6. HAEMORRHAGIC FEVERS – FILOVIRUS INFECTIONS

Different viruses cause haemorrhagic syndromes without great clinical specificity despite the variety of aetiological, ecological and epidemiological components involved (see Yellow fever, Dengue and Minor arbovirus diseases).

In the Far East, the haemorrhagic variety of dengue fever has aroused great interest in Southeast Asia, the Philippines, Oceania, and the Caribbean since 1956. It has not been reported in Africa.

The dreadful reputation of the haemorrhagic fevers stems mainly from the deadly epidemics of haemorrhagic fever with renal syndrome (HFRS) which raged in the Far East and remained mysterious until 1976-1977, when the hantavirus was identified as the aetiological agent. A milder form of this viral infection occurs in the Soviet Union under the name of haemorrhagic nephritis, with extensions into Scandinavia and even Belgium.

A series of haemorrhagic fevers, some of them transmitted by ticks, have also been identified in various foci across the huge Eurasian continent. Other haemorrhagic fevers caused by arenaviruses and spread by rodents have also made headlines in Latin American newspapers.

In Tropical Africa, Rift Valley fever, a severe zoonosis of sheep and cattle caused by a phlebovirus, had been implicated in sporadic cases of human infection before provoking major epidemics in Egypt in 1977-1978. Marburg virus disease, Lassa fever, and Ebola virus disease are other haemorrhagic fevers found in Africa. The first was the cause of epidemics among laboratory personnel working in Europe on African monkeys, the second affected health workers in Nigeria, while the third brought terror among the inhabitants and medical staff in northwestern Zaire and southwestern Sudan. Two other viruses involved in haemorrhagic syndromes elsewhere in the world, were also isolated in Tropical Africa. These are the chikungunya and Congo-Crimean viruses, both of which present potential risks and require constant monitoring.

Since November 1989, outbreaks of filovirus infection have been described among cynomolgus monkeys in the Philippines and in the States. A filovirus named Reston and later the Pennsylvania virus were found antigenically closely related to Ebola virus, although the antibodies reacted not always with the same strength. Humans seem able to survive Asian filovirus but not the African ones.

Haemorrhagic febrile syndromes may also be due to a number of other aetiologies, such as meningococcal septicaemia, septicemic plague, leptospirosis, tick fevers, rickettsial infection, measles, Delta hepatitis.

Haemorrhagic fevers are a serious public health problem in Central Africa. A surveillance system should therefore be organized to monitor all involved factors including the viruses and bacteria circulating in the area, the virus reservoirs and the possible vectors, as well as the immunological status in various age groups of the population. An up-to-date epidemiological record will make it possible to forecast shifts in the ecological balance and to undertake preventive immunization or vector control measures. Above all, it will help to quell mass panic and to avoid its tragic, painful, overwhelming and often costly consequences.
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HAEMORRHAGIC FEVERS

General introduction

The fevers that may be accompanied by a haemorrhagic syndrome, or are showing haemorrhagic symptoms or tendencies, are due to a wide range of factors. Moreover, the aetiology may even be multifactorial.

The habit of naming occasional or rare syndromes with esoteric geographical names or obscure layman's terms borrowed from local languages does not clarify medical knowledge, even if it does, it provides fertile ground for an adventurous, speculative mind.

These syndromes may be classified as follows:

- Bacterial origin: plague or the so-called Black Death, icterohaemorrhagic leptospirosis, meningococcal septicaemia with Waterhouse-Friederichsen syndrome (the meningococcus is called *capillarotrophic*), septic abortion, puerperal fever, and purpura fulminans developing in the course of streptococcus sore throat;
- Venomous origin: snake bite or insects bites as the Altamira haemorrhagic syndrome, attributed to sandflies active along the Amazon highway, and the haemorrhagic vesicular exantheme that strikes the inhabitants of Bolivia's high plateaux when they descend to lower altitudes;
- Intoxications: warfarin mixed by mistake with edible powders;
- Viruses: The syndromes of viral aetiology are the ones best known because of their epidemic outbreaks of variable magnitude.

The route of transmission differs:

by mosquitoes: yellow fever
dengue haemorrhagic fever
(DHF and DSS)
Rift Valley fever
Chikungunya fever

by ticks: Congo-Crimean haemorrhagic fever
Kyasanur Forest disease

by rodents: Lassa fever
Junin haemorrhagic fever (Argentina)
Machupo haemorrhagic fever (Bolivia)

The following pages will be limited to a discussion of the recent African haemorrhagic fevers of viral aetiology other than arbovirus infections and tick fevers, which are: Marburg virus disease, Ebola virus disease, Lassa fever and other filovirus. The first two are caused by Filiviridae, the last one by Arenaviridae.

In 1993 the State Health Department, the CDC Atlanta and the Navajo Division of Health reported an acute respiratory illness among Indians. The disease became widespread in USA. It is due to a newly recognized hantavirus. The disease was observed in individuals of 16 to 40 years of age and was marked, after 2 to 6 days fever with myalgia, nausea and vomiting, by progressing coughing and shortness of breath. Death followed in a high percentage of cases. There were bilateral interstitial infiltrations. The source of contamination was found in rodents (deer-mouse). It was prescribed to minimize exposure to rodents and their excretions, urine or dried excreta (Duchin et al., 1994).

A. MARBURG AND EBOLA VIRUS DISEASE

HISTORICAL BACKGROUND

1. Marburg virus disease

This haemorrhagic fever is still a puzzle. It was first described in August 1967, affecting 29 people – 23 in Marburg, 4 in Frankfurt, and 3 in Belgrade –, all of whom were working with fresh tissues and blood taken from vervet monkeys for vaccine production. None of the technicians in charge of the animal houses were affected. There were 3 secondary cases among contacts and 7 deaths among the primary cases.

The monkeys had been supplied by a reputable, well-organized firm employing some 500 trappers. The monkeys in the consignments had been captured near the Kyoga Lake (Uganda) and held at transit posts at Namasale, Kidera, and Ndolwa to be brought together in Entebbe before being shipped to Europe, the United States, and Japan by air. The 6-Days' War in the Middle East forced the plane to make an unplanned stopover at Heathrow Airport, where the monkeys were housed in the temporarily overcrowded facilities of the Royal Society for the Prevention of Cruelty to Animals. At the time 48 other species of animals and birds were also housed there. The monkeys were then sent on to their final destinations – 180 to Moscow and 100 to Germany and Yugoslavia. The breakdown of the latter consignment was 20 for Frankfurt, 73 for Marburg (3 of which were dead on arrival but not autopsied), and 4 for Belgrade; three other monkeys escaped at the airport but were subsequently recaptured.

The aetiology of the outbreak was established quickly after exclusion of the common bacterial (including leptospiral, rickettsial, and chlamydial infections) and viral
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causes (herpes viruses, cytomegalovirus, entroviruses and arboviruses). This was done by means of an intra-
peritoneal inoculation of citrated blood in guinea pigs and
passages after upsurges of fever. Each passage increased
the agent’s virulence until it became lethal, at which point
intracytoplasmic inclusion bodies could be detected. The
passage on cell cultures, such as Vero cells, was success-
ful. It produced a cytopathic effect in which the same
intracytoplasmic inclusion bodies were seen. The virus
has a distinctive appearance, with a characteristic dough-
nut or torus shape of considerable length (600 nm). It was
classified first among the toroviruses, then among the
rhabdoviruses, given its similarities with the rabies virus,
to finally be given its own identity as a Filovirus.

The transmission cycle seemed to be complete: typical
virus, animal reservoir, and human disease. The evidence
of transmission appeared even stronger as the first checks
on monkeys in the Kyoga Lake area of Uganda yielded
positive complement fixation results. These tests, which
were conducted using a rather crude antigen contained in
a non-purified liver-spleen tissue extract, gave a high pos-
itive reaction rate. Later tests with a purified viral antigen
showed that the earlier results were false positives. Mean-
while it was also shown that vervet monkeys were very
sensitive to the virus, with a high mortality, while the sur-
vivors developed antibodies.

This was the state of affairs when after eight years
an isolated case was diagnosed in South Africa in
1975. It probably originated in Zimbabwe and pro-
duced two confirmed secondary cases. The virus was
detected by electron microscopy in the tissues of the
first patient, who died.

Given the potential importance of this viral infection
and the possibility of tracing with great precision
the patient’s itinerary, an extensive epidemiological
investigation was organized. So could be determined the
place where he had been stung by an insect, as evidenced
by the erythematous, tender papule he presented, and the
place where he had been in contact with an animal (a
tame civet cat in the Kylebad National Park). All of the
information collected on site and the seroestes conducted
on specimens were completely negative. They were
applied on 356 people, 75 rodents (one of which was
positive for the Lassa virus), 39 cows, 8 horses, 14 dogs,
1 cat, and 140 spiders, mosquitoes, and flies.

In January 1980 an electrical engineer working for the
Nzoia sugar refinery near Bungoma in Kenya’s
Western Province fell sick, was hospitalized in Kisumu,
then transferred to Nairobi, because of the gravity of his
condition, where he died after having infected a young
Ugandan doctor in charge of the Intensive Care Unit.
The virus was isolated from this doctor, in whom the
course of the disease was marked by a significant rise
in his antibody level (from 1:4 to 1:256).

A thorough epidemiological investigation yielded
very few interesting facts. Out of 705 serum speci-
mens taken from contacts, the hospital staff, workmen
at the plant and from villagers, antibodies were
detected only on 3 nurses in a nearby hospital where
the patient had not been treated, on a laboratory tech-
nician, and the patient’s housekeeper. In addition,
close attention to possible signs of the disease led to
17 suspected cases over the next six months, but none
of them was confirmed by a specific antibody test.

Marburg virus disease made its fourth appearance in
1982, in an unemployed man from the vicinity of Fort
Victoria, Zimbabwe (the area where the first South
African patient had probably been infected); this man had
come to Newcastle to look for a job. Despite the fact that
he presented on his face scratches and a skull fracture
after a fall and an outbreak of P. falciparum malaria, the
seriousness of his febrile state and haematological values
prompted his transfer to the isolation unit of the hospital
at Rietfontein. The haemorrhagic symptoms became clear
and serological examinations pointed to Marburg virus as
the causative agent: indeed the patient’s specific antibody
level had risen from 0 to 1:256. The virus itself was not
isolated and the patient recovered, despite his serious
condition and the diversity of his ailments.

The disease is doubtless a fatal haemorrhagic fever
with a confounding sporadic pattern of appearance.
Secondary cases all recovered.

There are no tertiary cases. The monkey’s role is
merely that of an amplifier of occasional infection. The
known routes of transmission are the blood, sexual inter-
course, and the air. Serological studies using immuno-
fluorescence have shown that Marburg virus occurs in
Liberia, Central African Republic, Gabon, Kenya,
Sudan, and Zaire. It would be rash to try to make pre-
dictions for the future based on such meagre evidence.
The virus is not a new one. Will it continue to make spo-
radic appearances in man or, on the contrary, flare up
again as a deadly epidemic? Only the future will tell.

2. Ebola virus infection

The beginning of an epidemic of HFV in Zaire was
marked by the death of a schoolteacher at Yambuku (a
mission 90 km north of Bumba, in Equateur Province)
on September 8, 1976. The burial of this highly
esteemed figure was attended by a large crowd and
was an occasion for many contacts. The patient had
just finished a tour of inspection when he fell sick on
August 25 and was hospitalized with the diagnosis of
malaria, treated by chloroquine injections. He devel-
oped a gastro-intestinal bleeding on September 5. The
day after his funeral and for several days thereafter
new cases presenting haemorrhagic syndromes were hospitalized and died. A local investigation yielded a temporary diagnosis of typhoid fever and was followed up by a vaccination campaign.

In the interim after the death of a nun working as a midwife on September 14 after a five-day illness and of a missionary, the hospital’s chaplain had alerted these victims’ respective superiors. When a second nursing sister fell ill on September 23, she was transferred to Kinshasa for repatriation to Europe for health reasons. However, this was not possible and she died on September 30. Specimens obtained from this patient were sent to the Institute of Tropical Medicine, Antwerp, where the causative virus was isolated and identified.

This last patient also infected the two nurses who had cared for her, a Belgian, who fell sick on October 8 and died on October 14, and a Zairean who, due to a longer incubation period, travelled all over Kinshasa during her leave before dying on October 30.

The situation was becoming alarming. Doctors Ruppol and Raffier visited the territory where the epidemic had spread along a radius of more than 100 km. Abumombazi on the Ebola river marked its northern boundary. The population underwent a period of great fear, which was shared by the medical staff. Two patients from Yambuku transported to Bumba Hospital died there; no one dared touch them, forcing the doctors to bury the corpses themselves. No secondary cases developed in Bumba.

Meanwhile, an international committee (the International Commission for Acute Haemorrhagic Fever, ICAHF) had been set up and became operational on October 18, 1976.

At the same time, the attention of those concerned was drawn to the existence of a very similar epidemic in Maridi, in Sudan’s Equatoria Province.

The presumed first case of this epidemic had fallen sick at Nzara on July 26, 1976. Nzara’s hospital where the first cases of the epidemic were treated and died, had only 12 beds and rudimentary facilities. The first patient’s brother wanted to have him hospitalized in Juba, but his condition was so serious that he had to be treated at Maridi Hospital, on the road to Juba. The patient died and was buried in Maridi. He was already the fourth case suffering from the epidemic but the first case to be seen at Maridi. The brother and a nephew who had helped take the patient to Maridi fell ill in turn; both were treated at Nzara’s hospital by antimalarials and antibiotic injections. They were the fifth and sixth cases of the epidemic. They both died and infected the nurse who had cared for them and who was sent to Maridi’s hospital.

The two brothers had been employed in a nephew’s store on the edge of a major road; it was the usual stopping point for people and merchandise going back and forth between the area and Zaire. The store owner also fell sick and went to Khartoum via Juba. He died at Khartoum on August 30.

Tracing back the origin of the disease, it was found that a family friend, who was very popular in the area, often helped out in the store and served willingly as an interpreter. He had fallen sick on July 18 and had first been treated at home by relations, then hospitalized at Nzara on July 24, where he died on July 27. He was actually only the third case at Nzara where he worked in a cooperative cotton factory: two workers in this factory had died of haemorrhagic syndromes – the first one had fallen ill on June 27 and died on July 6; the second had been struck on July 12 and died on July 14. They were without a doubt the first and second cases of the epidemic. The connection with the Maridi epidemic emerged only after the third case (presumed the first) raised the question of a possible link with the Yambuku epidemic.

From this point on the epidemic began spreading rapidly through Nzara, but without any connections with the hospital. There was a total of 67 cases, with 31 deaths. Contacts could be traced back for 48 of the cases and 27 of the fatalities. The cooperative cotton factory was one of the sources of infection and contact; the third case was another source of infection.

Maridi Hospital, with its 120 beds and nursing school, was the epicentre of the epidemic. The original case was responsible for infecting a nurse, a maintenance man, an orderly, and a visitor. These subjects were hospitalized in different wards, thereby disseminating the infection and producing a second wave of the haemorrhagic syndrome among the patients, hospital staff, and visitors. The latter group spread the disease in the town of Maridi, where 153 people would be affected, leading to 76 deaths. In the hospital, 76 cases – mostly nursing students – were affected with 41 deaths. The epidemic thus struck 229 people, with a case-fatality rate of 117/229.

A second epidemic broke out in 1979, this time with Nzara Hospital as its origin. The original patient was once again a worker from the local cotton cooperative, aged 45. He died on August 5 of gastrointestinal bleeding. The conditions of hospitalization were so primitive that family, friends, and visitors had to dispose of the patients’ vomit and excrement and change the dirty linens, as well as taking care of preparing and burying the corpses. It therefore is not surprising that secondary cases occurred: it were a nurse, a hospital clerk, the patient in the next bed, and a visitor. These five patients were responsible for 34 additional cases – relatives and friends – and 22 deaths. This epidemic of a total of 64 cases, caused 27 deaths.
Meanwhile it had been ascertained that the third case of the first epidemic (1976), as well as the two women who escorted him, had been brought to Nzara Hospital by a passing Zairean truck driver who arrived in Bumba four days later. The two women fell sick on August 1 and 3 and died ten days later.

The truck driver was found on his way back. He had not been sick, nor, to his knowledge, had any of the other passengers. It became certain that rapid contacts between Nzara (Sudan) and Bumba (Zaire) were routine. It might be added that, all over the world, information on movements at border regions is governed by a code of silence and reserve. The identity of the mysterious patient who was first to be hospitalized at Yambuku but went on to die elsewhere was therefore never discovered.

Five cases of Ebola virus disease were seen in Kenya in 1980.

MAJOR CHALLENGES

1. Transmission

1.1. Agent

1.1.1. Marburg virus

This virus, isolated in 1967, appears under the electron microscope as a highly polymorphous, tubular structure with branching filaments that can bud into convolutions and other unexpected forms. It is 750 nm long and 80 nm in diameter, with only a single-stranded RNA genome at its core. The genome itself, isolated from the surface proteins, is not infectious.

It is cultivated easily on cell cultures, including Vero cells, on which it has a visible cytopathogenic effect, seen as inclusion bodies on light microscopy. It can be inoculated by intraperitoneal route in guinea pigs, producing fever. After several inoculations its incubation time is reduced and virulence increased to the point where the acclimatized virus will kill the guinea pigs. It has the same properties in hamsters. In contrast, mice are resistant to the infection, which however kills most monkeys.

Based on a superficial resemblance to rabies virus, this virus was first classified as a rhabdovirus. As antigenic properties were not the same, it was decided to classify this virus separately. Among others the name of Filoviridae has been suggested.

1.1.2. Ebola virus

Isolated in 1976, this virus is morphologically very close to, but antigenically different from Marburg virus. It has a complex, filamentous helical structure with branches, knots, and swirls. It is very long, measuring 200, 600, 1500 or more nanometres, so that its size may be expressed in microns, and has a 80 nm diameter; its core contains the RNA genome. It is visible by light microscopy.

Marburg antiserum has little or no effect on Ebola virus, confirming their different antigenic composition.

It is cultivated easily on various cell lines, including Vero cells, on which it has a cytopathogenic effect. It also grows in ordinary environment. The genome consists of a single-stranded RNA, but this unit is not infectious in the absence of viral proteins. To date, five proteins have been identified for the two viruses, but their roles are still unknown.

Some rather small differences have been detected in virulence, appearance of the plaques and organ invasion patterns, between viral strains from Sudan and from Zaire. Ebola virus is the most virulent and most invasive. These differences are not impairing true cross protection.

1.2. Sources of contamination

1.2.1. Marburg virus

The connection between the first epidemic and the vervet monkeys imported from Uganda was so evident that the monkey’s role as the source of the infection seemed certain. However the investigators had soon to change their ideas, because the monkeys were so receptive to the disease and had such a high mortality rate, that, far from being the reservoirs of the virus, they were actually its victims and unfortunate intermediaries. Furthermore, monkeys played no role in later outbreaks of the disease.

Investigations of a wide range of potential reservoirs were undertaken and became even more extensive when the first South African case had been detected. The investigation launched in connection with the Kenyan cases showed specific antibodies in 4 of the 136 monkeys examined (2.9%); furthermore the virus was never isolated from animals that did not have specific antibodies.

The origin of Marburg virus remains a mystery.

1.2.2. Ebola virus

The sources of the different original clinical cases have remained just as mysterious. As a virus with intracellular growth is presumed to have a reservoir, a large number of invertebrates (mosquitoes, bedbugs) and vertebrates were investigated, with special atten-
tion given to rodents and bats (*Tandaridae trevori*). The harvest was as negative as for Marburg virus. Besides this, an infection supposedly transmitted by haematophagous arthropods should logically have given rise to thousands of cases.

The significance of the presence of antibodies in free-ranging guinea pigs in Zaire and 3 (1.6%) of the 184 baboons examined at titres ranging from 1:32 to 1:288, points to no more than a probable common source of infection.

It has been hypothesized, that the virus is a plant virus. However, such a source of infection has never been seen in man and can be disregarded as pure fantasy. And thus the origin of contamination remains a mystery.

1.3. **Mode of transmission**

One must distinguish between two types of transmission:
- some infections develop, from an unknown source and remain confined to family circles;
- whereas others are maintained by human activities as in hospitals.

The former can take a subclinical or clinical course and may give rise to secondary cases, but die out in the third or fourth generation. The infections through hospital or extramural contacts create favourable conditions for a wild extension of the disease.

The risk of infection is determined for viruses by the number of particles per ml of the infected fluid or secretions. The concentration is low in the sputum, secretions, urine, and sperm (which carries the virus for long periods), but very high in the blood, hence the increased risk of nosocomial infections. Person-to-person transmission of the virus requires close contact, but hospital and dispensary work facilitates blood-borne transmission, especially if the working conditions are rudimentary. The virus remains in the blood for two weeks. A 100% mortality rate was reported for patients infected by non-sterile needles.

2. **Geographic distribution**

2.1. **Marburg virus**

The disease has been seen in Kenya, Zimbabwe, Natal (South African Union), and Uganda. Seroepidemiological surveys using IFAT (indirect fluorescent antibody test) have drawn attention to the frequency of seropositive cases in a population where the disease is not observed: Central African Republic 6%, Liberia 1%, Cameroon 0.6% (9 cases were positive on 1,434 examinees), Gabon, Sudan, Zaire. In Kenya antibodies have been found in vervet monkeys in captivity (4 cases positive on 136 or 2.9%).

2.2. **Ebola virus**

Haemorrhagic fever due to Ebola virus has been seen in Sudan, Zaire, and Kenya.

Seroepidemiological surveys carried out in Zaire confirmed suspicions that the virus was not all new and was fairly widespread. The Yambuku and Maridi outbreaks had led to its identification. The following seropositive rates were obtained: Tandala (Ubangi): children 14%, elderly 21%, women under 30 years of age 9%, and adult males 3%; Gemena (Ubangi) 18%; Kasongo (Maniema) 10%; Yambuku 8%.

Similar rates were found in other countries such as southwestern Sudan (5 to 30%), Cameroon (6%), Kenya (8 to 16%), Gabon (6%), Central African Republic (3.5 to 8% [17 on 499]), Casamance, Senegal (27%), northern Zimbabwe (3%). Antibodies have also been reported in Ethiopia, Guinea, and Nigeria. Rwanda seems unaffected.

In Kenya, 1 out of 184 baboons was found to be seropositive, but the colobus monkeys were negative.

3. **Clinical symptoms**

The symptoms of the Marburg and the Ebola virus and of the other filovirus diseases are similar. It is therefore logical to cover the clinical pathology of these two infections together.

After an incubation period of three to eight days for Marburg virus and four to sixteen days for Ebola virus (an average of seven days, but one to six days in the case of transmission by needles) there is sudden onset of a febrile attack. This abrupt onset is accompanied by a very marked discomfort, severe frontal and temporal headache, thoracic and lumbar-sacral myalgia, and sometimes pain in the orbits of the eyes. The second day the fever climbs to 39°C or more and reaches a plateau. Relative bradycardia occurs. The diagnosis may be missed because of pharyngeal discomfort due to a dry throat, painful swallowing, vomiting, and stomach cramps, all the more so as diffuse watery diarrhoea develops with up to a dozen bowel movements a day. The stools may be mucous-sanguineous. The picture is completed by chest pain of pleuritic type. Around the fifth to eighth day a non-prurigenous maculopapular exanthema appears across the buttocks, spreading towards the trunk, the limbs, and the face; it disappears with desquamation after three to four days. There may also be a dermatitis of scrotum or labia majora and exanthema of the palate and
TROPICAL DISEASES

tongue. The tonsils may be flecked with small whitish spots resembling tapioca.

The patient’s general condition deteriorates rapidly. The diarrhoea may be followed by dehydration. The face loses its expression, the patient is prostrate and uncooperative. Lymph gland enlargement may occur.

The abrupt severe attack is characterized above all by bleedings in the skin and mucous membranes, especially in the respiratory and gastro-intestinal tracts, and by epistaxis, petechiae, ecchymoses, bleeding gums, haemorrhagic conjunctivitis, haematemesis, melena, haematuria, vaginal bleeding and abortion. Their intensity varies with the individual. These manifestations show up in 30% of the Marburg cases and 70% of the Ebola cases.

The fever persists for 10 to 20 days, on average for two weeks. A second febrile attack may occur. The critical period comes around the fourth or fifth day when confusion and disturbed behaviour appear. Spontaneous haemorrhages bring a bleak prognosis. Disseminated intravascular coagulation (DIC), with the presence of fibrin degradation products, develops in a few patients. Cardiovascular involvement may compound the situation, leading to reversible or irreversible shock.

A number of other symptoms may accompany the clinical picture. These include jaundice, haemolysis, uraemia, hepatic coma, dehydration, acidosis, bacterial superinfections, CNS involvement, paresthesias, irritability, and mental confusion.

Hypovolaemic shock, accompanied by oliguria or anuria on the eighth or ninth day, is a sign that the end is near.

Major complications include broncho-pneumonia, myocarditis, orchitis, and uveitis.

The virulence of the virus is highly variable, explaining differences in the clinical course and severity of the symptoms from one patient to the other.

4. Diagnosis

4.1. Haematological findings

Leucopenia is the rule, with abnormal granulocytes (acquired Pelger-Huët pseudocytes), viral plasmocytoid lymphocytes, and thrombocytopenia.

Signs of DIC (Disseminated intravascular coagulation), increased levels of fibrinogen and fibrin degradation products, a prolonged prothrombin time, etc., may be observed.

4.2. Biochemical findings

Hypoproteinaemia, hypokalemia, elevated blood urea, elevated transaminases (SGOT or Aspartate aminotransferase, AST; SGPT or Alanine aminotrans-

ferase, ALT) glutamate dehydrogenase, sorbitol dehydrogenase, and glutaryl transpeptidase. The amylases may be elevated if the pancreas is involved.

The CSF is usually normal, although the protein concentration may be slightly elevated.

Proteinuria and haematuria will occur in the case of renal involvement.

It should be pointed out that these examinations were done mostly on Marburg virus cases. It is often unjustified to undertake highly diversified biochemical research on these infections, given the serious risks involved in handling the blood and serum.

4.3. Specific diagnosis

4.3.1. Isolation and identification of the virus

Isolation can be done in vivo by inoculating guinea pigs. This produces only a slight increase in temperature, until the virus’s virulence is increased to the point where it kills the animal. Adult and baby mice have also been used to isolate Ebola virus. Monkeys, which are very sensitive, present too many risks of transmitting the disease to the personnel.

In vitro inoculation is done on Vero cells without producing marked cytopathic changes, but it is possible to detect the characteristic intra-cytoplasmic inclusion bodies, which can be stained by haematoxylin and eosin. A quick diagnosis can be made by checking daily the cells both by Indirect Fluorescent Antibody Test (IFAT) and by electron-microscope (EM) scanning of the liquid.

The in vitro methods are the methods of choice, as they are simple and present fewer risks than inoculating animals.

Detection of the virus may be done by EM study of the blood (during the phase of acute viraemia); this enables to recognize the typical virions. The procedure may be facilitated by adding specific antibodies.

IFA tests can likewise be used to detect the virus in a thick blood film, as well as in the urine or saliva during an acute viraemic phase. As this procedure is not completely satisfactory for Ebola virus, ELISA might be another possibility.

4.3.2. Detection of specific antibodies

These antibodies appear later, towards the third week of infection. Complement fixation (CF) tests show that the antibody titre peaks after 2 weeks, then drops gradually, while the antibodies persist for two years. The IFAT is faster, easier, sensitive, and more specific. With IFAT antibodies appear a little earlier than with CF antibodies; they increase gradually to a peak and then drop to a lower level, at which they persist for years (see also below p. 1395 for cross-reactions with other filoviruses).
B. LASSA FEVER

HISTORICAL BACKGROUND

January 19, 1969, a 69-year-old American midwife working at Lassa, a large village with a population of 3,000 in the middle of the savannah of northeastern Nigeria, had a severe febrile attack with severe lumbar pain and with ulcerated pharyngitis, making swallowing very painful. This small town on the west bank of the Yedseram is located at the foot of the high plateaux of Cameroon, south of Lake Chad. It is one of those remote places where religious missions settle willingly. The Church of Brethren had founded there a hospital with a 62-bed capacity. The midwife devoted herself without rest to the population, taking advantage of her retirement to fulfill a desire of her youth and had remained a lifelong aspiration.

Her disease was resistant to antimalarials and antibiotics and gradually worsened. When petechiae appeared and the leucopenia became alarming, the decision was made on January 24, to take her to Mubi, from which she was taken by plane to Jos, capital of Benue State. She was hospitalized there at Bingham Memorial Hospital and died practically on arrival on January 26, 1969.

This patient started a series of infections. Two nurses who had attended her and, more specifically, cleared her painful throat of the secretions which were blocking it, fell sick in turn. One died on February 13, the other recovered. The head nurse, who took care of these two nurses, was also infected. She was sent to New York on a scheduled commercial flight with 80 other passengers and without the slightest precautionary measures. She was hospitalized at the Presbyterian Hospital of New York, Columbia University.

The causative virus was isolated by the scientists’ sera and various secretions, on Vero cells at the Yale Arbovirus Research Unit. The illustrious virologist Jordi Casals became infected in the course of this laboratory work; he owed his recovery to serum from the nurse who had overcame the infection.

This last episode, combined with the agonizing fears for Lassa’s nurses and midwives, alerted international public opinion. This hemorrhagic fever was given the name of plague of the year 2000, however, it came to light before the emergence of AIDS. As a result, the Lassa virus study was entrusted immediately in strict isolation in the CDC’s high security Laboratory in Atlanta. Such high precautions were justified as a Yale technician died of Lassa fever, although no contact had occurred with the virology lab. He might have been infected by his wife, a technician in the tissue culture team.

This dramatic outbreak struck 6 people, killing half of them. It was later ascertained that a parturient in Lassa hospital had been the source of this small epidemic.

Lassa fever made its comeback in 1970 at Jankwano Hospital in Jos, a densely populated mining centre in northeastern Nigeria. As it coincided with the waning phase of a major yellow fever epidemic (100,000 cases and a 40% case-fatality rate), the diagnosis was difficult.

The original case was a 25-year-old woman from Bassa, a village east of Jos, who was hospitalized with pneumonia. She was responsible for 28 cases and 13 deaths who followed. Twelve of these cases were definitely contracted at the hospital, confirming the risk of nosocomial contamination. Such was the bad luck of the unfortunate woman doctor who became infected accidentally during an autopsy, through a cut on her finger, and died of Lassa fever, drawing the public’s attention to this new epidemic.

A third epidemic broke out in 1972, this time at Zorzor Hospital, 30 km northeast of Monrovia (Liberia), between the 2nd and 20th of March. The first patient, who was hospitalized for threatened abortion, was an inhabitant of Zigriga, a village located some 30 kms north. She indeed aborted of twins in the fourth month of pregnancy. The midwife who attended her was generously spattered with blood, under more on some varicose ulcers, fell ill and died. A total of ten women, either hospitalized or working in the maternity ward, were affected; four would not survive the infection.

Serological examinations of 93 contacts with the original and the secondary cases, yielded 8 seropositive at complement fixation, revealing the existence of subclinical or mild cases.

The observations collected during this epidemic confirmed not only the important role of maternity wards and clinics as sources of nosocomial infections, but also the existence of clinically undetectable cases and the spread of the virus beyond Nigeria.

A Lassa virus infection was diagnosed in London in September 1972 in a patient from Panguma, Sierra Leone, confirming the virus’s spread in West Africa. Careful scrutiny of the medical files of Panguma and Tongo hospitals, located 150 kms from Zorzor, for the previous two-year period (October 1970 to October 1972) revealed 64 suspected cases, 54 of whom had never had contact with the hospital before falling sick. It was becoming obvious that the Lassa virus was circulating in the outside community.
A search for possible animal reservoirs was undertaken in this diamond-mining region. In the very high security laboratory of CDC in Atlanta 644 animals of all sorts, including 350 rodents, were examined. Of the rodents fourteen, all *Praomys* (*Mastomys* *natalensis*) were carrying the Lassa virus. The reservoir was thus identified. Systematic investigations ascertained the presence of the virus throughout West Africa and even beyond.

From 1975 onwards several case studies were published under the label *epidemic*, although these were simply instances of serious cases contracted by expatriate doctors followed by the identification of some infections in the people around them. Some good examples are provided by the micro-epidemics:

- at Onitsha (Nigeria), two German missionary doctors were infected by a 19-year-old male, while negative results were found in 101 contacts;
- at Zonkwa the case of an English physician working at the Hospital, who fell ill and died in London after being repatriated on a regularly scheduled flight; he had supposedly come in contact with two cases of fevers of unknown origin, a commonplace occurrence in Tropical Africa;
- in Vom (Nigeria), a pregnant woman doctor who was struck by the infection, together with three student nurses, one of whom died while the other two recovered; five other subjects were also found to be seropositive, while a number of cases must also be added who have been repatriated without further examinations and diagnosis;
- a large number of Lassa fever cases occurred also in Vom between 1974 and 1977: a deceased pregnant woman was at the origin of two secondary cases and of eight seropositive sporadic cases;
- at Bembereke, a woman doctor and a female laboratory technician, from whose serum the virus was isolated (the technician died), led to the detection of 2 seropositives among 88 contacts; five sporadic cases were also diagnosed.

To the list must be added the cases imported to the United States and to Europe. A lady Peace Corps volunteer, suffering of fever working at Mobai (Sierra Leone) was repatriated to Washington via London in 1976, accompanied by her husband and a nurse. The 552 people with whom she came into contact all remained seronegative.

In 1980 a Dutchman working for a cooperation organization at Ouagadougou (Burkina Faso) was repatriated to the Netherlands because of a febrile attack persisting for 13 days, accompanied by an exanthema and conjunctival injection but no signs of haemorrhages. He had a mild Lassa fever; all his contacts remained seronegative (Van der Heyde, 1980).

### MAJOR CHALLENGES

1. Transmission

1.1. Agent

The agent is a spherical virus of variable size (70 to 150 nm) with a cloudy appearance due to superficial microvillous projections. It is a double-stranded RNA virus that contains opaque grains formed by ribosomes incorporated from the host cell. These ribosomes are perhaps the key to the virus's persistence. Its grain-of-sand appearance resulted in its being classified with the arenaviruses (from the Latin word *arenosus*: like a grain of sand) along with the lymphocytic choriomeningitis, Junin, Machupo, Tacaribe, and other viruses.

It is easily inoculated in Vero cells, in which it has a cytopathogenic effect (CPE) in four to five days or less, yielding inclusion bodies which can be stained with May-Grünewald-Giemsa.

The virus can be inoculated intracerebrally, in newborn and adult mice. The guinea pig is very sensitive to it. The squirrel monkey can be infected by the IM route.

It was isolated in 1974 from *Mastomys natalensis* specimens captured in Nigeria and Sierra Leone.

A similar virus was isolated in Mozambique (Mopeia Velha) in 1977 from the tissues and organs of *Mastomys natalensis*. The virus reacted in Indirect Fluorescent Antibody Test (IFAT) with the diagnostic serum for Lassa virus, but did not react with specific monoclonal antibodies.

Another similar virus was isolated in 1981 from 20% (11 out of 55) of the *M. natalensis* (with chromosomal formula 2N = 32) studied in Zimbabwe, and 7% (1 out of 13) of the *Aethomys chrysophilus*.

The same occurred in Central African Republic, where the virus has been isolated eleven times from *Praomys*, of the sub-genus *Mastomys*, underscoring once more the difficulties epidemiologists have to face through frequent taxonomic modifications for rats.

Lassa virus seems to belong to an as yet incompletely elucidated complex. It is sensitive to Ribavirin.
1.2. Reservoir

*Praomys (Mastomys) natalensis* – the multimammate mouse – has been identified as the reservoir of the virus since 1974. This docile rat lives close to or with village houses. It is a commensal species that can be tamed and is raised easily in laboratory. It is 8 to 16 cm long with grey-brown fur. Its tail is shorter than its body. The species complex is identified by its characteristic 8 to 12 pairs of mammary glands. Its litters, requiring a gestation period of 23 days, often consist of 10 or more young. They reach maturity in 110 days and have an average life-span of 18 months. It has thus great proliferative abilities.

The species complex consists of two variants, with karyotype 2N = 32 and 2N = 38. In Sierra Leone, the former inhabits the forest, the second the savannah. Both karyotypes have been found infected.

Pre- or perinatal infection of *M. natalensis* leads to a viral infection that persists the animal’s entire life, with permanent excretion of large amounts of virus in the urine. *M. natalensis* tolerates Lassa virus, which has no pathogenic effects on it.

In Nigeria, *Rattus rattus* and *Mus minutoides* have been incriminated as other possible virus reservoirs.

1.3. Sources of contamination

*M. natalensis* – and perhaps other rodents – are the primary source of infection by this zoonosis. It constitutes the major risk of infection. Depending on the dose inoculated and the virus’ capacity for multiplication in the host it causes benign sporadic cases or serious infections (under more in pregnant women). The rodent’s urine and saliva carry the virus and may contaminate food or drink directly. When the rats are hand-captured they urinate out of fear, thus soiling the capturer’s hands. The virus may also be able to survive in dust and thus cause droplet infections.

Interhuman contamination seems to be at the root of most of the clinical cases, which often take the form of threatened abortion of which all expelled parts and excreta, secretions, or blood, are infective and can easily trigger a nosocomial transmission cycle (1/3 of the cases).

1.4. Mode of transmission

Knowledge of the interhuman and especially rodent-to-man transmission routes is still uncertain. Direct contact with relatives, doctors, nurses, or other health workers, blood, oral and pharyngeal secretions, vomitus, urine, soiled bandages, sheets and maternity ward linen are very dangerous. Contamination may be either direct or through dirty hands, wounds, scratches, insect bites and through injection puncture sites or the eyes. Indirect contact via infective dust particles suspended in the air is a possible but uncommon transmission pathway, given the rare tertiary infections or infrequent contamination among patients who had no direct contact with the infected individual or among hospital visitors.

2. Epidemiology

The micro-epidemic is invariably traced back to the hospitalization of a patient or parturient suffering from a fever from unknown origin. Such individuals give rise to secondary cases among the hospital staff or the other patients. Tertiary cases are rare and usually mild. Serotesting has proven that very mild, subclinical cases of Lassa fever are frequent. As such individuals are not hospitalized, they do not influence the specific mortality rates.

The 64 retrospective cases detected from the records of Panguna and Tongo Hospitals for a one-year period arose from non-hospital sources. The virus must have been circulating in the community, probably in a few families. Investigations showed the existence of silent viral sources.

The same can be said of the longitudinal study carried out by Monson et al. (1984) in Zorzor Hospital (Liberia). Twenty-two months of oriented searching resulted in the diagnosis of 44 cases, 14 of them by isolation of the virus and 37 on the basis of serological criteria. Five of the subjects were Guineans (the border is 5 kms away). Systematic screening for Lassa virus in all febrile cases affected over a month, revealed 6 cases or 13% of them. Moreover, some clusters of cases were also identified.

The transmission of the virus from rodents to man may occur through rodent catching, contamination of the hands or food by rat urine, etc. The means by which the enzootic is perpetuated in the rodent population (congenital, vertical, horizontal transmission?) is unknown.

More than 400 human cases have been recorded in all. The proportion of medical and paramedical staff in this total is very high: 9 doctors (3 deaths), 12 nurses and midwives (3 deaths), 3 laboratory technicians (2 deaths), and 20 hospital aides (7 deaths).

The clinical identified cases account for only a very small proportion of the cases of fever who were due to Lassa virus. They represent indeed, a very low percentage of Lassa virus infections, as for example, 3 to 11% of the febrile complaints studied in two villages.
in Sierra Leone were due to Lassa virus. The Mozambique-Zimbabwe variant has produced no clinical case to date.

3. Geographical distribution

The published clinical cases come from three West African countries – on the one hand Nigeria, and on the other Liberia and Sierra Leone, spilling over into Guinea. In Guinea, cases of Lassa fever are thought to have occurred in Telekore, as early as 1952. The infection may also be present along the Falémé river in western Senegal. One must add the case imported into the Netherlands from Ouagadougou (Burkina Faso).

It is worthwhile remembering that these small epidemics were diagnosed in remote areas served by mission or mining-company hospitals (as in Sierra Leone). A retrospective study of 94 usable serum specimens collected in New York from 124 missionaries yielded 5 complement fixation positives for the Lassa virus, that is, 5.3%. This is comparable to findings in 104 serum specimens that were collected from febrile patients in the mission hospitals and sent on for examination: this group yielded 6 (5.8%) seropositives. The missionaries had lived in Liberia, Ivory Coast, Nigeria (Jos Plateau), Mali, Burkina Faso, Central African Republic, and Zaire. It is inexcusable to fail to take these indications provided by Frame (1975) into account.

Other serological investigations yielded positive results in Ivory Coast, Burkina Faso (confirmed by the case imported into the Netherlands), Benin, Cameroon (0.15% of 1,252 serum specimens from Lomé, in the equatorial forest, and Poití, in the pre-Saharan savannah), and in Gabon (13% of 62 serum specimens from Lambarené, including 2 in children in the three to six years age group), Gambia and Ghana must also be added to this long list.

In contrast, all attempts to screen for antibodies in Kenya and Sudan (1,200 recruits representative of the entire area) remained negative. A more recent study, based on IFAT, has detected antibodies in 12 to 13% of the human serum specimens obtained from southwestern Sudan.

4. The disease

4.1. Clinical signs

The human infection by the Lassa virus takes the form of a lymphoreticulocytic tropism leading to disorganization of the lymphoid system and a weakening of the lymphocytic defences. All of the organs may be affected, but the pattern of symptoms is not specific.

The incubation period is estimated at three to sixteen days (an average of seven to ten days). Onset is slow and insidious, with the picture of malarial fever or a pseudo-influenzal syndrome, such as light fever, headache, chills, diffuse myalgia, malaise. The acute stage begins around the third to sixth day. The fever – of variable pattern (febrile with daily upward spikes, remittant at a high levels of 39-40°C, sometimes lower) – increases and persists for one to three weeks. It is accompanied by asthenia, lethargy, anorexia, and a state of prostration completely out of proportion to the level of temperature. Dysphagia developing in the wake of very painful pharyngitis due to inflammation of the tonsillar and pharyngeal tissues, is accompanied by whitish or yellowish exudative lesions and difficult-to-remove pseudomembranes, sometimes exhibiting vesicles and/or ulcerations. The tongue is furred, with oedema of the entire cavity. These disorders are accompanied by nausea, vomiting, and liquid stools. Diffuse colics in the right upper quadrant occur frequently. Distressing substernal aches, exacerbated by coughing or deep breathing, may be accompanied by pleural rhonchi or pericardial frémitus.

The macular or maculo-papular rash is slight. Extensive oedema affects the periorbital tissues, face, neck and internal organs (lungs, throat). Generalized lymphadenopathy is present.

There is a definite but light haemorrhagic tendency. There is excessive bleeding at the site of injections. Bleeding from the oral mucosa, intestines and vagina is seen. Spontaneous abortion is the rule and is often the reason for hospitalization.

The patient is prostrate, obtunibulated, intoxicated, and dehydrated. The blood pressure is low (<100 mm Hg), the pulse rate dissociated from the temperature, and bradycardia is the rule. The picture is completed by nervous system disorders such as trembling, nystagmus, vertigo, deafness (estimated as high as 20% appearing towards the 2nd or 3rd week of illness), confusion, and shock.

The convalescent period begins when the temperature returns to normal as of the second or fourth week. It is accompanied by asthenia and refractory alopecia.

From observations made in rural areas of Sierra Leone, the most useful diagnostic signs are facial oedema, conjunctivitis, purulent pharyngitis, vomiting, proteinuria, and abdominal tenderness. The probability of Lassa fever based on these signs is 80%, but the virus identification rate is actually only 44%. Besides, none of these symptoms or groups of symptoms is pathognomonic.
4.2. Diagnosis

4.2.1. Haematological findings

Leucopenia (<4,000), relative lymphopenia, and normal thrombocyte count. Prothrombin time is normal or only slightly abnormal.

4.2.2. Biochemical data

Following serum enzymes are elevated: SGOT or Aspartate aminotransferase, creatinine-phosphokinase, and lactic dehydrogenase. The aspartate amino-transferase titre is of definite predictive value: 150 IU at admission. A viraemia of 10⁴ LD 50 corresponds to a 14-fold higher risk of mortality.

In the event of dehydration, haemorrhage, or renal involvement, blood urea may be elevated.

The urine may contain proteins and granular cylinders.

There are no characteristic changes in the CSF.

4.2.3. Specific diagnosis

4.2.3.1. Isolation and identification of the virus

The virus may be isolated from the blood, serum, gargle liquid, throat swab, pleural liquid, or urine, which harbours the virus longer than the blood, and of course post-mortem tissues, including liver biopsy material. These specimens must be kept cold (refrigerator bags).

The method of choice is to inoculate Vero E6 cells and to use rapid identification techniques of the virus or the viral antigen. The virus can be identified after less than 24 hours of incubation in cell culture by Indirect Fluorescent Antibody Test (IFAT) after conjugation with specific antibodies. This can also be done using a urine centrifugation pellet, gargle liquid, blood leucocyte layer, skin biopsy, or conjunctival cells. ELISA may be used instead of IFAT.

Electron-microscope examination of the culture liquid or, better yet, after freezing-thawing this liquid, allows one to recognize the virus’s typical morphology.

IP inoculation in guinea pigs is possible. The animal’s temperature must be monitored and the blood screened for antibodies.

4.2.3.2. Detection of specific antibodies

The antibodies may be detected by IFAT as of the second week of illness using Lassa-infected cell preparations in the presence of fluorescein-conjugated anti-human serum. Positive serum will fluoresce green.

The diagnostic titres include an IgG titre of at least 1:512, and a positive IgM reaction, in addition to the fourfold increase in the titre of the convalescent as compared to the acute serum specimens. The ELISA method, which is especially appropriate for mass surveys, can also be used for similar screening purposes. Antibodies appear by complement fixation only after three or four weeks of illness and persist only two to five years.

To be significant the results must obviously show an increase in the titre of the convalescent compared with the acute serum.

4.3. Differential diagnosis

This is a concern chiefly for the first cases. All febrile diseases should be considered. Lassa fever is mostly mistaken for flu, typhoid fever, for the various types of septicaemia, malaria, and also the arboviruses diseases, including yellow fever, typhus, tick fevers and leptospirosis.

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C. INSTRUCTIONS FOR CARE OF PATIENTS WITH HAEMORRHAGIC FEVER

Instructions concerning the approach and precautions to take are more important than advice on therapeutic courses.

The patient must be isolated in a specialized department, isolation unit. The authorities must absolutely be informed of the case(s), even if there is no specific legislation.

Convalescent immune plasma should be administered as early as possible (definitely during the first week): 200 to 500 ml IV over 30 to 90 minutes. Interferon has such a wide range of action that it is difficult to give an opinion of its true usefulness. However, withholding interferon could result in medico-legal consequences, even lead to litigation. Administering heparin (10,000 U/24 hrs) is indicated only if disseminated intravascular coagulation (DIC) has definitely developed. In the absence of objective proof, it is better to refrain from administering heparin. However, fresh blood or platelet concentrate should be given. Finally, the patient’s water and electrolyte balance must be monitored and readjusted if necessary.

The main thing is to relieve the patient’s suffering as much as possible: oral hygiene every 2 hours, sedatives, antipyretics, anti-emetics, and antidiarrhoeals.
The blood pressure must be monitored. Daily intake of 1,800 calories is necessary.

The patient’s personal contacts, as well as the medical staff, must also be monitored (temperature checks).

Lassa virus is sensitive to ribavirin (Virazole®), the first synthetic non-interferon-producing antiviral triazole nucleoside. Its mechanism of action is not known (it is not virucidal nor enhances interferon production). The dose is 200 mg three to four times daily. This drug has no effect on the Marburg or the Ebola virus.

D. UPDATE FOR FILOVIRUS INFECTIONS

Morphology studies have placed the filoviruses in the Filoviridae family, named after their thread-like appearance under the electron microscope (Kiley et al., 1982). So far the filovirus family contained three members: the Marburg, the Ebola Zaire and the Ebola Sudan viruses. As described in more detail in the previous chapter, the Marburg virus was first isolated in 1967 from the tissues of African green monkeys (Cercopithecus aethiops) imported from Uganda. Two more members, Ebola Zaire and Ebola Sudan, of this unique family of viruses were isolated from patients in 1976 when simultaneous outbreaks of lethal haemorrhagic fever occurred among humans in Zaire and in Sudan. Since the last epidemic of Ebola in 1979 in Sudan (Baron et al., 1983) no more was heard of this virus. No association with monkeys was implicated and numerous ecologic studies failed to uncover the reservoir (van der Groen, personal communication). Small outbreaks and single cases of Marburg virus infections continue to occur sporadically in east, central and southern Africa (Gear et al., 1975; Teepe et al., 1983; WHO, 1990).

Since November 1989, outbreaks of filovirus infection have been described among cynomolgus monkeys (Macaca fascicularis) imported from the Philippines into quarantine facilities in Reston, Virginia and in Pennsylvania (CDC, 1989). The monkeys were flown from Manila, through Amsterdam to New York, then taken by truck to the Hazleton Research Products in Reston, Virginia. A filovirus Reston, closely related to the Ebola virus was isolated from 5 infected cynomolgus monkeys (Jahrling et al., 1990). Electron microscopic examination of MA-104 cells inoculated with serum from the infected monkeys revealed virus particles morphologically identical to the filoviruses. Immunohistochemical staining of liver of the infected animals using monoclonal antibodies specific for the Ebola virus, confirmed that the virus was Ebola like and not Marburg. A fourth member Reston of the filovirus family, was born. So far this was the first report of the first isolation of a filovirus from non-human primates. The natural reservoir host for Ebola Zaire or Ebola Sudan virus has been sought, but never identified. The finding of Jahrling and his co-workers, suggests that Asian macaques could function as a natural reservoir. Alternatively, these monkeys could have been exposed to the virus through contact with African monkeys during captivity. Extensive investigation at transit points in Amsterdam and New York did not implicate cross-infection of the monkeys by African primates (CDC, 1990). The pathogenicity of the Reston virus for cynomolgus monkeys was uncertain because a high rate of concurrent infection with Simian haemorrhagic fever virus, a known severe simian pathogen, was also observed (Jahrling et al., 1990). On November 28, 1989, a second shipment of cynomolgus monkeys from the Philippines, experiencing unusual mortality, had been received in Philadelphia, Pennsylvania.

An Ebola-related filovirus, Pennsylvania, was isolated from the liver tissue of one of these animals (CDC, 1990). As such, a fifth member of the filovirus family was documented. On March 11, 1992, a batch of 55 cynomolgus monkeys was imported into Italy from a monkey-breeding company in the Philippines. Eight animals died. Post-mortem examination revealed marked splenomegaly and widespread small haemorrhages in many tissues. Laboratory investigations have been conducted by the WHO Collaborating Centre designated at the Central Public Health Laboratory in London, United Kingdom. Viruses were isolated from 3 monkeys and confirmed as filoviruses by electron microscopy. They were shown to be antigenically related to the Ebola virus by indirect immuno-fluorescence using a well characterized monkey serum. Six weeks after importation of the monkeys no illness in associated humans has been reported. Further laboratory investigations are needed to show whether the same filoviruses are involved in both the Italian and United States outbreak.

Inspection of the animal facilities in the Philippines, including the facility that had supplied the monkeys in Virginia, did not identify unusual illness compatible with the Ebola virus disease in either workers or non-human primates. The infected animals had been captured from different remote areas. It is not clear yet, if
these animals were already infected in their natural habitat, or whether they became infected during their stay in the holding facilities in the Philippines. Serologic and virologic studies of animals and workers are under way in these and other facilities in the Philippines (CDC, 1990, 2).

These events have forced the Centres for Disease Control, Atlanta, United States to develop interim guidelines that update and modify the procedures used in the transportation and quarantine of non-human primates (CDC, 1990; WHO, 1990).

An immediate question to ask, was whether these newly described Asian filoviruses, Reston and Pennsylvania, had the same pathogenic potential to produce acute disease in various species of primate, including humans, as the African filoviruses, Ebola Zaire and the Sudan virus?

Previously, it has been documented that most of the non-human primates experimentally infected with African filoviruses Ebola and Marburg, have not survived (Fisher-Hoch et al., 1985). More recently 22 cynomolgus macaques and 20 African green monkeys (Cercopithecus aethiops) with filovirus infections were studied. Thirty-one were experimentally infected with African and Asian filoviruses, 6 were naturally infected during the Reston filovirus outbreak. Overall, African filovirus infections were more severe than Asian filovirus infections. For example none of the eight monkeys experimentally infected with Ebola Zaire survived, whereas 11 out of 15 monkeys infected with Reston virus survived. Animals surviving filovirus infection developed high titre, cross-reacting filovirus-specific antibody 14 to 21 days after infection, and this coincides with the virus clearance. Sudan virus infected monkeys reacted as strongly to the Reston antigen as to antigens prepared from the Sudan or the Ebola Zaire virus, whereas animals infected with the Reston virus reacted less strongly with the heterologous antigens. Healthy monkeys with low titre filovirus antibody may be regarded as uninfected (Fisher-Hoch et al., 1992).

That the Asian filoviruses have a lesser pathogenic potential in non-human primates is consistent with observations in accidental human infection. Filovirus-related illness has not been observed in any human who has been in contact with infected monkeys or their blood or tissues since monkeys infected with Asian filoviruses have been imported in the USA and Italy. However, in six of 178 persons tested, antibody to one or more filovirus antigens was found. Of the six persons, four – all animals handlers at one quarantine facility – had serologic evidence of recent infection (CDC, 1990, 3). It is likely that one out of the four was infected when he cut himself with a scalpel whilst examining the abdomen of a dead monkey. This monkey was subsequently shown by direct antigen capture enzyme linked immunosorbent assay and electron microscopy to be heavily infected with a filovirus. The worker developed a brief viraemia and seroconverted, but did not become ill. The mode of transmission for the other three is unknown (CDC, 1990, 4). The other two persons were seropositive at low titre and had evidence of post-infective state. They both have had contact with non-human primates. Asymptomatic infection has also been reported in the Philippines in persons working with sick cynomolgus monkeys in a facility from which monkeys have been dispatched to the USA (Miranda et al., 1991). These data support that Asian filoviruses can be transmitted to humans during care and management of quarantined animals, without causing disease in humans.

To further assess the health risk to humans posed by the presence of filoviruses in facilities for non-human primates in the United States, 550 persons with various levels of exposure to monkeys were tested for filovirus antibodies against Ebola Zaire, Sudan, Reston, and Marburg by an Indirect Immunofluorescence Assay (IFA) and a confirmatory Western Blot (WB) assay. Of these persons, 42 (7.6 %) were positive (IFA titre > 16, Western Blot confirmed) to one or more of the four filovirus test antigens used. For comparison, serum specimens from 449 persons from throughout the United States randomly selected from an adult primary-care outpatient population were tested. Of these, 12 (2.7 %) were positive (CDC, 1990, 2). In addition, low-titre IFA antibodies to filoviruses in human beings from many geographic locations have been documented (van der Groen et al., 1978). These include unlikely populations such as Cona Indians from Central America and Alaska (McCormick, personal communication) as well as Pygmees in South-East Cameroon (van der Groen, personal communication), Nigerian (Tomori, 1988) and Kenyan populations (Petit, personal communication).

Marburg and Ebola antibody positive serum specimens have also been documented among wild-life East African primates (Johnson et al., 1982). This background sero-positivity rate for filoviruses remains unexplained. One possibility is the antigenic cross-reactivity between the known filoviruses and another, as yet unrecognized non-pathogenic filovirus. The
second possibility is that these low-titred IFA positive sera were due to non-specific reactions. Further investigations are in progress to clarify this.

In summary, we can conclude that monkeys may be able to survive filovirus infection with minimal or no clinical disease, in contrast to infection with the African filoviruses. We should continue to be very alert for the potential danger of infection for imported monkey species, since they remain required for medical research, as well as all members of the filoviridae have for sure not yet been documented, and will not all be as benign as the recently reported Asian filoviruses.

P.G. Janssens and G. van der Groen

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HAEMORRHAGIC FEVERS


TEEPPE R.G.C., JOHNSON B.K., OCHENG D., GICHOCO A., LANGATT A., NGINDU A., KILEY M.,


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The author describes the epidemics that broke out in Lassa and Jos, Nigeria, in 1969; in Zorzor, Liberia, in 1972; and in Panguma, Sierra Leone, in 1973. The first showed a brief and due to hospital transmission, whereas the Panguma epidemic was observed in a rural community and lasted an entire year. He describes the clinical courses, changes in biological parameters, and pathological lesions revealed by post-mortems. He also gives some instructions for differential diagnosis, treatment and prevention.


This book contains the communications and discussions that took place at the international colloquium on Ebola virus fever and other haemorrhagic fevers that was held at the Institute of Tropical Medicine, Antwerp, in December 1977. The various aspects of Ebola virus infections that are examined include the disease’s clinical course and symptoms, the disease process, alterations in the patients’ laboratory parameters, the virus’s morphology and taxonomy, the disease’s epidemiology in Zaire and Sudan, epidemiological surveillance measures and plasmapheretic treatment trials. The book also tackles the problems posed by other haemorrhagic fevers, notably those caused by the Marburg, Lassa, and Crimean-Congo viruses and the South American fevers caused by the Junin and Machupo viruses. The last section considers public health aspects of haemorrhagic fever surveillance and control and transporting and isolating the patients and biological fluids and secretions.


This book consists of two parts, the first one devoted to the history and epidemiology of Lassa fever, and the second one devoted to the clinical description, treatment and prevention of the disease. The bibliography contains 155 references whilst the highly detailed eight-page table of contents doubles as an index.


This remarkable monograph covers exhaustively three formidable viral diseases originating in Africa that have given rise to epidemics in the past twenty years, namely, Marburg fever, Lassa fever, and Ebola virus fever. The author covers systematically the causal virus, treatment and prevention for each of these infections. A very complete bibliography is furnished for each aspect of these three diseases.