Viral haemorrhagic fevers

Report of a WHO
Expert Committee

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WHO EXPERT COMMITTEE ON VIRAL HAEMORRHAGIC FEVERS

Geneva, 19–23 March 1984

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Page 87, lines 34-37:

Delete  The Zaire strain is uniformly lethal to suckling mice and monkeys, while the Sudan strain, as well as Marburg virus, are not lethal to either of these. These differences are probably associated with...

Insert  The Zaire strain is uniformly lethal to suckling mice and monkeys, while the Sudan strain is not lethal to either of these. Marburg virus is lethal to monkeys, but not to mice. The differences between strains of Ebola virus are probably associated with...
VIRAL HAEMORRHAGIC FEVERS

Report of a WHO Expert Committee

The WHO Expert Committee on Viral Haemorrhagic Fevers met in Geneva from 19 to 23 March 1984. The meeting was opened on behalf of the Director-General by Dr F. Assaad, Director, Division of Communicable Diseases. He stated that haemorrhagic fevers had been of concern to WHO for a long time, because of their public health importance and their scientific interest. WHO had contributed to the efforts of Member States affected by these diseases and continued to do so. During the last two decades WHO had been able to cope with the unusual problems suddenly posed by the emergence of new viral haemorrhagic fevers in Africa such as Marburg virus disease, Lassa fever, and Ebola virus disease, with the assistance of international experts and because of the existence of a scheme for emergency aid during epidemics.

Coping with unusual epidemics is indeed one of the statutory functions of WHO, and the efficiency of the emergency aid scheme was reviewed in a meeting held in Geneva in 1981. The necessary laboratory support for this aid is provided though a network of WHO collaborating centres for reference and research. In addition to the existing network of centres, the particular safety problems associated with the handling of the agents of viral haemorrhagic fevers has necessitated the creation of a special network of WHO collaborating centres on special pathogens, which are equipped with facilities for high-level containment. These WHO collaborating centres for virus reference and research (special pathogens) play an important role in the study of the epidemiology and pathology of viral haemorrhagic fevers and in the provision of the expertise, laboratory services, and technical resources needed by WHO and the Member States for the prevention and control of outbreaks.

1. GENERAL BACKGROUND

The various viral haemorrhagic fevers all produce a similar severe clinical syndrome but have different etiologies and epidemiological characteristics. Some viral haemorrhagic fevers, such as yellow fever,
have been known for centuries, whereas the majority have emerged more recently either because of a new potential for spreading, such as dengue haemorrhagic fever in 1956 and Rift Valley fever in 1977, or as newly recognized diseases such as Marburg and Ebola virus diseases, which were first described in 1967 and 1976, respectively. In terms of the total number of cases, or yearly incidence, the viral haemorrhagic fevers are far less important than the major viral and parasitic diseases, such as measles or malaria, but they may cause special problems for public health services because of their epidemic potential, the often high case-fatality rate, and unusual difficulties in their treatment and prevention.

1.1 The common syndrome and its severity

The common syndrome is characterized by an incubation period of about 3–18 days, the gradual or sudden onset of systemic signs and symptoms lasting for about 3 days that may be similar to those associated with influenza or malaria, sometimes a short period of remission for a few hours, and later a sudden, rapidly deteriorating condition on the third or fourth day. Among the clinical features of this critical period, the tendency to bleed is most prominent—in particular cutaneous haemorrhages such as petechiae, ecchymoses, oozing at puncture sites, epistaxis, gum bleeding, and haemorrhagic conjunctivitis; internal bleeding is apparent as haematemeses, melaena, haematuria, and vaginal bleeding. The degree of haemorrhage, it should be emphasized, is highly variable among these diseases. A second prominent clinical feature is the cardiovascular involvement that leads to a shock syndrome which may be reversible or irreversible. Other complications may appear rapidly such as dehydration, uraemia, hepatic coma, haemolysis, jaundice, acidosis, or central nervous system involvement, as well as secondary bacterial infections.

The clinical picture may vary depending on the specific disease, the individual host response, and the particular virulence of the causative virus strain. The contribution of each of these factors is still largely unknown.

The case fatality rate of the viral haemorrhagic fevers may reach frightening levels—20–80% for yellow fever and 80% or more for Ebola virus disease. However, these figures are based on the most severe cases that are hospitalized, and the overall case-fatality rate is lower when milder cases are also included.
1.2 The etiological agents

The viruses causing the haemorrhagic fevers belong to different families and genera (Table 1). The special factors of virulence that enable them to cause the same severe syndrome is not well known. It is postulated that prior sensitization by an antigenically related virus induces a host immunopathological response; this, for example, may explain dengue haemorrhagic fever. On the other hand, in yellow fever and possibly other haemorrhagic fevers, the disseminated intravascular clotting and haemorrhagic disease may be the direct effects of the damage caused by the virus.

<table>
<thead>
<tr>
<th>Primary mode of transmission</th>
<th>Disease</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito-borne</td>
<td>Dengue haemorrhagic fever</td>
<td>Togaviridae Flavivirus</td>
</tr>
<tr>
<td></td>
<td>Rift Valley fever</td>
<td>Bunyaviridae Phlebovirus</td>
</tr>
<tr>
<td></td>
<td>Yellow fever</td>
<td>Togaviridae Flavivirus</td>
</tr>
<tr>
<td>Tick-borne</td>
<td>Crimea-Congo haemorrhagic fever</td>
<td>Bunyaviridae Nairovirus</td>
</tr>
<tr>
<td></td>
<td>Kyasanur Forest disease</td>
<td>Togaviridae Flavivirus</td>
</tr>
<tr>
<td></td>
<td>Orsk haemorrhagic fever</td>
<td>Togaviridae Flavivirus</td>
</tr>
<tr>
<td>Rodent-borne</td>
<td>Junin haemorrhagic fever</td>
<td>Arenaviridae Arenavirus</td>
</tr>
<tr>
<td></td>
<td>Machupo haemorrhagic fever</td>
<td>Arenaviridae Arenavirus</td>
</tr>
<tr>
<td></td>
<td>Haemorrhagic fever with renal syndrome</td>
<td>Bunyaviridae New genus (proposed)</td>
</tr>
<tr>
<td></td>
<td>Lassa fever</td>
<td>Arenaviridae Arenavirus</td>
</tr>
<tr>
<td>Unknown</td>
<td>Ebola virus disease</td>
<td>Filoviridae Filovirus*</td>
</tr>
<tr>
<td></td>
<td>Marburg virus disease</td>
<td>Filoviridae Filovirus*</td>
</tr>
</tbody>
</table>

*Proposed name.

It should be recalled here that a haemorrhagic syndrome may be seen in diseases other than those mentioned in Table 1 such as measles, lymphocytic choriomeningitis, chikungunya virus disease, hepatitis associated with the delta agent, leptospirosis, meningococcaemia, relapsing fever, septicaemic plague, typhoid fever, and Henoch-Schönlein purpura. Haemorrhagic smallpox will no longer be seen following the eradication of the variola virus.

1.3 The epidemiological disparity

There is a disparity among the viral haemorrhagic fevers in their mode of transmission and occurrence (rural, urban, limited to forest, etc.).

Several of these infections may be transmitted to man from different sources depending on the virus concerned (Table 2).
Epidemiologically, these are classified among arthropod-borne and rodent-borne viruses. However, the mode of transmission to man is still unknown for the Marburg and Ebola viruses. Some of the viruses responsible for haemorrhagic fevers have only one vertebrate animal reservoir, whereas others have several. When the animal reservoir is a domestic animal or an animal that may occasionally be found in houses, such as field mice, the risk of human infection is greater than when a wild animal is the reservoir. In certain cases a mammal acts as an amplifier of virus circulation, for example monkeys in the cases of yellow fever and Kyasanur Forest disease, and sheep and cattle in the case of Rift Valley fever, whereas the true reservoir that maintains the virus may be a different animal or is sometimes unknown. For certain diseases, the reservoir can be the arthropod vector itself, such as ticks in the case of Crimean-Congo haemorrhagic fever; the infection being maintained by transovarian transmission to some of the tick progeny. The same mechanism may be involved in the maintenance of mosquito-borne viruses in nature.

The occurrence in man may be sporadic, endemic, or epidemic, and this merely reflects the enzootic or epizootic transmission cycles in nature. Man-to-man transmission may occur, either by direct contact (Marburg and Ebola virus diseases, Lassa fever, Crimean-Congo haemorrhagic fever), through arthropods (dengue haemorrhagic fever, yellow fever), or man may be only a dead-end in the cycle.

The complexity and specificity of transmission cycles between particular reservoir animals explains the geographical distribution of these diseases, which is characterized by limited natural foci. However, epidemiological factors are not always straightforward or well understood. For instance, dengue occurs in all the tropical areas where its mosquito vector, *Aedes aegypti*, is found, but dengue haemorrhagic fever is seen only in East Asia, the Pacific area, and the Caribbean, and yellow fever, which has the same vector, only in tropical Africa and the Americas.

1.4 Variety of resulting public health problems

The etiological and epidemiological disparities give rise to different public health problems for each disease. However, all the diseases have some common problems that are related to medical care, containment measures, and prevention.
<table>
<thead>
<tr>
<th>Virus</th>
<th>Anthropo-borne</th>
<th>Unknown</th>
<th>Direct spread by contact with</th>
<th>Laboratory spread</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mosquito</td>
<td>Tick</td>
<td>Rodent excretions</td>
<td>Rodent excretions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Human blood or secretions*</td>
<td>Animal tissues and blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rodent excretions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Animal tissues and blood</td>
<td>Animal tissues and blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Laboratory manipulation*</td>
</tr>
<tr>
<td>Dengue haemorrhagic fever</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Crimean-Congo haemorrhagic fever</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Kyasanur Forest disease</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Omsh haemorrhagic fever</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Junin haemorrhagic fever</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Machupo haemorrhagic fever</td>
<td>X</td>
<td></td>
<td>rarely</td>
<td>X</td>
</tr>
<tr>
<td>Haemorrhagic fever with renal syndrome</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Lassa fever</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Ebola virus disease</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Marburg virus disease</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

*Venereal spread of Marburg virus has been described.
*Especially during the use of sonicators, centrifuges, and blenders.
1.4.1 Medical care

At present, there is no chemotherapy available for the treatment of the viral haemorrhagic fevers, although the drug ribavirin seems to offer a promising approach to the treatment of Lassa fever. The use of interferon has given only uncertain results. The use of immunoplasma or immunoglobulin has been effective only in cases of Junin haemorrhagic fever, and has produced uncertain results in other infections. Therefore, in general, medical care of these diseases is based mainly on symptomatic therapy aimed at maintaining the vital respiratory, cardiovascular, and renal functions. Minimum therapy includes the use of plasma expanders whenever there are plasma losses, or blood transfusion whenever haemorrhages are life-threatening. More sophisticated interventions are often needed, requiring intensive care units and expensive treatment based on frequent biochemical monitoring of vital functions.

1.4.2 Containment measures

Containment measures are required for infections such as Lassa fever, and Ebola and Marburg virus diseases, because of the possibility of man-to-man transmission. Very sophisticated equipment such as a Trexler's bed isolator or a transit stretcher isolator can provide an absolute biological barrier between the contagious patient and medical personnel. If this equipment is not available, medical personnel can be protected by wearing disposable protective clothing, including either a face shield and surgical cotton mask or a full-face biological mask, or by using a respiratory hood fitted with a high-efficiency particulate air filter. However, field experience has shown that strict adherence to good barrier nursing practice alone can provide a high degree of protection for medical personnel and other patients. It is important to have available a room for isolation and an ante-room separate from other hospital wards, and to make sure that the air does not flow from the isolation area to other rooms. It is even possible to improvise protective clothing using plastic bags. Wastes should be disposed of safely by heat or chemical treatment, for example with chlorine.

Less stringent precautions are recommended for Crimean-Congo haemorrhagic fever, Machupo haemorrhagic fever, and Junin haemorrhagic fever, but good barrier nursing practice should always be followed.
When obtaining and handling laboratory or necropsy specimens containment precautions must also be taken.
For some of the viral haemorrhagic fevers, containment measures include the protection of patients from mosquito (or any other arthropod) bites during the viraemic phase. This applies to dengue haemorrhagic fever, Rift Valley fever, and yellow fever.
Whenever direct person-to-person transmission may occur, containment measures also involve the quarantine of those considered to be the contacts of confirmed or suspected cases. This may result in a sudden influx of patients to be isolated and contacts to be quarantined and may overburden the medical services.
Containment measures may have international implications for the transport of patients and the movement of persons in and out of a contaminated area. There are no provisions in the International Health Regulations for viral haemorrhagic fevers, except yellow fever.
In some situations a cordon sanitaire may be considered; in other cases, however, this may not be possible or advisable, since it may cause unacceptable personal, social, economic, and political problems. An alternative is to reinforce the surveillance system by alerting medical personnel, especially primary health care workers, to look out for any suspect cases and to isolate them before they have the chance of infecting many contacts. Tracing all possible contacts is a difficult operation, particularly if a patient has travelled on public transport. Close contacts must be kept under medical surveillance, either at home or in hospital, until the end of the longest known incubation period. More remote contacts may stay at home, voluntarily limit their contacts, and report to a physician if any fever appears before the end of the incubation period of the disease.
A knowledge of the incubation period and the period of contagiousness is critical for the effective management of containment measures. These two periods are generally characterized by minima and maxima, with an average value.
Medical evacuation generally raises additional containment problems. Evacuation should not be undertaken without the approval of the health authorities of the place to which or through which the patient is to be transported. If there is any risk of person-to-person transmission, the personnel in contact with the patient during transport should be protected as described above for hospital personnel. Evacuation may cause additional stress to the patient and should be undertaken before the critical stage of the disease is
reached. However, as already mentioned, the diagnosis may be easily confused with malaria, influenza, or endemic infections during the early stages, but a decision has to be made by the third or fourth day, after which the patient’s condition may deteriorate rapidly. The medical evacuation of a patient with yellow fever, Rift Valley fever, or dengue haemorrhagic fever to a place where the mosquito vector exists is contraindicated, unless he or she can be strictly protected from any contact with the vector.

1.4.3 Preventive measures

At present, immunization against viral haemorrhagic fevers is available on a general basis only for yellow fever. Populations at risk can be protected for at least 10 years with an injection of 17D yellow fever vaccine. Immunization against yellow fever is especially recommended for health personnel who may be exposed to infective material, for example when taking blood specimens, analysing them, or performing a necropsy. Immunization against Rift Valley fever is available for those at high risk; however, the general immunization of domestic animals is recommended and preferred in order to decrease the risk for man.

The prevention of outbreaks of viral haemorrhagic fevers relies mainly on the vigilance of a surveillance system in areas of particular risk which should provide an early warning of suspect cases. Surveillance is based on the dissemination of appropriate technical information to “spotters” or medical personnel in health centres. Clinical criteria for the detection of cases should be circulated and prompt reporting channels established. According to local circumstances, it may be preferable to complement the existing passive surveillance system by periodic active surveys by a specialized team looking for suspect cases in hospitals and peripheral health centres. Systematic serological surveys may also detect the presence of unsuspected causal agents.

A contingency plan should be made to avoid a lack of resources which would aggravate any panic that might occur at the time of an unexpected outbreak. Such a contingency plan should specify those diseases considered to be a risk and provide for the rapid mobilization of experts, investigative teams, containment and medical care resources, laboratory support, specific control measures (vector control, immunization), and all the necessary logistic support. National emergency health services may be given
the responsibility for organizing this plan, together with a national committee for disasters. The mobilization of international aid, either on a bilateral basis or through United Nations agencies, should also be foreseen in the contingency plan. The World Health Organization is constitutionally committed to providing assistance to Member States facing epidemics and already has considerable experience in such situations. A WHO guide entitled *Emergency measures for the control of outbreaks of communicable diseases* is in preparation. Since laboratory specimens from cases of viral haemorrhagic fevers may be dangerous, a special network of WHO collaborating centres offers reference services for etiological diagnosis (see Annex 1). These specimens should be packaged according to the practice recommended by WHO.¹

The possibility that a “new” virus may arise should also be foreseen in the contingency plan, since this exceptional situation has already occurred—twice—in 1967 with Marburg virus disease and in 1976 with Ebola virus disease. This is an additional reason why WHO should be informed and laboratory specimens from any suspect case should be sent as soon as possible to one of the specialized WHO collaborating centres listed in Annex 1.

2. MOSQUITO-BORNE HAEMORRHAGIC FEVERS

There are three major mosquito-borne haemorrhagic fevers: dengue haemorrhagic fever, yellow fever, and Rift Valley fever. These are notable among the haemorrhagic fevers because of their ability to cause large, often unpredicted epidemics. During the epidemics the majority of cases are usually undifferentiated fevers and a minority are haemorrhagic disease. Other mosquito-borne diseases such as chikungunya virus disease may also present haemorrhage as a clinical feature but these diseases are rarely fatal and a haemorrhagic complication is very unusual. Dengue haemorrhagic fever, yellow fever, and Rift Valley fever have unique features that will be described in the following sections.

2.1 Dengue haemorrhagic fever

2.1.1 Historical background

The clinical syndromes associated with the four distinct serotypes of dengue virus infection include the classical form of dengue and the haemorrhagic fever with concomitant shock and high mortality. The dengue-like syndrome and dengue viruses have been prevalent in tropical Africa and the Americas for a number of years, and in the past outbreaks have been recognized in Europe bordering the Mediterranean Sea, in Australasia, and in South-East Asia and the Western Pacific regions. The first outbreak of dengue haemorrhagic fever was recognized in the Philippines in 1953, where dengue virus types 2, 3, and 4 were isolated; the second was in 1957 in Thailand, where subsequently all four types of dengue virus were isolated. However, there is some evidence that cases with the characteristics of dengue haemorrhagic fever were observed during epidemics in Greece and South Africa during the 1920s.

2.1.2 Etiology

Dengue viruses belong to the Togaviridae family and Flavivirus genus. They are 50-nm diameter spheres. There are four serotypes of dengue virus, and although they can all stimulate both group- and type-specific antibodies, the immunity induced by one type of virus is only partially protective against another.

2.1.3 Epidemiology

Since 1953 dengue haemorrhagic fever has become one of the most important causes of morbidity and mortality among children in South-East Asia. Confirmed epidemics of this disease have been reported in 10 tropical countries—namely, Burma, Cuba, Democratic Kampuchea, Indonesia, Lao People's Democratic Republic, Malaysia, Philippines, Singapore, Thailand, and Viet Nam, while reports of similar cases have been received from southern China, India, and Sri Lanka. In these countries 750 000 people are reported to have been hospitalized in the past 25 years, with at least 20 000 deaths. Sporadic cases of the disease have been reported from the South Sea islands in the Western Pacific.

In 1981 an outbreak of dengue haemorrhagic fever caused by dengue virus type 2 occurred in Cuba and resulted in 158 deaths.
This followed a dengue virus type 1 infection that had been associated with a low morbidity and no mortality between 1977 and 1980. The epidemic in 1981 mostly affected children under 15 years of age although adults were also affected; the adult deaths accounted for about one-third of the total number of fatalities.

Transmission. Dengue virus is transmitted to man by mosquito bite. Man seems to be the reservoir of the virus, although it has also been isolated from naturally infected monkeys, which may play a role in sylvatic transmission.

Vectors. *Aedes aegypti* is the most important vector, being a domestic mosquito that feeds on man. Rapid urbanization often results in the proliferation of the small peridomestic breeding sites such as discarded tyres and tins as well as an inadequate provision of water supply and disposal facilities. The mosquitoes breed in relatively clean water in domestic water containers or water vessels. Female mosquitoes feed on man during the day, are infected with dengue virus, and transmit viruses to man after an 8–10-day extrinsic incubation period. Mechanical transmission of the virus may also occur if the infected mosquito immediately feeds on another person. Recent experimental studies have shown that transovarian transmission can occur in a very small percentage of infected female mosquitoes; however, it is not clear whether or not such transmission occurs in nature.

Other vectors with possibly less epidemic importance are *Aedes albopictus, A. polynesiensis,* and *A. scutellaris* complex.

Groups at risk. It is apparent from epidemiological studies carried out during the 1960s and 1970s, and from subsequent, albeit sporadic, reports that cases of dengue haemorrhagic fever may occur in:

(a) infants less than 1 year old with primary dengue infection;
(b) children about 3–7 years old with secondary infections (the greatest number of cases are in this category);
(c) adolescents or young adults with secondary dengue infection;
(d) children or adults with primary infection (this type of case is relatively rare but carefully studied cases have been reported).

In Thailand all four types of dengue virus have been recovered from patients with dengue haemorrhagic fever, while in some
countries, including Cuba, dengue virus type 2 seems to be more important, and in others, such as Indonesia, dengue virus type 3 seems to be more prevalent. Epidemiological studies have also shown that in some study areas the number of cases of dengue shock syndrome associated with dengue haemorrhagic fever could be as high as 30 cases for every 100 cases of secondary dengue infection. In one study, the relative risk of developing the shock syndrome was 163 times greater for children with secondary infection than for those with primary infection. The ratio of dengue haemorrhagic fever to infection is higher in females than in males over 4 years old, i.e., the rate of occurrence of the disease has not been found to be related to the number of exposures to infected mosquitoes in male and female subjects.

The risk factors predisposing individuals to dengue haemorrhagic fever are not well understood. Epidemiological studies have shown that in epidemics in South-East Asia, the majority of cases occur in children who have secondary dengue infection and this observation has led to the hypothesis that pre-existing dengue antibodies in these children may represent an important risk factor.

Several prospective studies carried out in recent years have given an indication that sequential infections which end with an infection by dengue virus type 2 have resulted in cases of dengue haemorrhagic fever in Thailand and in at least one epidemic in 1981 in Cuba; while in Indonesia, sequential infection ending with infection by virus type 3 has resulted in cases of the disease. Of the children with previous virus type 1, 3, and 4 infections, some were found to have moderately high titres of cross-reacting dengue virus type 2 neutralizing antibody; when subsequently these children were infected with dengue virus type 2, their disease was mild. In children who exhibited no or low titre heterotypic dengue virus type 2 neutralizing antibodies the disease that manifested during the secondary dengue virus type 2 infection was severe.

2.1.4 Clinical diagnosis

After the mosquito injects dengue virus into the skin, there is a latent period of 4–5 days that is followed by fever, headache, and malaise. In classical dengue fever, platelet counts, serum level of liver enzymes, and complement levels are normal. No coagulation defect is found. A rash may appear, usually as the fever is subsiding. In certain geographical areas, such as Thailand or India, classical
dengue fever has to be differentiated from chikungunya virus disease.

In dengue haemorrhagic fever, after 4–5 days' incubation, the illness begins with a sudden rise in temperature. The fever persists for 2–7 days and then falls to a normal or subnormal level. The fever may be accompanied by non-specific constitutional symptoms. After the lowering of the temperature or sometimes before this, haemorrhagic manifestations may be observed most commonly in the skin; these include positive tourniquet test, easy bruising and bleeding at venepuncture sites, and petechial haemorrhage. Petechial rash is frequently observed even during recovery. The liver is variably enlarged, the enlargement occurring usually during the febrile period. Therefore, this enlargement is unlikely to be due to shock or cardiac failure. Jaundice is extremely rare. Convulsions may be observed, and in a number of cases encephalopathy has been diagnosed. However, the cerebrospinal fluid usually shows no significant change.

In a number of cases of dengue haemorrhagic fever, beginning in the late febrile period, patients may go through a severe hypovolaemic shock with haemoconcentration, indicating that there is a generalized increase in vascular permeability with an outpouring of water, electrolytes, and proteins of low relative molecular mass, such as albumin, into the interstitial spaces. This phenomenon is called dengue shock syndrome and is associated with a very high mortality rate. The plasma volume may be less than 20% of normal. Red blood cell volume, extracellular fluid volume, and total body water content show no significant changes. If patients are managed properly during this shock by the correction of plasma volume and survive this critical phase, fluid returns to the intravascular compartment and clinical recovery is rapid. Most children who do not recover die 24–48 hours after shock.

A clinical classification for confirmed cases of dengue haemorrhagic fever has been adopted as follows:

(a) Grade I—fever accompanied by constitutional symptoms and positive tourniquet test;
(b) Grade II—same as grade I but accompanied by spontaneous bleeding;
(c) Grade III—circulatory failure manifested by rapid and weak pulse, narrowing of pulse pressure (20 mmHg [2.7 kPa] or less) or hypotension;
(d) Grade IV—profound shock with undetectable blood pressure and pulse.

2.1.5 Laboratory diagnosis

The low platelet count found during grades I and II with some degree of haemoconcentration can be used to differentiate grades I and II from classical dengue.

Laboratory findings in cases of dengue haemorrhagic fever with or without shock may vary according to the individual but important changes that can help in the diagnosis are mild depression or elevation of white blood cell count with relative lymphocytosis or monocytosis, haemoconcentration, thrombocytopenia, hypoalbuminaemia, hyponatraemia, and elevated transaminase and blood urea nitrogen levels.

The more severe cases such as those with dengue shock syndrome show a prolonged prothrombin-time with low levels of coagulation factors II, V, VII, IX, and XII. Fibrinogen levels are also lowered and this is related to an increased consumption of fibrinogen. Small amounts of fibrinogen split products can be detected. Special studies have shown that blood levels of complement C1q, C4, C5–8, and C3 proactivator are decreased and C3 catabolic rates are increased. The degree of complement activation and hypofibrinogenenaemia varies in direct proportion to the grade of the disease.

Antigen detection from acute specimens such as peripheral blood leukocytes is made by the immunofluorescence method, the peroxidase technique, or countercurrent immunoelectrophoresis.

Virus isolation can be made from acute sera, plasma, leukocyte buffy coat, or organs at autopsy. The material is inoculated preferably into mosquitoes or mosquito cell culture and infection is indicated by positive immunofluorescence using polyclonal or monoclonal antibody in the direct or indirect immunofluorescent antibody (IFA) test. Monoclonal antibodies specific for each of the four types of dengue virus are used in this test to determine the type.

Sero logical tests for dengue include the haemagglutination-inhibition (HI) test, the complement fixation (CF) test, the neutralization test and immunofluorescent antibody test (IFA) or the enzyme-linked immunosorbent assay (ELISA) to detect IgM
and IgG dengue antibodies. The serological reaction in a primary infection is usually quite specific. There is a rapid anamnestic response in secondary infections with broad cross-reactions making the specific serological identification of the second infecting dengue virus difficult or impossible.

2.1.6 Pathology

There is no pathognomonic lesion in dengue haemorrhagic fever; however, when a large number of cases are reviewed certain patterns emerge. Gross findings may reveal some degree of haemorrhage in the skin and subcutaneous tissue; in the heart especially, flame-shaped subendocardial haemorrhage is found in the left ventricular septum; it is also found in the mucosa of the nose, mouth, and gastrointestinal tracts, and in the liver. The amount of haemorrhage, however, is not excessive. Serous effusion with a high protein content, mostly albumin, is commonly present in the pleural and abdominal cavities. At the light microscope level, the blood vessels show no significant changes in the vascular walls. Occasional capillaries and venules in affected organ systems may show perivascular haemorrhage and perivascular infiltration by lymphocytes and mononuclear cells. In adolescents and young adults, haemorrhage has been observed to be relatively more severe than in young children, and morphological evidence of intravascular clot formation in small vessels has been recognized. The lymphoid tissue in most fatal cases, with the exception of infants who have the primary dengue infection, shows increased activity of the B-cell system, with active proliferation of plasma cells and lymphocytoid cells, and very active germinal centres. There is evidence to indicate the proliferation of large immunoblasts, and a considerable turnover of lymphocytes manifested by a reduction of the white splenic pulps, lymphocytolysis, and marked lymphocytic phagocytosis. A recent study using current knowledge of immunopathology has shown a decreasing number of cells in the T lymphocyte-dependent area in the lymphoid organs.

In the liver there is focal necrosis of the liver cells, Councilman bodies, and swelling with hyaline necrosis of the Kupffer cells. Proliferation of mononuclear leukocytes and rare polymorphonuclear leukocytes in the sinusoids and occasionally in the portal areas is observed. The lesions in the liver resemble those of experimental yellow fever in monkeys with spotty focal necrosis after
about 72–96 hours not yet accompanied by extensive parenchymal cell damage.

Antigen-containing cells are found in the liver, spleen, thymus, lungs, and lymph nodes. These are the Kupffer cells, sinusoidal-lining cells, phagocytic reticulum cells, and alveolar-lining cells. In the fatal cases in infants that have primary infection there are more antigen-bearing cells and an increased quantity of the antigen compared with the levels found in children with secondary dengue infection.

Pathological studies have been made on bone marrow, kidney, and skin using biopsy material. Depression of bone marrow elements was observed; this rapidly improves when the fever subsides. Young megakaryocytes were observed to proliferate and lodge in the blood vessels of the visceral organs. The kidneys showed immune-complex type of glomerulonephritis that cleared in about 3 weeks, leaving no residual changes. Skin rashes showed perivascular oedema of the terminal microvasculature in the dermal papillae with infiltration of lymphocytes and monocytes. Antigen-bearing mononuclear phagocytes could be localized in the vicinity of the microvasculature of the dermal papillae. Complement C3, IgM, and fibrinogen deposits have also been found on the walls of the microvascular beds.

2.1.7 *Pathophysiology*

The major pathophysiological changes that occur lead to two effector pathways. One results in haemorrhage, which is due to thrombocytopenia, defects of coagulation factors, some degree of vasculopathy, and, in a number of cases, disseminated intravascular coagulation. The other leads to a generalized increase in vascular permeability with leakage of water, proteins of low relative molecular mass, and electrolytes from the vascular compartment. Hypovolaemia and consequently hypovolaemic shock develops giving rise to the dengue shock syndrome.

The characteristics of shock in dengue haemorrhagic fever in children have been well defined. It is mainly hypovolaemic in nature and caused by the loss of plasma proteins of low relative molecular mass, water, and some electrolytes into the interstitial spaces, while haemorrhage, i.e., leakage of erythrocytes from the blood vessels, is not an important process. Hypovolaemic shock is demonstrated by high haematocrit values, indicating peripheral haemoconcentration,
a large amount of fluid in serous cavities with a high level of the albumin fraction, and concomitant hypoalbuminuria in the plasma. At autopsy it has also been shown that the volume of serous fluid collected in the body cavities far exceeds the volume of fluid administered parenterally. By dilution techniques and \textsuperscript{131}I-labelled human serum albumin, polyvinylpyrrolidone, and globulin, plasma volume has been shown to begin to move out of the vascular compartment towards the end of the febrile period and this shift is pronounced during the defervescence period (shock period). Sometimes the plasma level is 20% or more below normal. Extracellular fluid volume and total body water content show no deviation from normal, and red blood cell volume shows no significant change. During the recovery stage after shock, mild hypovolaemia may occur in some cases; thus the plasma volume replacement therapy may have to be conducted carefully to prevent cardiac overloading during recovery when fluid returns into the vascular compartment.

While hypovolaemia is the primary cause of shock in children, the situation in adolescents and adults is less clear. There seems to be more haemorrhage in this group of subjects and more evidence of disseminated intravascular coagulation. Severe thrombocytopenia, prolonged partial thromboplastin time, low prothrombin time, rapid euglobulin clot lysis, increased fibrinogen degradation products, and a low fibrinogen level are the characteristics observed in this group of patients. The red blood cell volume has not been carefully studied although isolated results indicate a normal or low haematocrit value, suggesting that red blood cell loss may be a significant contributing factor to hypovolaemia in addition to the leakage of plasma volume, or may even be the major contributing factor. This group of patients deserves further careful study.

The mediators that lead to several of these pathophysiological events have been alluded to but remain to be identified. Much work is needed in this area.

2.1.8 Pathogenesis

The pathogenesis of dengue haemorrhagic fever may be considered to result from direct cellular injury by dengue viruses or from immunologically mediated injury, or a combination of both mechanisms. Since there are at least two clinical syndromes associated with dengue infection i.e., classical dengue and dengue
haemorrhagic fever, and since a large number of subjects who manifest the dengue shock syndrome have secondary infection, the idea has arisen that immunopathological injury, occurring as a consequence of sequential infection, may be important in the pathogenesis of the shock syndrome associated with dengue haemorrhagic fever.

It is only recently that the possible sites of dengue virus replication in the human body have been recognized. Recent work has shown at autopsy that cells of the mononuclear phagocyte series in different reticuloendothelial organs contain dengue antigen, that circulating monocytes can support the replication of dengue viruses, and that entry of virus is promoted by immune complexes formed between anti-dengue antibody and dengue virus. These latter antibodies, when circulating at non-neutralizing concentrations or when directed at viral epitopes that do not produce neutralization, are termed infection-enhancing. The more cells infected, the more severe the disease, and these enhancing antibodies help to promote virus infection of cells.

It has also been shown in unrelated experimental systems that activated monocytes may release substances that can activate the complement system and the clotting system, and also modify vascular permeability. This, in combination with the enhancement phenomenon, makes monocyte–dengue virus interaction important in dengue haemorrhagic fever.

This postulate may explain the phenomenon of dengue haemorrhagic fever that occurs in infants whose mothers are immune to dengue. These infants may be infected with dengue virus when the maternal neutralizing IgG antibody that passes through the placenta is below optimal level and the enhancing antibody is also at its most effective level. The pathogenesis of the disease in children with secondary infection may also be explained on this basis.

2.1.9 Complement activation

It has been shown by an international study group that in the acute stage of dengue haemorrhagic fever, the serum levels of complement fractions C1q, C3, C4, C5–8, and C3 proactivator were depressed, this depression being more pronounced in severe cases of
the disease. The C3 catabolic rates were shown to be elevated. These findings suggest that there is a complement consumption process via the classical pathway and perhaps via the alternate pathway as well. It has also been postulated that this may result in the formation of C3a and C5a, which are potent anaphylotoxins and which may greatly increase the vascular permeability either directly or via the histamine system. In cases of dengue haemorrhagic fever associated with primary infection, severe complement activation can also be demonstrated. Recently, using crossed immuno-electrophoresis (CIEP) on sera drawn serially from patients with acute infection, C3-splitted products (C3sp) were detected by comparing these C3sp peaks with control peaks that are formed when complement activation occurs after the treatment of sera with insulin. C3sp products were detected only in patients with grades III and IV dengue haemorrhagic fever from whom sera were obtained at the time of admission. This method which permits quantitative measurement of C3 conversion shows that the amount of C3sp found often exceeded 30%, and was even higher in patients with profound shock. With improvement in the clinical status, the C3 level returns to normal. The ease and speed of the crossed immunoelectrophoresis technique, which could also differentiate native C3 and C3sp, make this an important tool to investigate further the mechanism of complement activation and the temporal relationship between the circulation of dengue viral antigen(s), dengue viral antigen antibody complex, and C3sp products. This method could also be applied in clinical situations to identify potential shock victims and to monitor the result of the treatment of shock in individual patients. Using the same approach, but with an in vitro system and normal human sera it has been shown that dengue viral antigen from different sources, including mouse brain antigen, LLC-MK2 infected cell culture, infected cultured human monocytes, as well as in vitro preformed dengue antigen–antibody complex, could activate the complement C3. This suggests that, in patients, the circulating dengue virus or virus antibody complexes may themselves activate the complement system. Further, in vitro studies using blocking of the classical and alternative pathways by heat and chelating agents and restoration with calcium and

magnesium ions have shown that dengue viral antigen activates the complement system via the alternate pathway, while viral antibody complex activates the complement via the classical pathway.

2.1.10 Treatment

Treatment of dengue haemorrhagic fever consists of general measures such as adequate fluid intake by mouth, paracetamol to treat fever, and close monitoring for shock by blood pressure measurements, and haematocrit and platelet studies. For parenteral fluid therapy, glucose (5% in water), and physiological saline are recommended, the dose of these solutions being adjusted to the clinical requirements. If acidosis occurs, a solution of sodium bicarbonate (0.167 mole/litre) should be given. Haematocrit determination should be used to guide the fluid replacement. Plasma expanders are used for volume replacement. Fresh whole blood transfusion may be needed in cases with significant bleeding such as in adolescents or in adults.\(^1\)

2.1.11 Research needs

In addition to the research needs and recommendations cited at the end of this document concerning rapid methods of diagnosis and pathogenesis, the following two areas of research apply specifically to dengue haemorrhagic fever:

(1) Development of methods for the rapid detection of complement split products which could be an indicator of shock.

(2) Laboratory methods to determine the infection sequence, including the identification of both the first and second infecting viruses, when only material obtained during the second infection is available (blood, serum, plasma, leukocytes).

2.2 Yellow fever

Yellow fever is a viral disease that is endemic in tropical regions of the Americas and Africa and is transmitted by certain mosquitoes from monkey to man and man to man. Numerous outbreaks have occurred in the past and continue to occur, causing major health problems in these regions.

2.2.1 *Historical background*

The first reliable descriptions of outbreaks date from the seventeenth century, both in Africa and the Americas. One of the most important discoveries concerned the role of the mosquito *Aedes aegypti* as the vector by Carlos Finlay; this was confirmed by the Walter Reed Commission in Cuba in 1901. Eradication of the vector *Aedes aegypti* eliminated the disease from urban centres in the Americas. The virus was isolated in 1927 in Africa. The strain from Senegal was passaged in mouse brain and is the origin of the “French neurotropic virus”. An isolate from Ghana was maintained in monkeys and is known as the Asibi strain. In the early 1930s, F.L. Soper discovered yellow fever cases in parts of Brazil where *Aedes aegypti* was absent and described a jungle cycle of transmission of the disease by mosquitos between monkeys, and from monkeys to man.

2.2.2 *Etiology*

Yellow fever virus is an arthropod-borne virus belonging to the *Flavivirus* genus (formerly Group B) of the family Togaviridae. It shares group-specific antigens with other members of the genus (e.g., in Africa: Zika, West Nile, Wesselsbron, dengue, Uganda S, Spondweni, Banzi, and other viruses).

2.2.3 *Epidemiology*

The epidemiology of yellow fever is basically the same in Africa and the Americas but there are also several differences.

*Distribution.* In Africa, yellow fever is prevalent between 15°N and 10°S. The endemic zone, defined by the presence of neutralizing antibody, includes the whole or part of a total of 28 countries. In the Americas, yellow fever is, at present, maintained enzootically in tropical forests, for example, those of the Amazon region, and the Orinoco and Magdalena Valleys (between 10°N, and 40°S). Yellow fever has been reported from 10 New World countries, with most of the cases coming from Bolivia, Brazil, Colombia, and Peru. The yearly incidence of cases on both continents during the period 1965–83 is given in Table 3.
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Transmission

(a) The Americas. In the enzootic areas of South America, yellow fever virus is believed to move in waves through populations of susceptible monkeys, with periodic invasions to other areas. Marsupials may also act as hosts where the monkey population is too sparse to sustain transmission.

Mosquitos of the genus Haemagogus, especially Haemagogus H. janthinomys, are considered to be the primary vectors of jungle yellow fever. These day-biting mosquitos are found predominantly in the forest canopy, although they can be captured on the forest floor, particularly in gallery forests with low and spaced trees. Haemagogus may follow people for almost 300 metres out of the forest, and it bites both inside and outside houses. Thus, the virus cycle dependent on this mosquito is basically sylvatic although not exclusively confined to the forest.

The mechanism of maintenance of yellow fever virus during the dry season is not understood. Transovarian transmission of the virus in Haemagogus has been recently demonstrated in the laboratory, but no evidence of this type of transmission was found in naturally occurring Haemagogus populations during extensive studies carried out in Trinidad after the 1978-79 outbreak.

In the past, yellow fever spread periodically at 5–9-year intervals from the enzootic Brazilian Amazon forest into central and western Brazil causing significant outbreaks among the population in areas where gallery forests were interspersed with savannah-like vegetation.

The virus also migrated through Central America periodically from 1948 to 1957. These movements appear to have ceased or at least diminished.

The reasons for the reduction in the spread of the disease are not clear, but it has been suggested that deforestation may control the spread of the virus.

Yellow fever virus has the ability to reappear in certain areas after long intervals of quiescence. The outbreaks recorded in Colombia and in Trinidad in 1978-79 after a period of 19 or more years, and in 1981 in Bolivia, after 30 years are examples of this phenomenon.

(b) Africa. The Rockefeller Mission in Bwamba County, Uganda, in the 1930s proved that yellow fever virus was transmitted between monkeys in the forest canopy by the Aedes africanus mosquito (sylvatic yellow fever). Monkeys infected in the forest also raided banana plantations in nearby villages and were bitten by A. simpsoni.
mosquitos which breed in plant axils and afterwards bite man. In villages, the virus was transmitted from person to person by *A. aegypti*, if present, or by *A. simpsoni* (urban yellow fever).

This pattern, typical of East Africa, was observed in Ethiopia in 1960–62 in an epidemic when out of a population of 1 million there were about 100,000 cases, of which 30,000 died. There were fewer cases in the Didessa Valley, where man was infected by *A. africanus* on entering the forest, than in the Omo Valley, where man was infected by *A. simpsoni* in villages.

There are several variations of this pattern in Africa. Other *Aedes* species are vectors of yellow fever west of Cameroon, where *A. simpsoni* does not bite man. Monkey-to-monkey transmission by *A. africanus* in the western Congolese–Guinean rain forest at times affects man as well. The virus radiates from the forest cycle to riverine forests penetrating into the savannah, where alternative vectors transmit the disease. This is the emergence zone of West Africa, where alternative vectors such as *Aedes luteocephalus*, *A. furcifer*, and *A. taylori* are responsible for sporadic cases, for outbreaks, and sometimes for man-to-man transmission. Natural transovarian transmission occurs in these mosquitoes, accounting in part for the persistence of the virus. At long intervals the cycle may extend even further during the rainy season to the semi-arid zones where *A. aegypti* and urban non-immune populations combine to produce large epidemics.

For long-term maintenance of the virus, the monkey appears to be a transient virus amplifier while the mosquito is the African reservoir, being infected for life and able to transmit the virus to its progeny.

Groups at risk. In the Americas, colonists and temporary workers from non-endemic zones, as well as the native-born population from enzootic areas engaged in agricultural and forest activities are those at most risk of contracting the disease.

Of special concern has been the occurrence of yellow fever in close proximity to urban centres infested with *Aedes aegypti* and the consequent risk of the urbanization of jungle yellow fever. Several of the outbreaks of urban yellow fever documented in the past were traced to persons who had been infected in forested areas and had brought the disease into the city. No proven cases of urban yellow fever have been documented in South America since 1942, and, undoubtedly, the elimination of the disease from urban centres has
been due to effective immunization programmes and to A. aegypti control/eradication activities. The re-establishment and rapid growth of A. aegypti populations in extensive areas of South America has reintroduced the threat of urbanization of sylvatic yellow fever. In several instances patients with yellow fever have been hospitalized in the cities of Brazil and Colombia which are highly infested with A. aegypti. The reason why the virus did not urbanize in these areas is not clear, but the patients concerned may no longer have been viraemic.

In Africa there is some indication that repeated infections with certain other flaviviruses closely related antigenically to yellow fever virus, such as Zika, Wesselsbron, and Uganda S, might provide some degree of cross-protection by the age of 15 years. It has been found experimentally that in monkeys the viraemia produced after challenge with yellow fever virus is lower if the monkey has been previously immunized with dengue, Zika, or Wesselsbron virus. However, whether dengue immunity would result in cross-protection against yellow fever in man is not certain, especially since yellow fever immunization does not protect against dengue.

Reporting is poor in Africa; however, it has been shown that during an important period of epizootic activity in riverine forests seroconversions occurred in the child population of nearby villages without any evidence of clinical outbreaks. This may be interpreted as a sylvatic cycle with low virus virulence that can be enhanced after passage through man-to-man transmission cycles.

Epidemics occur at irregular intervals in Africa and, in populations where the disease has not occurred for several years, there may be a large number of cases. The death rate among severe cases that are hospitalized may reach 80% but it is generally thought to be about 15% if less severe cases are taken into account or about 1% if inapparent infections are included. Since the mosquito vectors are present in the villages, all age groups are affected, even infants less than 6 months old if they are not protected by maternal antibodies. Since immunization is not carried out in children under 6 months old, these infants are especially at risk. Both sexes are equally affected by the disease.

2.2.4 Clinical diagnosis

Subclinical yellow fever infection is common; the incubation period is between 3 and 6 days. Clinical forms can be graded into
four categories according to severity: (1) Very mild: patients experience only transient fever and headache that persists usually for a few hours. (2) Mild: the fever and headache are more pronounced and may be accompanied by nausea, epistaxis, Faget's sign, slight albuminuria, and subclinical elevation of bilirubin. The illness usually lasts from 2 to 3 days. Epigastric pain, backache, general body pain, vertigo, vomiting, and photophobia may also be present; some patients exhibit a prolonged period of asthenia. (3) Moderately severe: the fever is higher, the headache and backache are more severe, and the nausea and vomiting are more intense. The course is diphasic. In the second phase, jaundice, albuminuria, and oliguria are usually present. Black vomitus, melaena, or uterine haemorrhage may occur. The fever persists for about one week. (4) Malignant: all the classical signs and symptoms of the disease are present. In general, death occurs between the 6th and 8th days of illness; however some patients may die within 3–4 days or, alternatively, there may be late death, 10 or more days after onset.

Death occurs after 1 or 2 days of coma, or suddenly after an attack of haematemesis in hypothermia and shock. The overall mortality rate among hospitalized patients may be as high as 40–50%. Survivors exhibit long-lasting immunity.

In Ethiopia, a number of confirmed cases during the 1959 epidemic showed only fever and meningeal signs and symptoms, similar to cases that were observed during the Nuba mountain epidemic in Sudan in 1940. Again in Ethiopia, during the 1960–62 epidemic, fulminant forms were observed in one region, with no hepatic or renal signs, death occurring after 2–3 days, whereas only the classical form was seen in other regions.

Although there is no great difficulty in diagnosing yellow fever during an epidemic, sporadic cases may be confused with malaria or influenza when they are mild, or with hepatitis or other haemorrhagic fevers when they are severe. Ebola virus infections were confirmed serologically during the yellow fever epidemic in the Gambia in 1978 and there is a possibility that Orungo virus may have caused haemorrhagic complications during a yellow fever outbreak.

2.2.5 Laboratory diagnosis

Histopathology, virus isolation, and serology are used in the laboratory diagnosis of yellow fever.
Histopathology. Liver biopsy is absolutely contraindicated in the living patient, because of the danger of haemorrhage. Liver samples from fatal cases may be obtained by abdominal incision, or by the use of a viscerotome, or a large calibre biopsy needle.

Specimens for histopathology should be fixed in 10% formalin, preferably no longer than 2 weeks before embedding. Frozen sections of liver may be stained with Sudan III and haematoxylin–eosin to obtain a rapid presumptive diagnosis. Paraffin sections can give a more accurate diagnosis within 48 hours.

The interpretation of the results is sometimes difficult and can be made only by an experienced histopathologist. In typical cases, the histological diagnosis is based on an eosinophilic degeneration of the hepatocytes leading to the formation of Councilman bodies, predominant midzonal necrosis, and microvacuolar steatosis. However, other infections such as those caused by Lassa, Marburg, Ebola, and Congo viruses, as well as viral hepatitis and leptospirosis may produce a similar histopathological picture. The indirect fluorescence test may be useful to detect yellow fever antigen in liver sections, particularly in those cases in which typical histopathological lesions are not present.

Virus isolation. Blood must be obtained within the first 3–4 days of infection. Virus can also be obtained, post mortem, from the liver until 10–12 days after onset. For shipment, specimens may be preserved at +4°C for a short period of time (preferably not longer than 24 hours) or preferably preserved frozen. If this is not possible, the specimen should be placed in a 50% glycerol solution and shipped at ambient temperature.

Virus isolation can be performed by inoculation of suckling mice and/or vertebrate or insect cell cultures. Isolation by intrathoracic inoculation of mosquitoes is highly sensitive. The virus is detected and identified by immunofluorescence and/or complement-fixation tests.

The most frequently used cell cultures are those of Aedes aegypti (clone C17), A. albopictus (clone C6–36), and A. pseudoscutellaris (AP 61). After inoculation and incubation for 3–4 days, the cells are examined for virus by immunofluorescence. Cell cultures are more sensitive than suckling mice for the isolation of yellow fever virus and are of comparable sensitivity to the intrathoracic inoculation of mosquitoes. In addition, the use of insect cell cultures is more convenient since they can be transported to, and used in, the field at ambient temperatures.
Yellow fever type-specific monoclonal antibodies can be used in the indirect immunofluorescence test for virus identification.

*Serological tests.* The haemagglutination inhibition (HI) test detects antibodies that usually appear early (within the first week after onset). In cases of primary yellow fever infection, a specific diagnosis is sometimes possible by this method. However, the HI antibody response broadens with time, and cross-reactions with other flaviviruses may make interpretation difficult.

The complement-fixation (CF) test is more specific than the HI test. CF antibodies appear later (during the second week after onset) and may decline relatively rapidly to low levels 6–12 months after infection.

The neutralization test is the most specific. Neutralizing antibodies appear early (during the first week after onset) and last for many years (probably life). Various techniques for the measurement of neutralizing antibody may be used, including plaque-reduction assays in cell culture and tests in mice.

The enzyme-linked immunosorbent assay (ELISA) is used to detect both IgG and IgM antibodies. In a primary infection, specific IgG antibodies are regularly found; IgM antibodies are also highly specific and usually present.

Radial haemolysis-in-gel has also been used to detect yellow fever IgG antibodies.

The direct detection of yellow fever antigen in serum by means of the ELISA technique has been demonstrated experimentally. The limit of sensitivity of the assay applied to viraemic monkey serum is approximately $10^{3.3}$ plaque-forming units (pfu) per 0.05 ml.

2.2.6 *Pathogenesis and pathophysiology*

Most of the information available on the pathogenesis of yellow fever comes from experimental studies carried out in rhesus monkeys. Antigen is first detected in Kupffer cells by immunofluorescence, 24 hours after inoculation. There is also evidence suggesting that mononuclear leukocytes play an important role in replication. Lymphocytic necrosis is seen in the germinal centres (B-cell region) of the spleen and in the lymph nodes. Between 24 and 48 hours after the onset of infection, glycogen is reduced in the hepatocytes and at 72 hours many Kupffer cells are degenerating, and the alteration in the hepatocytes is more evident. Infection of
the hepatic parenchyma probably occurs by the haematogenous route and by direct spread from liver macrophages.

Little is known about the physiopathological events that occur during yellow fever infection in man. Liver and kidney injury are conspicuous but the mechanisms involved are still unclear. Similarly, there is no explanation for the fatty degeneration of myocardial fibres uniformly present when the heart is involved.

The nature of the haemorrhagic manifestations seems complex. It is not known whether virus-mediated damage of the vascular endothelium occurs. Studies undertaken by Brazilian investigators suggest that disseminated intravascular coagulation may be present. Thus severely ill patients may exhibit marked thrombocytopenia, prolonged blood coagulation time and prothrombin time, reduced levels of fibrinogen, and poor clot retraction. Moreover the coagulation factors II, V, and VII, plus factors X, VIII, IX, XII, and XIII, may be depressed in these patients; however, fibrinogen/fibrin split products have not been demonstrated. A decreased synthesis of coagulation factors due to liver injury probably also plays an important role in the production of haemorrhagic complications.

Rhesus monkey model studies have shown a rapid evolution of hepatic and renal disease mimicking the human illness, but they should be complemented by investigations on therapeutic intervention measures. Also, in view of the more rapid evolution and outcome of infection in monkeys, it is crucial to determine the pathophysiological parameters in the human disease.

2.2.7 Treatment

The treatment of yellow fever is mainly supportive. Bed rest is very important. Clinical manifestations of the first phase of illness can be treated at the primary health care level. This includes administration of antipyretics (e.g., paracetamol), anti-emetics, and analgesics.

The oral administration of fluids in the form of salt solutions with glucose is helpful to prevent or treat dehydration. The appropriate management of patients during the period of intoxication requires hospitalization; great care should be observed, however, when a patient is removed to a hospital. The blood pressure, temperature, arterial pulse, and the amount of fluids lost in urine and vomitus should be carefully monitored. Microhaematocrit and haemoglobin determinations are helpful to guide fluid replacement. The urea,
creatinine, transaminases, sodium, and potassium serum levels, and prothrombin time, should be determined together with sodium, potassium, and creatinine concentrations in the urine. Red and white blood cell counts and platelet counts should be performed. Arterial blood gas determinations are helpful in monitoring acid–base status.

Blood transfusion may be required, especially when extensive bleeding occurs. Plasma substitutes can be used when blood is not readily available. The potential usefulness of vitamin K in large doses deserves further study. Heparin treatment in cases compatible with disseminated intravascular coagulation has been unrewarding.

Appropriate treatment should be instituted when electrolyte disturbance and acidosis are present. Peritoneal dialysis may be beneficial in cases of renal failure. Vigorous intravenous administration of fluids, which may include blood, plasma, or plasma substitutes should be instituted when there is shock. The use of antibiotics is often necessary to combat secondary bacterial infections. Appropriate antimalarial drugs should be administered when malaria infection is present. The use of antiviral drugs such as ribavirin should be evaluated through careful clinical trials.

2.2.8 Surveillance, prevention, and control

Surveillance. A programme of surveillance of yellow fever should be instituted in countries where the disease is endemic, for the early detection or prevention of outbreaks. It should be based on clinical and serological monitoring of the human population and whenever possible should include mosquito and monkey surveys.

For clinical surveillance in man the broadest possible case definition should be used, i.e., febrile illness with jaundice. Since other diseases may cause jaundice, the use of a more specific case definition, which helps to focus on yellow fever, may be substituted; for example, jaundice with albuminuria or jaundice with haemorrhagic manifestations. Most infections with yellow fever virus do not result in jaundice; however, this feature of the disease is useful as a basis for surveillance since the other clinical signs are nonspecific. Most countries have a system for reporting cases of hepatitis, which can be adapted to provide information about the occurrence of yellow fever. The monthly incidence of, and deaths from, hepatitis should be examined by geographical region or administrative area. Data indicating possible cases of yellow fever
include an unusual hepatitis case-fatality rate: in general the fatality rate for viral hepatitis and other causes of jaundice that may be confused with viral hepatitis is less than 1%, whereas the fatality rate of hospitalized yellow fever cases is high, usually greater than 30%. Comparisons of incidence and mortality data from different hospitals or geographical regions provide important information, since yellow fever outbreaks may be quite localized.

In certain areas, particularly in the zone of emergence, annual serological surveys of children less than 2 years old may provide important information on a recent circulation of yellow fever virus.

In tropical America, viscerotomy and histopathological examination of liver specimens from fatal cases has been extremely useful in the surveillance of yellow fever whereas other laboratory methods such as virus isolation and serology rarely provide a specific diagnosis. In Africa, it has proved to be difficult to obtain permission for autopsy or viscerotomy. In addition, many terminally ill patients are removed from hospital before death. Despite the difficulties involved, this diagnostic technique is useful and should be encouraged.

Mosquito surveillance, although often very informative as regards enzootic or epizootic circulation of yellow fever virus among monkeys in the forest, is feasible only on rare occasions because there are usually no entomologists available or no local facilities for the isolation of the virus. However, surveillance of *Aedes aegypti* must be carried out in communities at risk or in cities in the endemic zone, and control measures must be instituted when indicated by the mosquito density (house index, container index, or Breteau index). Indices that have been shown to be associated with outbreaks include a house index of 4–35 or above, a container index of 3–20 or above, and a Breteau index of 5–50 or above. The susceptibility of *A. aegypti*, and possibly other potential vectors, to the principal insecticides that may be required for their control should be determined regularly (possibly as frequently as every 6 months). Standard WHO techniques should be used. It is important that countries fulfil their obligations under the International Health Regulations\(^1\) regarding the maintenance of ports and airports free from insect vectors, with particular emphasis on Article 19 which states:

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"1. Every port and the area within the perimeter of every airport shall be kept free from Aedes aegypti in its immature and adult stages and the mosquito vectors of malaria and other diseases of epidemiological significance in international traffic. For this purpose active anti-mosquito measures shall be maintained within a protective area extending for a distance of at least 400 metres around the perimeter."

A density survey of A. aegypti is therefore an essential component of effective surveillance to implement the International Health Regulations.

In the Americas, certain monkey species (Ateles) are very susceptible to yellow fever; an unusual number of deaths among them indicates a yellow fever epizootic and can act as a warning of a possible human epidemic. Other monkey species in the Americas and in Africa are less susceptible and generally do not die when infected with yellow fever virus. Serological surveys in monkey populations or sentinel monkeys may be used to detect a recent circulation of the virus in forests.

Prevention. The 17D yellow fever vaccine has been used for almost 50 years for the prevention of yellow fever. This is a live attenuated vaccine obtained by the inoculation of chick embryos and the lyophilization of embryo juice harvested before the embryos are 12 days old. Although it is preferable to prepare the vaccine in avian-leukosis-free eggs, epidemiological inquiries have failed to find any evidence of increased cancer incidence in persons immunized with a vaccine prepared in contaminated eggs. The vaccine is administered subcutaneously in 0.5-ml doses to adults and children older than 6 months. Mild post-immunization febrile reactions may occur in 10% of vaccinees. Encephalitis reactions are very rare and out of more than 100 million immunizations carried out fewer than 20 cases have been described in children less than 6 months old. As a precautionary measure, and when there is no risk of exposure to yellow fever virus, immunization may be postponed until the child is 1 year old. Although no teratogenic effects have been ascribed to yellow fever vaccine, immunization should also be avoided during pregnancy if there is no risk of exposure.

Immunity begins to appear on the 7th day and there is evidence that it may last for at least 35 years in 97% of vaccinees and possibly for life. However, the validity of the international vaccination certificate has been maintained at 10 years. To be valid, the
certificate must mention the use of a vaccine prepared at an institute recognized by the World Health Organization.

Countries were A. aegypti exists may stipulate a quarantine period of 6 days for non-immunized persons coming from endemic countries. Vaccination certificate requirements for international travel are published annually by WHO.

Control. The recognition of an increased incidence of fatal hepatitis (from hospital reports), the diagnosis of even a single case (by histopathology, serology, or virus isolation), and any reports of suspected cases of yellow fever require immediate epidemiological investigation to determine whether the virus is active in human, mosquito, and monkey populations.

Investigation in human populations is based on clinical diagnosis of suspect cases and laboratory confirmation by the isolation of virus or serological methods to detect the presence of antigen or antibodies. This will determine the attack rate in different population groups, the infection rate (subclinical and overt disease), the geographical extent of the infected area, and the trend as regards the spread of the epidemic. Isolation of virus is necessary not only to confirm the etiology of the disease but also to obtain material for further research. As already mentioned, histopathological examination of liver is useful but may not be always conclusive. Serological detection of specific IgM antibodies provides the quickest proof of recent infection. Determination of affected population groups is necessary to establish a strategy for rapid immunization.

Since immunity appears after a delay of 7 days following immunization, mosquito control is necessary to limit the transmission of the virus during this period. Therefore, an entomological investigation is needed to determine the vector species involved, its biological characteristics, its particular mode of contact with the human population, its breeding sites, and sensitivity to insecticides. The latter must be determined in order to provide effective control.

2.2.9 Research needs and recommendations

(1) To develop and produce a safe and effective 17D cell culture vaccine, with improved stability and availability and less reactogenicity.
(2) To investigate the factors that regulate the periodic appearance of yellow fever in certain areas; to determine whether the virus is reintroduced into, or persists in, such areas during interepidemic periods and if so what are the mechanisms involved.

2.3 Rift Valley fever

2.3.1 Historical background

Rift Valley fever is an acute febrile disease of cattle, sheep, and man that is caused by a mosquito-borne virus. The virus was first isolated following the inoculation of susceptible sheep with blood from an infected newborn lamb during an epizootic north of Lake Naivasha, Kenya. The disease in sheep was characterized by fever, abortion, loss of appetite, and listlessness. Death occurred in nearly 100% of young sheep. The disease in cattle was similar, but the mortality rate was lower. Epizootics have more recently been recorded in Kenya (1968), South Africa (1951, 1953, 1955–59, 1969–70, 1974–75), Namibia (1955, 1974–75), Zimbabwe (1955, 1957–58, 1968–70, 1978), Uganda (1960), Mozambique (1969), Sudan (1973, 1976), and Zambia (1974–75). Self-limited human febrile disease in veterinarians, butchers, and shepherds has long been recognized. During an outbreak of Rift Valley fever in South Africa in 1975, cases of encephalitis and fatal haemorrhagic disease were noted in man for the first time.

In 1977, Rift Valley fever caused a massive epizootic/epidemic in Egypt. This was the first account of the disease spreading north of the Sahara Desert; it was also the first record of a significant level of illness and death in human populations. A total of 18,000 cases with 598 deaths was reported from Egypt. Retrospective serological studies established that infection had not occurred in Egypt prior to 1977 and that the infection rate in sheep, cattle, buffalo, and man was greatly in excess of the reported number of cases. About one-third of the people in Sharqiya and Qalyubiya Governorates developed antibodies to the virus. Infection and disease recurred in Egypt in 1978, principally in areas not affected in 1977. After 1978 the number of cases rapidly diminished and no definite evidence of virus transmission has been found in Egypt since 1980.
This potential of the virus to spread and cause devastating animal and human disease has raised urgent questions about the epidemiology of the disease and its possible spread to new areas.¹

2.3.2 Etiology

The virus causing Rift Valley fever is a member of the genus Phlebovirus of the family Bunyaviridae. It is serologically related to the Naples and Sicilian types of sandfly fever virus; it grows readily in mice and in tissue cultures, and is sensitive to heat, detergents, and acid pH.

2.3.3 Epidemiology

Distribution. The virus or its antibody has been found in all countries and territories south of the Sahara where testing has been carried out, and, since 1977, in Egypt. The virus has not yet been reported outside of Africa.

Transmission. The virus is transmitted during epizootics and epidemics by mosquitoes; it undergoes a short incubation period in the insect. The virus may also be transmitted to man by contact with the aborted fetuses of sheep and cattle or with the blood and other tissues of infected sheep and cattle during slaughter. In addition, it is possible that mechanical transmission by arthropods takes place, since viraemia levels in sheep may reach 10¹⁰ LD₅₀/ml. There is very little epidemiological or experimental evidence for transmission by contact with, or by eating, infected meat or dairy products.

Vectors. At least 26 species of mosquito have been implicated as potential vectors by virus isolation or by laboratory experiments. Of these, Culex theileri and Aedes caballus are considered to be major epizootic vectors. The epidemic in Egypt during 1977–78 was also probably mosquito-borne. Although C. pipiens was implicated epidemiologically, few isolates were obtained from these mosquitoes.

Epizootics start during heavy rains or, as in Egypt, when mosquitoes become prevalent around irrigation and drainage canals.

Mosquitoes are consistently abundant in years of disease. In Kenya, South Africa, and Zimbabwe the disease breaks out suddenly in multiple foci over a wide range of lowland and upland plateau grasslands. Transmission appears to stop during the colder months, but resumes, as in Egypt, in the subsequent year. There may be intervals of several years between outbreaks.

**Vertebrate hosts.** Sheep and cattle serve as vertebrate amplifying hosts during epizootics. Viraemic people may also amplify transmission although this has not been proved. It is not known how the Rift Valley fever virus is maintained during inter-epizootic periods, nor is the natural vertebrate host, other than domestic animals, known.

Extensive attempts to find a forest cycle have been unrewarding. Antibody is rare or absent in monkeys, forest rodents, birds, and other animals. The virus has only rarely been isolated from forest arthropods. The disease appears to arise in the grasslands shortly after heavy rains. There is preliminary experimental evidence in Kenya that the virus may be maintained in *Aedes lineatopennis* by transovarian transmission but this needs to be confirmed.1 If so, one can hypothesize that the Rift Valley fever virus is transmitted through the egg, where it remains during several years of dry weather until flooding occurs. When the infected progeny emerge they transmit the virus to sheep and cattle. This would explain the long periods between epizootics, the emergence in virtually the same location in successive outbreaks, and the multiple foci of transmission during an epizootic. It would also explain the paucity of antibody in vertebrates between outbreaks.

**Groups at risk.** During the epidemic of 1977–78 in Egypt all age groups of both sexes were infected. During outbreaks in sub-Saharan Africa, high-risk groups included shepherds, veterinarians, and butchers, all of whom were exposed to sheep and cattle or to their tissues. Laboratory infections are also common, presumably occurring by the aerosol route of infection.

Other than exposure to animals and laboratory aerosols, the primary risk factor is exposure to mosquitos.

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1 LINTHICUM, K.J. et al. unpublished data, 1983.
2.3.4 Clinical diagnosis

There are four clinical syndromes: undifferentiated fever, haemorrhagic fever, encephalitis, and retinitis with blindness. Inapparent infection is common. Most patients have undifferentiated fever lasting 2–7 days. In a small percentage of cases hepatitis with haemorrhagic disease develops on about the fourth day of fever; many of these patients die. In another small group of patients, encephalitis develops after an afebrile period; this is rarely fatal but there may be sequelae. In a third group of patients blindness occurs about 2 weeks after the febrile illness as a result of either unilateral or bilateral retinitis. Macular and paramacular exudates and haemorrhagic lesions of the fundus are observed. The blindness is usually not permanent.

2.3.5 Laboratory diagnosis

Viraemia ranges between $10^7$ and $10^{10}$ pfu per ml of blood in man, sheep, and cattle. Thus a rapid diagnosis can often be made by antigen detection in serum. The agar gel precipitin test or the antigen-detection ELISA are recommended. Patients and animals develop HI, ELISA, neutralizing, IFA, and CF antibodies after the first week of infection and often by the fourth day after onset of fever. The neutralization test is a relatively specific reaction, but can be done only with live virus and this is not recommended in laboratories outside enzootic areas because of the risk that the virus might escape from the laboratory and infect domestic animals.

After day 4, when viraemia has ceased, an early and rapid diagnosis can also be made by the capture IgM ELISA technique, which measures specific IgM in serum indicating a recent Rift Valley fever infection.

2.3.6 Pathogenesis

The virus infects vertebrates via the bite of a mosquito or by inhalation of animal blood in aerosols. After an incubation period of about 3 days, viraemia and fever are always observed.

Rift Valley fever in experimental mice, rats, sheep, and sometimes in man is a fulminating disease characterized by hepatic necrosis, vasculitis, and death. The disease presumably results from the direct invasion of hepatocytes and endothelium by the virus. In disease-resistant rats, administration of cyclophosphamide did not
materially alter the course of the disease, suggesting that the host immune system is not a major factor in the pathogenesis of the disease.

Encephalitis is found in animals surviving the hepatic stage of the illness. This may indicate that the virus consists of both hepatotropic and neurotropic populations and whichever population dominates will determine the outcome. The virus is found in the brain of experimental animals with encephalitis, and specific IgG and IgM have been reported in the cerebrospinal fluid of patients from Egypt. This indicates that the virus probably multiplies in the brain and has a direct pathogenic effect. This does not rule out, however, an immunopathological lesion.

2.3.7 Treatment

There is no specific treatment for Rift Valley fever. It is not necessary to isolate or place in quarantine persons suspected of being infected. These patients, however, should be protected from arthropods and should not be moved to non-infected areas where vectors are present until after the viraemic period.

General supportive treatment is indicated, including blood, plasma, and electrolyte replacement if necessary, as well as nursing care.

The roles of immune plasma, interferon, and other antiviral agents in the prevention or treatment of infection in man have not been investigated.

2.3.8 Surveillance, prevention, and control

Surveillance for Rift Valley fever involves the detection of seroconversion in sheep, cattle, and man, observation of excess abortion in livestock with demonstration of virus or antigen in the fetus, and case-finding in persons living in or coming from enzootic zones. Arthropod surveillance may also assist in predicting an outbreak. Persons with haemorrhagic disease, encephalitis, or blindness that is otherwise unexplained should be treated as suspected cases of Rift Valley fever. The mutual cooperation of public health and veterinary authorities is important.

Cases of Rift Valley fever should be reported immediately to WHO and the Office Internationale des Epizooties. When an outbreak occurs, animals should be immunized to control virus
transmission both to other animals and to man. A live attenuated vaccine and an inactivated tissue culture vaccine are available for veterinary use. The live vaccine is produced in mouse brain and is administered in a single inoculation. In laboratory experiments the live vaccine has caused abortions in animals, although in practice, abortion has not been a serious obstacle to its use. The inactivated vaccine requires at least two inoculations with frequent boosting doses. It is the only vaccine recommended for use in non-enzootic areas since there is a risk of reversion to virulence of the live-attenuated product.

An inactivated tissue culture vaccine for human use has also been produced for the immunization of high-risk personnel such as laboratory staff, veterinarians, and the military. Being an experimental vaccine, it is not available for travellers or for large-scale application during epidemics.

Other control measures recommended are ultra-low-volume spraying of malathion, which rapidly kills adult mosquitoes, and avoidance of slaughter of sick animals, a measure that minimizes the spread to man via the blood of sick and dying animals.

2.3.9 Research needs and recommendations

In addition to research needs and recommendations on rapid diagnosis, epidemiology, and pathogenesis mentioned at the end of this report, there is, in relation to Rift Valley fever, a need to:

1. search for the virus in the eggs and larvae of mosquitoes, both to verify the findings in Aedes lineatopennis in Kenya and to expand these observations to other geographical areas;
2. maintain serological and disease surveillance of sentinel herds of sheep and cattle in known epizootic areas; and
3. develop new veterinary vaccines including improved live-attenuated and subunit types.

3. TICK-BORNE HAEMORRHAGIC FEVERS

There are three major tick-borne haemorrhagic fevers: Crimean-Congo haemorrhagic fever, Kyasanur Forest disease, and Omsk haemorrhagic fever. The viruses causing Kyasanur Forest disease and Omsk haemorrhagic fever are related antigenically to the tick-borne flaviviruses, particularly members of the tick-borne
encephalitis complex, but in both infections, the haemorrhagic disease is more prominent than the neurological form. Crimean-Congo haemorrhagic fever infection has been identified in eastern Europe, China, and Africa but the other two diseases have a limited geographical distribution.

3.1 Crimean-Congo haemorrhagic fever

3.1.1 Historical background

The first well-documented outbreaks of this disease were recorded in the summers of 1944 and 1945, when over 200 cases of a serious, acute, febrile illness accompanied by severe haemorrhagic manifestations occurred in the USSR in the steppe region of the western Crimea. Many of the cases were among troops of the Soviet Union who were helping with the harvest. At first this disease was called acute infectious toxicosis, but it was later renamed Crimean haemorrhagic fever. Virus strains were isolated from blood samples taken from patients with acute disease and from the tick *Hyalomma marginatum marginatum*.

It was later realized that a similar disease had been known for many years in other areas of the USSR, particularly the Central Asian republics, and the same syndrome has since been described in areas of the USSR bordering the Black and Caspian Seas and in Bulgaria and Yugoslavia.

The virus strains causing Crimean haemorrhagic fever were later shown to be antigenically and biologically closely related to Congo fever virus, first isolated in 1956 in the Belgian Congo (Zaire) from the blood of a febrile child. Further investigations have shown the virus to be widespread in eastern, western, and southern Africa, Asia, and eastern Europe; however, only sporadic cases of the disease have been recognized in Africa. Hazara virus, isolated in Pakistan, has also been shown to be serologically related.

3.1.2 Etiological agent

The virus causing Crimean-Congo haemorrhagic fever is a member of the *Nairovirus* genus of the Bunyaviridae family.
3.1.3 Epidemiology

Distribution. Enzootic foci of the virus occur mainly in steppe, savannah, semi-desert, and foothill biotopes where one or two *Hyalomma* species are the predominant ticks parasitizing domestic and wild animals. The disease occurs over a large area of the world. It has been reported from Afghanistan, Bulgaria, China, Hungary, Iraq, Islamic Republic of Iran, Pakistan, Soviet Central Asia, Syrian Arab Republic, the United Arab Emirates, southwestern USSR, and Yugoslavia. It is widespread in East and West Africa and was also recently reported in South Africa and Zimbabwe. In addition, the virus or antibody has been found in Egypt, France, Greece, and India.

Vectors. Although the virus has been isolated from 25 different species or subspecies of 1-, 2-, or 3-host ticks, including *Boophilus*, *Dermacentor*, *Rhipicephalus*, *Amblyomma*, *Argus*, *Ixodes*, *Haemaphysalis*, and *Hyalomma*, members of the genus *Hyalomma* are the most important vectors in Asia, Africa, and Europe.

Vertebrate hosts. Both domestic and wild animals are probably involved in the maintenance cycle of the virus. It is possible that domestic animals (sheep, goats, and cattle) act as amplifying hosts during the epizootic season. In the USSR the disease is sharply seasonal, the peak incidence occurring in June and July.

Groups at risk. Agricultural workers (especially those involved in animal husbandry), campers, and the military are the groups most at risk.

3.1.4 Clinical diagnosis

After an infective tick bite, the incubation period is about 5–12 days. The illness begins abruptly with fever, chills, malaise, irritability, headache, and severe pains in the limbs and back, followed by nausea, vomiting, and abdominal pain. Fever is continuous but may be remittent and sometimes biphasic, resolving by crisis and lysis after 8 days. The face and neck are flushed and oedematous, the conjunctiva and pharynx are congested and there is oedema of the soft palate. The mouth is dry and the breath has a foul odour. Patients are depressed and somnolent. In most cases a fine petechial rash begins on the back and then covers the entire
body. The liver is enlarged in about 50% of cases but the respiratory system is unaffected. A haemorrhagic exanthem appears on the soft palate and uvula early in the illness and other bleeding manifestations, including haematemesis, rash, and melaena appear on about the fourth or fifth day in over 75% of patients. Leukopenia and severe thrombocytopenia are common. Large purpuric areas caused by subcutaneous extravasation of blood sometimes occur. Bleeding occurs—in descending order of frequency—from the nose, gums, buccal mucosa, stomach, uterus, intestines, and lungs. Gastric and nasal haemorrhages often lead to death. Involvement of the central nervous system is seen in 10–25% of cases and is usually associated with a poor prognosis; it includes neck rigidity, excitation, and coma. The case fatality rate has reached as high as 30–50% in nosocomial outbreaks (hospitals, laboratories, etc.), usually due to shock, secondary blood loss, or intercurrent infection. Subclinical infections are rare.

The severe disease seen in Europe is not generally observed in Africa. Laboratory infections are common and a fatal case has been reported in a laboratory worker in Uganda. In South Africa the disease was first recognized in 1981, when a Caucasian child was infected by a tick bite in a wooded area and subsequently died in hospital with severe bleeding complications. Subclinical infections are common throughout East and West Africa.

Nosocomial infections associated with high levels of mortality have been reported in recent years from Dubai, Iraq, and Pakistan. Crimean-Congo haemorrhagic fever was not suspected in any of the three index cases and medical and nursing staff were infected by prolonged contact with the patients’ blood and secretions.

3.1.5 Laboratory diagnosis

The virus causing Crimean-Congo haemorrhagic fever may be isolated in a variety of cell lines although Vero, CER, and LLC-MK₂ are the cell lines of choice. It may also be isolated in suckling mice following intracerebral inoculation.

Detection of antigen in the inoculated cells is best performed by immunofluorescent antibody (IFA) techniques with appropriate antisera.

Serological diagnosis is most easily performed by the IFA test on virus-infected cell monolayers. Alternative methods, more cumbersome and less sensitive, are the complement fixation (CF) test
and agar gel diffusion. The mouse or plaque-reduction-neutralization test is more sensitive and specific but requires a properly contained virology laboratory. Sera from man and many animal species contain non-specific neutralizing substances, removably by ether. The neutralization test results are unreliable unless ether extraction has been carried out. A rapid, reliable ELISA test is also available for Crimean-Congo haemorrhagic fever.

3.1.6 Pathogenesis

The pathogenesis of this disease is not well understood. The virus preferentially invades the capillary endothelium and reticuloendothelial cells. Associated with infection, there is evidence of thrombocytopenia, leukopenia, increased partial thromboplastin time, and the presence of fibrinogen degradation products leading to the disseminated intravascular coagulation that in turn causes shock and haemorrhage.

3.1.7 Treatment

Cases of Crimean-Congo haemorrhagic fever require special attention (see section 8.2 on management of patients) when the patient develops bleeding. A patient without bleeding complications may require only palliative treatment such as analgesics and antipyretics.

3.1.8 Surveillance

Human population. The objective of surveillance for Crimean-Congo haemorrhagic fever is the early detection of cases, especially sporadic cases, and also the diagnosis of early suspected cases in potential outbreaks, since these may cause nosocomial outbreaks if not managed correctly. Preventive and clinical health workers, if not familiar with early clinical and laboratory diagnosis, are likely to misdiagnose such cases as influenza-like illness, gastroenteritis, or surgical emergencies. Such workers should be made aware of the existence of foci of the disease. Appropriate precautionary measures should be taken when handling blood samples, such as avoiding splashing, securing the bottles with tape, and putting them in a second container. It must be emphasized that the blood from patients with this disease is highly infectious. Areas where the disease
is prevalent should have a laboratory designated to carry out virus isolation, rapid detection of antigen, and serological procedures such as CF, HI, IFA, and ELISA tests.

Serological surveys of populations should be carried out in 4 age groups: 0–9, 10–19, 20–29, and 30 years.

*Vector population.* Vector surveillance is necessary for the following reasons:

(a) to obtain information on the distribution of vectors;
(b) to determine their ecology, their absolute and relative density, and their susceptibility to insecticides;
(c) to demonstrate the presence of virus in the vectors.

The virus is maintained in nature by transtadial and transovarian transmission within the tick population and by horizontal transmission between tick vectors and various domestic and wild animals. Man is infected by the accidental bite of an infected ixodid tick. *Hyalomma* spp. ticks remain the major vector of the virus. Studies should be carried out on this vector particularly on tick populations from areas from which the disease has been reported earlier and also from areas where *Hyalomma* ticks are abundant and virus isolations have been made from them.

### 3.1.9 Prevention and control

Control measures are mainly aimed at reducing the population of tick vectors. This is particularly necessary when new areas of land are developed for agricultural, industrial, or housing schemes. Labour forces working in such areas are at a high risk of developing Crimean-Congo haemorrhagic fever. A suitable acaricide should be selected and applied prior to the development of such tick-infested areas. HCH has been found to be a suitable acaricide, but others are also available. Tick repellents are also of value and should be used by workers involved in clearing forest and digging ground for construction programmes.

*Control of nosocomial spread.* Once a clinical diagnosis of Crimean-Congo haemorrhagic fever virus infection with bleeding manifestations has been made, the patient concerned should be moved to the isolation area of the hospital. Transporting a bleeding patient is hazardous and may spread the disease, causing a
nosocomial outbreak. The syringes, needles, and other materials used on an infected patient should be thoroughly disinfected by heat or chemicals after use. Special barrier nursing should be practised. The fatality rate is especially high among patients who have caught the infection through nosocomial spread. When an outbreak has been confirmed, a thorough clinical, virological, serological, and entomological survey should be undertaken to assess the extent of the epidemic area and the source of the index case.

Immunoprophylaxis. In Bulgaria, an inactivated mouse-brain vaccine has been used to immunize workers. However, no standardized vaccine for Crimean-Congo haemorrhagic fever is available; efforts should be made to develop such a vaccine for use among persons involved in the investigation of disease as well as health personnel and those occupationally exposed to the disease.

3.1.10 Research needs and recommendations

(1) Trials of specific immune plasma and/or antiviral drugs should be carried out in areas where Crimean-Congo haemorrhagic fever is common, when such areas are located.

(2) Modern molecular biology techniques should be used to develop a candidate vaccine for Crimean-Congo haemorrhagic fever.

(3) Research on tick biology, using modern biological techniques, should be encouraged.

3.2 Kyasanur Forest disease

3.2.1 Historical background

Early in 1957 there were reports of fatal epizootics in wild monkeys in forested areas of Shimoga district, Karnataka (formerly Mysore) State in India, associated with outbreaks of disease in villagers who lived in and around the forest fringe. Local inhabitants called the affliction “monkey disease” because of the known association with dead monkeys. The disease was later named after the locality—Kyasanur Forest—from where the virus was first isolated.
3.2.2 Etiology

Kyasanur Forest disease virus is a member of the *Flavivirus* genus of the Togaviridae family. It is antigenically related to other tick-borne flaviviruses, particularly the viruses causing Far Eastern tick-borne encephalitis, and Omsk haemorrhagic fever.

3.2.3 Epidemiology

The virus has a complex life-cycle involving a wide variety of tick species, particularly *Haemaphysalis spinigera* in its nymphal stages. Man is an incidental host and plays no part in virus transmission. Small mammals, particularly porcupines, squirrels, and rats are the main reservoirs of the virus. Birds and bats are less important hosts. The silent enzootic situation was perhaps dramatically altered by man's need for more land, both for grazing and other agricultural purposes. Cattle were put to graze around the forest and thus provided *Haemaphysalis* ticks with a new and plentiful source of blood meals, which in turn resulted in a population explosion among the ticks. Cattle are very important in maintaining tick populations but play no part in virus maintenance. The ticks feed on other mammalian species such as monkeys, which show marked viraemia and an illness from which they may die. The monkeys are recognized as amplifying hosts for the virus. Seasonal epidemics of Kyasanur Forest disease have been associated with epizootics in monkeys, the most important being the black-faced langur (*Presbytis entellus*) and the South Indian bonnet macaque (*Macaca radiata*).

The disease is restricted to four districts (Shimoga, North Kanara, South Kanara, and Chikamagaloor) in Karnataka State, India. The epidemic patterns indicate irregular and unpredictable spread of the virus since its recognition in 1957. Earlier, the disease was found to be limited mainly to an area around the original focus covering about 800 km². Newer foci have since been recognized covering over 6000 km². The latest outbreak during 1982-83 seems to have been the largest. A part of the Nidle forest of some 400 hectares was clear-felled by the forest department to make room for a cashew plantation. The labour force brought from the neighbouring areas and some villagers noticed a few dead monkeys in the forest by late October and the first cases of the disease in man were reported from December 1982 onwards.
3.2.4 Clinical and clinicopathological diagnosis

The incubation period is estimated to be between 3 and 8 days. The disease appears with a sudden onset of fever, headache, and severe myalgia, with prostration in some patients. The acute phase lasts about 2 weeks. Gastrointestinal disturbances and haemorrhages occur in severe cases. There is no abdominal pain except in patients in whom there is gastrointestinal bleeding. The liver is not palpable but the tip of the spleen may be felt, most often in patients with generalized lymphadenopathy. Bronchiolar involvement occurs in some patients and results in a persistent cough and abnormal physical signs in the lungs. In some patients (with blood-tinged sputum) serious signs of lung involvement have been recorded that were considered to be the precursors of pneumonia.

There is a diphasic disease course in a number of patients. The second phase is characterized by mild meningoencephalitis after an afebrile period of 7–21 days. It is manifested by a return of fever, severe headache followed by neck stiffness, mental disturbance, coarse tremors, giddiness, and abnormal reflexes.

Convalescence is generally prolonged lasting for up to 4 weeks. The case-fatality rate has been estimated to be around 5–10%.

The virus is extremely infectious and therefore is hazardous for laboratory workers; more than 100 persons are known to have suffered from laboratory-acquired infections. The clinical features are not significantly different from those described for natural infections except that the disease is generally milder and, to date, no case has proved fatal.

Limited haematological and biochemical investigations have been carried out, particularly during the early years. Leukopenia has been found to be an almost constant feature of the disease, mediated in all probability through antileukocyte antibodies. Thrombocytopenia of variable degree has also been found to be an important feature. Thromboagglutinins have been found in the circulation of the majority of patients. In contrast to the findings in haemorrhagic fevers from the USSR and the Korean peninsula, there is no evidence of gross capillary damage apart from those incidental to thrombocytopenia.

Albuminuria appears in most cases during the acute febrile stage. Granular casts have been occasionally observed. In most cases, the cerebrospinal fluid is clear with no increase in cells or alterations in
proteins, chlorides, or sugar. In patients developing the second phase with meningeal signs, there is an increase in both cells and proteins.

The histopathological findings from 3 fatal cases in man showed similarities to the observations reported for other haemorrhagic fevers. It was concluded that the histopathological changes, in both man and monkeys, appear to be mild in comparison with the severity of the clinical illness. The haemorrhagic phenomena that were seen in some cases of the clinical disease were not adequately explained by the histological appearance of the organs or blood vessels examined.

3.2.5 Etiological diagnosis

Unlike many other arboviruses, the Kyasanur Forest disease virus has a prolonged viraemia of about 10 days or more. Of the sera collected during the first 8 days of illness about 95% contained the virus. The serological response of man to infection with this virus was studied in 104 fever patients, all of whom had demonstrable viraemia; the patterns of serological response were not unlike those seen with other flavivirus infections. The response depended upon whether the current infection was the first encounter with a flavivirus (primary) or whether there had been a previous infection with one or more flaviviruses (secondary). For secondary infections, specific serodiagnosis was difficult. Both the neutralization test and the agar gel diffusion test are specific; the latter is also useful in the field. The agar gel diffusion test is less sensitive than the neutralization test. For primary infections, haemagglutination inhibition tests or single radial haemolysis tests seem to be satisfactory.

3.2.6 Treatment

There is no specific treatment for Kyasanur Forest disease. Supportive and symptomatic treatment should be provided, such as analgesics for myalgia and headache, intravenous fluids for dehydration, and blood transfusions if there is haemorrhaging. During convalescence, rest and an adequate diet seem to be the major requirements. It is not necessary to isolate the patients.

3.2.7 Surveillance

A surveillance system has been set up by the Karnataka state government that records the number of cases and deaths suspected
of being due to Kyasanur Forest disease. Isolation of the virus from sera taken during the acute phase is sometimes carried out, but only on a small number of suspected cases.

The majority of human infections, with the exception of those acquired in the laboratory, are transmitted exclusively in and around the forest. During forest clearing operations, rodents and monkeys with their tick ectoparasites migrate to other areas, thus enlarging the focus of natural infection. Attempts should be made to detect the virus or antigen in ticks and a serological survey of rodents should be carried out in virgin forest areas that are to be cleared.

3.2.8 Prevention and control

On forest tracks used by man, the number of ticks can be controlled by spraying with acaricides. This, and the application of effective repellents for the personal protection of the forest labourers, may be implemented in restricted areas where monkey deaths occur.

It is proposed that the population at risk should be immunized with inactivated-killed Kyasanur Forest disease vaccine prepared with chick embryo fibroblast.

3.3 Omsk haemorrhagic fever

3.3.1 Historical background

Omsk haemorrhagic fever is an acute febrile disease. Its characteristic features are: viral etiology, natural focality, haemorrhagic symptoms in patients, and a relatively benign course.

Cases of the disease were first reported in 1944–45, although there is some evidence that similar cases appeared also in 1941–43, in rural areas north of Omsk (Western Siberia). In 1945 and 1946 during two outbreaks of Omsk haemorrhagic fever there were more than 200 and 600 cases, respectively, and in subsequent years, clinical, epidemiological, and etiological features of the disease were studied by a group of medical specialists from Omsk and Moscow. As a result of these studies it was concluded that Omsk haemorrhagic fever is a specific viral haemorrhagic disease different from other known viral haemorrhagic fevers.
3.3.2 Etiology

The virus that causes Omsk haemorrhagic fever is antigenically close to the tick-borne encephalitis virus and other members of this group. Taxonomically it belongs to the genus *Flavivirus* of the Togaviridae family (Group B arboviruses). The virus is pathogenic for white mice, in which it has neurotropic characteristics, and for several types of cell culture. The virus has been isolated from patients' blood taken during the acute period of illness and from the tick *Dermacentor pictus*. Antibody to this virus is usually detected in serum samples from convalescent patients.

3.3.3 Epidemiology

Cases of Omsk haemorrhagic fever have been registered in wet grasslands of the forest-steppe areas that are rich in lakes. Initially these cases were observed in 3 districts of Omskaja oblast (Omsk region) north of Omsk and since 1950 in adjacent districts of Novosibirskaja and Tyumenskaja oblasts (Novosibirsk and Tyumen regions) in the western part of Siberia. No other natural foci of the disease have been detected. Cases of the disease appear mainly from April to October (with occasional single cases in November and December). The seasonal morbidity curve has two peaks—in May and in August–September. Transmission is via the bite of infected ticks. There is apparently no direct man-to-man transmission, and no hospital outbreaks or intrafamily cluster outbreaks have been observed. Ticks can be carried to man by dogs. The population groups at risk are agricultural workers in the focal area and collectors of mushrooms or wild berries. Both sexes may be affected and the age range of cases is 5–70 years; most cases occur, however, among active workers. It has been demonstrated experimentally that the virus can survive for about 3 days in goats and can be found in their milk for a few days.

The reservoir of the virus and its vector in nature is the tick *Dermacentor pictus*; there is some indication that the tick *D. marginatus* may also act as a reservoir and vector of the virus in some parts of the Siberian natural focus. The reservoir status was confirmed by the isolation of the virus from *D. pictus*. In addition, there is a marked correlation between the seasonal dynamics of disease morbidity and tick population size and activity. Transovarian transmission of the virus has been shown in *D. pictus*. A biological cycle of unknown complexity, involving small
mammals and ticks, is known to exist. *D. pictus* parasitizes some 37 different species of mammal and Omsk haemorrhagic fever virus is pathogenic for a number of these species. Muskrats (*Ondatra zibethica*) that were introduced into the region some 60 years ago for hunting purposes, have been shown to play an important role in virus transmission in several natural foci. This animal seems to be capable of transmitting the virus by direct contact and it has been shown that single cases of Omsk haemorrhagic fever observed during the winter months occur in persons who have been hunting muskrats and treating their carcasses.

### 3.3.4 Clinical features

The incubation period varies; it is usually between 3 and 7 days but can be from 1 to 10 days. The onset is sudden, with a period of fever (39–40°C) lasting for 5–12 days. In 30–50% of cases a second febrile wave appears 10–15 days after the beginning of the illness. The second febrile period is often more severe than the first one. In typical cases there is headache, meningeismus, vomiting, and exanthema of the palate; epistaxis, haematemesis, and melena may occur but nasal, enteric, lung, and uterine haemorrhage predominate. Hyperaemia of the skin (face, upper parts of the body) and mucous membranes (particularly conjunctival hyperaemia) is typical of this disease. Blood analysis shows leukopenia, thrombocytopenia and plasmocytosis. During the second febrile period meningeal symptoms, pneumonia (in about one-third of the cases), and nephrosis may develop.

Usually the patient recovers fully after several days (or a few weeks in severe cases), but convalescence may be prolonged, with often weeks of persistent weakness; there are no sequelae. Transient hair loss in convalescence is frequently seen. According to various reports, the case-fatality rate is variable, but it is generally low, 0.4% to a maximum of 2.5%.

### 3.3.5 Diagnosis

Diagnosis is usually based on laboratory investigations, including isolation of virus from blood taken during the acute phase of illness and seroconversion detected in paired serum samples by virus neutralization. Newer techniques such as the immunofluorescence test and ELISA are also available.
3.3.6 Pathogenesis and pathophysiology

There are no morphological changes typical of Omsk haemorrhagic fever. The characteristics common to other haemorrhagic fevers are a generalized increase in vascular permeability, extravasation, and perivascular infiltration with thrombi in small vessels. The pathogenesis is determined by the degree of vascular damage, thrombocytopenia, and bleeding in the brain, kidney, endocardium, myocardium, stomach, and intestines. Oedema of the brain causes sensory changes. Hypotonia can lead to collapse and shock in serious or fatal cases, particularly in the first week of illness. Haemosiderin deposits are found in the Kupffer cells of the liver.

3.3.7 Surveillance, prevention, and control

Since the disease has not been very active in recent years with only sporadic cases reported, there is no need for special surveillance programmes, but the medical authorities should be vigilant especially for any increase in morbidity suggesting an outbreak. Anti-tick control measures are not justified at present. However, workers in focal areas should wear specially designed nylon-net-like protective clothes for general protection against ticks. Repellents may be used for short-term protection when exposure to ticks is particularly likely.

*Immunoprophylaxis.* A specific formal-inactivated vaccine has been developed from the brain tissue of white mice infected with Omsk haemorrhagic fever virus. Despite its highly protective effect, this vaccine has not been in use for many years because of adverse reactions that have occurred as a result of the high concentration of brain-tissue components.

4. RODENT-BORNE HAEMORRHAGIC FEVERS

Among the rodent-borne haemorrhagic fever viruses are the arenaviruses — Lassa, Junin, and Machupo — causing respectively, Lassa fever, and Junin and Machupo haemorrhagic fevers. Another group of viruses, of which Hantaan is the type virus, belong to the Bunyaviridae family. They produce haemorrhagic fever with renal syndrome and have been isolated only recently. The latter group
may cause particular problems for public health services because of their epidemic potential, the high case-fatality rate, and the unusual difficulties in their diagnosis, treatment, and prevention. A vaccine against Junin haemorrhagic fever is in preparation.

Hantaan and Hantaan-like viruses are distributed throughout most of the world and cause both severe and mild illness. The clinical diagnosis of the mild form of the disease in man is difficult, and the real prevalence of the disease is probably much higher than has been reported. In parts of the world where the disease has not yet been diagnosed, physicians should now look out for it.

Lassa fever is endemic in large areas of West Africa; but further investigations are required. Lassa-related viruses have been isolated from central and southern Africa, but it is not yet known whether these viruses can cause disease in man.

4.1 Lassa fever

4.1.1 Historical background

Lassa fever is perhaps the most publicized of all the viral haemorrhagic fevers. The first recognized victim was an American nurse who was infected at a small mission station in Lassa township in Nigeria in 1969. This was the start of a dramatic episode, during which two other nurses died, one of whom was evacuated to the USA in a commercial airliner with no strict containment precautions during transport.

4.1.2 Etiology

Lassa virus belongs to the family Arenaviridae and is one of 4 members of this family that are pathogenic for man. The arenaviruses are generally associated with chronic infection in rodent hosts. Lassa virus is a double-segmented RNA virus that is pleomorphic, with a diameter of 80–150 nm. It is an enveloped virus and is generally sensitive to lipid solvents and detergents.

4.1.3 Epidemiology

**Distribution.** Illness or serological evidence of infection with Lassa virus has been demonstrated in numerous West African countries, including Burkina Faso, Central African Republic,
Gambia, Ghana, Guinea, Ivory Coast, Liberia, Mali, Nigeria, Senegal, and Sierra Leone. Between 3 and 11% of febrile illness in two villages studied in Sierra Leone were caused by Lassa virus. Recently fluorescent antibodies against Lassa virus were demonstrated in 12-13% of human sera taken in the western part of the Sudan, without evidence of human illness related to Lassa fever. Limited human serological studies carried out in Mozambique have also shown antibodies against a locally isolated, Lassa-related virus.

Vectors and reservoirs. Lassa and Lassa-related viruses have no known arthropod vector. Lassa virus has been isolated from the multimammate rat *Mastomys natalensis* in Nigeria and Sierra Leone. Lassa virus has also been found in both the 32- and the 36-chromosome types of *M. natalensis*. In 1977, a second arenavirus was isolated from *M. natalensis* in Mozambique. Interest in the Mozambique virus was stimulated by the finding that it caused asymptomatic infection in rhesus monkeys and that they were subsequently shown to have acquired clinical resistance when challenged with otherwise lethal Lassa fever virus. In 1981, six strains of Mozambique virus, identified by the use of specific monoclonal antibodies to the agent, were recovered from the visceral tissues of *M. natalensis* in Zimbabwe. Immunofluorescent antibodies to Mozambique virus were found in *M. natalensis* and *Aethomys chrysophilus*. In the Central African Republic, 14 Lassa-related virus strains were isolated from *Praomys* spp.

The use of a battery of monoclonal antibodies has shown four identifiable, geographically distinct subtypes of Lassa virus: Nigeria, Sierra Leone/Liberia, Central African Republic, and Zimbabwe/Mozambique. Lassa strains collected in any one given area have shown a remarkable similarity.

Transmission. The pathogenesis of virus infection in *Mastomys* shows that pre- or perinatal infection results in the animals remaining infected throughout life and excreting large amounts of infectious virus in their urine. In Sierra Leone villages it has been observed that the people are surrounded in their houses, both day and night, by virus-infected rodents that excrete large amounts of virus. Since many of the houses are closed during the day, there is a simulated nocturnal environment for nearly 24 hours a day. This
allows rodents to move around freely and results in urine being deposited in beds, on floors, and in food supplies.

Transmission to man occurs throughout the year. There is a general correlation among antibody prevalence in man, the numbers of *Mastomys* found in the village, and the proportion of these rodents excreting virus. Man-to-man infection also occurs and some evidence suggests that it may happen more frequently than was thought previously. Transmission by aerosol is possible, but not well documented.

*Groups at risk.* From a three-year study carried out in Sierra Leone, it became evident that in many villages a substantial proportion of the population is infected with Lassa virus during their lifetime, often from an early age. There is little difference in the mortality rate between age groups. The case-fatality rate in two hospitals studied in Sierra Leone was 16.5%, with no difference in mortality between the sexes. Those at risk are human populations living in West Africa in rural areas or in urban centres, densely populated by *Mastomys* species.

The major risk factor is contact with the excreta of rodents infected with Lassa virus. In Lassa fever it is important to be aware that the virus is difficult to neutralize with convalescent sera and that it can be isolated despite a high level of serum antibody. This, when considered together with the important observation that in man fluorescent antibody was lost at a measurable rate, raises the question whether people with and without immunofluorescent antibodies, but previously infected with Lassa virus are still protected or are sensitive to reinfection.

4.1.4 Clinical diagnosis

A case of Lassa fever is defined as a person with a febrile illness accompanied by one or more of the following: isolation of Lassa virus, a 4-fold rise in antibody to Lassa virus, an IgG titre of at least 1:512 on admission, and a positive IgM titre.

Lassa virus causes a disease that may vary in severity from subclinical to fatal. The incubation period varies from 3 to 16 days and the disease begins insidiously with non-specific symptoms, including fever.

Field studies in Sierra Leone have shown that the best single predictors of Lassa fever are facial oedema, conjunctivitis, purulent
pharyngitis, vomiting, proteinuria, and abdominal tenderness. For patients with purulent pharyngitis and proteinuria, the probability of having Lassa fever is 80%. However, these symptoms only identify 44% of Lassa fever patients. No single symptom or combination of the above-mentioned symptoms clearly differentiates Lassa fever infection from other viral infections.

The absence of any specific symptom in the early stage of the disease makes it difficult for the clinician to make a diagnosis and hence a decision concerning isolation and treatment of patients. Lassa fever may be confused in the early stages with many other febrile illnesses such as malaria, influenza, typhoid fever, septicaemia, and other severe infectious diseases. Although not apparent clinically, quantitative measurements of viraemia and of the level of circulating liver enzymes (aspartate aminotransferase) clearly have a high predictive value regarding the ultimate outcome of Lassa fever infection. Patients who, on the day of admission to hospital, have more than $10^4$ ID$_{50}$ of virus and 150 IU of aspartate aminotransferase in the blood have 14 times more chance of dying than those who meet neither of these criteria. The white blood cell count cannot be relied upon to differentiate Lassa fever from bacterial infection, and extremely high white blood cell counts have been encountered in Lassa fever patients.

No relationship has been established between clinical outcome and the time of appearance or titres of IgM or IgG anti-Lassa virus antibodies detected by immunofluorescence. The studies in the two hospitals in Sierra Leone have shown that Lassa fever infection is probably the most important single medical cause of death in adults and a major cause of adult admissions. The case-fatality ratio in hospitalized cases of Lassa fever was 16%. An estimate of the overall case-fatality rate for Lassa fever in the eastern part of Sierra Leone was 1–2%.

4.1.5 Laboratory diagnosis

The primary isolation of Lassa virus can be successfully performed in the continuous cell line, Vero E6, in a high-security laboratory. Until now, no neutralization test has worked satisfactorily. As yet, no rapid diagnostic test for antigen detection in the patient or infected animal is available. However, making acetone-fixed touch smears followed by the immunofluorescent antibody (IFA) test using monoclonal antibodies against Lassa virus
seems to be a promising procedure for the direct detection of antigen in animal tissues; and the immunofluorescent antibody test using inactivated cells on slides as the antigen source is the method of choice for serological diagnosis.

4.1.6 Pathogenesis and pathophysiology

The most consistently observed lesions in patients with Lassa fever are hepatocellular, adrenal, and splenic necrosis, and adrenal cytoplasmic inclusions. Neither these lesions, nor other milder and less consistently observed lesions such as myocarditis, renal tubule injury, and interstitial pneumonia, are severe enough to be regarded as the cause of death in Lassa fever.

The central nervous system does not show any specific lesions. Viral titrations demonstrate a high virus content in the liver, lung, spleen, kidney, heart, placenta, and mammary gland. Because of this apparent lack of pathological lesions despite the presence of a widely disseminated viral infection, further investigation of humoral inflammatory mechanisms is needed. The high and persistent viraemia during the febrile stage of the illness, the delay in the development of neutralizing antibodies, and the simultaneous presence of both virus and specific antibodies also suggest that fatal Lassa fever may result from a severely compromised cell-mediated immune function. At present this concept is speculative and will require further confirmation. Severe cases of Lassa fever, however, differ from cases of both Junin and Machupo haemorrhagic fevers, although these infections are caused by other members of the arenaviridae. In the latter diseases viraemia is of a lower titre and is not predictive of clinical outcome; and antibodies, including neutralizing immunoglobulins, are not formed until viraemia has disappeared and the acute disease is resolved. Lassa virus may appear in the urine, initially in high concentration, during the late stage of the febrile illness and has been found during convalescence, up to 70 days after the onset of illness.

4.1.7 Surveillance

Active surveillance is carried out in certain areas of Sierra Leone, and transmission from rodent to man is found throughout the year. In the areas studied there are an estimated 200 hospitalized Lassa fever cases every year. Man-to-man transmission also occurs and
some evidence suggests it may happen frequently. These surveillance programmes include case-finding, serological surveys, and attempts to detect the virus in rodents.

4.1.8 Prevention

Attempts to prepare a Lassa fever vaccine have so far been unsuccessful. Passive immunization with convalescent-phase plasma has not been effective. The use of chemoprophylaxis with ribavirin has shown promise and should be considered.

4.1.9 Control

In theory the elimination of Lassa-virus-infected rodents in an endemic area could reduce significantly the spread of the disease. However, studies in Sierra Leone have shown that it would be impossible to achieve its complete eradication.

In Sierra Leone, barrier nursing in a single room or an open ward, using protective clothing including rubber gloves, has proved successful in preventing the spread of infection to hospital staff and other members of the patient’s family. This practice should be viewed with caution for application elsewhere; there is a substantial level of immunity in the local population, but stricter measures may be necessary in a non-immune population. Although 10 cases of Lassa fever have been imported into Europe and North America there has been no evidence of person-to-person spread. The risk of airborne transmission appears to be slight. However, large amounts of virus can be detected in the blood and other body secretions of patients and great care must be exercised by the clinical staff looking after a patient, and by laboratory staff dealing with specimens. Virological work with Lassa virus should preferably be carried out in laboratories with maximum containment facilities.

Whenever possible a patient with suspected Lassa fever should be nursed locally, using approved basic techniques.

4.1.10 Treatment

The division of Lassa fever patients into generally fatal and non-fatal groups is crucial to the design of therapeutic trials in this disease. Patients having more than $10^4 \text{ID}_{50}$ of virus per ml of blood and more than 150 IU of aspartate aminotransferase per ml of blood
have only a 20% chance of survival. It is to these patients that initial experimental therapy should be directed because the potential benefits would be greatest among them. Experiments have shown clearly that guinea-pigs and monkeys survived challenge with virulent Lassa viruses when they were treated with immune plasma rich in neutralizing antibodies. The titre of neutralizing antibodies present in the plasma is critical for the final outcome of the treatment. The generally low neutralizing antibody content and the difficulty of determining the neutralization titre in convalescent plasma taken from patients complicates the use of plasma in the treatment of Lassa fever in man.

Ribavirin, an antiviral drug, showed a beneficial effect when tested in monkeys infected with Lassa virus. A 10-day course of treatment with ribavirin given early in the disease was beneficial to some Lassa patients in an endemic area in Sierra Leone. The drug was given intravenously on the first 4 days of the disease (60 mg/kg/day) and subsequently orally (30 mg/kg/day).

4.1.11 Research needs and recommendations

In addition to the research needs and recommendations on rapid diagnosis, epidemiology, and pathogenesis mentioned at the end of this report, the following points apply specifically to Lassa fever.

(1) Evaluation and standardization of serological tests (neutralization, IFA, ELISA) between various laboratories is desirable.

(2) It seems appropriate to continue trying to improve regimes for the therapy of Lassa fever patients with ribavirin.

(3) The collection of human immune plasma for specific therapeutic trials should be continued. The search for alternative practical means of obtaining immunoglobulin with enhanced neutralizing antibody titres suitable for use in man should be encouraged.

(4) The feasibility of preparing a Lassa fever vaccine should be investigated.

(5) The prognostic value of the level of viraemia has already been established; now the prognostic value of antigenaemia should be determined, using a rapid, simple test applicable under field conditions.
Further epidemiological studies must be undertaken in order to answer the following question: are the Mozambique and Central African Republic types of virus capable of producing serious, acute disease in man?

4.2 Junin and Machupo haemorrhagic fevers

4.2.1 Historical background

Junin and Machupo haemorrhagic fevers (also known as Argentinian and Bolivian haemorrhagic fevers, respectively) are severe systemic viral diseases caused by Junin and Machupo viruses. Outbreaks of these diseases were first recorded about 30 years ago. The case-fatality rates of these two rodent-borne haemorrhagic fevers may be as high as 30%.

4.2.2 Etiology

The etiological agents of these two diseases are two closely related arenaviruses, Junin and Machupo viruses, that are associated with cricetine rodents in which they produce chronic infections with persistent viraemia and virus shedding in the saliva and urine.

4.2.3 Epidemiology

Distribution. In Argentina, outbreaks of Junin haemorrhagic fever have been recorded every year since 1958 and a total of more than 20,000 cases has been notified. Furthermore, during the last 20 years a progressive extension of the endemo-epidemic area of the disease has occurred. In 1958, all the cases of the disease originated from a relatively small area in the north-west of Buenos Aires Province. The disease is now endemic in the richest agricultural regions of the provinces of Buenos Aires, Santa Fe, Córdoba, and La Pampa. Seroepidemiological surveys conducted in different places in the endemic area of Junin haemorrhagic fever have shown a 4–6% Junin antibody prevalence in the rural inhabitants. From this finding, it can be estimated that over 90% of the population of rural endemic areas remain susceptible to Junin virus infection since they lack specific antibodies against this virus. This observation is relevant in relation to the need for preventive measures, such as the development of an effective vaccine against Junin virus.
Outbreaks of Machupo haemorrhagic fever were first detected in the town of San Joachim in the Beni region in Bolivia. Presumably because of effective rodent control, no new cases of this disease have been notified since 1975.

Transmission. The precise mechanisms of transmission of the Junin and Machupo viruses from rodents to man remain unknown. Careful studies indicate that any participation in this process by arthropod vectors is highly unlikely. However, the persistent shedding of virus in the saliva and urine of chronically infected rodents, with the consequent contamination of the environment, strongly suggests that direct transmission occurs. Junin and Machupo haemorrhagic fevers are usually not contagious diseases, but a few well-documented instances of man-to-man transmission exist. Virus is always present in the blood during the acute period and its presence in bloody secretions, mucous membranes, or the urine of some patients may result in occasional person-to-person transmission.

Vectors and reservoirs. Different species of cricetine rodent are the natural hosts of the etiological agents of these haemorrhagic fevers. Calomys callosus is the reservoir of Machupo virus, while C. musculinus and C. laucha are the principal hosts of Junin virus. No arthropod vectors have been demonstrated in either disease.

Another interesting finding concerns the activity of the virus of lymphocytic choriomeningitis in rodents and man in the endemic area of Junin haemorrhagic fever. Isolation of lymphocytic choriomeningitis virus from Mus musculus captured in Argentina near Pergamino and in the province of Córdoba and the demonstration of serological activity in them were followed by the demonstration of lymphocytic choriomeningitis virus infection in patients with a presumptive clinical diagnosis of Junin haemorrhagic fever. The simultaneous activity of Junin virus and lymphocytic choriomeningitis virus, two arenaviruses pathogenic for man, in rodents and man from the same geographical area provides a unique opportunity to study several aspects of these diseases.

Groups at risk. The rodent reservoir of Junin haemorrhagic fever is distributed in rural areas and the group at risk of infection is the rural population particularly the males (85% of cases). Although there are cases in all age groups, the disease is more common
between 20 and 60 years of age. On the other hand, the rodent host of Machupo haemorrhagic fever lives in and around the houses of small towns and the sex distribution of the disease is almost even. Laboratory workers handling Junin and Machupo viruses are another group at risk.

4.2.4 Clinical diagnosis

Susceptibility is general. The incubation period of Junin haemorrhagic fever is 10–14 days. Early recognition of the disease is of crucial importance so that treatment with immune plasma can begin.

Early and rapid diagnosis of both diseases can be made by a combination of a careful analysis of the epidemiological history, the presence of certain signs and symptoms, and a few simple clinical laboratory tests. Only the symptoms and signs that may be present during the first 6 days of evolution will be considered. They are listed according to their relative frequency and importance for the differential diagnosis.

Symptoms. The onset is insidious. The almost constant absence of productive cough, sore throat, or nasal congestion is helpful in differential diagnosis with respect to acute respiratory infection. Symptoms include moderate hyperthermia (37.5–38.5°C), malaise, and anorexia. There is headache with retro-orbital pain and dizziness, lumbar pain which may be severe, and muscular pains, particularly in the lower extremities. Gastrointestinal symptoms include epigastric distress, nausea, and vomiting.

Signs. Signs include conjunctival congestion; periorbital oedema; flushing of the face, neck, and upper part of the chest; congestion of the gums (with a red line at their junction with the teeth and a tendency to bleed either spontaneously or under slight pressure); congestion of the pharynx (with increased vascular markings, small vesicles, and petechiae in the soft palate); moderate and painless enlargement of the lymph nodes (particularly in the laterocervical regions); petechiae (usually present in the axillary regions); decreased muscular tone and deep-tendon reflexes (particularly in the lower limbs); fine tremor of the hands and tongue; relative bradycardia and orthostatic hypertension. Hepatomegaly, splenomegaly, and jaundice do not occur.
4.2.5 Laboratory diagnosis

In both diseases there is leukopenia (1000–2500/mm³) and thrombocytopenia (40 000–80 000 mm³). The sedimentation rate is normal and there may be haemoconcentration. Proteinuria may be massive (more than 10 g being excreted per day), and cylindruria is observed. In Junin but not in Machupo haemorrhagic fever there are round or oval cells with cytoplasmic vacuoles in the urinary sediment.

The virological diagnosis is established by the isolation of Junin or Machupo virus from suspected cases. Viraemia occurs during the whole of the acute febrile period and virus can also be isolated from autopsy materials in fatal cases. Isolation of these viruses in suckling mice and/or guinea-pigs may take 2–4 weeks. Isolation in cell culture monolayers (Vero, BHK), in combination with immunohistochemical methods, such as an immunofluorescent antibody test or a peroxidase-antiperoxidase test means that positive results can be obtained in 1–3 days. These two techniques and electron microscopy are useful in establishing a rapid diagnosis in tissues obtained from fatal cases.

Cocultivation on Vero cell monolayers of lymphomononuclear cells obtained from the peripheral blood of cases of Junin haemorrhagic fever has recently been shown to increase the chances of isolating Junin virus from the blood of patients.

Indirect immunofluorescence and plaque reduction tests on Vero cells detect specific antibodies earlier and are very useful for the selection of immune plasma donors, using the criterion of antibody persistence. Complement fixation has disadvantages, such as the late detection and short persistence of complement-fixing antibodies. In addition, these antibodies cross-react with other arenaviruses (Tacaribe group), and in cases of Junin haemorrhagic fever 10% of the results are false negatives when this method is used. In this respect, complement fixation antibodies against Junin or Machupo viruses will rarely be detected for more than 2 years after the disease, while immunofluorescent and neutralizing antibodies are present in 90% of cases of Junin haemorrhagic fever for up to 15 years after the infection.

Monoclonal antibodies against Junin virus have recently been obtained and may prove useful for a better serological diagnosis of infection. An ELISA test is also being developed to detect Junin virus antigens.
4.2.6 Pathogenesis

The pathogenesis of Junin and Machupo virus infections is not immunologically mediated since direct damage occurs as a result of the action of proteolytic enzymes released by the cells invaded by the virus. Several studies in cases of Junin haemorrhagic fever in man and in animals experimentally infected with Junin virus have shown that the virus replicates in haematopoietic and lymphatic organs. It has been shown in vivo and in vitro that Junin virus multiplies in human mononuclear phagocytes and there is some evidence that these cells play a direct role in the activation of the clotting and complement systems, as well as in the rest of the pathological disturbances resulting from infection.

4.2.7 Surveillance

Notification of suspected cases of Junin haemorrhagic fever and serological diagnosis with acute and convalescent serum samples from notified cases are carried out in the four provinces of Argentina included in the endemic area. For this purpose, as well as for the admission and treatment of patients, there are 12 centres covering the endemic area. The etiological diagnosis is made at one of four laboratories in the endemic region by indirect immunofluorescent antibody tests with spots and reagents prepared and distributed by the National Institute for Studies on Haemorrhagic Virus Diseases, where viral isolation from acute blood samples and tissues from autopsy is performed in containment laboratories.

4.2.8 Prevention and control

In Bolivia, infected Calomys are found in and around houses and towns in domestic and peridomestic habitats and this means that rodent control is practicable. However, in Argentina the rodent hosts of Junin virus are only found in the agricultural areas, and they are widely distributed over a very extensive rural region, where the rodent population cannot be effectively controlled by trapping and killing. Alternative methods are being investigated, but ecological control of Junin haemorrhagic fever is very difficult. The production of an effective vaccine is a more practical approach and development work is already in progress. The candidate attenuated strain of Junin virus in certified cell cultures is more attenuated and less neurovirulent for guinea-pigs than any other known attenuated
strain of Junin virus. The primary and secondary vaccine seeds are free of adventitious agents. Neurovirulence tests in rhesus monkeys are in progress, as well as potency and protection studies. After certification, field trials should be conducted.

4.2.9 Treatment

The efficacy of immune plasma in the treatment of Junin haemorrhagic fever has been proved in a controlled therapeutic study. The results shown in Table 4 indicate that this form of treatment results in a marked reduction in disease mortality if given within the first 8 days of illness. Viraemia is neutralized by the administration of immune plasma. In a recent study of the role of the interferon system in Junin haemorrhagic fever, very high levels of circulating alpha interferon were detected in acute serum samples obtained on hospital admission of patients. A drastic reduction of interferon levels was observed after transfusion of immune plasma. It is hypothesized that alpha interferon is produced by Junin virus-infected macrophages, and that the neutralizing effect of immune plasma eliminates the viral replication that was inducing interferon synthesis. In this respect, it is worth mentioning that circulating antibodies against Junin virus are detected at about the same time as clinical improvement begins when endogenous interferon titres are also low. The close correlation between the detection of circulating Junin antibodies and clinical improvement is further evidence to support the hypothesis of a mechanism of direct viral damage in the pathogenesis of Junin haemorrhagic fever.

An association between treatment with immune plasma and a late neurological syndrome has been demonstrated. Usually the patient develops fever, headache, and a cerebellar syndrome after an illness-free interval, 4–6 weeks following the acute period of the infection. Although the great majority of cases improve without sequelae in a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>Mortality</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Died</td>
</tr>
<tr>
<td>Immune plasma</td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>97</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>188</td>
<td>17</td>
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few days, occasional cases with severe neurological alterations, and even death, have been observed. Without exception, there is a latent period of 2-3 weeks between the recovery from the acute phase of the disease and the onset of this late neurological syndrome. No correlation has been observed between the severity of the disease, viraemia, or other clinical or laboratory manifestations of the disease and the occurrence of the late neurological syndrome. Attempts to isolate virus from the blood and cerebrospinal fluid have been consistently negative. Nevertheless, a moderate increase is found in the number of cells in the cerebrospinal fluid, where there are also antibodies against Junin virus. Although these findings suggest an immunological basis, several mechanisms can be postulated to explain the association between treatment with immune plasma and the late neurological syndrome. Until the pathogenesis is elucidated, it must be considered that this syndrome is generally benign and that immune plasma is the only form of treatment at present available for Junin haemorrhagic fever.

4.2.10 Research needs and recommendations

In addition to research needs and recommendations on rapid diagnosis and epidemiology cited at the end of this document, the following apply specifically to Junin haemorrhagic fever.

1. Seroepidemiological surveys should be conducted to determine the prevalence of Junin virus in rodents and man in the endemic and neighbouring regions. This information is needed to establish the prevalence of Junin virus and hence to select the places and population samples to be included in vaccine field trials.

2. Investigations on the pathogenesis and treatment, as well as on the late neurological syndrome seen in cases of Junin haemorrhagic fever treated with immune plasma, should continue.

3. Further support is needed to ensure the production and trials of the candidate attenuated Junin virus vaccine.

4.3 Haemorrhagic fever with renal syndrome

4.3.1 Historical background

This disease may have existed in Asia for at least the last 1000 years since there is a suggestive description of haemorrhagic fever
with an associated renal syndrome in a Chinese medical book that was written about A.D. 960.

Various haemorrhagic fevers with a very similar syndrome have been reported throughout Eurasia: (i) as haemorrhagic nephrosonephritis or haemorrhagic fever with renal syndrome in the Soviet Union, with several thousand cases reported annually since 1913; (ii) as Songo fever or epidemic haemorrhagic fever, first recognized in 1913 in China, with more than 32,000 cases reported in 1981; (iii) as nephropathia epidemica in Scandinavia, with several hundred cases reported annually since 1934; (iv) as epidemic nephritis or epidemic haemorrhagic fever in Eastern Europe, since 1934; (v) as Korean haemorrhagic fever since 1951, and (vi) as epidemic haemorrhagic fever in Japan, since 1960. Epidemics involving thousands of cases of a similar disease of unknown etiology occurred among soldiers in Lapland and Manchuria during past wars. It was postulated in 1962 that all the diseases listed above might be caused by a single etiological agent.

Haemorrhagic fever with renal syndrome was first described in the Tula Region of the USSR in the 1930s, and viral origin was proved by passage in human volunteers in the Soviet Union in 1940.

During the Korean War more than 3000 United Nations troops stationed in the demilitarized zone developed what was considered by western physicians to be a new, rare disease and it attracted a great deal of attention throughout the world. It was a haemorrhagic disease terminating in shock, renal failure, and death in about 10–15% of cases. The causative agent of this outbreak was first isolated in 1976 from the rodent Apodemus agrarius and in 1978 from patients. The etiological agent was propagated in a human cell culture line, and it was named Hantaan virus after the Hantaan river, which runs near the 38th parallel across the Korean peninsula.

A close etiological relationship was established by antibody studies between this disease and haemorrhagic fever with renal syndrome in the USSR, nephropathia epidemica in Scandinavia, and epidemic haemorrhagic fever in China and in Japan.

The WHO Working Group on Haemorrhagic Fever with Renal Syndrome, which met in Tokyo in 1982, recommended that the above-mentioned diseases with different names should all be referred to as “haemorrhagic fever with renal syndrome”.¹

4.3.2 Etiology

Hantaan and related viruses are spherical, RNA viruses, 85–110 nm in diameter. Morphological, physicochemical, and serological studies have shown that these are new members of the family Bunyaviridae.

4.3.3 Epidemiology

Distribution. Seroepidemiological surveys and documented case reports show that Hantaan and Hantaan-related agents are widely distributed throughout much of the world, as demonstrated by the presence of antibodies against the agents in sera from man, urban rats, and laboratory rats.

There are at least two forms of disease, mild and severe. The severe form is common in Asian countries. In the Republic of Korea several hundred cases occur annually in rural and urban areas with a case-fatality rate of approximately 5%. In Japan 116 cases with one death have been reported since 1976, the origin of infection being laboratory rats infected with Hantaan virus in the animal rooms of 19 medical centres, and in the 1960s about 150 patients were hospitalized in Osaka City with 3 deaths. In China, 30 000 hospitalized cases were reported in 1980 and 42 000 cases in 1981, with a 7–15% case-fatality rate; recently mild forms of the disease were recorded in some cities. In the USSR about 11 000 cases of the disease have been reported during the past 5 years.

The majority of cases in Europe are mild. Several hundred cases of haemorrhagic fever with a syndrome very similar to mild forms of the Korean disease have been reported annually as nephropathia epidemica in Scandinavia (Finland, Norway, Sweden), and as epidemic nephritis in Eastern Europe (Bulgaria, Czechoslovakia, Hungary, Romania, Yugoslavia). Several cases of nephropathia epidemica were recently reported in Belgium, France, and Greece.

Antibodies against Hantaan or Hantaan-related viruses were detected in human sera in the Americas (Alaska, Brazil, Canada, Colombia, USA), the Western Pacific and South-East Asia (Burma, China (Taiwan), Hong Kong, India, Malaysia, Philippines, Thailand), Africa (Central African Republic, Egypt, Gabon, Nigeria, Uganda), and some Pacific islands (Fiji, Hawaii).

Antibodies against Hantaan or Hantaan-related virus were detected also in urban rat sera in the Americas (Argentina, Brazil, and the USA, including Hawaii), the Western Pacific Region (China,
Fiji, Hong Kong, Japan, the Republic of Korea, Malaysia, Philippines, Thailand) and the Eastern Mediterranean (Egypt).

Transmission. Large quantities of virus are excreted in the saliva, urine, and faeces of infected mice, Apodemus agrarius. Excretion persists in the saliva and faeces for at least 1 month and in the urine for 12 months. Horizontal transmission of the virus among Apodemus mice has been demonstrated. In these experiments Apodemus mice were infected by inoculation. Non-infected Apodemus mice caged together with the infected Apodemus for several days acquired infection beginning 10 days after initial exposure. Results were the same when ectoparasitized and clean animals were used in these experiments. The main route of infection in Apodemus mice is via the respiratory tract and infection can be transmitted via the saliva, urine, or faeces of infected Apodemus mice. Transmission of the virus among house rats and experimental animals of other species requires to be further studied. There is no evidence of direct man-to-man transmission of the virus in hospitals.

Reservoirs. The reservoir of Hantaan virus in the rural endemic areas in the Republic of Korea is Apodemus agrarius coreae; in Finland and west of the Ural mountains, the reservoir is Clethrionomys glareolus; and in the urban areas of the Republic of Korea, Rattus rattus and R. norvegicus. Antigen was detected in 16 different rodent species and 4 different insectivorous species in the USSR. Colonized experimental rats act as a dangerous reservoir of Hantaan and related viruses and these have been responsible for several outbreaks of haemorrhagic fever with renal syndrome among laboratory animal-room personnel at research institutes in Belgium, Japan, and the Republic of Korea.

Vectors. The identification of Hantaan virus as a proposed member of the family Bunyaviridae suggests that it may be an arthropod-transmitted virus, but that role has not yet been confirmed. It has been hypothesized that Hantaan and Hantaan-related viruses can be transmitted by ectoparasites harboured by various field rats. Until now, Hantaan virus has not been isolated from arthropods.

Groups at risk. The disease appears to affect most frequently persons in the age group of 20–50 years; cases in children under 10
years old are rare. Although the disease occurs in both sexes, the figures accumulated so far show a significantly higher prevalence in males. The victims are primarily farmers and soldiers on duty at field stations.

The following groups of personnel are also at increased risk of infection by Hantaan and related viruses:

(a) health personnel working in laboratories where research into the disease is going on;

(b) animal-room workers;

(c) rodent breeders.

Diagnostic tests for the detection of infection with Hantaan and related viruses require access to infected rodents, tissues from infected animals, or infected cell cultures. Caution is required in handling infected materials because of the risk of laboratory infection and the possible escape of virus into the surrounding community.

Studies on infected animals (trapping, bleeding, autopsies, inoculations) and the passage of virus in tissue culture are very dangerous and require extreme care to protect laboratory personnel. Laboratory rodents used for biomedical research should be housed in quarters that prevent any contact with wild rodents, and breeding colonies should be regularly tested to confirm that there is no infection. When new breeding stock is introduced into an animal house, or imported from overseas, care should be taken to ensure that these animals are free from infection.

4.3.4 Clinical diagnosis

Serological tests for haemorrhagic fever with renal syndrome have confirmed that the clinical disease produced by the Hantaan virus is one with diverse and protean signs and symptoms. Its major manifestations are fever, headache, pain in the back and abdomen, flushed face, prostration, vomiting, proteinuria, and haemorrhagic phenomena. Death from shock and renal failure occurs in 10–15% of cases. The incubation period is generally 2–3 weeks, but may vary from 4 to 42 days.

In the Republic of Korea approximately 30% of patients show a mild clinical course without haemorrhagic phenomena or
proteinuria, about 50% exhibit a moderate course, while 20% have severe forms of the disease. In severe cases the disease usually passes through five phases: (a) febrile phase; (b) hypotensive phase; (c) oliguric phase; (d) diuretic phase; and (e) convalescent phase. These phases are based on characteristic clinical, laboratory, and pathophysiological features.

The symptoms associated with Hantaan or Hantaan-related virus infection are diverse and it is very difficult to diagnose moderate and mild forms of the disease on clinical grounds alone. The symptoms of infection with Hantaan-related agents in many parts of the world where haemorrhagic fever with renal syndrome is not yet known to exist may be mild or even different from typical forms of Korean haemorrhagic fever and nephropathia epidemica. Therefore, specific serological testing for the diagnosis of Hantaan or related virus is recommended.

4.3.5 Laboratory diagnosis

It is impossible in the individual case with moderate to mild clinical symptoms to diagnose Hantaan virus infection on clinical grounds alone, and even in severe cases an accurate clinical diagnosis of this disease can be difficult. In a limited study, a correct clinical diagnosis was made in only 50% of seropositive Korean patients.

Serological tests: Specific serological diagnosis of haemorrhagic fever with renal syndrome is made by demonstrating a rise in titre of specific immunofluorescent (IFA) and neutralization (N) antibodies against Hantaan and related viruses in sera collected twice during the course of illness at an interval of one week. IFA and N antibodies appear during the first week of symptoms, reach a peak at the end of the second week, and persist for as long as 34 years. Specific antibodies to the virus can be detected even in mild and subclinical forms of the disease.

IFA antibodies against Hantaan and related viruses can be detected using virus-infected A-549 and E6 cells; if these are not available, virus-infected Apodemus lung sections can be used as substrate. With Apodemus lung sections care should be taken that there is no contaminating reovirus or other murine agent. Specific fluorescence appears as discrete pinpoint granules distributed throughout the cytoplasm of the cells. Recently, ELISA, radio-immunoassay (RIA), immune-adherence passive haemagglutination
(IAPH), and plaque-reduction neutralization tests have been developed.

*Virus isolation.* Isolation of Hantaan and related viruses is possible by inoculation of *Apodemus* mice or *Clethrionomys* voles or Vero E6 cells with blood and serum taken during the early stages of infection. *Apodemus* mice infected with the virus do not show overt signs of disease, but the viral antigen can be recognized in the pulmonary tissue by the indirect fluorescent antibody technique. Hantaan virus kills suckling mice if it is inoculated intracerebrally and viral antigen can be found in the brain, lungs, and other tissues of dead mice.

E6 cells infected with the virus do not show any cytopathogenic effect. Recently, the causative agent of nephropathia epidemica, and laboratory rat and wild rodent strains of Hantaan-related virus were isolated in E6 cells in independent laboratories throughout the world.

Immunoelectron microscopy and physicochemical studies with Hantaan and related viruses have shown that they are new members of the family Bunyaviridae. Monoclonal and polyclonal antibodies against Hantaan virus indicate that there are several serotypes of Hantaan virus known at present and monoclonal antibodies can be used as immunological reagents for the classification of this new group of viruses.

4.3.6 *Pathogenesis and pathophysiology*

Little is known of the pathogenesis of haemorrhagic fever with renal syndrome in man and there is no primate model of this disease. The primary sites of virus multiplication in patients are the vascular endothelial cells. Pathological findings have been observed in the kidneys, heart, pituitary glands, adrenal glands, and other organs at autopsy. The characteristic findings are haemorrhagic manifestations and kidney impairment. Immune complexes have been demonstrated in kidney biopsy material during illness.

4.3.7 *Surveillance*

Improved surveillance of this disease is needed. Because of limitations in the supply of reagents and the fact that few laboratories in the world have the facilities to work safely with the
virus, the increase in surveillance has been slow. Until 1982, laboratories in the Republic of Korea and the USA undertook to assist with seroepidemiological studies aimed at defining the prevalence of haemorrhagic fever with renal syndrome in different countries and detecting the presence of the agent in wild rodent and laboratory stocks. Laboratories in Belgium, Finland, Japan, Sweden, and the USSR are now participating in the surveillance programme for the disease.

4.3.8 Prevention and control

Control of laboratory rodent infection by Hantaan virus has posed a difficult problem. Since the virus is not transmitted vertically the use of the technique of cesarean delivery by laboratories propagating rodent colonies is recommended. In addition, serological screening of laboratory rodents imported from parts of the world where the disease is known to occur will help prevent the introduction of infection into uninfected colonies.

Rodent control measures in rural areas are expensive and difficult to maintain over long periods since it is impossible to eradicate the reservoir of the virus from nature. In urban areas, however, rodent control is feasible and should be encouraged. Basically, control depends upon reducing the contact between man and rodent excreta; however, with the adaptation of strains to cell culture it should prove possible to develop a vaccine that could be given to high-risk population groups.

4.3.9 Treatment

The management of patients with haemorrhagic fever with renal syndrome is supportive and based on an understanding of the pathophysiology of the disease. Treatment of the febrile phase consists of bed rest, sedation, analgesics, and the maintenance of fluid balance. Overhydration should be avoided. Hypotension is counteracted with volume expanders such as salt-free albumin. In the oliguric phase fluid should be restricted to the volume needed to compensate for fluid loss. A potassium-binding resin can be given if hyperkalaemia occurs. Dialysis may be necessary in severe cases. In the diuretic phase, particular attention must again be paid to the adequate replacement of fluid and electrolytes.
4.3.10 Research needs and recommendations

In addition to the research needs and recommendations on rapid diagnosis, epidemiology, and pathogenesis mentioned at the end of this report, the following apply specifically to haemorrhagic fever with renal syndrome.

(1) Surveillance by virus isolation and seroepidemiology is urgently needed to determine the distribution of Hantaan and related viruses in the world and their correlation with illness in man; surveys of wild urban rats and laboratory rats in all parts of the world are also needed.

(2) Efforts should be made to develop an animal model of the clinical disease in man that will permit studies of pathogenesis and comparison of the virulence of different strains.

(3) Vaccines should be developed for use in breeding colonies of rats and in man.

(4) Because of initial positive results in the prophylactic treatment with ribavirin of animals infected with Hantaan virus, the use of this drug to eliminate Hantaan virus or prevent its spread in animal colonies should be investigated.

(5) Research on the control of rodents through ecological and other means should be promoted.

(6) The sensitivity and specificity of the several available serological tests such as IFA, ELISA, RIA, and IAPH should be carefully evaluated in comparison with plaque neutralization tests in order to assess the reliability and specificity of the different tests.

5. HAEMORRHAGIC FEVERS WITH UNKNOWN RESERVOIRS

5.1 Marburg virus disease and Ebola virus disease

There are two relatively recently recognized viral haemorrhagic diseases in Africa, Marburg virus disease and Ebola virus disease, for which the reservoirs are unknown. The endemic areas of these diseases overlap and yet the two viruses are not antigenically related.

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Morphologically and genetically, however, they can both be placed in a new family of viruses; the name Filoviridae has been proposed for this family.

5.1.1 Historical background

Marburg virus disease was the first of these two new diseases to occur, causing an epidemic of 31 cases in Europe in 1967, with cases being reported from the cities of Marburg and Frankfurt in the Federal Republic of Germany and Belgrade in Yugoslavia. Subsequently 3 cases occurred in South Africa in 1975 and another in 1982; 2 cases occurred in Kenya in 1980. Nine of these 37 patients died.

In 1976, there were simultaneous outbreaks of a highly lethal haemorrhagic disease in north-west Zaire and south-west Sudan. During the course of these epidemics over 500 cases occurred with approximately 400 deaths. Ebola virus was discovered to be the cause of this outbreak. Ebola virus disease has subsequently recurred in Sudan in 1979, and sporadic cases have been reported in Zaire in 1978 and in Kenya in 1980. Recently it was shown that the virus isolated in 1976 from Sudan and from Zaire, although antigenically related, was biologically, genetically, antigenically, and biochemically distinguishable.

5.1.2 Etiology

Marburg virus was first isolated during the 1967 epidemic in Europe. It was found to be morphologically distinct from any other known mammalian virus. Although it is pleomorphic and capable of taking on many strange shapes, its primary infectious unit is a rod approximately 750 nm long and 80 nm in diameter with a central core 50 nm in diameter containing the viral genome. The viral genome is not infectious in the absence of the outer proteins.

Ebola virus was isolated in 1976 and was immediately shown to have the same morphological characteristics as Marburg virus, but was found to be antigenically unrelated. Further study has shown that it has a primary infectious unit about 900 nm long, although like the Marburg virus its pleomorphism often produces lengths of up to 1400 nm. Again like the Marburg virus, it has a diameter of 80 nm with a central core containing the viral genome.
To date, 5 primary proteins have been identified in both viruses, the functions of which are mostly unknown. Both viruses have single-stranded RNA genomes that are not infectious without at least some of the accompanying proteins.

5.1.3 Epidemiology

Distribution. Outbreaks of Marburg virus disease have occurred in 3 and possibly 4 countries in Africa. In the first outbreak, *Cercopithecus* monkeys from Uganda were shown to harbour the virus. In the second outbreak the primary case was believed to have acquired the disease in Zimbabwe while travelling with a companion. However, acquisition in northern South Africa could not be excluded. The index case in 1980 acquired the disease in western Kenya, and the patient in South Africa in 1982 again may have acquired the disease in Zimbabwe, where he was travelling prior to his illness.

Serosurveys using the immunofluorescent antibody assay in the Central African Republic, Gabon, Kenya, Sudan, and Zaire have found individuals with antibody to Marburg virus, but patients with the disease have been identified only in Kenya, probably because of the lack of a surveillance system.

To date, Ebola virus disease has been associated with only three countries: the virus has been isolated in Sudan and Zaire, and one case was confirmed serologically in Kenya in 1980. However, serosurveys in other countries suggest that Ebola virus, or a closely related virus, may occur in countries such as the Central African Republic, Ethiopia, Guinea, and Nigeria. In addition, populations with antibody have been identified in the western hemisphere, although the significance of this is not yet understood.

Transmission. The source of the primary transmission to man is not yet known. Although Marburg virus disease was first transmitted to man from African green monkeys, and was originally known as "African green monkey disease", there is no proof that this monkey is indeed the reservoir.

Man-to-man transmission is the major area of concern with Marburg virus disease. It is this mode of transmission that gives the virus epidemic potential and strict precautions must therefore be taken. All of the 8 secondary cases of Marburg virus disease resulting from man-to-man transmission have occurred following close
contact with infected patients, either via infected fluids or secretions or through sexual contact. Marburg virus has been isolated from semen on at least two occasions, and Ebola virus on one occasion, as late as two months after primary infection.

There is no evidence for the aerosol transmission of Marburg virus in man, or of transmission through fomites or through simple casual encounters. There is some evidence for aerosol transmission to monkeys. The potential for spread within hospitals, however, must be emphasized since this is a very likely place for close contact with infectious material to occur.

**Vectors and reservoirs.** Intensive investigations of rodents, insects, bats, and over 100 other mammalian species during and between the several epidemics of Ebola and Marburg virus diseases have failed to identify, either serologically or by virus isolation, any candidate reservoir of either virus. Immunofluorescence techniques have shown vervet monkeys and baboons from Kenya to possess Ebola virus antibody; however, the significance of this is unclear. The severe disease observed in monkeys during the 1967 Marburg virus disease outbreak and the general sensitivity of several monkey species to small doses of the Zaire strain of the virus make monkeys poor candidates as reservoirs. The finding of Ebola virus antibody in domesticated guinea-pigs in north-east Zaire is unlikely to implicate this species as a natural reservoir, but rather as an intermediate host, since guinea-pigs are not found in the other areas where this virus occurs. The source of these viruses in nature remains a mystery.

**Groups at risk.** No risk group or risk factor is known for primary exposure to either Ebola or Marburg virus disease. The most complete epidemiological picture available is that of epidemic disease, mostly resulting from nosocomial and intrafamilial spread. The picture is likely to be very different from that of a sporadic endemic disease. In Zaire in 1976 only 20% of cases occurred in children less than 15 years old, a group normally expected to represent nearly 50% of the population. The largest proportion of cases occurred in females of child-bearing age (35%), probably because of the transmission of disease to these women in prenatal clinics. The outbreak in Sudan was similar, with a predominance of adult cases probably because of the nosocomial origin of most of them. In the 1979 epidemic, which primarily spread intrafamilially,
there were only 2 cases in children, probably because there was little close contact between the children and active cases. There was a predominance of female cases because of the major role played by women in nursing care. In fact, hospital personnel and family members who participate in nursing care at home or who help in preparing the body for burial are at very high risk. Close contact increases the risk of infection by five times when compared with the risk associated with more casual contact such as shaking hands or simply touching a patient. The seasonality of sporadic disease is unknown.

For Marburg virus disease the epidemiological picture is similar because of the dominating feature of person-to-person spread between close contacts.

Apart from the groups cited above and their risk factors, another factor, noted primarily in Zaire in 1976, was exposure to inadequately sterilized needles. Injections by nurses or itinerant curers are common in many rural areas of the world, but this practice carries with it a high risk of contracting many infectious diseases, and this was the situation in Zaire in 1976, where the mortality among cases exposed to the disease via needles was 100%.

5.1.4 Clinical features and diagnosis

The course of the illness in both Marburg and Ebola virus infections is very similar, though epidemics of Ebola virus disease tend to be more severe and have a much higher mortality rate.

The incubation period of Marburg virus disease in the European outbreak was 3–9 days, while the incubation period of Ebola virus disease in Africa ranged from 3 to 18 days and was usually around 7 days. The incubation period for needle exposure to Ebola virus was 6 days with a range of 1–5 days.

The onset of Marburg and Ebola viral infection is abrupt, with shivering and a rapid rise in temperature accompanied by severe headache, backache, generalized aching in muscles and joints, and malaise. Central abdominal pain and nausea are presenting features in some cases, but gastrointestinal disturbance commonly commences about the third day of illness with loss of appetite, nausea, vomiting, and diarrhoea. The stools are watery and may contain mucus and blood. Profuse diarrhoea may continue for several days and lead to dehydration.
An erythematous maculopapular rash commonly emerges on the trunk after 3–8 days and quickly spreads to other parts of the body, ultimately becoming confluent. The rash fades after 3 or 4 days and is followed by fine desquamation. The erythematous stage of the rash is easily overlooked in dark-skinned patients though the subsequent desquamation is usually obvious. Swallowing is often painful, the throat is inflamed, and small transparent lesions resembling tapioca granules may be seen on the soft palate. About half of the patients have inflamed conjunctivae and some complain of photophobia.

The fever reaches a peak after 3–4 days and is sustained at a high level for at least a week before falling by lysis. The duration of the febrile phase varies from 10 to 20 days and is commonly between 14 and 16 days. Some patients may have a secondary rise in temperature. Within 4–5 days from the onset of illness the patient's condition usually becomes critical with extreme lethargy and alteration in the mental state. Many become sullen with aggressive or negative behaviour, while those who will die may become restless and confused before sinking into a deep coma. A few have convulsions; others complain of paraesthesia.

About half of the patients with Marburg virus disease have spontaneous bleeding that is particularly troublesome at needle-puncture sites. A very high proportion of patients with Ebola virus infection have severe bleeding, especially from the respiratory and gastrointestinal tracts. Abortion with massive haemorrhage was common in pregnant women during the Zaire outbreak of 1976. Spontaneous bleeding usually commences towards the end of the first week and is a striking feature in fatal attacks. The cause of the bleeding remains obscure. In many cases the platelet count is low and the prothrombin time prolonged. A few patients have evidence of disseminated intravascular coagulation with an increase in fibrin degradation products.

Liver function tests may show evidence of hepatocellular damage, but frank jaundice is rare. Several patients with Marburg virus disease have had elevated levels of serum amylase suggesting some pancreatic involvement. Electrocardiography may reveal evidence of myocardial damage. Many patients have proteinuria and oliguria and most of the fatal cases develop renal failure.

If death occurs it is usually between the 8th and 17th day, very often on day 8 or 9. Survivors face a prolonged period of convalescence with anorexia, loss of weight, excessive fatigue, and
loss of hair. Some patients have persistent psychological problems. Some have testicular atrophy and it is noteworthy that both Marburg and Ebola viruses have been recovered from semen during late convalescence. Uveitis has been reported and virus has been isolated from the anterior chamber of the eye 83 days after onset of the illness.

The case-fatality rate of Marburg virus disease in the Federal Republic of Germany was 22%. Mortality in Ebola virus disease in Africa was much higher; it was about 90% during the Zaire outbreak in 1976 and the earlier stages of the Sudanese epidemic that same year. Severe bleeding, renal and circulatory failure, and pronounced cerebral disturbance are prominent features in fatal attacks. The illness is exceptionally severe in pregnant women.

The sudden onset of the illness in both Marburg and Ebola virus infections contrasts with the more insidious onset of Lassa fever, but clinical diagnosis is not usually possible until the characteristic rash emerges. In the pre-eruptive stage of the illness malaria, typhoid fever, typhus, septicaemia, and other viral infections should be considered and appropriate tests performed.

5.1.5 Laboratory diagnosis

The white blood cell count may be very low and the blood film may show atypical plasmacytoid lymphocytes and polymorphonuclear leukocytes with an acquired Pelger-Huet anomaly. Thrombocytopenia is a common finding. The erythrocyte sedimentation rate is usually low. Serum levels of enzymes such as aspartate transaminase, alanine transaminase, and amylase may be elevated. The blood urea content is high; the protein and potassium levels are low. The prothrombin time may be prolonged and there may be evidence of disseminated intravascular coagulation. Unless special laboratory facilities are available it is seldom possible or justified to undertake extensive laboratory tests. Management tests should be directed to the control of water and electrolyte balance and haemorrhage.

*Virus isolation.* Ebola and Marburg viruses have been isolated in cell cultures and by animal inoculation. Both viruses replicate well in cell cultures, and it is the method of choice, being both simpler and safer than animal inoculation. The most universal cell line for
these viruses is Vero, with the most sensitive being the E6 Vero cell clone available from the American Type Culture Collection.

Material to be inoculated may be blood, serum, tissue from biopsy or autopsy, and effusate. It should be collected early in the course of illness, and inoculated as soon as possible to maximize the likelihood of isolation. If inoculation is delayed, storage at \(-20^\circ\text{C}\) or \(-70^\circ\text{C}\) is preferable; however, storage for 3–4 days at 4 °C will often be sufficient to allow isolation of virus. Both viruses produce a cytopathogenic effect in Vero cells, although the strains from Sudan often need 1 or 2 passages before the effect is seen. It is better, however, to use specific antiserum and remove some cells from the tube or flask on the seventh to tenth day after inoculation and search for virus by indirect immunofluorescence methods. More details are given in the general section on laboratory diagnosis (section 6.3).

Serological tests. The method currently recommended for diagnosis is indirect immunofluorescence. Antigens are prepared in collaborating centres by harvesting E6 Vero cells, inactivating infectivity, and placing a layer of cells in a small well on a microscope slide, where they are fixed and kept at \(-20^\circ\text{C}\) until use. These slides are available from WHO collaborating centres. Further details are provided in section 6.3.

Diagnosis of Ebola or Marburg virus disease is confirmed by a rise in specific antibody in a patient, or by an antibody titre of 1 : 64 or greater with a corresponding titre of specific IgM of 1 : 8 or more, both accompanied by a compatible illness. In the event of a suspected case being identified on the basis of serological diagnosis, an aliquot of serum should be forwarded to one of the WHO collaborating centres for confirmation. As with virus isolation, a serum specimen should be obtained as early in the illness as possible and then a convalescent serum obtained 1–2 weeks afterwards in order to attempt demonstration of a four-fold rise in IgG antibody.

Rapid diagnosis. At present, the most rapid method of diagnosis available is the demonstration of IgM antibody. In the absence of this, demonstration of antigen in tissue by the immunofluorescent antibody test (IFA) or electron microscopy is feasible under certain conditions. The least rapid methods are virus isolation and demonstration of a 4-fold rise in IgG antibody level. No established method of rapid antigen detection is available.
5.1.6 Patient management

Strict isolation precautions are essential to prevent the spread of infection from body fluids. Attendant staff must wear protective clothing and all waste must be rendered safe before disposal. Great care must be exercised when taking blood samples for investigation or when giving injections. Routine autopsy should not be performed but samples of tissue may be taken post-mortem using a suitable biopsy needle.

5.1.7 Treatment

The use of convalescent serum has been advocated for the treatment of Marburg and Ebola virus infections, but no controlled trial has been carried out to establish its value and there is very little scientific evidence available to support its use. Interferon appears to delay the onset of viraemia in rhesus monkeys experimentally infected with Ebola virus but it has no effect on the eventual outcome.

Treatment is otherwise aimed at relieving symptoms and maintaining the water and electrolyte balance. Attempts should be made to control bleeding by the infusion of platelets, fresh frozen plasma, and factor concentrates. Blood transfusion may be necessary. The use of heparin has been recommended to diminish damage caused by disseminated intravascular coagulation, but its use is controversial and great care must be taken to monitor its effects. Renal failure may necessitate peritonal dialysis.

5.1.8 Route of infection

All of the evidence available at present suggests that infection occurs via direct contact with infected materials. It seems likely that direct inoculation of the virus through tiny openings in the skin or mucous membranes is the primary route of infection. Although one or two possible instances of sexual transmission have occurred, these do not prove that virus may enter otherwise intact mucous membranes. The low rate of transmission to persons with casual contact suggests that aerosol transmission is not efficient, if it occurs at all.
5.1.9 Pathophysiology

Both Ebola and Marburg virus diseases produce sustained viraemias, with serum levels reaching $10^3$–$10^6$ TCID$_{50}$/ml in the case of Ebola virus disease in Zaire. The levels produced by the Sudan strain of the virus are substantially lower than those produced by the Zaire strain.

*Marburg virus disease.* Extensive pathological studies were undertaken during the 1967 epidemic. The gross pathological description included evidence of haemorrhaging in the skin, mucous membranes, soft tissues, visceral organs, and bowel. Although focal necroses were observed in many organs the most extensive lesions were found in the liver, lymphatic system, testes, and ovaries.

Hepatocellular necrosis is the most prominent feature of the disease. The distribution of the necrosis tends to be random and focal, with the cell damage at any one focus being synchronous. Small inclusions and Councilman-like bodies are prominent. Fibrin thrombi are seen in the renal glomeruli suggesting disseminated intravascular coagulation. In the brain, there is histological evidence of diffuse inflammation and interstitial oedema.

There is an important component of vascular permeability and shock associated with this disease. The underlying mechanisms of these phenomena are not understood, but, along with the observed lesions in the liver, kidneys, and brain, they are the keys to understanding the pathophysiology of this disease.

*Ebola virus disease.* The pathology of Ebola virus disease is largely indistinguishable from that described for Marburg virus disease. There tend to be fewer and less severe lesions in the disease caused by the Sudan strain of virus than in that caused by the Zaire strain. In monkey models, marked neutrophilia, depletion of lymphocytes, and early failure of platelet aggregation precede a consumption coagulation with a micro-angiopathic haemolytic anaemia, thrombocytopenia, and failure of prostacyclin production by the vascular endothelium.

There are distinct biological, genetic, and biochemical differences between the Sudan and Zaire strains of the virus. The Zaire strain is uniformly lethal to suckling mice and monkeys, while the Sudan strain, as well as Marburg virus, are not lethal to either of these. These differences are probably associated with the significantly
higher level of mortality observed with the Zaire strain, as well as
with the relatively more extensive pathological damage observed
with the Zaire strain in man.

5.1.10 Surveillance

Countries contiguous to areas where it is known that Marburg
and Ebola diseases occur should at least maintain passive
surveillance programmes for cases in man. This should include the
education of field medical personnel in the important features of the
two diseases.

Active surveillance mechanisms are, however, advisable in
countries where the disease is known or thought to occur. Back-up
by a competent virus laboratory is essential. Acute and convalescent
blood specimens from suspected cases should be taken, observing all
the necessary precautions, and forwarded to such a laboratory. In
the event of laboratory confirmation, serologically or by virus
isolation, an appropriately constituted epidemiological team should
begin on-the-spot-investigations of the movements and contacts of
the patient with the aim of identifying the possible source(s) of
infection. Support can be sought from WHO or a WHO
collaborating centre at this stage if necessary.

In countries where the disease is known to have occurred, special
serological and ecological surveys in the affected localities should be
carried out to determine the prevalence of the disease and the
possible natural reservoirs.

5.1.11 Prevention and control

Since the natural reservoirs of Marburg and Ebola viruses are
unknown, no control activities can be carried out in Africa.
Nosocomial spread has, however, been a marked feature of both
Marburg and Ebola virus diseases. Hospital management of patients
therefore requires special attention. In endemic areas the patients
should be nursed principally in the medical units in the locality
where the diagnosis has been made. In non-endemic areas the
patients may either be nursed in the hospital where the diagnosis has
been made or transferred at an early stage to a designated high-
security unit according to national facilities and policies. If the
patient is nursed locally, an expert national team should be available
to help and train the staff in the local hospital.

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In the absence of information about the natural reservoirs of Marburg and Ebola viruses, it is a common practice to quarantine imported monkeys.

5.1.12 Research needs and recommendations

In addition to research needs and recommendations on rapid diagnosis, epidemiology, and pathogenesis mentioned at the end of this report, the following apply specifically to Marburg and Ebola virus diseases.

(1) More extensive surveillance is needed to detect cases of sporadic acute disease in Africa. This should include establishing mechanisms for the collection and storage of specimens for laboratory confirmation of disease. Such surveillance should be integrated with a renewed effort to identify the natural reservoirs of Marburg and Ebola viruses.

(2) Regional laboratory competence in Africa should be strengthened. This will assist in producing a rapid diagnosis in the event of an outbreak. It will offer laboratory support for extensive outbreak investigations, and it will assist in the routine surveillance of these diseases.

6. LABORATORY ETIOLOGICAL INVESTIGATION IN MAN, VECTORS, AND RESERVOIRS

6.1 Introduction

Rapid progress in biology has resulted in the development of many laboratory tests that are simple enough to be applied in areas outside the usual diagnostic or research laboratory. These tests are important in several ways, whether applied in the field or in the reference laboratory: (a) to establish the validity of the clinical diagnosis, (b) to identify true cases of infection for the purpose of contact surveillance, and (c) to identify the specific reservoirs or vector and the extent of their infection.

For the laboratory investigation of one or several cases of viral haemorrhagic fever the laboratory must be capable of identifying any of those diseases already known, as well as of isolating and identifying new agents. If the laboratory is not adequately prepared for the identification of new agents then the chances of such an
identification will be decreased and will depend primarily on good luck, rather than on anticipation and proper preparation.

The purpose of this section on laboratory support is to provide a general survey of the various laboratory techniques that may be valuable in the investigation of viral haemorrhagic diseases; some of the methods have proved useful for some diseases and should be attempted for other diseases when the opportunity arises.

6.2 Collection of laboratory specimens for specific etiological diagnosis

The epidemiologist or someone designated by him should take responsibility for the proper collection and handling of specimens to ensure that they reach the microbiological laboratory in good condition, accompanied by the correct documentation, and with the minimal risk of accidental exposure of persons to the contaminated materials. All specimens should be collected in sterile containers and must be carefully labelled with appropriate identifying data. If only a few samples are obtained, each specimen should be labelled with the patient’s name, age and sex, and the date of collection; if many samples are obtained, a numbering system should be used. A separate document should be prepared (in duplicate, using carbon paper) with the assigned number and the following minimal information: name, age, sex, address, name of hospital, date of collection, date of onset of illness, and history of immunization (if appropriate). Labels should be waterproof and an indelible pen or soft pencil used. For specimens from animals, labelling with an animal number and demographic and epidemiological data is required.

(1) Blood should be collected with or without anticoagulant under aseptic conditions, preferably using an evacuated specimen tube (Vacutainer) or disposable syringe and needle. Care should be taken so that the health worker drawing or processing the blood is not infected. Gloves and a visor (preferable) or a mask plus goggles are recommended. Face protection is especially important if the sample is centrifuged. The blood of acute cases of all viral haemorrhagic fevers may contain virus, sometimes at a high titre. Therefore the specimen tubes, syringes, and needles should be disinfected immediately after use. Serum can be separated from the clot after allowing the clot to retract and the cells to settle in the refrigerator. Sera should be stored in aliquots to avoid several freezing and
thawing processes. The clot may also be retained for isolation attempts or culture. For bacterial cultures, 5–10-ml samples of whole blood are placed in an appropriate culture medium.

(2) Necropsy samples. Tissues are preserved both for isolation or culture and in fixative (formol, glutaraldehyde) for histopathological and electron-microscopical studies.

All specimens for isolation attempts or culture must be processed immediately or maintained at a low temperature until returned to the laboratory. Storage or transport at 4°C (wet ice, “cold dogs”, mechanical refrigerator) is acceptable for only a few (2–3) hours, after which freezing at a very low temperature (dry ice, liquid nitrogen) is required. A few viruses (e.g., cytomegalovirus) do not withstand freezing and specimens suspected of containing these agents should be kept at 4°C.

(3) Serological specimens. Confirmatory serological diagnosis depends upon the collection of appropriately timed serum samples. Serum should be obtained as early as possible during the acute phase of illness and then at least 10–14 days later. In the case of the non-vector-born haemorrhagic fevers, in which antibody titres may rise late, a third sample should be collected 3–4 weeks after onset.

(4) Transport. The expeditious and safe transport of specimens from the field to the diagnostic laboratory is a difficult problem, especially when the outbreak occurs in a remote area. The establishment of a reliable method of transport should be one of the aims of the epidemiological team. It is generally preferable to deliver by hand specimens that are critical to the investigation or that contain especially dangerous agents, rather than to rely on an intermediate, uncontrolled carrier (e.g., the mail, public transport services, etc.).

The proper packaging and refrigeration of specimens for transport is extremely important to avoid loss and potential exposure of persons en route. Specific guidance on transport may be found in a WHO publication1 (see also section 6.7).

If epidemics are preceded or accompanied by sickness or death of domestic animals, rodents, or birds, then samples of blood, throat swabs, and stools should be taken from the sick animals, and autopsy material should be collected from recently dead animals (including blood, hair, spleen, brain, heart, lungs, and bones). The

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precautions in the collection, labelling, handling, and transport of these materials to the laboratory are the same as for human specimens.

For virus isolation, 20 g of tissue are homogenized in 100 ml of a 20–25% animal serum solution (20–25 ml serum/100 ml buffer), then centrifuged to free it from tissue fragments, and the supernatant is used for inoculating animals or tissue cultures. Tests for bacterial sterility should be used routinely. Antibiotics may be added to the serum solution. The presence of virus in the diagnostic sample is confirmed by the appearance of specific signs in inoculated animals or cytopathogenic changes in the cells of the tissue culture. In the absence of these signs the presence of specific virus antigen can be detected by the immunofluorescent antibody (IFA) test or by the enzyme-linked immunosorbent assay (ELISA).

6.3 Methods

6.3.1 Virus isolation

General methods. The cell lines most commonly used for the primary isolation of the heterogenous group of viruses causing viral haemorrhagic fevers are summarized in Table 5. The general strategy is not to wait until a cytopathogenic effect is seen, but to detect the presence of the viral antigen by means of an indirect immunofluorescent antibody test (IFA), using high-titre specific monoclonal antibodies or hyperimmune reference antisera. With this technique some of these viruses can be detected less than 24 hours after inoculation. It is recommended that attempts should be made to detect the presence of antigen on days 1, 6, and 12 after inoculation. It is clear that the commercially available continuous cell line Vero E6 (American Type Culture Collection) is very suitable for the primary isolation of these viruses. It is worth while to try the Vero E6 cell culture for viruses that have not previously been tested. At present, the technique most often used for antigen detection by the IFA method in infected cells is to scrape off the cells from the culture dish, resuspend them in 5% fetal calf serum (5 ml serum plus 95 ml saline) and drop them on to multispot slides. After drying and acetone fixation (10 min) the reference sera are added and the antibody test performed. Direct detection of the antigen in the inoculated cells without scraping them off the dish appears to be a promising technique. It would be worth while to try methanol
Table 5. Cell lines used for the primary isolation of the viruses causing haemorrhagic fevers

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cell line</th>
<th>Monoclonal antibody available</th>
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</thead>
<tbody>
<tr>
<td>Mosquito-borne</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>C6-36, AP61 (Aedes)</td>
<td>+</td>
</tr>
<tr>
<td>Dengue haemorrhagic fever</td>
<td>Toxorhynchites TRA 294</td>
<td>+</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>Aedes C6-36, AP61</td>
<td>+</td>
</tr>
<tr>
<td>Tick-borne</td>
<td>E6</td>
<td>+</td>
</tr>
<tr>
<td>Crimean-Congo haemorrhagic fever</td>
<td>CER</td>
<td>+</td>
</tr>
<tr>
<td>Kyasanur Forest disease</td>
<td>Vero</td>
<td></td>
</tr>
<tr>
<td>Omk haemorrhagic fever</td>
<td>Chick embryo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macaca (Cynomolgus)</td>
<td></td>
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<tr>
<td></td>
<td>heart cells</td>
<td></td>
</tr>
<tr>
<td>Rodent-borne</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junin haemorrhagic fever</td>
<td>E6</td>
<td>+</td>
</tr>
<tr>
<td>Machupo haemorrhagic fever</td>
<td>Vero</td>
<td>+</td>
</tr>
<tr>
<td>Lassa fever</td>
<td>E6</td>
<td>+</td>
</tr>
<tr>
<td>Haemorrhagic fever with renal syndrome</td>
<td>E6</td>
<td></td>
</tr>
<tr>
<td>Haemorrhagic fever unknown origin</td>
<td>E6</td>
<td>+</td>
</tr>
<tr>
<td>Ebola virus disease</td>
<td>E6</td>
<td>+</td>
</tr>
<tr>
<td>Marburg virus disease</td>
<td>E6</td>
<td></td>
</tr>
</tbody>
</table>

fixation of infected monolayers in the wells of microplates and then perform the IFA or ELISA tests directly on the infected cells. This would be useful especially for those viruses, such as Hantaan virus, which cause no cytopathogenic effect. Primary isolation of some viruses, like Omk haemorrhagic fever virus, is accomplished in baby mice. The mosquito-borne viruses are isolated by direct inoculation of mosquitoes or into continuous cell lines derived from mosquitoes. These cultures grow at ambient temperature and therefore the primary isolation of virus can be attempted directly in the field.

Where cell lines are not available, the primary isolation of most of the viruses that cause haemorrhagic fevers can be made in animals. Infant mice are the preferred animal, except for Hantaan and Hantaan-like viruses, for which rats should be used. Guinea-pigs are the animal of choice for the primary isolation of Lassa, Ebola, and Marburg viruses. “Touch preparations” as described in section 6.3.3 can accelerate the detection of antigen in the inoculated animals.

*Virus isolation from arthropod vectors.* The viral haemorrhagic fevers transmitted via anthropod vectors tend to occur in areas where the ecological conditions are suitable for these vectors to
breed in large numbers. Periodic testing of pooled entomological material for virus infection is valuable in monitoring the activity of these viruses.

In addition, during epidemics it may be desirable to collect arthropod vectors from in and around the affected houses and localities and test them for the presence of the virus.

(a) Mosquito collections. Resting collections are made with an aspirator from houses or animal sheds. Mosquitos attracted to animal or human baits are similarly collected from baited traps. Under certain situations, light traps may give good results. The collected mosquitos are held alive in Barraud cages for at least 24 hours before identification is attempted, and they may be transported to the laboratory alive if this is within a day or two. If there is a longer delay before transport, it is necessary to anaesthetize the mosquitos and to make suitable pools after identification. For transport to the laboratory for virus isolation, these pools may be held in dry ice or preferably in liquid nitrogen refrigerators.

(b) Tick collections. Generally ticks are collected at the larval and nymphal stages by “flag-dragging” in forested areas or in grazing grounds—i.e., by pulling a piece of cloth over the forest floor or over the grass.

Engorged ticks are picked off specific mammal/bird hosts—cattle, sheep, monkeys, rodents, etc.—using a pair of forceps. They are stored in screw-capped vials and transported to the laboratory for virus isolation, where after anaesthesia they are identified and suitably pooled.

For virus isolation, the arthropods are ground in a mortar, and suspensions are made in a suitable diluent containing antibiotics. These suspensions are centrifuged with all the precautions necessary and the clear supernatant fluid is inoculated into animals or cell cultures. The infected host system is observed for specific periods for sickness or cytopathic effect. Wherever indicated, fluorescent antibody tests may also be carried out to detect the viral antigen.

Virus isolation from rodents. The collection of rodents in the endemic areas of viral haemorrhagic fever should be carefully planned, and carried out preferably by field workers who are immune to the disease. For non-immune field workers the wearing of masks is recommended to prevent accidental infection by contact with rodent excreta. Rodents may be bled in the field, then marked
and released or preferably brought to the laboratory as soon as possible and kept in an animal isolation room. Subsequently, the rodent is anaesthetized, blood is taken from the heart for the antibody test, and various organs are removed aseptically for the isolation of virus. Fresh organs should be kept in vials in a freezer (below \(-65^\circ\text{C}\)) until tested.

The isolation of virus from an antigen-positive rodent is carried out by the inoculation of a 10% suspension of tissue into a susceptible animal host and onto susceptible tissue culture cells which support the multiplication of the virus. Blind passages of inoculated animal tissue and tissue culture cells with a suspension of antigen-positive tissues are recommended, since the virus may grow slowly and needs passage to adapt to multiplication in cells. Sometimes, cocultivation of a piece of tissue thought to contain the virus increases the possibility of isolation. Rodent tissues should be tested, using polyvalent antibody, for murine viruses (especially reovirus) which may react specifically or non-specifically with human sera.

6.3.2 Serological tests

**Immunofluorescent antibody test (IFA).** The indirect IFA test is more sensitive than the direct IFA test and purified commercial reagents are available. The indirect IFA test has been used for the demonstration of both antigen and antibodies of the viruses that cause viral haemorrhagic fevers. Spot slides are prepared in one of two ways.

- (a) Tissue culture cells infected with a virus (60% infected cell + 40% normal cells) grown on the spot-slide give a clearer fluorescence spot than a smear preparation of infected cells on a slide.
- (b) A cryostat section of fresh infected tissue on a slide is almost as good as (a). Reovirus is one of the normal flora found in the tissues of rodents that may yield false positive results.

Antigen slide preparations can be stored at \(-65^\circ\text{C}\) for at least 1 year without loss of antigenicity. The antigen preparations are usually fixed with acetone before testing.

The sensitivity of the IFA test is increased by the use of high-titred antiserum against virus, purified fluorescein isothiocyanate conjugate, and careful complete washing.
Antiviral monoclonal antibodies are available as immunological reagents and can be used to differentiate the various strains among a group of viruses. Broadly-reacting monoclonals or pools of monoclonals may be used to detect group reactivity.

Neutralization tests. The antibodies detected by the neutralization test play an important part in the natural defence mechanism against these viral diseases and are usually the most specific.

The reagents (the known virus and the test or reference sera) are mixed together and allowed to react for a certain incubation period which is usually 1–2 hours at 37°C or overnight at 4°C. The serum–virus mixture is inoculated into either experimental animals or suitable cell cultures. Neutralizing ability is measured in terms of the death or survival of the infected animals or the presence or absence of cytopathic effect in the infected cell cultures.

A plaque reduction test has been found to be a more sensitive method of measuring the neutralizing ability and is based on a 50% or greater reduction in the number of plaques obtained. It is necessary to use reference positive and negative sera as controls.

Complement fixation (CF) and haemagglutination inhibition (HI) tests. Complement fixation antigens can be prepared from the brain of newborn mice or hamsters and from tissue cultures infected with an appropriate virus. To protect against infectious aerosols, the antigen should be inactivated prior to processing, or the procedure should be carried out in a safety cabinet. For the preparation of complement fixation antigen by the acetone-sucrose method the brain tissue is homogenized in an aqueous sucrose diluent. The homogenate is extracted twice by acetone at 4°C, the sediment dried under vacuum, and used as an antigen. Antigens are stored lyophilized.

The complement fixation test is usually less specific than the neutralization test. A 4-fold or greater rise in titre when paired sera are studied is usually considered a presumptive diagnosis.

For the haemagglutination inhibition (HI) test the antigen is usually prepared in the same way as the complement fixation antigen. The interpretation of the test results is similar to that for the complement fixation test. The specificity of these reactions may differ with different viruses.
ELISA tests. Standardized indirect antigen capturing ELISA techniques for the determination of IgG-type antibodies against yellow fever, dengue, Rift Valley fever, and Crimean-Congo haemorrhagic fever viruses are in use.

For most viruses beta-propiolactone-inactivated antigen extracted from mouse brain or liver is used. For Rift Valley fever virus a mixture of three monoclonal antibodies is used to precoat the plates. In addition, indirect ELISA techniques using the anti-IgM, anti-μ fragment for IgM antibody determination are standardized and currently used for yellow fever, Rift Valley fever, Crimean-Congo, and Kyasanur Forest disease IgM antibody determination. The reagents in limited quantities as well as the protocols to perform the tests can be obtained for yellow fever, Rift Valley fever, and Crimean-Congo haemorrhagic fever viruses (Yale Arbovirus Research Unit, Box 3333, New Haven, CT, USA); yellow fever and dengue viruses (Vector-borne Virus Division, Centers for Disease Control, Fort Collins, CO, USA); and Kyasanur Forest disease virus (National Institute of Virology, Pune, India). For the other haemorrhagic fever viruses the ELISA tests are still in the process of development. For Lassa fever antibody determination, the indirect antigen capture technique using a standard amount of either gamma-irradiated or beta-propiolactone-inactivated extracellular Lassa virus antigen seems promising, but the inactivation process may cause some reduction in sensitivity. Gamma irradiation is preferable, when available. For Ebola and Marburg viruses, direct coating of the solid phase with extracellular inactivated Ebola or Marburg antigen gives better results than the indirect standard antigen ELISA technique. The immunofluorescence assay using inactivated B6 cells infected with Lassa, Marburg, and Ebola viruses is by far the most sensitive to detect IgG-type antibodies. Further standardization and evaluation of these tests are necessary.

6.3.3 Rapid antigen detection

Immunofluorescence methods. These methods are used to detect antigen in tissue, either frozen or fixed, or in cells derived from blood, urine, or other fluids.

Biopsy or autopsy tissue may be frozen, sectioned, and placed on a microscope slide and subjected to a search for antigen, using specific antisera or monoclonal antibodies in an indirect or direct immunofluorescent antibody test (the indirect test may be the more
sensitive). A simpler method that is especially useful in identifying antigen in animal tissues of vectors is a "touch preparation" made by lightly and briefly touching the cut surface (after blotting) of a piece of tissue on to a microscope slide (several touches can be made on a single slide), which is then fixed in acetone and stained by the usual direct or indirect techniques. While there is more experience with animal tissues, these techniques can also be used in human cases of disease. Essential elements in this test are good quality, high-titre reagents and the use of uninfected tissue and non-antibody-containing serum to control for non-specific staining from the conjugated antiserum.

Antigen-detection ELISA. The ELISA technique described in section 6.3.2 can also be used to detect antigen in human tissues and in mosquitos. For the haemorrhagic fever viruses, a titre of $10^4$ pfu per ml or greater is usually required for detection. More sensitive tests are needed to broaden the applicability of the ELISA.

Electron microscopy. The electron microscope is obviously limited in use to the reference laboratory, but it can play and has played a role in the rapid identification of virus in tissue preserved in either formalin or glutaraldehyde. This has been particularly successful for Ebola and Marburg virus infections, but less so for other diseases, such as Lassa fever and yellow fever.

Agar gel diffusion precipitation. The agar gel diffusion precipitation (AGDP) test is the test of choice for the detection of Rift Valley fever antigen during outbreaks in sheep and cattle. It is a simple test that requires only agar and a glass slide plus a reference immune serum. The test is not very sensitive, but since titres of virus may be as high as $10^{19}$ pfu per ml of serum and amniotic fluid of sheep, it will detect antigen in most acutely ill animals during an epizootic.

6.3.4 Pathology

In the Americas, outside the endemic zones of Junin haemorrhagic fever and Machupo haemorrhagic fever, the role of light microscopy in specific etiological identification is limited mostly to yellow fever. The use of the viscerotome in South America retains its time-honoured value as a simple, inexpensive, first-line diagnostic
system for yellow fever. In Africa, the difficulty of distinguishing the
liver pathology of the African haemorrhagic fevers from yellow fever
makes the post-mortem examination of liver less useful than it might
otherwise be.
Techniques have now been developed for antigen detection in
formalin-fixed tissue, given that the formalin is reasonably well
buffered and that the tissue is not left in the formalin for more than
2–3 weeks before being embedded in paraffin. For this technique thin
sections (3–4 μm) are cut and placed on a microscope slide, treated
with enzymes such as protease or trypsin, and then stained by
indirect immunofluorescent techniques.

6.4 Reagents
Specific antisera and antigens are available in limited quantities
from the WHO Collaborating Centres for Virus Reference and
Research (Special Pathogens) listed in Annex 1. Some reagents are
also available from many of the WHO Collaborating Centres for
Arbovirus Reference and Research, and for haemorrhagic fever with
renal syndrome (HFRS) from the WHO Collaborating Centre for
HFRS Reference and Research. Korea University Medical College,
Seoul, Republic of Korea. Table 6 lists some of these reagents and
their availability, but it is not intended to be complete and is subject
to change as reagents are exhausted or new reagents become
available.

6.5 Field laboratory investigations for unknown agents
Field laboratories must be capable of serologically identifying
and confirming known agents and searching for new agents if a
known agent cannot be confirmed.

6.5.1 General approach
Field investigations involve specimen collection and storage for
eventual virus isolation, serological testing of cases in order to
determine as quickly as possible their validity, and the provision of
many routine clinical laboratory tests such as complete blood count,
urine analysis, blood-film examination, and stool examination for
blood or parasites. Often autopsies will have to be done by the
physician and laboratory staff in the field. In addition, local health
Table 6. Availability of some reagents for the diagnosis and identification of viral haemorrhagic fevers

<table>
<thead>
<tr>
<th>Virus</th>
<th>Reference inactivated antigen or spot-slides</th>
<th>Reference antibody</th>
<th>Monoclonal antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow fever</td>
<td>1, 5, 3</td>
<td>1, 5</td>
<td>5</td>
</tr>
<tr>
<td>Dengue haemorrhagic fever (4 virus types)</td>
<td>1, 5, 3</td>
<td>1, 5</td>
<td>5</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>1, 2</td>
<td>2, 3</td>
<td>1</td>
</tr>
<tr>
<td>Crimean-Congo haemorrhagic fever</td>
<td>1, 2, 4</td>
<td>1, 2, 3, 4</td>
<td>1, 2</td>
</tr>
<tr>
<td>Kyasanur Forest disease</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Omek haemorrhagic fever</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Junin haemorrhagic fever</td>
<td>1, 2</td>
<td>1, 2</td>
<td>2</td>
</tr>
<tr>
<td>Machupo haemorrhagic fever</td>
<td>2</td>
<td>1, 2</td>
<td></td>
</tr>
<tr>
<td>Haemorrhagic fever with renal syndrome</td>
<td>2, 3, 6</td>
<td>2, 3, 6</td>
<td>2</td>
</tr>
<tr>
<td>Lassa fever</td>
<td>2, 3</td>
<td>2, 3</td>
<td>2</td>
</tr>
<tr>
<td>Ebola virus disease</td>
<td>2, 3</td>
<td>2, 3</td>
<td>2</td>
</tr>
<tr>
<td>Marburg virus disease</td>
<td>2, 3</td>
<td>2, 3</td>
<td>2</td>
</tr>
</tbody>
</table>

Sources:
1 – Yale Arbovirus Research Unit, Box 3333, New Haven, CT 06510, USA.
2 – Special Pathogens Branch, Centres for Disease Control, Atlanta, GA, USA.
3 – Institut de Médecine Tropicale "Princesse Louise" , B-2000 Antwerp, Belgium.
4 – Institute of Poliomyelitis and Virus Encephalitides, Moscow Oblast 142 700, USSR.
5 – Vector-borne Virus Diseases Division, Centers for Disease Control, Fort Collins, CO 80522, USA.
6 – Korea University Medical College, Seoul 110, Republic of Korea.
7 – National Institute of Virology, Pune, India.

Authorities will often require local staff to have some training in the collecting, handling, and testing of specimens and in the safety procedures involved in dealing with contaminated material. All these functions must be considered when a field outbreak investigation is planned.

6.5.2 Equipment and supplies

There is no substitute for prior planning for the eventuality of an outbreak investigation. Trying to remember all the materials needed when an emergency investigation is in preparation will inevitably result in something critical being forgotten. In addition, the country or agency (such as WHO) requesting the assistance should find out as much as possible about the availability of such fundamental services as water and electricity in the area of the outbreak. This will assist greatly in the decisions regarding what equipment and supplies should be taken.

Supplies for collecting any type of diagnostic material must be included. A source of low-temperature storage (liquid nitrogen in long-life tanks is preferable) must be included in order to store material. However, taking along a supply of dry ice will assist in the
collection and shipping of all diagnostic specimens to the reference laboratory, a very important first step in ensuring that a new agent will be sought as rapidly as possible in the reference laboratory.

Despite the expense involved, taking a fluorescence microscope (with at least a 50-watt mercury lamp), a small generator, and a portable plastic isolator should be considered.

6.5.3 Specimen collection and storage

While it is usually better to collect too many specimens than too few, specimens that are poorly collected and stored are of no value, so close attention must be paid to the techniques of collecting, labelling, and storing specimens. Blood specimens will provide material for both virus isolation and serology. Blood taken for serological purposes may be kept in the cold overnight and separated the following day to avoid centrifugation. The material for virus isolation must be put into liquid nitrogen or dry ice as quickly as possible to maximize the chance of success. Tissues must also be preserved as quickly as possible for virus isolation, and some should be fixed for light or electron microscopic study. Taking a liver specimen with a liver biopsy needle as soon as possible after death should be the minimal procedure for etiological diagnosis with the least risk. However, for diseases where the physiopathology is not known or is limited, or in the case of the sudden appearance of a new agent, useful information will be obtained from an autopsy performed with full safety precautions.

6.6 Laboratory safety

Laboratory accidents and infections related to viral haemorrhagic fevers have been caused mainly by accidents with syringes, contamination from or exposure to blood and other body fluids, and aerosols. In general, these accidents may be prevented by insistence on good laboratory practice and accepted techniques in conjunction with appropriate and correctly used laboratory equipment and facilities. It should be emphasized that good technique is the most important preventive measure. The principles of laboratory safety for working with viral haemorrhagic fever specimens are outlined below:¹

¹ A more detailed discussion can be found in: Laboratory biosafety manual. Geneva, World Health Organization, 1983.
(a) mouth pipetting should be prohibited;
(b) gloves should be worn for all procedures that may involve direct contact with blood or other body fluids;
(c) laboratory coats, gowns, or uniforms should be worn in the laboratory and removed before leaving;
(d) care must be taken in the decontamination and disposal of syringes and needles—disposable units are recommended;
(e) all technical procedures should be performed so as to minimize the creation of aerosols and droplets;
(f) the use of biological safety cabinets and other primary containment devices (centrifuge safety cups, etc.) is advisable for work where there is a high risk of creating aerosols and droplets, i.e., centrifuging, blending, vigorous mixing, etc.;
(g) spills should be cleaned up promptly by covering with a cloth or paper towel soaked in disinfectant—an effective disinfectant solution is sodium hypochlorite (10 g of available chlorine per litre);
(h) bench-tops and other work surfaces should be decontaminated at least once a day—here again, an effective disinfectant solution is sodium hypochlorite (5 g of available chlorine per litre).

The exterior of specimen containers may be wiped with the same solution:

(i) all potentially contaminated materials used in laboratory tests (including laboratory coats, gowns, etc.) should be decontaminated before disposal or reprocessing, preferably by autoclaving;
(j) all workers should wash their hands after handling specimens, after removing gloves and other protective clothing, and when leaving the laboratory;
(k) in the case of accidental infections, cuts and abrasions, the worker should remove gloves, wash hands, apply skin disinfectant if appropriate, report to the first-aid room and, if necessary, consult a physician.

Evidence currently available suggests that under normal conditions the majority of viral haemorrhagic fevers are not spread by aerosol infection. However, when biological safety cabinets are not available, work with new or unknown agents should be carried out wearing respirators.

Laboratory containment requirements are predicated on classification of infective microorganisms by risk group. WHO has adopted the following system (see the Laboratory biosafety manual mentioned above):

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Risk group I (low individual and community risk): A microorganism that is unlikely to cause human disease or animal disease of veterinary importance.

Risk group II (moderate individual risk, limited community risk): A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposure may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited.

Risk group III (high individual risk, low community risk): A pathogen that usually produces serious human disease but does not ordinarily spread from one infected individual to another.

Risk group IV (high individual and community risk): A pathogen that usually produces serious human or animal disease and may be readily transmitted from one individual to another, directly or indirectly.

After classifying the microorganism, the appropriate laboratory facility can then be selected using the scheme shown in Table 7.

The handling, transfer, and shipment of improperly packed specimens and infectious agents carries a risk of spreading the infection to all those directly engaged in, or in contact with, any part of the process. Improper handling within the laboratory endangers not only the staff directly concerned but also administrative, secretarial, and other support personnel. The transfer of materials between laboratories or institutions increases the risk to the public and to airline and postal personnel.

6.7 Packaging requirements

It is recommended that infectious substances and diagnostic specimens are packaged in three layers: (a) a primary watertight receptacle containing the specimen; (b) a secondary watertight receptacle enclosing enough absorbent material between it and the primary receptacle to absorb all of the fluid in the specimen container in case of leakage; and (c) an outer package that is intended to protect the secondary package from external damage and water, while in transit. It is important to tape securely to the
<table>
<thead>
<tr>
<th>Risk groups</th>
<th>Laboratory classification</th>
<th>Laboratory features</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low individual and community risk May be handled in basic laboratory</td>
<td>(a) General microbiology laboratory</td>
</tr>
<tr>
<td>II</td>
<td>Moderate individual* risk and limited community risk May be handled in basic laboratory</td>
<td>(a) General microbiology laboratory (b) Class I or Class II biological safety cabinets</td>
</tr>
<tr>
<td>III</td>
<td>High individual risk but low community risk Requires a containment laboratory</td>
<td>(a) Restricted entrance through double-door vestibule, changing room, or air-lock (b) Interior surfaces waterproof, penetrations sealed (c) Hand-washing facilities (d) Windows sealed, doors self-closing (e) Rodent-proof (f) Autoclave available in laboratory area (g) Mechanic-exhaust negative-pressure air-flow system that provides directional flow (h) Class I or Class II biological safety cabinets or flexible film isolator</td>
</tr>
<tr>
<td>IV</td>
<td>High individual and community risk Requires maximum containment facilities</td>
<td>(a) Separate building or isolated zone of laboratory building (b) Controlled-access double door (c) Sealed, waterproof, interior surfaces to provide for fumigation (d) Rodent-proof (e) Double-door pass-through autoclave (f) Liquid waste (showers, toilets, sinks, hand-wash, etc.) decontamination system (g) Hand-washing facilities (h) Controlled, mechanical, air-flow system: negative pressure, directional flow, maintained by individual interlocked supply and exhaust systems High-efficiency particulate air filters in exhaust system (i) Class III biosafety cabinet or flexible-film isolator to same standards or to suit laboratory</td>
</tr>
</tbody>
</table>

*Where large volumes or high concentrations are used or where aerosol production is continuous the agents should be promoted to Risk group III.

outside of the secondary container one copy of the specimen data forms, letters, and other information that identifies or describes the specimen. Another copy should be sent by airmail to the receiving laboratory and a third copy retained by the sender. In this way, the receiving laboratory can identify the specimen and decide on the best method for handling and examining it. A detailed description of
packaging and shipping recommendations can be found elsewhere.\(^1\)

Infectious substances are classified as dangerous goods. Packages containing such substances must bear the infectious substance (biohazard) label. The specimen should be sent only to laboratories with the necessary containment facilities to deal with it.

The International Air Transport Association (IATA) Shipper's Declaration for Dangerous Goods must also be completed for the shipment of specimens by either airfreight or airmail.

The Universal Postal Union (UPU) requires that containers for the international shipment of non-infectious diagnostic specimens and other biological materials bear the standard international violet-coloured label “matières biologiques périssables” ( perishable biological substances).

7. FIELD OPERATIONS FOR SURVEILLANCE AND CONTROL

7.1 Disease surveillance

The early disclosure of the existence and extent of disease activity and prompt reporting are of crucial importance so that appropriate containment measures can be carried out. The late recognition and reporting of outbreaks of disease result in continued transmission and delay the start of the clinical, epidemiological, and laboratory investigations that are essential to provide the necessary data for the design of appropriate methods of prevention and control. Surveillance teams should be assembled and should consist of locally recruited staff trained on the spot and supervised by one or more epidemiologists. All team members must be fully aware of the measures they should take to protect themselves, and they should be provided with protective equipment. Their task is to collect the specimens that are to be sent to laboratories to confirm a suspected diagnosis as well as to prescribe emergency isolation measures. They

should be provided with standard forms for case and control evaluation and for village surveillance reports. Adequate transport facilities and a means of rapid communication are essential for the investigation and control of outbreaks.

The first information on an outbreak may come from epidemiological surveillance and early warning systems or from other sources such as veterinary services, laboratories, or, as happens frequently, from public rumours among the population spread rapidly by information media. An epidemic may also be first detected by a village official or local administrator who senses that a problem exists.

Corroborative inquiries from different sources will indicate whether any credibility can be given to the information. The situation may necessitate a rapid site visit by a competent person who should have both clinical and epidemiological experience with the suspected disease. It is important that he or she is aware of all the possible diseases that the rumour may concern and that some laboratory specimens are collected to confirm the tentative clinical diagnosis. The analysis of first data may appear to give positive evidence of a certain disease. However, differential diagnosis should be given every attention and a sufficient number of cases should be examined. Immediately the outbreak is confirmed, field operations should be instigated.

For efficient investigation and containment measures a clear definition of cases and contacts must be applied. The wording of the case definition is very important since it should assist the case-finding procedures of the field investigation teams. Thus the case definition should satisfy two exigencies: it must be precise enough, but not too exclusive.

7.1.1 Organization of field operations

The organization of field teams may be simple in countries where access to the focus of the epidemic is easy. It is more complicated and requires more detailed attention when access to the focus is difficult. An example of this more complicated organization is outlined here. Field teams have two functions to carry out simultaneously: investigation and control. The organization of field teams involves the following: selection of personnel; instruction of team leaders; equipment and logistic support; safety precautions; medical evacuation.
Instruction of field operators. Teams of investigators should be given precise directives, which must include: the safety precautions to be observed and the methods necessary for case-finding, contact-tracing, special investigations, and the collection and shipment of laboratory specimens.

Case-finding. Hospital and community surveys should be carried out to detect the earliest suspected cases, using the established case-definition and procedures. Standard survey forms help to ensure the accuracy and rapidity of investigations.

The identification of cases is based on the case-definition that has been adopted. A case is recorded as: confirmed, probable, or suspected.

Contact-tracing. For each case of viral haemorrhagic fever there will be a number of contacts—family members, friends, health personnel, travellers, and others. Some of these, such as family, school, employment or hospital contacts, can be readily identified, while others may be difficult to trace (such as market or travel contacts) and this presents the epidemiologist with tremendous problems.

A contact is defined (for Lassa fever, Ebola and Marburg virus diseases, and Crimean-Congo haemorrhagic fever—according to the USA Centers for Disease Control) as a person who has been exposed to an infected person or his/her secretions, excretions, or tissues in such a way as to be at risk of acquiring the infection. For the infections mentioned above this includes anyone who has been associated with an infected person at any time from onset of fever to 3 weeks later, in any of the following ways:

—shared the same residence;
—had face-to-face contact (within 1 metre) with the patient;
—had skin or mucous membrane contact and/or an accident with a syringe (or other penetrating injury) contaminated with the patient’s secretions, excretions, blood, or tissues.

For yellow fever, Kyasanur Forest disease, Omsk haemorrhagic fever, Rift Valley fever, dengue haemorrhagic fever, and haemorrhagic fever with renal syndrome there is no need to trace the face-to-face contacts or persons who have not (or might have not)
shared the same possible exposure to the vector (room) or handled blood material of the patient in the laboratory.

The persons identified as contacts should be instructed to check their temperature and report immediately to the designated health authorities any increase in temperature or the appearance of any symptoms of ill health. Operational criteria should be set if limitation of movement of the person under surveillance is requested, or if isolation is thought necessary.

*Special investigations.* Whenever there is some evidence of a common source of infection, special investigations must be carried out in order to confirm this suspicion. Possible sources may be: arthropods, vertebrate animals, food and beverages, and the environment.

Collection and processing of human specimens should be carried out as described in sections 6.2 and 6.3.

*Vectors and vertebrate reservoirs.* A list of arthropod vectors and vertebrate reservoirs of viral haemorrhagic fevers is given in Table 8. When these infections are suspected or confirmed an active entomological and vertebrate animal investigation is essential to determine the specific vector and host species involved and to formulate appropriate control methods.

During the investigation of outbreaks the principal man-biting mosquitos and suspected vertebrates should be collected, identified, and processed for virus isolation to confirm their role as vectors and hosts. Techniques for the collection of specimens are described in section 6.3.

Different models of traps are available for the capture of rodents. The type of trap and bait used will determine the success of domestic and field rodent capture. The correct identification of species may require a specialist. For example, *Mastomys natalensis* differs in its ability to transmit Lassa fever according to whether it belongs to the 32- or 36-chromosome type. The mode of transmission of the disease to man depends on his contact with the rodent population. This contact may be either direct—as for hunters or animal care workers—or indirect via contaminated dust or food inside houses. The viruses are mainly excreted in the saliva and urine. The capture of rodents to provide specimens for laboratory examination requires that adequate safety precautions are taken.
<table>
<thead>
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<th>Virus</th>
<th>Arthropod</th>
<th>Vertebrate host</th>
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<td>A. theileri</td>
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<td>C. musculinus</td>
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<td>with renal syndrome</td>
<td>Rattus spp.</td>
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7.2 Vector control—principles and strategies

7.2.1 Mosquito control

The techniques for controlling the arthropod vectors of haemorrhagic fevers are well known and documented. This is true both for long-term preventive measures and for emergency measures for dealing with outbreaks. An understanding of the strategies, concepts, and constraints of vector control is, however, very important for the proper planning of operations.

For the most part, the control strategy for yellow fever in Africa and jungle yellow fever in South America is the rapid immunization
of the population at risk. However, during emergencies it is important at the same time to reduce the vector population wherever possible in order to reduce further the number of cases and check the spread of the disease. Thus the effective aerial application of insecticides as an ultra-low-volume formulation is of special importance, particularly in urban or periurban areas where Aedes aegypti breeds abundantly, in the forest galleries (in Africa), in banana plantations surrounding congregations of human populations, and for the control of epidemics and enzootics of Rift Valley fever.

Emergency vector control should be under the responsibility of an interdisciplinary committee with broad powers to mobilize rapidly resources of manpower, spraying equipment, insecticides, and transport required, and to plan and direct emergency control operations.

Emergency vector control as a part of the strategy of control of these diseases requires that operations are begun early enough before the epidemic reaches its peak, or as soon as the surveillance data (concerning both the disease and the vector) indicate a build-up in the number of cases or the density of vectors. When such a build-up is detected, a state of national alert should be declared and WHO informed of the situation. Vector control measures should then be implemented. It is important that surveillance of disease transmission and vector density is continued so that the efficiency of control measures can be assessed.

In the urban and periurban settings, organized source reduction and larviciding may also be important additional measures. Planning and assessment of these measures is based on the use of the following mosquito density indices:

1. **Premises (house) index** is the percentage of houses, including peridomestic areas, examined that have larvae of *Aedes aegypti* in at least some containers.
2. **Container (receptacle) index** is the percentage of water-holding containers examined that contain larvae of *A. aegypti*.
3. **Breteau index** is the total number of containers with larvae of *A. aegypti* per 100 houses.

The above remarks on emergency control of vectors also apply to outbreaks of dengue haemorrhagic fever. A good example of a concerted vector control effort can be cited from the Caribbean, where the spread of dengue and dengue haemorrhagic fever...
culminated in the 1981 epidemic. Some 344,203 cases of dengue, approximately 10,000 cases of dengue haemorrhagic fever, and 158 deaths were reported in Cuba. A thoroughly organized and executed programme of *Aedes aegypti* control took place using space-spraying against adult mosquitoes, larviciding, and source reduction. The premises index after 7 treatment cycles was 0.005.

In countries of the South-East Asia and Western Pacific Regions, dengue viruses are transmitted by a number of vectors, primarily *Aedes aegypti*, but also *A. albopictus*, and *A. polynesiensis*. Unfortunately, elimination of *A. aegypti* has become virtually impossible because of the water-storage habits of the population and the improper disposal of urban waste. Even a reduction of the number of mosquitoes to a level that would suppress transmission of virus has become difficult.

Within the context of the new emphasis on the role of primary health care, there is the possibility of involving the community in vector control at the household level. In addition, with preventive vector control measures and source reduction by use of larvicides such as temephos and space sprays to control the build-up of *A. aegypti* densities, it should be possible to organize the long-term control of vectors. Health education activities promoting participation of community leaders, schoolteachers, students, agricultural cooperative staff, religious leaders, and the mass media should be a part of this programme for the long-term control of *A. aegypti*.

Innovative techniques should be tried and evaluated for dealing with urban water-retaining debris such as old tyres, the development of appropriate covers for water-storage jars, and the use of biological control agents such as *Bacillus thuringiensis* H-14 under certain conditions.

**Techniques for mosquito control.** The most important element in the emergency control of mosquitoes is the rapid reduction of the target adult mosquito population in order to stop or reduce transmission quickly. Chemical insecticides are applied as space-sprays, frequently supported by larvicides and sometimes also by residual treatments.

Space-spraying involves the application, in a short period, of a quick-acting formulation to cover an epidemic area. Flying vectors acquire a lethal dose by colliding with the droplets. Two forms are used: thermal fogs and ultra-low-volume (ULV) aerosols and mists.
Thermal fogs are produced by equipment that vaporizes the insecticide, usually dissolved in oil, by injecting it into a high-velocity stream of hot gas. Ultra-low-volume applications spray fine mists of concentrated liquid insecticide at rates of less than 460 l/km². Thermal fogs are sometimes more effective in ground applications, among dense housing, and where vegetation is close to houses. Ultra-low-volume aerosols are applied by aircraft and are used in many urban areas. The use of ultra-low-volume applications is greatly affected by the weather; they are usually applied in the early morning or evening, during atmospheric inversion near the ground.

Space-spraying equipment is described in the WHO publication, *Equipment for vector control* (1974). Thermal foggers deliver a blast of visible fog; mist blowers deliver large droplets as a cold mist; ultra-low-volume aerosol applicators deliver very fine droplets and are relatively cost-effective for aerial applications.

For aerial application, small aircraft are flown at 160 km/h 30 m above the ground with a swath spacing of 50–100 m. Two applications are made at 3–7-day intervals. Safeguards for non-target animals and beehives are required as is monitoring of effectiveness in killing mosquitos.

Ground application with vehicle-mounted equipment is practical where good roads exist. One machine can cover up to 2000 houses per day. Inhabitants should be instructed to open windows and doors. The vehicle is driven cross-wind at 5–15 km/h. The swath is usually 60–90 m wide and wind velocities should be below 10 km/h. *Aedes aegypti* control should be carried out during the daytime.

The efficacy of malathion aerosols, applied from the ground using a vehicle-mounted LECO ultra-low-volume aerosol generator, at a target dosage of 43.8 l/km², with two treatments 3 days apart, yielded a 99% reduction in the number of adult *A. aegypti* in a town of 15 000 persons near Bangkok, Thailand.

Portable back-pack application of insecticidal mists is also effective in areas where vehicle-mounted equipment cannot be used.

Larvicides for longer-acting effect are used to control urban *A. aegypti*. The larvicides do not rapidly reduce transmission but can assist in keeping populations at low levels over sustained periods. Temephos and methoprene are used in drinking-water for the control of *A. aegypti* larvae. Sprays may also be applied by power sprayers or vehicle-mounted mist blowers.

Perifocal spraying of residual insecticides is used in South America and might be useful in Africa for the control of domestic
A. aegypti. This is selective spraying in which the residual spray is applied by a hard-compression sprayer to the inside and outside walls of containers and any walls close to the container up to 60 cm each side and above it. Non-potable water (but not drinking-water) is also treated. DDT and HCH are used in areas where A. aegypti is not resistant to them.

7.2.2 Tick control

Ticks are vectors of some of the viral haemorrhagic diseases, but there is a complex ecological relationship between the ticks and their hosts that makes tick control difficult. The control of tick-borne diseases is further complicated by transovarian transmission of the disease agent from one generation of ticks to another. Because of the extensive areas involved, tick control using acaricides may not always be cost-effective. However, when only small areas are involved and in special situations, such control may be feasible. The clearance of sites where ticks may survive (haystacks, brush piles, leaf litters, long grass at forest edges and forest paths) is recommended for the control of Hyalomma marginatum. Depending on the level of susceptibility of particular tick populations to chemicals, 5% DDT, 0.5% lindane, 0.25–0.48% bendiocarb, 5% carbaryl, 0.5% diazinon, 2% malathion, or 1% pirimiphos-methyl may be used for indoor or outdoor treatments. Aerial spraying has not proved to be very effective. Tick repellents are also available for the treatment of clothing for use under special circumstances, namely deet (diethyltoluamide), butopyroxonoxyl, or permethrin. Rodent control may reduce the tick population and the spraying of cattle to kill ticks was effective in the USSR for the control of tick vectors of Crimean-Congo haemorrhagic fever.

7.2.3 Resistance to insecticides

For the effective use of insecticides, it is essential that information is available on the level of susceptibility of the local vector to the insecticides proposed for use. Summaries of this information are available from WHO.1 Where necessary, susceptibility tests on the

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vector population should be routinely carried out. The methods to be used for these tests are available on request from WHO.¹

7.2.4 Legislation and vector control

Ideally one should aim for community involvement in vector control, but there are very often difficulties in achieving this. Some countries, e.g., Singapore, have been able to enact special legislation that has been helpful in achieving a high level of control of domestic vector species, such as *Aedes aegypti*. Naturally such legislation will only work in areas where vector breeding habitats are created by the activity or negligence of man. Punitive measures, such as fines imposed on the householder if he allows mosquito breeding in or around his premises can, under some conditions, help in encouraging community participation.

7.2.5 Rodent control

Different rodenticides may be used for rodent control in addition to trapping. The selection of the rodentine and the amount that is required as a single dose or in multiple doses varies according to the target animal and should be determined by a specialist. Experience in South America has shown that it is possible to control populations of *Callomys callosus* effectively and economically, reducing them to a level that resulted in a marked decrease in the incidence of Machupo haemorrhagic fever. This rodent is a peridomestic species that readily enters houses and is therefore easy to control. Conversely, the hosts of the Junin virus, *Callomys musculus* and *C. laucha*, are found over very large areas, and efforts to maintain control of these populations and thereby interrupt the transmission of Junin haemorrhagic fever would probably not be effective. This is also true for the savannah areas where *Mastomys natalensis* populations are the reservoirs of Lassa fever. While temporary control might be obtained during epidemic outbreaks of the disease, continued suppression of populations to a level at which transmission would cease would be extremely difficult to maintain. On the other hand, sustained, effective, and economical control of populations living in and around village dwellings can be obtained by enlisting the active participation of the inhabitants and by

¹ Division of Vector Biology and Control, World Health Organization, Geneva, Switzerland.

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providing them with simple and effective control methods or materials. Control of sylvatic reservoirs of haemorrhagic fever with renal syndrome would be difficult unless such control coincided with areas in which the rodent hosts were of economic importance in agriculture so that long-term, repeated control could be made cost-effective. However, the control of urban hosts of haemorrhagic fever with renal syndrome is entirely feasible.

Each epidemiological and ecological situation must be considered separately before any decision is made on rodent control. The distribution and prevalence of infection in the rodent population, their habits and densities, as well as their susceptibility to different control methods, must all be taken into account.

7.2.6 Training

In order to ensure the cost-effective use of material and manpower resources, it is important that all those involved in rodent and vector control are trained and periodically retrained. Regular training courses for the operators and trainers should be organized with the emphasis being on the proper and effective use of rodenticides, insecticides, and equipment for vector control.

8. DIAGNOSIS AND MANAGEMENT

8.1 Clinical diagnosis

In the early stages of most viral haemorrhagic fevers there are no distinguishing features and the illness may closely mimic such conditions as malaria, septicaemia, influenza, or other severe virus infections. Gastrointestinal disturbance is common in many viral haemorrhagic fevers and haemorrhage is a prominent feature in severe cases. Circulatory disturbance and shock may prove troublesome and even fatal, while renal failure and coma commonly presage death. It is noteworthy that there is a wide spectrum of severity in these infections and many attacks are too mild to be recognized clinically.

For some of the viral haemorrhagic fevers the nature of the illness may be suspected by characteristic clinical features. For example, the onset of symptoms is usually sudden in Rift Valley fever, Crimean-Congo haemorrhagic fever, Omsk haemorrhagic fever, Kyasanur
Forest disease, haemorrhagic fever with renal syndrome, and Ebola and Marburg virus diseases, and sudden onset is also characteristic in yellow fever. In others, including dengue haemorrhagic fever, Lassa fever, and Junin and Machupo haemorrhagic fevers, the onset is usually insidious. Other specific clinical features include jaundice in 20–30% of cases of yellow fever, a painful ulcerated throat and extreme lassitude in Lassa fever, and a characteristic erythematous maculopapular rash appearing around the fifth day in patients with Ebola and Marburg virus diseases.

8.2 Management of patients

8.2.1 Assessment of patients with suspected viral haemorrhagic fever

Because many viral haemorrhagic fevers have no distinguishing features, at least in the early stages, it is virtually impossible to make a firm diagnosis on clinical grounds alone. It is therefore necessary to pay particular attention to epidemiological evidence when assessing a patient from an endemic area with an unexplained fever.

Patients in an endemic area. The assessment of a patient with an unexplained fever in an endemic area should be based on an analysis of the epidemiological and clinical features of the illness. When laboratory facilities are not available it may be justifiable to observe the therapeutic response to antimalarial drugs and selected antibodies. The level of isolation selected, whether at home or in hospital, should be appropriate to the characteristics of the endemic disease.

Patients with suspected viral haemorrhagic fever should be transferred outside an endemic area only in exceptional circumstances. The movement of patients within an endemic area should be kept to a minimum. When it is considered essential to transport a patient within or from an endemic area protective measures should be taken that are appropriate to the epidemiology of the local disease.

When febrile patients from endemic areas are admitted to hospital with possible viral haemorrhagic fever, malaria should be excluded by examining a blood smear and giving antimalarial treatment. The blood film should be rendered safe by dipping it in a 10% solution of formol in buffered saline. If malaria has been
excluded, further clinical laboratory investigations may be required to establish a diagnosis. Tests for this purpose should be carried out under safe conditions.

*Patients in a non-endemic area.* Travellers from an endemic area who arrive with an unexplained fever, or who develop a fever within the quarantine period after departure from an endemic area, should also be assessed on the epidemiological and clinical evidence, then classified into one of the following three categories according to the level of suspicion and dealt with accordingly.

(a) *Minimal suspicion.* Patients who have come from major cities where a viral haemorrhagic fever has not been reported to be endemic should be admitted to standard isolation in hospital, or may be confined at home when there is no immediate threat to life. The patient may be transported in an ambulance taking the standard precautions for infectious diseases. Specimens may be sent to routine laboratories. Close contacts should be identified but need not be placed under surveillance.

(b) *Moderate suspicion.* Patients from small towns or country districts in an endemic area should be regarded with more suspicion, especially if the onset and course of the illness are consistent with a dangerous viral haemorrhagic fever, and they should be admitted to an isolation room with filtered negative-pressure ventilation and separate facilities for dealing with contaminated waste. Ambulance crews should wear protective clothing. Specimens should be taken with the greatest care, securely packaged, and dispatched to a laboratory for processing in a high-security cabinet. If malarial parasites are not found and the fever continues, these patients should be transferred to isolation areas of maximum security for further observation and investigation. Close contacts should be identified but need not be placed under immediate surveillance.

(c) *High suspicion.* Patients from rural areas and towns where a dangerous viral haemorrhagic fever is known to be endemic, and cases among medical and nursing staff from country hospitals, contacts of confirmed cases, and laboratory staff processing dangerous material should be admitted directly to a designated high-security unit. Ambulance crews should wear full protective clothing and possibly respirators. The use of aircraft or ambulance transit isolators should be considered for long journeys. Specimens should be taken with the greatest care, securely packaged, and dispatched
by special messenger to a designated maximum-security laboratory. Close contacts should be placed under surveillance.

8.2.2 Management policy in a general hospital

Patients with an unexplained fever from an endemic area are likely to attend the accident and emergency department of a general hospital, and it is undesirable that they should be admitted without preliminary screening to exclude the possibility of a viral haemorrhagic fever. The receptionist should inquire routinely about recent travel, and those who have become ill during the quarantine period following departure from an endemic area should be directed to a separate room for assessment. Those who fall into the categories of minimal or high risk should be dealt with accordingly. Those in the moderate-risk group require careful clinical and epidemiological assessment and blood films should be examined to exclude malaria. If it is considered necessary to admit such patients, it is essential that they should be nursed in single rooms with strict isolation techniques. Special care must be taken with laboratory specimens.

8.2.3 Isolation facilities in designated units

The designated unit should have the following basic facilities and policies:

(a) The unit should be self-contained and separated from the rest of the hospital.
(b) Access should be restricted to authorized personnel and a record should be kept of anyone entering the unit.
(c) There should be mechanical ventilation to maintain a negative pressure within the possibly contaminated areas and exhaust air should be filtered.
(d) All waste must be rendered safe before disposal.
(e) The unit must be easy to clean and there must be provision for sealing the area for fumigation.
(f) Changing rooms and shower facilities should be available for the staff.
(g) There should be an emergency generator to supply electric power.
(h) Facilities should be provided within the unit for patient-management tests and for radiology.
(i) An area should be designated for the disinfection of ambulances.

(ii) Patients in the intermediate category should be nursed in single rooms by staff wearing protective clothing. Respirators are not necessary.

(k) Patients in the maximum-risk category should be nursed in flexible-film, negative-pressure isolators or by staff wearing full protective clothing, including respirators or ventilated hoods.

(l) Staff should be kept under surveillance for 21 days after their last exposure to a confirmed case. Their general state of health and their temperature should be recorded each day.

8.2.4 Isolation of patient

Once admitted into a maximum security unit, the patient should be strictly isolated until a firm diagnosis has been established and viral haemorrhagic fever has been excluded, or until the fever has subsided and it is apparent that the course of the illness has not been consistent with that of a viral haemorrhagic fever, or until laboratory tests have proved negative. If an admitted patient is subsequently found to have malaria, strict isolation should be continued until there has been a satisfactory response to treatment; experience has shown that malaria may coexist with other diseases.

Patients with confirmed viral haemorrhagic fever may continue to harbour the virus for many weeks after the onset of illness and should not be released from isolation until blood and urine cultures have been shown to be negative. With Marburg or Ebola virus disease, virus may persist in the semen and other body fluids for long periods. In these circumstances strict isolation is probably unnecessary but advice should be given to prevent the spread of infection.

8.2.5 Laboratory investigations

Diagnostic tests are essential to establish the cause of illness in a patient with suspected viral haemorrhagic fever. These tests fall into three distinct categories:

(a) Tests for viral haemorrhagic fever. Virus cultures and antibody studies should be undertaken only in designated maximum-security laboratories.
(b) Tests may be required to exclude other causes of unexplained fever. These investigations may include routine haematology, blood films for malaria, blood cultures, stool and urine examinations, and serological tests for bacterial, rickettsial, and other viral infections. Such investigations should be undertaken only in a designated laboratory or in a patient-management isolator within a designated high-security unit.

(c) Patient-management tests are most conveniently carried out within a flexible-film laboratory isolator sited in the high-security unit. Haematological and biochemical investigations required for the optimal management of the patient may include: haematology, clotting studies, blood grouping, estimation of urea, electrolytes, and glucose, liver function tests, and urinalysis.

The general principles of collection, transport, and handling of laboratory specimens are described in section 6 of this report. Transport of specimens within the hospital from the bedside to the clinical laboratory should be carried out following the same precautions as are required for external transport.

8.2.6 Treatment

General. Skilled nursing and careful attention to water and electrolyte balance are essential for a favourable outcome in severe infections, and even the severely ill patient may survive given skilled basic care. Whenever possible, patient-management tests should be available to monitor treatment. It is essential that the team of nurses and doctors looking after the patient should be well trained to ensure that there is no spread of infection.

During the febrile phase of the illness, symptomatic relief may be obtained by tepid sponging, but antipyretics such as salicylates are contraindicated. Analgesics may be necessary for the relief of pain and mild sedation to control restlessness.

Specific. Convalescent plasma has proved to be of value in the treatment of Junin haemorrhagic fever providing it contains sufficient neutralizing antibody and is given within the first 8 days of illness. About 10% of patients receiving this convalescent plasma develop a temporary disturbance of cerebellar function. Convalescent plasma has not been shown to have therapeutic value in other haemorrhagic fevers. In experimental animals, convalescent
plasma has not been shown to have prophylactic value in either Marburg or Ebola virus infections. Limited randomized trials of Lassa fever plasma with a high antibody titre have not so far been shown to have any therapeutic value.

A trial of ribavirin in cases of Lassa fever has shown that it is beneficial when given during the first 6 days of illness. It has also been demonstrated to be of value when given prophylactically in animal experiments, and the use of this drug should be considered in the event of accidental exposure to Lassa fever infection or haemorrhagic fever with renal syndrome.

For Junin haemorrhagic fever and Lassa fever, very high levels of alpha interferon occur naturally in severe cases. Therefore, there appears to be no rational basis for the therapeutic use of alpha interferon in these two diseases.

8.2.7 Complications

Shock. Patients, especially those with dengue haemorrhagic fever, should be kept under close observation for signs of shock. In the early stages of shock the pulse becomes weak and rapid and the blood pressure falls. The patient is restless and the skin feels cold and clammy. In advanced shock the pulse and blood pressure may not be detectable. In children the loss of fluid from the intravascular compartment may be more important than haemorrhage, although the opposite may be true in adults. Depending on the underlying causes, shock may be treated with an infusion of glucose-saline and plasma expanders or with transfusions of whole blood.

Haemorrhage. The cause of haemorrhage in most of the viral haemorrhagic fevers is not understood. There may be evidence of vascular damage, thrombocytopenia, prolongation of the prothrombin time, and disseminated intravascular coagulation. In the event of serious bleeding, the use of fresh platelets, fresh frozen plasma, or coagulation-factor concentrates should be considered, although the value of such preparations has yet to be established for most viral haemorrhagic fevers. These preparations should be used with caution when there is evidence of concomitant disseminated intravascular coagulation. Transfusion of fresh blood may prove helpful in controlling haemorrhage and transfusion of stored blood may be necessary to restore the circulation. The use of heparin for the treatment of disseminated intravascular coagulation is
controversial. Heparin has been advocated particularly for the
treatment of Crimean-Congo haemorrhagic fever, but great care
must be taken and its use must be carefully monitored.

*Renal failure.* Renal failure may be treated by peritoneal dialysis
but stringent precautions must be taken to prevent the spread of
infection to hospital staff and the dialysate must be sterilized before
disposal. Haemodialysis would be very hazardous during the
viraemic phase of most viral haemorrhagic fevers and should not be
attempted except in haemorrhagic fever with renal syndrome or
yellow fever.

8.3 Research needs and recommendations

1. National policies should be devised to deal with imported
cases of potentially dangerous viral haemorrhagic fevers.
2. Individual hospitals should prepare contingency plans to
deal with suspected cases.
3. General practitioners and hospital staff should be reminded
regularly that care is essential when dealing with patients from
endemic areas with unexplained pyrexia.
4. Hospital units should be designated to investigate and nurse
suspected or confirmed cases under conditions of safety. Staff should
regularly receive the necessary training.
5. Laboratories should be approved and designated for
diagnostic and patient-management tests.
6. Further research is required to evaluate antiviral drugs—
including ribavirin, interferon, and immune plasma—in the
treatment and prevention of viral haemorrhagic fevers.

9. GENERAL RESEARCH NEEDED
AND RECOMMENDATIONS FOR
VIRAL HAEMORRHAGIC FEVERS

9.1 Epidemiology

Research is needed:

(a) to determine the real geographical distribution of the viral
haemorrhagic fevers, in particular of Lassa fever, Ebola and
Marburg virus diseases, Crimean-Congo haemorrhagic fever, Rift
Valley fever, and haemorrhagic fever with renal syndrome, as well as the prevalence of different clinical forms of the haemorrhagic fevers;

(b) to investigate the possible role of as yet unknown reservoirs for some of the viruses known to produce haemorrhagic fevers;

(c) to determine the degree of person-to-person transmission of several of the viral haemorrhagic fevers;

(d) to determine the impact of changing ecological factors on the dynamics of the viral haemorrhagic fevers.

9.2 Pathogenesis

Research is needed:

(a) to define the precise mechanisms leading to haemorrhagic diathesis in each of the viral haemorrhagic fevers;

(b) to establish the role of the immune system in the pathogenesis of each disease;

(c) to determine the mechanisms leading to shock, renal failure, and neurological disturbance observed in some of the haemorrhagic fevers.

9.3 Laboratory diagnosis

Research is needed:

(a) to develop methods for the rapid virological identification and specific serological diagnosis of the viral haemorrhagic fevers, with special emphasis on their application in endemic regions;

(b) to support the development, production, and distribution of reagents and reagent kits for the diagnosis and surveillance of viral haemorrhagic fevers, and to provide training in the use of these reagents.

9.4 Management of cases

Research is needed to review periodically the real value of currently accepted measures for the management of cases.

9.5 Health education

Research is needed to support public health education programmes in countries where these diseases are endemic and in
countries where they may be introduced. These programmes should be integrated with primary health care facilities.

9.6 Emergency action

In view of the emergencies caused by outbreaks of viral haemorrhagic fevers, WHO should develop a contingency plan to secure, at the global level, a rapid mobilization of expertise, vaccines, and other supplies and equipment that may be necessary at short notice.

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Annex 1

WHO COLLABORATING CENTRES FOR VIRUS REFERENCE AND RESEARCH (SPECIAL PATHOGENS)

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