basilar rales. The abdomen was soft without organomegaly. However, there was epigastric and right subcostal tenderness. Pregnant females occasionally went into premature labour. Males occasionally manifested orchitis.

LABORATORY TESTS

The laboratory facilities were minimal and no extensive examinations were performed. White blood counts, when studied, were elevated and platelets were reportedly decreased in a few severe patients. Also, proteinuria and microscopic haematuria were observed in a few patients. Spinal fluid was taken from one patient and was clear on gross examination.

DISCUSSION OF CLINICAL MANIFESTATIONS

In summary, the disease was clinically characteristic. It began as a nonspecific influenza-like illness, but soon became unusual both in its severity and with the addition of gastrointestinal disturbances, chest pain, and haemorrhagic manifestations. Its high mortality and slow recovery rate were remarkable.

The differential diagnosis of a single case of haemorrhagic fever in rural Africa is difficult and includes protozoal, bacterial, and viral diseases. However, the etiological possibilities are more limited when there is a cluster of cases with an influenza-like prodrome and subsequent haemorrhagic diathesis. The possibilities are truly few when person-to-person transmission is observed among contacts of acute cases—especially hospital staff. With an outbreak of a tropical haemorrhagic fever transmitted among close contacts, Lassa fever (*5*, *6*), Marburg virus disease (*3*, *4*), and Ebola haemorrhagic fever are the most likely candidates.

There are more similarities than dissimilarities between Lassa fever and Ebola haemorrhagic fever. The vomiting, chest pain, epigastric pain, and rash of Lassa fever are almost identical to those of seen in the Sudan outbreak of Ebola haemorrhagic fever. However, Lassa fever almost always has an insidious onset, and pharyngitis is always pronounced. In addition, the conjunctivitis in Lassa fever is often severe with periorbital swelling and discomfort. In the Sudanese outbreak of Ebola haemorrhagic fever, although the conjunctivae were often injected, they caused few or no symptoms. There is diarrhoea in both diseases but it is more profuse in Ebola infections. With these similarities in mind, the difference between these two diseases is not always clear. The most marked difference is the pharyngitis, which should be weighted heavily in making an early provisional diagnosis before laboratory information is available.

Marburg virus disease and the simultaneous outbreak of Ebola in haemorrhagic fever in Sudan and Zaire, both caused by a morphologically identical virus, are even more difficult to differentiate. The only possible difference is chest pain, which was rare in the two Marburg outbreaks reported to date (*3*, *4*), but was frequent in the Sudan outbreak of Ebola fever. However, it was uncommon in Zaire and might be a variable finding, and possibly not be useful in differentiating between Marburg and Ebola infections. The remaining symptoms of both diseases are identical.

Although few laboratory data exist from the Sudan outbreak of Ebola haemorrhagic fever, there is no reason to suspect any differences between it and the other two diseases. The few patients with elevated white blood cell counts observed in Maridi were

tested relatively late in the disease. The usual finding of leucopenia with a marked shift to the left was probably present in Sudan but not documented. From our clinical examinations we would expect there to be ECG signs of myocarditis or pericarditis, and enzymatic signs of pancreatitis, as described for Lassa fever and Marburg virus disease (*3*, *4*, *5*, *6*). Also, the disseminated intravascular coagulopathy (*4*) seen in these diseases should be as similar chemically as it is clinically.

Therefore, excepting the painful pharyngitis in Lassa fever, the three diseases (Lassa fever, Marburg virus disease, and Ebola haemorrhagic fever) are clinically very similar. Unfortunately, the treatment of the diseases (immune plasma) is virus specific, and an accurate diagnosis must be made before the appropriate plasma can be chosen. Unless further study reveals a distinct geographical distribution for each disease, the virus laboratory will have to be relied upon to determine the true diagnosis.

PATHOLOGY

In Sudan, the high mortality rate and the alarmingly high rate of infection among Maridi hospital staff necessarily curtailed clinical laboratory investigations. Medical staff were understandably reluctant to carry out any post mortems. At the time of the WHO investigation team's visit to Sudan, the only histopathological study of the disease was limited to three liver biopsy specimens obtained in Zaire (*7*). Two limited post mortems were carried out by WHO investigation team members at Maridi, and tissues were removed for virological, histopathological, and electron microscopic study. The histopathological results are reported here.

MATERIALS AND METHODS

Because of the risk of infection, each post mortem was carried out by only two personnel, who wore full protective clothing and biological respirators. Full precautions as suggested by Simpson (*8*) were observed throughout.

Case 1 was that of a young adult female, aged 21 years. The body was autopsied on 17 November 1976, approximately 4 hours after death.

Case 2 was that of an adult male, aged approximately 28 years. The body was autopsied on 18 November 1976, approximately 7 hours after death.

Samples of liver, spleen, kidney, heart, and lung were removed in approximately 1-cm cubes, and placed immediately in a 10% formol–saline solution in sealed tubes. A small biopsy sample of brain was removed from Case 1 and treated similarly. Formol-fixed tissue was blocked after 3 months fixation. Paraffin sections were cut at 5 and 7 μm and stained with haematoxylin and eosin; where appropriate, sections were also stained for reticulin and iron pigment, or with periodic acid—Schiff (PAS), or methylgreen-pyronin.

MACROSCOPIC FINDINGS

No haemorrhages or exudates were visible in the body cavities. Some autolysis was observed in both cadavers, but particularly in Case 2.

In both cases the liver was moderately enlarged, congested, and friable. Blood poured out freely on section, leaving the organ a dull yellow colour. The spleen was also moderately enlarged and purple in colour. The cut surface was rough on section, and bulged out of the capsule. Congestion of other abdominal organs was noted, but otherwise they appeared normal. The pleura was normal, and the lungs, greyish in colour, did not display any obvious areas of consolidation. The heart appeared to be normal.

HISTOPATHOLOGY

Case 1

Liver. The most prominent feature was a focal, vacuolar degeneration affecting mainly the periportal and mid-zonal areas of the parenchyma. The vacuoles were mostly small or medium sized (Fig. 3), but there were a few large ones. In the absence of unembedded tissue for fat stains, it was difficult to say how much of the vacuolation was due to fat or to intracellular oedema (other authors have reported fat). The reticulin fibres were intact. Some isolated parenchymal cells, and a few small inconspicuous clusters of cells, had undergone eosinophilic degeneration (Fig. 3), the nucleus being replaced in some cases by a nonrefractile, eosinophilic body. The eosinophilic cells were nearly always adjacent to a portal tract, and in, or adjacent to, an area of vacuolar degeneration, though many of the vacuolated areas were free of eosinophilic necrosis. There was also noneosinophilic necrosis of some parenchymal cells immediately adjacent to portal tracts. The centrilobular areas were congested, and the

hyperaemia extended in places into the mid-lobular areas, but not into the vacuolated areas, which were all relatively ischaemic. The portal tracts were somewhat enlarged, and contained accumulations of lymphocytes. Intermixed with these were some large unidentified cells (monocytoid or reticular cells possibly) and a few eosinophils and plasma cells. In these areas nuclear debris was present as coarse lumps or fine granules, intracellular or extracellular. This appeared to be derived from the lymphocytes, whose nuclei were often shrunken, pyknotic, or crescent shaped. One or two amorphous clumps of nuclear material were seen in sinusoids. Many Kupffer cells were swollen with foamy cytoplasm containing PAS positive material and granules of iron pigment. There was some slight cholestasis. There were two dead schistosome ova in the portal tracts, and clumps of pigment in the parenchyma that were probably due to the schistosomiasis. One microfilaria was seen in a capillary.

Spleen. The lymphoid follicles were moderately depleted, though some were of normal size, and in only one was there a germinal centre. Necrosis and nuclear debris was present in this one centre but not elsewhere. The follicles were studded with large, pale, hypertrophied reticular cells. Plasma cells were not numerous. The pulp was engorged with blood, and contained much blood pigment, especially in the zone around the lymph follicles, but there were no haemorrhages.

Kidney. Glomeruli and tubules were quite well preserved, and there was no hyperaemia. There was a small amount of proteinaceous precipitate, but no red cells, in Bowman's spaces and tubules. There was an interstitial cellular infiltrate, which consisted of lymphocytes and plasma cells, with scanty eosinophils and a number of cells with large dark nuclei of uncertain origin; among these were some immature plasma cells. In these areas there was pyknosis, and extracellular nuclear debris that appeared to represent the destruction of all infiltrating cell types. But whereas the infiltrate was greatest near the junction of the cortex and the medulla (Fig. 4), pyknosis and debris was most marked in the medulla (Fig. 5).

Lung. The only conspicuous finding was a marked thickening of the alveolar walls due to proliferative accumulations of alveolar cells. There were some macrophages present, and a few small areas of infiltration with polymorphs, lymphocytes, and plasma cells. In these situations pyknosis and nuclear debris were present. There was no intra-alveolar exudate, cellular or fibrinous. Bronchioles were nornal.

Heart. In one block of auricle there was a mild focal interstitial myocarditis with some infiltration of lymphocytes, myocytolysis, and oedema. Pyknosis was not seen. The ventricular myocardium was normal except for mild interstitial oedema.

Brain. There was probably some small increase of glia cells, but no glial nodes. In other respects the brain, as seen with routine histological stains, was normal.

Purulent exudate with many polymorphs was present in one small specimen. The site was not identifiable.

Case 2.

Liver. The liver was essentially normal. There were no degenerative changes or necrosis as in Case 1. No inclusions could be detected. Hyperaemia was not significant and Kupffer cells were not swollen. There was some fibrosis of portal tracts, not remarkable in an African. The only abnormality was a light infiltrate of lymphocytes and plasma cells in the portal tracts, but without pyknosis or nuclear debris.

Spleen. The lymphatic tissue was severely depleted and almost devoid of lymphocytes. The lymphoid follicles were either completely lacking or else, with few exceptions, they were replaced by a thin perivascular cuff of plasma cells (Fig. 6). Among these there were also immature plasma cells, but lymphocytes were either scanty or absent altogether. Pyknosis and nuclear debris were not seen. Plasma cells, mature and immature, were numerous also in the red pulp, which was moderately hyperaemic.

Kidney. In many of the glomeruli, the Bowman's space was partly filled with a variable amount of a proteinaceous precipitate which in some cases was profuse (Fig. 7). Scanty red cells were also present. The glomerular tufts were fairly normal. The proximal convoluted tubules, loops of Henle, and some of the distal convoluted tubules were affected by granular degenerative changes progressing to complete necrosis (Fig. 7). The lumens of the more intact tubules were filled with precipitate. Granular and globular calcified deposits were present in some tubules, and were sometimes large enough to have destroyed the tubular epithelium. In the interstitial tissue there were capillary hyperaemia and a focal plasma cell infiltrate. Lymphocytes were scanty or absent. Pyknosis and nuclear debris were not seen.

Lung. In the one block of tissue available, there was a proliferative thickening of the alveolar septa as in Case 1. In the sub-pleural region, but not elsewhere, there was an infiltrate of plasma cells, lymphocytes and mononuclear cells. Pyknosis and nuclear debris were not seen.

Heart. The ventricular myocardium and interstitial tissue appeared normal.

DISCUSSION

The outbreak of viral haemorrhagic fever in Zaire and the Sudan in 1976 has been shown to be a variant of Marburg virus disease designated as Ebola haemorrhagic fever. These two diseases are similar (see above, pages 257-258, and references *3*, *4*), and the two viruses are morphologically identical, though immunologically distinct (*7*, *9*). On the evidence of the liver biopsies of Johnson et al. (*7*), and of the two autopsies reported here, the pathology of the two conditions is shown to be essentially similar, though it cannot yet be said to be identical. The pathology is that of an acute viral infection, and although it has characteristic features, it is not distinctively diagnostic.

The essence of the pathological process in Marburg virus disease in man is necrosis with a minimal inflammatory response, affecting first the liver and lymphoid system, later the pancreas, testis, adrenal, pituitary, thyroid, kidney, and skin. In lymphoid tissue the lymphocytes are replaced by plasma cells and monocytoid cells. The haemorrhagic state observed clinically is represented histologically by hyperaemia in most instances (*10*). In two cases with encephalitis there were glia nodules surrounded by a necrotic zone (*11*).

In Ebola haemorrhagic fever three post-mortem biopsies showed a picture of focal eosinophilic necrosis similar to that of Marburg virus disease livers (*7*). The liver of one of our cases was consistent with the previous descriptions, though eosinophilic necrosis was not a conspicuous feature. In the other case, the liver, surprisingly, was almost unaffected. There was renal tubular necrosis in this second case similar to the situation in some Marburg cases. The most consistent aspect of our two cases was the destruction of lymphocytes or their replacement by plasma cells. In Case 1, pyknosis and nuclear debris were seen in four organs at sites where lymphocytes were present, and appeared to be due to destruction of lymphocytes, though plasma cells were present also. In Case 2, there was most marked depletion of lymphocytes in the spleen, with a compensatory increase of plasma cells. Focal infiltration in other organs was predominantly, if not exclusively, due to plasma cells, and pyknosis and nuclear debris were not seen at any site in this case. This would seem to imply that lymphocytes are the main, though not necessarily the only, source of nuclear debris in affected tissues in our cases. Gedigk et al. (*10*) state that plasma cell substitution of lymphocytes is a feature of the late stage of Marburg virus disease.

For these reasons we have reported all small basophilic bodies (other than colonies of coccobacilli) as nuclear debris. Many of them were extracellular, the staining reactions were appropriate, and it was difficult to identify any non-nuclear material among the debris. However, it is quite possible that it masked basophilic inclusions of viral origin, and most authors have thought that inclusions were present, though the inclusions of Johnson et al. (*7*) were eosinophilic. Inclusions that stain red with Macchiavello have been demonstrated in the liver by workers using animals experimentally infected with Marburg virus (*12*) and Ebola virus (*9*). Most human post-mortem material is unsuitable for use with this stain, and we were unable to obtain satisfactory results with it in our cases. Johnson et al. (*7*) demonstrated the Ebola virus in their human necropsy livers by electron microscopy, and similar particles have been demonstrated under the electron microscope in our material (*13*). Zlotnik (*12*) thinks that the virus inclusions only become visible by light microscopy if they become coated with glucoprotein or impregnated with calcium, as happens in guinea-pigs and hamsters. It may be, therefore, that the only reliable way of demonstrating viral bodies in human tissue is electron microscopy.

VIROLOGICAL AND SEROLOGICAL STUDIES

This section describes virus isolation attempts on acute human material, and post-mortem specimens collected by the joint WHO/Sudanese investigation team, and serological studies on human sera collected from convalescent patients, case contacts, and members of the local populations of Nzara and Maridi who were believed not to have been exposed to infection with Ebola virus during the outbreak.

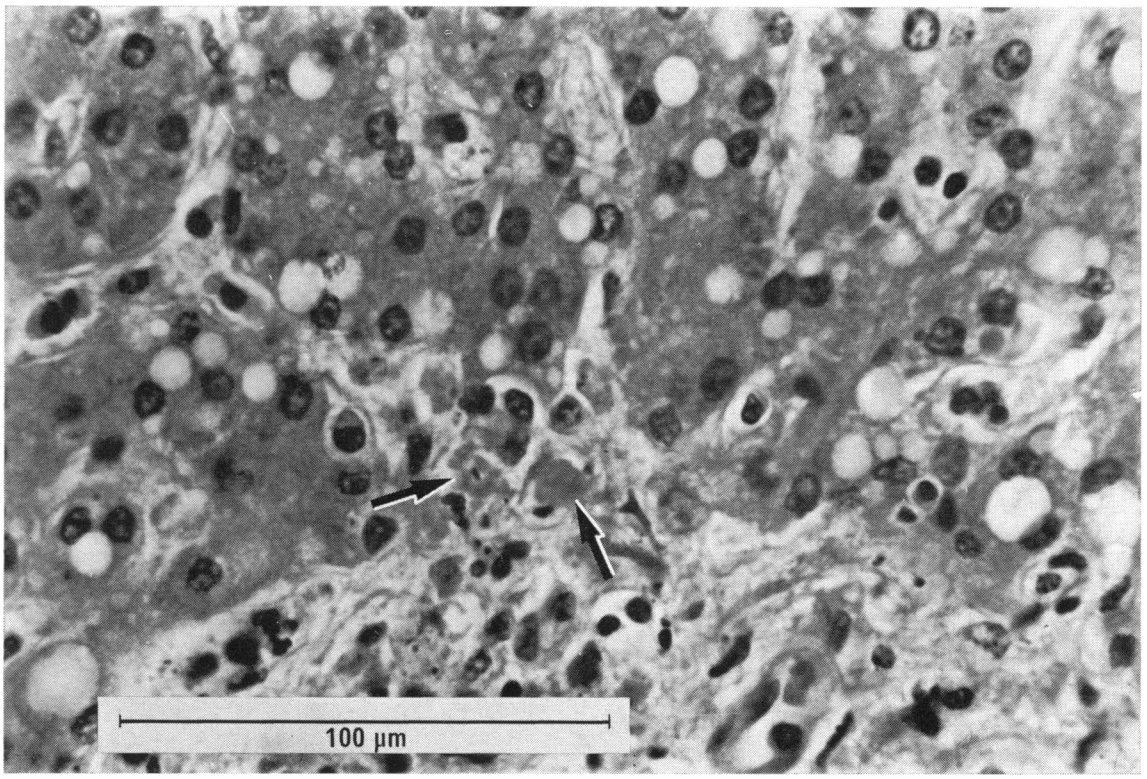

Fig. 3. Liver. Vacuolar degeneration and eosinophilic necrosis (arrows) on edge of portal tract. Note infiltrating cells and nuclear debris in proximity to the eosinophilic necrosis. (Case 1).

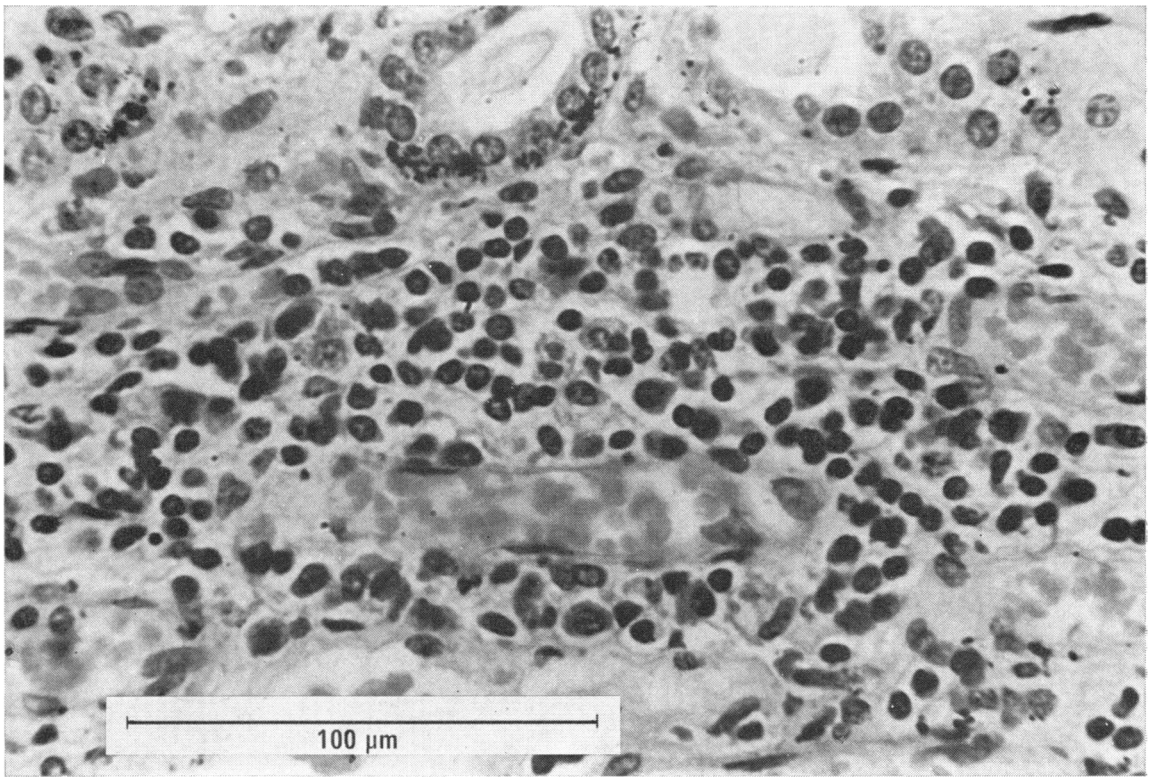

Fig. 4. Kidney. Interstitial infiltrate of lymphocytes and other chronic inflammatory cells near corticomedullary junction. There is little nuclear disintegration. (Case 1).

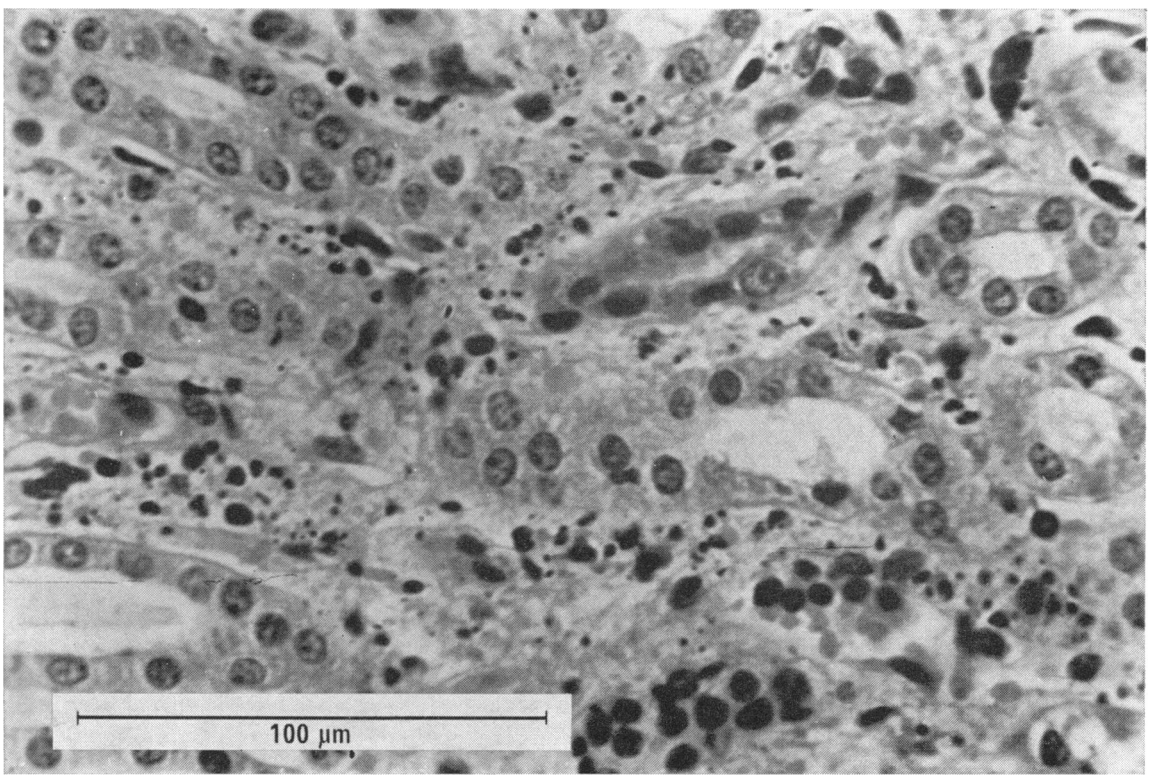

Fig. 5. Kidney. Nuclear disintegration and debris in area of interstitial cellular infiltrate in medulla. (Case 1).

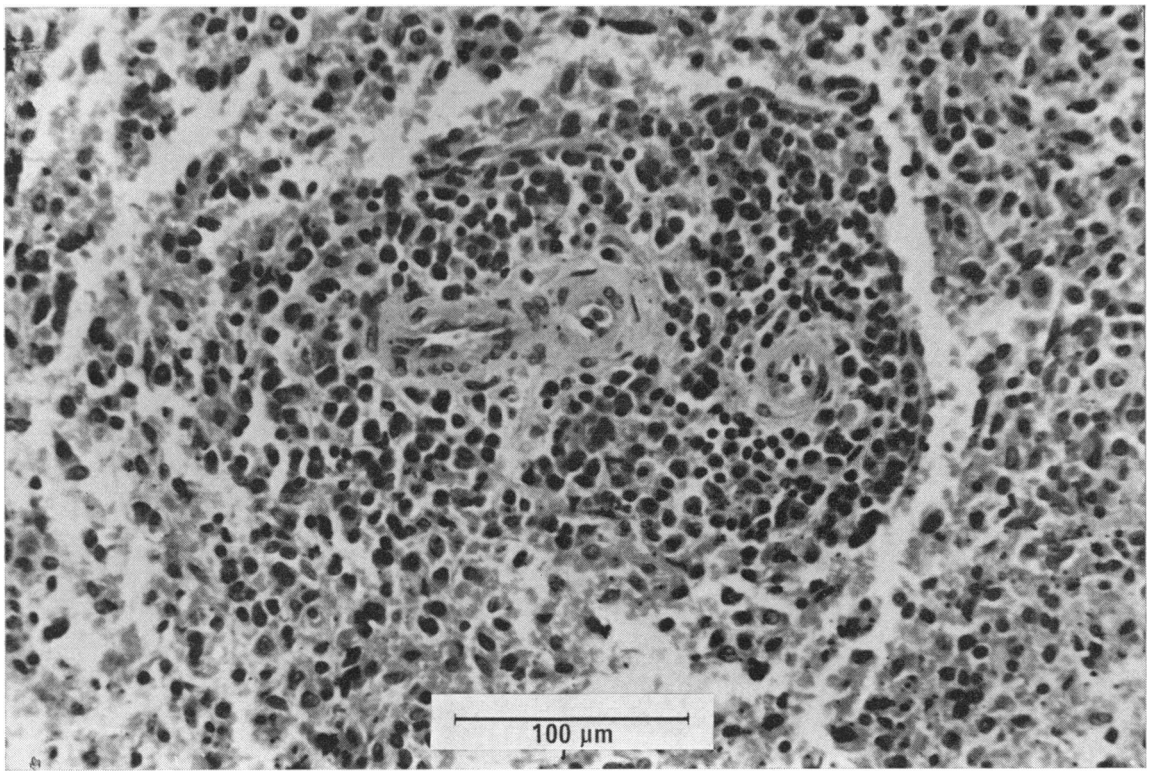

Fig. 6. Spleen. Lymphatic nodule around arteriole. About 90 % of the cells are plasma cells. (Case 2).

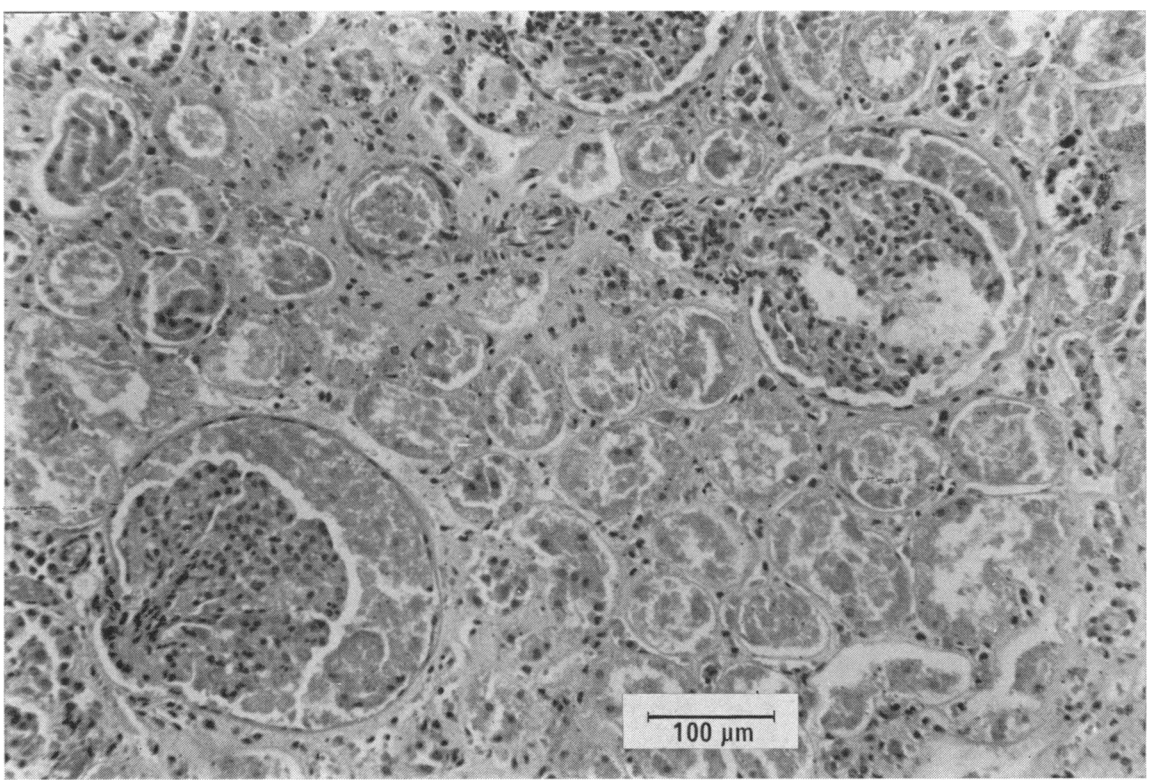

Fig. 7. Kidney. Area of tubular necrosis and exceptionally profuse precipitate in Bowman's spaces. (The small specks are formalin pigment, not nuclear debris or inclusions. (Case 2).

MATERIALS AND METHODS

Acute samples

Blood, throat swabs, and urine samples were collected within five days of the onset of illness from eight acutely ill patients in Maridi Hospital isolation wards. All eight patients were febrile, grossly dehydrated, and displayed many of the clinical features described.

In the isolation wards, and when handling samples, team members wore protective clothing and biological respirators as suggested by Simpson (*8*). Blood samples were collected using disposable syringes and needles, and were immediately injected into Vacutainer tubes. Throat swabs were placed in sterile saline in screw-capped polypropylene serum tubes (Nunc); urine samples were collected from bed-pan samples in similar polypropylene tubes. Specimen containers were washed on the outside with 2% formol–saline solution, and placed in plastic bags for removal from the isolation area. Swabs, needles, and syringes were immersed in 2% formol–saline, placed in plastic bags inside a bucket and taken for immediate incineration. Protective clothing was removed on leaving the isolation area; disposable items were incinerated, and reusable gowns were decontaminated by boiling.

After clotting, blood was centrifuged in sealed Vacutainer tubes in the field laboratory, and serum aspirated off into polypropylene tubes. All acute samples were stored in liquid nitrogen prior to dispatch to the Microbiological Research Establishment, Porton, England.

Postmortem samples

Small pieces of liver, spleen, heart, lung, kidney, and bone marrow were collected in polypropylene tubes, and stored in liquid nitrogen.

Convalescent plasma

Aliquots of immune plasma were collected using a modified electrophoresis technique. As no centrifuge was available, plasma and cells were separated by gravity only over a 2–3 h period.

Sera from contacts

Blood samples were collected using disposable syringes and needles, and stored before centrifugation in sealed Vacutainer tubes. In Nzara, samples were collected in the cotton factory, and in homesteads around the township; in Maridi, samples were collected in the hospital grounds, schools, and surrounding homesteads. After centrifugation, sera were stored in polypropylene tubes, and stored at —5°C. For dispatch they were placed in liquid nitrogen; at the Microbiological Research Establishment they were stored at —20°C until tested.

Laboratory studies

All manipulations involving potentially infectious material were carried out in the Maximum Security Laboratory at the Microbiological Research Establishment.

Virus isolation attempts

Ten percent suspensions of postmortem tissues were prepared in a pH 7.2 phosphate buffered saline containing 0.75% bovine plasma albumin (Armour Fraction V), penicillin, and streptomycin. Tissue suspensions, acute serum samples, urine, and throat swab eluates were inoculated into cultures of an African green monkey cell line (Vero) and Dunkin-Hartley strain guinea-pigs, following the procedures described by Bowen et al. (*9*).

Immunofluorescent technique

The indirect immunofluorescent test was similar to that described by Wulff & Lange (*14*). Vero cells were prepared in 25-cm^2 plastic flasks (Nunc) and maintained in Leibovitz medium (L15) containing 2% fetal calf serum at pH 7.2–7.4. Each flask was inoculated with 1 ml of Ebola virus suspension consisting of 10% guinea-pig liver and spleen. The medium was changed on the second day to reduce toxicity caused by guinea-pig tissue. As indicated by daily immunofluorescence antibody (IFA) testing of replicate preparations, flasks were incubated at 37°C until 30–40% of the cells had become infected, usually at 6–7 days.

For slide preparation, media was first decanted off the infected cells, and the cell sheet washed three times in a pH 7.2–7.4 phosphate buffered saline (PBS). The cell sheet was detached with 2 ml of 0.2% trypsin in versene; the cell suspension was removed and diluted in an equal volume of PBS containing 3% calf serum to inactivate the trypsin/versene activity. The suspension was centrifuged at 500 *g* for 5 min, the supernatant discarded, and the cells resuspended in PBS. This procedure was repeated twice and the cells were finally suspended in PBS containing 0.2% of bovine serum albumin to a concentration of approximately 5×10^5 cells/ml. The suspension was seeded into the wells of polytetrafluoroethylene-coated glass slides each having twelve

6-mm wells. Each well received one drop of cell suspension. The slides were allowed to dry under an ultraviolet lamp for 20 min followed by 10 min fixation in chilled acetone.

Sera were examined for the presence of Ebola virus antibodies by an indirect immunofluorescent method (*14*). Duplicate dilutions of all test sera were made in PBS, and sera were screened at dilutions of 1 : 4 and 1 : 8. Known human positive and negative sera were included as controls, and PBS controls were also used. All test and control sera were screened on both infected and uninfected slides. Diluted sera were placed in slide wells, and held in a moist chamber at 37°C for 30 min, and then washed in PBS for 5–10 min. Slides were then dried in air, and a drop of fluorescein-labelled rabbit anti-human IgG conjugate (Wellcome) at dilutions of 1 : 20 to 1 : 25 applied to all wells. The slides were again held at 37°C in a moist chamber for 30 min, and then washed three times in PBS for 2–4 min on each occasion, and finally rinsed in distilled water for 30 seconds. Slides were then dried in air and examined under a Reichert Fluorvar microscope using an HBO 50 W mercury-vapour burner. Sera were accepted as positive only if clear fluorescence was observed at dilutions $\geqslant$1 : 8. Sera that were positive only at a dilution of 1 : 4 were regarded as equivocal and graded as negative.

RESULTS

Virus isolation

Two strains of Ebola virus were isolated from acute human sera from two patients in the Maridi isolation wards. One was from a 12-year-old schoolgirl (collected on 5 November 1976), who died 3 days later; the other, collected on 8 November 1976, was from an 18-year-old male student, who later recovered. The isolations were made in guinea-pigs inoculated intraperitoneally. The guinea-pigs developed a febrile illness 5 days after inoculation; passage of guinea-pig blood to further guinea-pigs produced a similar febrile illness but a fatal infection was produced in guinea-pigs only after 5 passages. The isolates were later successfully cultured in Vero cells, and identified as strains of Ebola virus by immunofluorescence.

No isolates were obtained from other patients' sera or from any of the throat swabs, urines, or post-mortem tissues. Virus particles were, however, demonstrated by electron microscopy in liver sections from two fatal cases (*13*).

Table 5. Results of IFA tests on convalescent patients' sera

Patients	No. of survivors	No. tested	No. positive
Hospital staff			
Medical assistant	1	1	1
Nurses and midwives	9	8	7
Student nurses	17	14	11
Cleaners	4	4	3
Miscellaneous staff	4	3	3
Total	35	30	25
Non-hospital staff	69	18	17
Overall total	104	48	42

Serology

Altogether, 104 surviving Maridi patients were identified. All of them had been diagnosed as suffering from Ebola haemorrhagic fever on clinical grounds alone; 35 were members of the Maridi hospital staff and the remaining 69 were Maridi residents, some being relatives of hospital staff. Of the 35 surviving hospital staff, 30 were bled for serological studies; 25 (83%) of them had detectable IFA antibodies. Only 18 of the 69 patients who were not hospital staff were willing to be bled; 17 of these patients had detectable antibodies. Altogether 42 of the 48 patients diagnosed clinically as having been infected, and who were bled, had antibodies against Ebola virus (Table 5).

Among the probable and possible case-contacts, hospital staff were considered to be those most at risk. A total of 159 members of the staff had given no history of severe febrile illness during the epidemic. Sera from 64 of these were tested for Ebola virus antibodies. The results are shown in Table 6. Seven staff members, including 4 nurses, a cleaner, a toilet cleaner, and a water carrier had evidence of infection. None of the 3 doctors attending sick patients had antibodies.

Of the Maridi population who had been in contact with known cases of infection, 102 were tested. The majority of them were close family contacts, and several had helped to nurse sick relatives during their illnesses. Twenty-two had demonstrable antibodies. Close questioning revealed that 9 of these close contacts had some evidence of febrile illness without manifesting severe disease. Twenty-nine members of

Table 6. Results of IFA tests on sera from Maridi Hospital staff contacts.

Healthy contacts	Number bled	Number positive
Doctors	3	0
Nursing staff (including tutors, midwives, theatre staff)	35	4
Drivers	5	0
Cleaners	11	1
Toilet cleaner	1	1
Water carrier	1	1
Laboratory assistant	1	0
Messengers	3	0
Gardeners/carpenters	4	0
Total	64	7

a Maridi school were tested as a control group. None of them was thought to have had any contact with a known or suspected case of Ebola virus disease. However, 3 schoolboys had antibodies. None of them had any history of recent illness.

In Nzara, 37 surviving patients diagnosed clinically as having had Ebola haemorrhagic fever were identified, and 31 of them were bled for antibody studies. Only 6 (19%) had demonstrable antibodies, and none had antibody levels greater than 1 : 32. Among close family contacts, only 1 person out of 78 tested had antibodies, although 5 had a titre of 1 : 4, which was not accepted as positive.

Sera from 109 cotton factory workers were tested. Seven members of the staff had antibodies, with the highest proportion, 3, in the cloth room and the adjacent store. Three workers in the weaving section had antibodies, while only 1 member of the 28 spinning-section staff who were bled was positive. Antibody levels ranged from 1 : 16 to 1 : 64, and none of the 7 workers gave a history of any recent illness.

DISCUSSION OF SEROLOGICAL STUDIES

No really satisfactory serological test has yet been devised to detect and quantify antibodies against either Marburg or Ebola viruses. Early workers (*15, 16, 17*) used complement fixation tests to detect antibodies to Marburg virus following the outbreak in 1967. They used crude antigens derived from guinea-pig and monkey tissues but found them to be unreliable because they lacked specificity. Malherbe & Strickland-Cholmley (*18*) compared antigens derived from animal tissues with those prepared in monkey kidney cell cultures and found both types to be unsatisfactory. Slenczka & Wolff (*19*), and Slenczka et al. (*20*) found that antigens derived from chronically infected Vero cells were much more satisfactory but still had doubts about their sensitivity.

Neutralization tests have been carried out in guinea-pigs and tissue culture systems, but have proved costly in animals, cumbersome, time consuming, and generally very unsatisfactory. Siegert & Slenczka (*17*), and ourselves have had some success with neutralization tests in Vero and BHK21 cells based on counts of intracytoplasmic inclusion bodies, but the results were difficult to reproduce. The problem is that no really obvious cytopathic changes can be readily demonstrated in any of the cell culture systems used so far, although Johnson et al. (*7*) have claimed that some cytopathic changes are discernible in Vero cells. However, these workers, like ourselves, have based their serological results on immunofluorescent tests. Immunofluorescence with Marburg virus was described in BHK21 by Carter & Bright (*21*) but recent work on Marburg and Ebola viruses has been based on the indirect immunofluorescent technique described by Wulff & Lange (*14*) for use with Lassa virus. The method is more sensitive than complement fixation and appears to be quite specific. It certainly distinguishes between Marburg and Ebola viruses (*7*). Wulff & Conrad (*22*) believe that the method could be used successfully for survey work, since, at least with Marburg virus, antibodies can be detected by this method five years after infection.

The diagnosis of Ebola virus infection was confirmed by the indirect immunofluorescence test in 42 of 48 Maridi patients who had been diagnosed clinically. The results with several of these sera were confirmed by a parallel test kindly carried out at CDC, Atlanta, by Dr Patricia Webb. There can be little doubt that the other 6 patients, in whom no antibodies could be detected, had been infected with the same virus since they all had febrile illnesses accompanied by haemorrhages and symptoms as described. The lack of detectable antibodies in 25 of 31 Nzara patients was surprising and disappointing especially as all of them had had illnesses clinically indistinguishable from proven cases of Ebola fever. The Nzara patients were bled at times ranging from

6 to 17 weeks after the onset of their illnesses. It may be that their antibody levels had fallen below those detectable by the immunofluorescent test. In view of earlier results with Marburg virus, this possibility seems unlikely. However, antibody levels in proven cases of Ebola infection in Maridi did fall quickly. Of 23 patients bled in November 1976 and again in January 1977, 18 had distinct falls in antibody levels ranging from 2- to 5-fold. Later, antibody levels were often detected at the lowest acceptable dilution of 1: 8. Two patients actually became negative. These results could possibly be explained if the immunofluorescent test were directed towards detecting IgM antibodies alone. Unfortunately the test was specifically directed to detect IgG and therefore no satisfactory explanation for the lack of detectable antibodies can be advanced. Obviously further work needs to be carried out on developing more sensitive test systems.

The finding of Ebola virus antibodies in 25 (19%) of 131 Maridi case-contacts, and in 7 (19%) of 64 hospital staff contacts indicates that Ebola virus can cause mild illnesses and even subclinical infections. It also supports the view advanced above (pages 253-254) that Ebola virus is not readily transmissible from person to person except where there is close and prolonged contact with a patient suffering from severe disease. Clinical observation of cases occurring late in the epidemic showed that frank haemorrhagic manifestations were less apparent. There may have been some attenuation of Ebola virus resulting from repeated passage in man. Observations in guinea-pigs infected with isolates obtained later in the outbreak may confirm this.

The antibody results with sera collected from the Nzara cotton factory staff indicate that 9 (37%) of the 24 staff of the cloth room and the adjacent store were infected. The levels of antibody, ranging from 1 : 16 to 1 : 256, indicate a fairly recent infection, although the 7 members of staff who were bled in November and were shown to have been infected gave no history of illness. These results point very strongly to the cotton factory having been a prime source of infection. The results of virus isolation attempts and serological studies on rodents and bats collected in the factory are awaited with interest.

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RÉSUMÉ

FIÈVRE HÉMORRAGIQUE D'EBOLA AU SOUDAN, 1976

Une importante poussée de fièvre hémorragique (appelée ultérieurement fièvre hémorragique d'Ebola) s'est produite au Soudan méridional entre juin et novembre 1976. Il y a eu en tout 284 cas: 67 à Nzara, première ville touchée, 213 à Maridi, 3 à Tembura et 1 à Juba. A Nzara, la poussée paraît avoir commencé chez les travailleurs d'une fabrique de cotonnades. A Maridi, l'ampleur de l'épidémie s'explique par la transmission de la maladie dans un grand hôpital, siège d'une intense activité. La transmission exigeait un contact étroit avec un cas aigu, et elle a été en général associée au fait de soigner un malade. La période d'incubation était de 7 à 14 jours. Nzara pourrait avoir été la source de l'infection d'une épidémie similaire dans la Zone de Bumba, au Zaïre, mais l'existence d'un lien entre les deux poussées n'a pas pu être prouvée.

Lors de cette flambée épidémique, la fièvre hémorragique d'Ebola s'est présentée comme une maladie clinique de caractère exceptionnel, avec un taux élevé de létalité (taux général de 53%) et un temps de guérison prolongé pour les sujets qui ont survécu. Commençant par un syndrome d'allure grippale, avec fièvre, céphalées et douleurs articulaires et musculaires, la maladie provoquait rapidement de la diarrhée (81% des cas), des vomissements (59%), des douleurs thoraciques (83%), une gorge sèche et douloureuse (63%) et des éruptions (52%). Des manifestations hémorragiques fréquentes (71%), ont été observées dans la moitié des cas qui ont guéri et dans presque tous les cas mortels.

L'autopsie de deux malades décédés a été effectuée en novembre 1976. Les observations histopathologiques qui ont été faites rappellaient celles que l'on peut faire lors d'infections virales aiguës et, tout en étant caractéristiques, elles ne permettaient pas un diagnostic exclusif. Ces observations étaient très analogues à celles que l'on fait dans les cas d'infection à virus Marburg, avec des foyers de nécrose hépatique se colorant à l'éosine, destruction des lymphocytes et leur remplacement par des plasmocytes. Dans un cas, on a observé une nécrose des tubes urinifères.

Deux souches de virus Ebola ont été isolées chez des malades en phase aiguë à l'Hôpital de Maridi lors de l'enquête de novembre 1976. Des anticorps anti-virus Ebola ont été détectés par immunofluorescence chez 42 des 48 malades ayant fait l'objet d'un diagnostic clinique à Maridi, mais seulement chez 6 malades sur 31 à Nzara. On examine la possibilité que l'épreuve par immunofluorescence indirecte ne soit pas suffisamment sensible.

A Maridi, des anticorps ont été mis en évidence chez 19% des contacts des cas, tant à l'hôpital que dans la population locale. Très peu parmi les contacts ont fait état d'antécédents morbides, ce qui indique que le virus Ebola peut provoquer des infections bénignes ou même infracliniques. Les travailleurs employés dans les ateliers de la fabrique de Nzara semblent avoir été infectés dans une proportion de 37%, ce qui fait penser que cette fabrique pourrait être la première source d'infection.

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