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Review article

Viral haemorrhagic fevers: properties and prospects for treatment and prevention

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Introduction

Many haemorrhagic fevers of viral origin have come to the attention of virologists within the last half century. Although many are comparatively rare, reports of high mortality together with potential ease of introduction between continents by the rapid development of air transport has resulted in the term 'exotic' being applied to many of these agents. In all probability, agents such as Marburg, Lassa and Ebola occur as natural infections of animals and nonhuman primates within endemic areas. It should be recognized, however, that haemorrhagic fevers resulting from virus infection have been known for centuries. Among these, yellow fever and small pox are examples where epidemiological control measures such as vector control or vaccination have proved effective. However, the most recent examples of outbreaks of haemorrhagic fevers have paralleled certain changes in many habitats. Man makes increasingly strenuous efforts to increase agricultural production and to provide associated energy needs by the application of new technology. Thus, ecosystems become irreversibly changed in the name of progress. This has inevitably altered the population dynamics and behaviour of animals persistently infected with viruses.

The term haemorrhagic fever was first used by Russian and Asian investigators examining what is now recognized as haemorrhagic fever with renal syndrome (HFRS). All share many clinical features, in particular damage to the capillary and haematopoietic systems accompanied by leukopenia, thrombocytopenia and fever. Although these similar clinical syndromes suggest a common tissue tropism or host response to infection, many of these viruses widely differ in their properties and resulting classification. In Table 1 it can be seen that the majority of these possess RNA genomes. Yet the diverse strategies of replication between members of different families imply fundamentally different approaches for any chemotherapeutic ap-

TABLE 1

Viruses causing haemorrhagic fever in man

Family	Virus	Average genome size/polarity	Primary route of transmission
Flaviviridae	Yellowfever Dengue	4.0×10^6/ positive strand	Mosquito-borne
Arenaviridae	Lassa Junin Machupo	4.2×10^6/ negative strand	Aerosol/food contamination
Bunyaviridae	Rift Valley fever Congo-Crimean Hantaan[b]	4.0×10^6/ negative strand	Zoonosis Tick-borne
Filoviridae[a]	Marburg Ebola	4.0×10^6/ negative strand	Unknown

[a] Classification name to be confirmed.
[b] Causative agent of Korean haemorrhagic fever (HFRS).

proach to treatment of acute infection. For example, Flaviviruses are known to replicate independently of the host cell nucleus whereas both the Arenaviruses and the agent of Ebola contain RNA sequences that require the synthesis of complementary copies prior to the expression of virus-specific products within the infected cell (negative polarity). It is known that certain Arenaviruses require an intact host cell nucleus for active virus replication [4]. Much is known regarding the molecular biology of Bunyaviruses, to which family belong the agents of Rift Valley fever, Congo-Crimean haemorrhagic fever (CCHF), and Korean haemorrhagic fever, a form of HFRS. In both the Arenaviridae and Bunyaviridae genetic information is segregated on respectively 2 or 3 discrete pieces of single-stranded RNA of different sizes. Yet again the agents of Marburg and Ebola (filoviruses) appear to contain only a single species of negative strand RNA.

Before reviewing the more important viral haemorrhagic fevers, it should be emphasized that, with the possible exception of yellow fever and Dengue, sera and other biological fluids from suspected or confirmed cases of severe haemorrhagic-like illness should only be handled in suitably equipped high security laboratories. This has in turn considerably hampered the thorough laboratory investigations required for understanding these pathogens and preventing the diseases caused by them.

Flaviviridae

Yellow fever

Yellow fever is a zoonotic infection of man and still constitutes the most important cause of viral haemorrhagic fever, despite the effectiveness of mosquito eradication in reducing the incidence of infection, and the availability of an effective vaccine. It is a

disease principally of primates and is transmitted from man to man by *Aedes aegypti*. Although elimination of this vector has almost completely eradicated urban yellow fever, sporadic epidemics still occur both in South America and Africa. Recently, epidemics of yellow fever occurred in Senegal and Ethiopia, the latter causing about 30 000 deaths [93]. Control by vector eradication alone is difficult owing to maintenance of a sylvatic cycle involving monkeys and forest-dwelling mosquitoes of both *Aedes* and *Haemagogus* species.

In man yellow fever varies from an inapparent infection to a fulminant disease which is invariably fatal [83]. Next to the hepatitis viruses A and B yellow fever virus is the most important hepatotropic virus. However, it also affects the spleen, kidneys and heart.

Following an incubation period of 3–6 days, the sudden onset of illness is characterized by fever, rigors, and headache. After 3–4 days, the fever temporarily subsides before the illness develops into either an icteric or anicteric state. The appearance of jaundice indicates a poor prognosis, with bleeding and haematemesis occurring concomitantly. Death may occur a week later, although in the fulminant disease, death is hastened to within 3 or 4 days of the onset of jaundice. The anicteric infection occurs much more frequently and the prognosis is generally good. A disease profile of jaundice and haemorrhagic manifestations make accurate diagnosis of yellow fever difficult, even during an epidemic, and virological or histopathological investigation is always recommended.

Serological diagnosis of yellow fever is generally accomplished by either haemagglutination inhibition, complement fixation, or neutralization analysis of serum samples taken 3–4 weeks apart. Although useful in providing epidemiological data, care must be taken, as cross-reactivity may occur with antibodies directed against other Flaviviruses such as West Nile, Zika and Wesselsbron viruses. Direct isolation of virus by the intracerebral inoculation of mice may be achieved during the early acute stages of the illness.

The yellow fever virion is a 40 nm diam. spherical particle with an outer, ether-sensitive envelope similar in appearance to other Flaviviruses. Despite the availability of information regarding the epidemiology, pathology and prevention of this disease, remarkably few studies have attempted to analyze the chemical composition of this virus. The genome appears to be single-stranded RNA and the outer virion coat protein resembles that of Dengue virus [78]. Although it is possible, using polyclonal animal sera, to differentiate between strains of African or South American origin, there appears to be remarkably little difference between the antigenic compositions of the different viruses, a property distinct from Dengue virus. However, Schlesinger et al. [78] have recently characterized a panel of monoclonal antibodies with varying degrees of specificity for 17D and/or Asibi strains of yellow fever virus.

The development of a safe, attenuated vaccine (17D strain) grown in chick embryos represents one of the landmarks in medical virology. This vaccine produces few, if any, complications and provides long-lasting immunity. An alternative neurotropic vaccine developed at the Pasteur Institute (Dakar) produced a number of cases of post-vaccination encephalitis and is now little used.

Administration of the 17D yellow fever vaccine results in appearance of circulating

antibody, detectable for as long as 19 years [39,74], but it is possible that protection persists beyond the eventual disappearance of neutralizing antibodies. The vaccine is safe providing it is prepared by internationally standardized methods [94]. The 17D seed virus is the result of over 200 successive passages in mouse embryo cells, whole chick tissues and chick embryo tissues devoid of nervous tissue [76]. Recent improvements include the removal of avian leukosis virus originally introduced by the use of chick embryos. However, the use of approved diploid cell lines as a substitute for the growth of yellow fever vaccine has yet to be thoroughly investigated and there is a paucity of data regarding the stability of lyophilized vaccine.

Little is known regarding the immunochemical properties of yellow fever, whether vaccine or wild-type isolates. A recent study of 17D yellow fever vaccines produced using RNA oligonucleotide mapping has failed to reveal differences between all vaccines produced worldwide from the 17D-204 substrain [61]. Vaccines produced from the commonly used alternative 17DD substrain showed substantially greater genotypic variation, and this study showed that the attenuated yellow fever virus may undergo minor variations in only one or two egg passages. These studies are also complemented by the finding that monoclonal antibodies produced against the 17D virus [78] are able to discriminate between the 17D-204 and 17DD vaccine substrains.

Dengue viruses

Dengue is a widespread and important Flavivirus infection. Four distinct serotypes are circulating, predominantly in Southeast Asia, Africa, India, the Pacific and the Caribbean. Epidemics are common in the rainy season when the *Aedes* mosquito multiplies. There is no evidence of an animal reservoir. Uncomplicated 'classical' Dengue is a febrile illness usually of adults and adolescents, accompanied by severe joint pains, headache, retroorbital pain and rash. Many patients under the age of 15, however, develop a haemorrhagic fever with a possible shock syndrome and fatal outcome [77]. Many of these fatal cases appear to have been previously infected with a subtype other than that which caused the complication. This has given rise to the hypothesis that Dengue haemorrhagic fever and shock result from an immunopathological process whereby the second infection by heterologous virus causes an anamnestic response of the Dengue subgroup-specific antibodies [41]. Despite the high titre of these antibodies, virus fails to be neutralized by the heterologous antibodies, resulting in the formation of infectious virus–antibody complexes. Activation of complement by these immune complexes would in turn liberate toxic substances resulting in vasodilation and possible hypovolaemic shock. This hypothesis has been extended to infants in Southeast Asia who develop haemorrhagic manifestations as a result of primary Dengue infection during the first year of life. In such circumstances, it has been proposed by Halstead that passive transfer of maternal antibodies or an enhancing immune factor may result in infectious virus–antibody complexes in the circulation of infected infants if the factor has been diluted out to a critical, nonneutralizing concentration at the time of primary infection[41].

Not all cases of Dengue haemorrhagic fever can be explained in terms of prior exposure to a heterologous subtype. Rosen (1977) has pointed out that severe haemorrhage and shock can occur in primary infections in adults, and that these complica-

tions are rarely seen outside Southeast Asia despite the fact that, in an endemic area, more than one serotype may be circulating at any one given time. An alternative explanation is that Dengue virus strains differ in virulence and that the more virulent strains are associated with the more severe clinical forms restricted to Southeast Asia. At present, however, there are no biochemical or laboratory data to substantially support this concept of variation in virulence between Dengue isolates. It should also be noted that asymptomatic infections are frequent. During an epidemic they may account for nearly 70% of all infections [40].

An experimental Dengue type 2 vaccine has been prepared by attenuation in monkey kidney cells. This resulted in a stable temperature-sensitive clone which produced small plaques and was passaged further in foetal rhesus monkey cells [27]. The use of such a vaccine is complicated as a result of increased antibody titres being seen in individuals previously exposed to other Flaviviruses, e.g., yellow fever. Volunteers previously immunized with the 17D yellow fever vaccine were found to develop mild viraemia and transient subclinical infection after receiving the candidate Dengue 2 vaccine [3]. This makes possible limited clinical trials in populations with no previous exposure to Flaviviruses to assess the protective efficacy of this temperature-sensitive vaccine strain. The question remains, however, as to the respective merits and disadvantages of the widespread use of a monovalent vaccine, especially in areas where other, heterologous viral subtypes coexist.

Arenaviridae

Arenaviruses are a group of enveloped single-stranded RNA viruses. Nearly all of the members so far described cause acute or persistent infections of rodents in either Africa, Europe or the Americas. Certain Arenaviruses cause severe haemorrhagic disease in man, notably Lassa, Machupo and Junin, whereas others cause no apparent disease in man despite causing persistent infections in rodent species. Acute haemorrhagic disease due to Machupo and Junin viruses represent serious public health problems in Bolivia and Argentina, respectively, and Lassa virus has achieved public notoriety owing to its association with severe febrile illness among missionaries and travellers returning from rural parts of West Africa. All the evidence so far available suggests that the morbidity of Lassa and South American haemorrhagic Arenavirus infections results from the direct cytolytic action of these agents. This is in sharp contrast to the immunopathological basis of 'classical' lymphocytic choriomeningitis (LCM) disease seen in adult mice infected with LCM virus, often regarded as the prototype of the family. For a general review of Arenaviruses, see Howard and Simpson [42].

Diseases produced in man by infection with either Junin, Lassa or Machupo viruses are very similar. The onset of illness is insidious with chills, malaise, headache, pain behind the eyes and in the muscles, and nausea followed by fever, conjunctival infection, and suffusion, exanthema and oedema of the face, neck and upper thorax. Petechiae and lymphadenopathy are common. The differential diagnosis includes malaria, typhoid, yellow fever, influenza and measles. Prostration out of proportion

to the degree of pyrexia has been described as the single most suggestive feature of Lassa fever. After a few days, the patient's condition becomes appreciably worse, with the development of hypotension, oliguria, haemorrhages from the gums and nose, haematemesis, haematuria and melaena. Oliguria may turn to anuria and pronounced neurological manifestations may develop. Death may result from anaemic coma or hypovolaemic shock caused by plasma leakage. In Lassa infections, pronounced pharyngitis with ulcerative lesions on the tonsils is a frequent finding. Case fatality rates in individual outbreaks have varied from 5% to 40%. Junin and Machupo are rarely transmitted from person to person, unlike Lassa, which has a formidable reputation resulting from frequent man-to-man transmissions. This may correlate with the low titre of circulating virus in patients with Argentinian and Bolivian haemorrhagic fevers, in contrast to the high titre found in cases of Lassa infection. Inapparent and subclinical infections with Lassa are now believed to be quite common, but inapparent infections with Junin and Machupo viruses are comparatively rare.

Negative-staining electron microscopy of Arenaviruses shows the presence of pleomorphic particles ranging in diameter from 80 to 150 nm. The virus envelope is formed invariably from the plasma membrane of infected cells. Little is known about the internal structure of the Arenavirus particle, although thin sections of mature and budding viruses clearly show the ordered and often circular arrangement of host ribosomes typical of this virus group. All evidence obtained so far indicates that the Arenavirus genome consists of at least 2 discrete pieces of single-stranded RNA with different sizes, with a total molecular weight estimated in the range $3.2–4.8 \times 10^6$ [70]. Considerable evidence is now available confirming that the electron-dense 20–25 nm particles visible within purified virions represent associated host RNA and may account for up to 50% of the total RNA content [89] and that they are not required for virus replication [55].

Arenaviruses contain a major nucleocapsid-associated protein of molecular weight 54 000 to 68 000 together with one or two glycoproteins in the outer virus envelope [42,55]. Although the nature of Arenavirus surface antigens has yet to be defined, it is likely that the surface glycoproteins play an important role in eliciting a protecting antibody response. Indirect evidence of this is the finding that sera containing neutralizing antibodies to LCM virus also react with both glycoproteins present in the outer envelope [17].

Overwhelming evidence of early infection in lymphoreticular cells indicates that Arenaviruses may interfere with host defences during acute infection. Murphy et al. [65] showed that huge numbers of virus particles were present in the lymph nodes, thymus, and spleen of Machupo-infected *Calomys callosus* rodents. Large numbers of virus particles associated with the plasma membrane of large lymphoblastoid cells were also seen. Similar studies have also been performed with Tamiami, an Arenavirus infection of the cotton rat [66]; in the latter study, megakaryotes were also infected. This finding suggests a possible general relationship between platelet function and the pathogenesis of Arenavirus haemorrhagic fevers. Histologically, the liver is the major organ involved in Lassa virus infection; there is a range of severity induced by this virus [92]. The degree of inflammatory cell infiltration is small and unrelated to

the extent of hepatocellular damage and eosinophilic necrosis of individual hepatocytes is a constant finding, and the nonzonal distribution of necrosis distinguishes Lassa virus hepatitis from the classical lesion of yellow fever.

The mechanism by which Arenaviruses cause disease in man are not fully understood. There is no evidence that either immunopathological or allergenic processes play any part in causing disease and it appears much more likely that direct virus damage to cells is the cause. Postmortem studies on patients who died following Junin virus infection have shown generalized lymphadenopathy with endothelial swelling in capillaries and arterioles in almost every organ, together with lymphocyte depletion in the spleen. There are many similarities in the pathological lesions found in man following Junin, Lassa and Machupo virus infections. Focal nonzonal necrosis in the liver has been described in all 3 conditions [22,34,92] associated with Kupffer cell hyperplasia, erythrophagocytosis, and acidophilic necrosis of hepatocytes.

Each member of the Arenaviruses possesses some antigenic relationship to other members of the group, although the degree of cross-reactivity largely depends on the assay system used [16,21].

The assay systems currently being used to determine antigenic relationships among the Arenaviruses are therefore clearly not of sufficient sensitivity and new techniques are urgently needed. Those showing promise are radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) using either human or animal antisera, although these methods have yet to be fully evaluated. A recent study has shown the potential of using monoclonal antibodies as diagnostic reagents [18]. Among a panel of 46 antibodies raised against LCM virus, 5 were found to cross-react with Lassa virus. This cross-reactivity was limited to antibodies against the internal nucleocapsid component of LCM virus. None of these reagents reacted with Arenaviruses isolated in the New World.

Control of human Arenavirus infection is now becoming a real possibility, with several lines of investigation showing encouraging results. This is also superimposed upon successful control of the rodent vectors in the case of Bolivian haemorrhagic fever. However, this is unlikely to be achieved with Lassa and Junin infections. Prevention may be possible by either the attenuation or inactivation of wild-type virus or by exploring the extent of protection that may be offered by other antigenically related Arenaviruses that do not cause clinical illness in man. In addition, the relatively low yields of virus in the blood during acute Machupo or Junin infection make the use of both immune plasma and chemotherapy a possibility.

In Argentina, therapeutic trials have been carried out with immune plasma for some time. Maiztegui et al. [58] have reported encouraging results with immunotherapy carried out within 8 days of the onset of disease. Viraemia levels were lowered and there was a dramatic improvement in survival rate. One problem that requires further attention, however, was the finding of a late neurological syndrome in a proportion of patients treated with immune plasma. Although occasionally severe, in general such relapses are benign and self-limiting. They may have an immunopathological basis. Cerebellar involvement in the pathogenesis of Arenavirus infection has also been seen in rhesus monkeys infected with Machupo virus [29]. Although many animals infected with this virus succumb to a fatal haemorrhagic illness, many of the survivors develop

severe encephalitis. Passive protection of animals with specific immunoglobulin is successful in preventing the acute haemorrhagic illness, but late neurological complications are enhanced and may result in death of the protected animals.

Development of any Arenavirus vaccine is likely to proceed slowly in view of the extreme caution one has to observe owing to the high mortality associated with naturally acquired infections and the lymphotropism shown by these pathogens. In the case of Junin, leucopenia is another critically important consideration. However, the guinea pig-adapted XJ strain of Junin has been repeatedly passaged in suckling mice and further cloned in rabbit kidney cells (XJ-Cl_3 strain). Although this variant grows only to low yield in cell culture, it was shown by De Guerrero et al. [26] that virulence for guinea pigs had been lost whilst still retaining immunogenicity of sufficient potency to confer protection against challenge. This material has been boosted by a further passage in mouse brain and used in a limited trial to immunize 636 persons at risk from natural Junin infection. Greater than 90% seroconversion was recorded in the absence of deaths or serious illness. Some evidence of protection among the vaccines is available, but the source of the vaccine together with imprecise details as to its derivation and biological properties has prevented extensive use of the Junin XJ-Cl_3 vaccine. Recently, however, the development of attenuated Junin vaccine has been examined afresh and a candidate vaccine strain is reported to be undergoing laboratory tests (Barrera Oro, J. and Eddy, G.A., personal communication).

Lassa virus infection of rhesus monkeys produces a disease clinically similar to that observed in man. Fever develops by day 7 and a viraemia rapidly develops prior to death. In contrast, the related Mopeia virus from Mozambique does not cause a febrile illness in monkeys and antibodies develop which cross-react by immunofluorescence with Lassa viral antigens. These animals are subsequently protected against challenge from the virulent Lassa virus [46]. Although it has been suggested that Mopeia virus may prove a candidate Lassa vaccine, further information is required on the pathogenicity of this agent for man. Some isolates of Mopeia virus may cause a severe febrile illness in monkeys resembling Lassa fever (Lloyd, G., personal communication).

Ribavirin, also known as Virazole, is a synthetic nucleoside analogue with a broad spectrum of antiviral activity against viruses with both RNA and DNA genomes, and may be administered orally to patients in a dose of 800 mg daily for a month without evidence of toxicity [62,81]. Jahrling et al. [43] found that ribavirin inhibited the growth of Lassa virus in tissue culture infected at low multiplicities of infection. In addition, a pronounced antiviral effect was seen in infected rhesus alveolar cultures infected at all multiplicities and stimulated experiments in vivo. None of 8 infected monkeys treated with ribavirin died and the onset of a much reduced viraemia was delayed by several days.

Filoviridae

Both Marburg and Ebola viruses are indigenous to Africa and are extremely virulent. Great care is required in the handling and examination of suspected or

known positive fluids and this caution hampered serious investigation of the properties prior to the establishment of high security laboratories. Although both agents show minimal antigenic cross-reactivity, they are morphologically similar, most often appearing as long tubular structures. Although initially thought to structurally resemble the rhabdoviruses, new data on both these agents now make a separate family status desirable. The name 'filovirus', meaning thread-like, has been proposed and the criteria for such a classification are summarized by Kiley et al. [48].

Marburg virus

Marburg virus disease, commonly but incorrectly named 'green monkey' disease, is a severe distinctive haemorrhagic febrile illness of man, first described in 1967, when 31 cases with 7 deaths in Germany and in Yugoslavia were traced to direct contact with the blood, organs, or tissue cell cultures from a batch of African green monkeys (*Cercopithecus aethiops*) previously caught in Uganda [60]. Several secondary cases occurred in hospital personnel by contact with the blood of patients. One further case was apparently transmitted by sexual intercourse 83 days after the initial illness, and virus was isolated from the serum. The case fatality rate was 29% for the primary cases, although no deaths occurred in 6 secondary cases. The possible origin of the infection was investigated subsequent to the 1967 outbreak. Complement fixing antibodies to the Marburg virus were reported by a laboratory in the U.S.A. to occur in many African monkeys, but conflicting results were obtained in other surveys. Furthermore, before 1967 many thousands of monkeys had been handled in biomedical laboratories without such outbreaks, and, in addition, experimental infection of monkeys proved uniformly fatal. Despite extensive efforts with both Marburg and Ebola viruses, the natural cycle of transmission of these viruses in various species of animals and the origin of infection remains unknown.

The clinical illness characteristically begins with sudden fever, malaise, headache, and myalgia. Gastrointestinal symptoms soon appear and include nausea, vomiting and often watery diarrhoea. A maculopapular rash appears between the 5th and 7th day of illness and is most marked on the buttocks, trunk and the outer aspects of the upper arms. Conjunctivitis is common. In most patients liver function is impaired during the 2nd week of illness, but jaundice has not been observed. Renal damage manifested by proteinuria, oliguria and rising blood urea levels has been observed. The patients develop a tendency to bleed, particularly from the gums and from needle punctures. Severe haemorrhages may occur, particularly into the gastrointestinal tract.

The first recognized outbreak of disease subsequent to the one initiated in the laboratories in Europe occurred in South Africa in February 1975. The primary case was a young Australian man who had hitchhiked through Rhodesia. He died in a Johannesburg hospital and shortly afterwards his female travel companion and one of the nurses who had cared for him fell ill with the same disease. The women recovered. Virological studies showed that this outbreak was caused by the Marburg virus, and virus was cultured from fluid aspirated from the anterior chamber of the eye 80 days after the onset of the illness, evidence that the Marburg agent can persist in the body for at least 2–3 months after the initial infection [37].

Gross morphological changes found at autopsy were related to haemorrhages in the gastrointestinal tract and the lungs. The principal histological lesions were limited to the liver, kidneys and lungs with the liver as the primary target organ [38]. The kinetics of viral maturation in the liver have been investigated in vervet monkeys [64]. Early in the course of infection, many hepatocytes contained discrete cytoplasmic matrices which increased in number and complexity by the 7th day. Masses of uniformly packed filaments, arranged in parallel array, were found in these inclusions. These filaments or cylinders developed into the core structure of the virus as maturation occurred by budding through plasma or intracytoplasmic membranes.

Ebola

Between August and November 1967, outbreaks of severe and frequently fatal viral haemorrhagic fever occurred in the equatorial provinces of Sudan and Zaire, causing widespread international concern. In Nzara, Sudan, 33 of 70 cases were fatal, and in Maridi, also in the Sudan, the epidemic caused 290 cases, 117 of which were fatal. Of the 230 members of the staff in Maridi Hospital, 76 were infected and 41 died. In Zaire there were 237 cases, including 211 deaths [28]. During laboratory investigations carried out to identify the virus, a member of the laboratory staff of the Microbiological Research Establishment, Porton Down, England contracted the disease but recovered [35].

The prototype of the virus strains isolated has been named Ebola, after a small river in Zaire that flows north of Yambuku, the village of origin of the patient from whom the first isolate of the virus was obtained. It has been established that the viruses isolated both from northern Zaire and southern Sudan are morphologically related but antigenically distinct from Marburg virus [12,44,68]. The clinical picture associated with Ebola virus infection was found to be indistinguishable from Marburg disease. The incubation period ranged from 4 to 16 days, with an average of 7 days. The illness in Zaire was associated with fewer respiratory symptoms than in the Sudan and had a shorter clinical course and a higher fatality rate. Disseminated intravascular coagulopathy was a major feature of the disease, and bleeding occurred in the majority of cases, mainly from the gastrointestinal tract. Jaundice was present in about 5% of the patients who died in Zaire. The disease occurred in all age groups, with a predominance in adults. Transmission from person to person required close contact, particularly with blood or body fluids. The disease may be a zoonosis, but the natural host reservoir is unknown, although, as in Lassa fever, Ebola virus probably has an animal reservoir.

Tissues from 2 autopsies carried out on patients with Ebola virus infection who died in the Sudan and 3 specimens from patients in Zaire revealed that the histological lesions in the liver were identical to those of Marburg virus infection. The most prominent feature was focal necrosis of hepatocytes affecting principally the periportal and midzonal areas. Areas of coagulative necrosis of variable size were also common. Single or multiple eosinophilic inclusion bodies, ranging in size from 5 to 25 μm were found in hepatocytes. Electron microscopy revealed large aggregates in dying hepatocytes and in the bile canaliculi [30]. Precursors of the cores of this virus were found within intact hepatocytes, and these were aligned as membrane-bound aggre-

gates. The complete virions, formed by incorporation from membranes from the surface through which the cores extruded, were mainly in the long tubular form. Many tubular forms had enlarged terminals or 'heads' and some branched and torus forms were identified.

Bunyaviridae

Rift Valley fever

Rift Valley fever, or enzootic hepatitis, is a viral infection that primarily affects sheep and cattle, causing many deaths in pregnant and newborn animals. The natural disease occurs only in Africa and the infection is mosquito-borne. The causative agent was first isolated in 1930 after an outbreak of enzootic hepatitis in cattle near Naivasha in the Rift Valley of East Africa [25]. Since then, large epizootics have occurred at frequent intervals throughout Eastern, Central and Southern Africa. Many infections have occurred in man, particularly through direct contact with infected animals or carcasses. In the 1950 epizootic in South Africa, as many as 20 000 human cases may have occurred, although there were no fatalities. However, there have been several deaths associated with more recent epizootics in South Africa [88]. The virus appeared in 1977 in the Sudan and in 1977–78 was active toward the lower reaches of the Nile in Egypt. A large number of human infections have occurred in these later outbreaks and more than 90 deaths were reported [1]. Laboratory infections have also arisen probably as a result of aerosol transmission of the virus from contaminated fluids and tissues from infected sheep and cattle, although secondary infections are rare.

The Rift Valley fever virion is spherical, with an average diameter of 100 nm and a core component of 80–85 nm. Morphologically it resembles other members of the Phlebovirus genus. The outer envelope contains approximately 160 morphological subunits that project 5–8 nm from the virion [33], and that probably represent the 2 externally situated glycoproteins in the molecular weight range 60 000–75 000 found in other members of the Bunyaviridae family [10]. Particles are found within infected cells adjacent to the smooth endoplasmic reticulum, either as multitubular complexes or contained within a single large vacuole. Inclusion bodies consisting of rods and fine granules are also seen within the host cell nuclei, together with aggregates of fine or coarse granules in the cytoplasm. The genome of Bunyaviruses consists of single-stranded RNA consisting of 3 unique subunits with an approximate total molecular weight of 4×10^6 [10]. By analogy with other members, the genome segment of intermediate size is likely to contain the gene coding for the surface glycoprotein molecules. The disease in man usually follows a transient febrile course, with severe headache and myalgia developing 3–7days after exposure [88]. Complications include facial inflammation, encephalitis and involvement of the eye with macular degeneration and temporary or permanent blindness. Haemorrhage (e.g., gastrointestinal) together with jaundice indicate a poor prognosis. Autopsy of cases in which haemorrhage occurred revealed hepatic necrosis, either widespread or localized in the periportal and midzonal areas, tubular necrosis of the kidneys, pulmonary congestion

and haemorrhages in the stomach and colon. Lambs are highly susceptible to experimental infection and may die from massive necrosis of the liver within 36 h of infection. Mice die with hepatitis within 3 days. Other laboratory rodents are also readily infected. Monkeys develop a mild fever. The pathological lesions are principally those of massive hepatitis in lambs and focal lesions of the liver in older sheep. Eosinophilic bodies are present and resemble the Councilman bodies characteristic of yellow fever infection. Areas of haemorrhagic necrosis may be present in the liver and tubular necrosis in the kidneys.

Numerous isolates have been obtained from mosquitoes, in particualar from *Culex pipiens* during the recent outbreaks in Egypt. Almost certainly sheep, cattle and buffalo and other vertebrates act as primary reservoirs of the virus. Rift Valley fever is of considerable economic importance in Africa and the Middle East when epizootics occur [85]. It is likely, therefore, that virus spread may be limited by effective immunization of sheep and cattle within and around endemic areas. Several candidate vaccines have been developed. For example, virus passaged in suckling mouse brain has been shown to be effective in raising antibodies and a live neurotropic vaccine has been developed for veterinary use [5]. However, such neuroadapted vaccines are unsuitable for use in man and as a result Randall et al. (1962) investigated the use of virus grown in monkey kidney cells subsequently inactivated with formalin. Vaccinated individuals developed long-lasting levels of neutralizing antibodies in the absence of detectable complement fixing and other antibodies normally found in the sera of humans acutely infected with wild-type virus. However, one individual with a low level of antibodies was not fully protected against a subsequent exposure to live virus [9] and it has been noted that Rift Valley virus vaccine may have a teratological effect on the offspring delivered to pregnant ewes inoculated with the vaccine [23].

The process for developing an inactivated RVF vaccine has recently been modified by the use of virus passaged in diploid foetal rhesus monkey cells [45]. Two studies have reported the ability of this vaccine to induce a neutralizing antibody response in individuals given 3 doses over a period of 4 weeks [45,67]. However, antibody levels had waned by 6 months indicating the need for successive booster doses at regular intervals. Clearly there is a need to improve considerably on the potency of Rift Valley fever vaccines of this nature.

Congo–Crimean haemorrhagic fever (CCHF)

CCHF was first recognized in Africa in 1956 and is identical to the agent of Crimean haemorrhagic fever recognized 12 years previously [20]. The disease is transmitted by ticks and has been repeatedly isolated from small mammals. Although the virus is found extensively in Africa, and occasionally in Europe, recent sporadic outbreaks in the Middle East have rekindled interest in this virus.

All of the recent outbreaks have been hospital-associated. The first, in Pakistan, occurred following surgery on an infected patient and 2 of 5 secondary cases died from the disease [95]. Similar secondary infections occurred among staff attending patients admitted to hospital in Baghdad and Dubai, thereby establishing CCHF as an 'exotic' viral pathogen in countries around the Persian Gulf [80,96].

The illness generally follows a biphasic course, early nonspecific symptoms being

followed after the 6th day of illness by haemorrhage from the nose, mouth and gastrointestinal tract. The appearance of large ecchymotic areas on the limbs is a particularly noticeable feature in many cases. Despite widespread haemorrhage accompanied by marked thrombocytopenia and leukopenia, there is no evidence of disseminated intravascular coagulation [6]. By the absence of cellular inflammation in the liver and other organs together with an apparent depletion of lymphoid cells the disease closely resembles yellow fever [6]. The tropism of CCHF virus for lymphoid cells would correlate with similar findings in Lassa, Marburg, Ebola and Dengue, and may represent a common feature in the development of haemorrhagic manifestations.

At present, no information is available regarding the potential treatment and prophylaxis of CCHF.

The virus of CCHF is serologically distinguishable from Hazara virus, the other member of this group. Hazara virus was originally isolated from a tick collected off a mountain vole trapped in Pakistan [7] but is not known to cause overt clinical illness in man. Recent biochemical analysis has shown that Hazara virus is composed of a major nucleocapsid protein (M_r = 52 000) and 3 major glycoproteins with molecular weights of 84 000, 45 000 and 30 000 [36]. This is similar to the polypeptide composition of other members of the Nairovirus genus to which CCHF virus also been assigned [11]. As Hazara virus elicits cross-protection in mice against challenge with CCHF (cited in [36]), it is conceivable that analysis of the relevant antigenic determinants may provide for the development of a polypeptide vaccine against CCHF using Hazara virus as a source of antigen.

Korean haemorrhagic fever

An epidemic form of haemorrhagic fever with renal syndrome (HFRS) was observed in the Korean war zone during the early 1950s and was subsequently described as affecting over 3 000 individuals during a 4 year period [82]. In addition to haemorrhagic manifestations, toxaemia and marked proteinuria, with lower nephron-nephritis and other renal disorders, were typical features of acute human infection. On average, the incubation period is from 2 to 3 weeks and mild forms without haemorrhagic manifestations and proteinuria are common. Approximately 20% of infected individuals show severe illness. Cases continue to be recognized in the Korean peninsula and elsewhere in Asia to the present day. Nephropathia epidemic in Scandinavia is a similar haemorrhagic fever but the clinical picture is dominated by an acute tubular and interstitial nephritis [49]. Both geographically isolated fever syndromes have recently been described as having a common aetiology, and there is an additional relationship with haemorrhagic fevers reported from Japan, China and the Soviet Union [82]. The zoonotic nature of the infection, implying transmission from feral rodents, has been established. In 1976, Lee and colleagues [52] demonstrated by immunofluorescence the presence of specific antibody in cases of Korean haemorrhagic fever using the lung tissue of field mice (*Apodemus* sp.) as a source of viral antigen. Sera from patients with Scandinavian nephropathia epidemica similarly react with virus-infected *Apodemus* lung tissue [84] and recent work has shown that bank voles (*Clethrionomys* sp.) are chronically infected in the endemic region of nephropathia endemica [15].

The causative agent of Korean haemorrhagic fever has recently been isolated by passage in A549 cells and is referred to as Hantaan virus. Morphologically, virus particles of approximately 80 nm diam. are seen by electron microscopy and serologically a close relationship has been found between Hantaan virus and members of the Tete group of the arbovirus family Bunyaviridae [56,91, Johnson, K.M., personal communication]. Many of the viruses in the Tete group have been isolated from birds trapped in tropical and subtropical regions, but none have hitherto been associated with human disease [8]. Other serological groupings of Bunyaviruses, however, contain members of medical importance, for example, the agent of the CCHF group and the virus of Rift Valley fever. Although there is no detailed information on the properties of Hantaan virus and its precise immunochemical relationship with other members of the Bunyaviridae family, there is evidence that antigenic differences exist between Hantaan virus isolates obtained from different endemic regions. Infectivity for mice remains stable at pH 7.0–9.0, but complete inactivation occurs at pH 5.0 and below. Although relatively stable at room temperature and on freezing in the presence of bovine albumin, the virus is inactivated rapidly at 37°C [51].

Serial samples from patients acutely infected with Hantaan virus have been found to contain significant levels of circulating immune complexes [99]. With regard to other viral haemorrhagic fevers only in Dengue have immune complexes been implicated as playing a pathogenic role [86]. The long incubation period of several weeks together with elevated immunoglobulin levels soon after the onset of clinical disease also point to the immunopathological nature of acute infection in man. Immunoglobulin and C3 deposits are also found in the glomerular and tubular regions of the kidney. Examination of liver biopsies has shown that immune complex-like electron-dense deposits are occasionally seen in the glomerular basement membrane up to 5 years following acute infection [50]. However, the exact role of immune complexes in acute infection and the associated nephritis has not been investigated further.

Hantaan virus is found as a chronically infecting agent in feral-rodents trapped in geographical zones where the disease is endemic [53]. In addition, the virus produces a persistent asymptomatic infection in Wistar and Fisher strains of laboratory rats [54]. Virus is detected in kidney, liver and salivary glands of infected animals, a finding similar to the distribution of the virus in feral rodents. Importantly, outbreaks of Korean haemorrhagic fever-like illness with accompanying renal dysfunction have occurred among research scientists and technicians handling rats chronically infected with this virus in both Japan [87] and Europe (Johnson, K.M., unpublished information). The exact origin of the infection in these animal populations remains unknown, but has heightened concern among investigators of haemorrhagic disease that infection of laboratory rodents may be more ubiquitous than otherwise suspected. In addition, zoonotic infection arising from exposure to chronically infected feral or urban rodents may be a significant cause of renal dysfunction in cases where infection occurs in the absence of haemorrhagic manifestations. At the time of this writing, no information is available as to the potential protective efficacy of immune globulins. Attempts to induce active immunization will clearly have to await further investigations into the extent of antigenic variation and immunochemical properties of Hantaan virus.

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