Arthropod-borne
and rodent-borne
viral diseases

Report of a
WHO Scientific Group

World Health Organization
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WHO SCIENTIFIC GROUP ON ARTHROPOD-BORNE AND
RODENT-BORNE VIRAL DISEASES

Geneva, 28 February – 4 March 1983

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<th>Abbreviation</th>
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ARTHROPOD-BORNE AND RODENT-BORNE VIRAL DISEASES

Report of a WHO Scientific Group

The WHO Scientific Group on Arthropod-borne and Rodent-borne Viral Diseases met in Geneva from 28 February to 4 March 1983. In opening the meeting on behalf of the Director-General, Dr S.K. Litvinov, Assistant Director-General, commented that since the last WHO Scientific Group met in 1966 to discuss arboviruses and human disease, the number of arthropod-borne and rodent-borne viruses had risen from 200 to 460, of which 97 were known to be pathogenic for man. The Organization had focused mainly on haemorrhagic fevers in Africa, yellow fever, dengue haemorrhagic fever, Japanese encephalitis, and, more recently, Rift Valley fever and haemorrhagic fever with renal syndrome. A primary goal of WHO had been to assist nations in coping with new diseases and the public health problems they caused. Thus, WHO had geared itself to provide emergency aid in the face of epidemics. The Organization would continue to encourage and coordinate high-quality scientific research relevant to the diagnosis, management, and control of viral diseases and looked forward to receiving the Group’s recommendations on research priorities to guide its future programme.

1. INTRODUCTION

The term “arthropod-borne viruses” was introduced in 1942 to describe members of a group of animal viruses that multiplied in an arthropod and were transmitted to a vertebrate host. The viruses have since been shown to belong to a number of different taxonomic groups, some of which include viruses not transmitted by arthropods. On the recommendation of the International Subcommittee on Viral Nomenclature in 1963, the term “arbovirus” was officially endorsed for the arthropod-borne viruses.

The terms “arthropod-borne” and “rodent-borne” viruses refer to the ecological factors and conditions governing their transmission.
in nature. Most of the viruses considered in this report are included in families and genera of the universal system of classification on the basis of their morphological, morphogenetic, physical, chemical, and replicative properties, but the ecological terms retain considerable practical usefulness.

The names given to the individual agents were at first those of the disease, e.g., yellow fever virus, dengue virus, louping ill virus, type B encephalitis virus of Japan, and equine encephalomyelitis virus. Later, place names were incorporated in several instances, e.g., Western, Eastern, and St Louis encephalitis were accepted as names of viruses to distinguish several apparently distinct agents causing the single disease syndrome of encephalitis. As a result of attempts to isolate and identify agents of diseases, a large number of other isolates were made, and in most cases, the viruses were given names reflecting the source or locality of isolation.

Arboviruses are viruses that are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by haematophagous arthropods or through transovarian and possibly venereal transmission in arthropods; the viruses multiply and produce viraemia in the vertebrates, multiply in the tissues of arthropods, and are passed on to new vertebrates by the bites of arthropods after a period of extrinsic incubation.

This definition is similar to one stated in 1967 in the report of a WHO Scientific Group (1), but it has now been modified to recognize the importance of vertical (transovarian or venereal) transmission in arthropods to the basic maintenance of some arboviruses.

A rodent-borne virus is one maintained in nature by direct intra-and/or interspecific transmission from rodent to rodent without the participation of arthropod vectors. Virus infection is usually chronic in at least a portion of the rodent population and transmission may occur through one or more of the following means: direct contact, salivary or venereal secretions, milk, urine, or intrauterine infection. Host immune responses to infection may be suppressed but acute illness rarely results from infection. Virus is transmitted indirectly to man, usually via the urine or saliva of the chronically infected rodent.

As with any definition, practical considerations dictate that exceptions should be made and that flexibility should be maintained. Viruses are found in bats, rodents, or other vertebrates, whose
transmission by arthropods has not as yet been proved. These viruses are none the less included in the *International catalogue of arthropod-borne viruses* (2, 3) because they are frequently isolated in laboratories dealing with arboviruses and they will be considered in this report. There is another set of viruses, isolated from arthropods and serologically related to rabies virus in the genus *Lyssavirus*. These fit the definition of an arbovirus whereas rabies virus clearly does not. Another set, Marburg and Ebola viruses, will be considered together with rodent-borne viruses although their natural reservoir and mode of transmission are not known but it is strongly suspected that they are harboured by rodents. It must not be assumed that genuine arboviruses can be transmitted only by arthropods; it is known that under certain circumstances certain arboviruses have been transmitted by inhalation, ingestion, and other mechanisms.

During the past 15 years, the vast majority of zoonotic viruses have been placed in the universal system of classification. A remarkable correlation between placement in genera and placement as arboviruses or rodent-borne viruses has emerged. Among the Togaviridae, for instance, all members of the genus *Alphavirus* and nearly all of the genus *Flavivirus* are arboviruses. In the family Bunyaviridae, all four currently recognized genera are composed of arboviruses. The genus *Orbivirus* in the family Reoviridae is composed of arboviruses. These taxa demonstrate distinctive biological properties, undoubtedly linked to evolution, that govern ecological behaviour. These properties may relate to the ability to attach both to arthropod and vertebrate cells, to utilize enzymes common to both arthropods and vertebrates, or to replicate over the wide range of temperatures encompassed by arthropods and vertebrates. It is a challenge of the future to define these properties.

2. CLASSIFICATION OF ARTHROPOD-BORNE AND RODENT-BORNE VIRUSES

2.1 Antigenic properties and classification

The antigenic classification of arboviruses and rodent-borne viruses stems from observations during the 1950s of several scientists and brought together definitively by Casals (4). The haemagglutination-inhibition (HI), complement fixation (CF), and
neutralization (N) tests were used primarily for antigenic classification. The relative specificity of the tests varied depending on the genus. For flaviviruses, the HI test was found to be the most cross-reactive, followed by the CF and then the N test, which was type-specific. The HI test with alphaviruses was less cross-reactive than it was with flaviviruses, but still useful to show group relationships.

The characteristics of cross-reaction and specificity of viruses within a genus are different and are determined by the specificity of each component antigen or epitope on the virus. Alphaviruses have a type-specific, a complex-specific and a group-specific antigen. Bunyaviruses have segmented genomes. The small RNA encodes the nucleocapsid protein, which is responsible for the major CF reaction. The middle-sized RNA encodes the glycoproteins, which are responsible for N and HI reactions. Reassortment of viruses in nature can provide different viruses with the same small RNA segment. These viruses react differently in the N and HI tests and share a common CF reaction. The reverse may also be true. It is thus not possible to generalize among bunyaviruses or other genera in the Bunyaviridae about the specificity of each test. A similar case holds for the orbiviruses, which have 10 or 12 segments. Many different orbivirus serotypes share a common CF antigen. Viruses of one genus do not cross-react serologically with viruses of another.

Monoclonal antibodies have been made to viruses in each of the major genera of arboviruses and rodent-borne viruses. These have been used to identify differences in subtypes and varieties of viruses, a procedure that formerly required antibody absorption, kinetic HI, N, or monospecific sera from wild animals or animals bled very early after immunization.

Recent advances in sequencing RNA directly, or after converting it to a complementary sequence of DNA (cDNA), have opened the possibility of direct comparison of the genomes of closely related viruses. The currently used classification system, however, is still based on reactions in serological tests. The description of viruses conventionally followed isolation of the agent, identification with disease, and the development of serological tests to define the incidence of infection, the range of susceptible host animals, and the duration of immunity.

The central role of the serum-neutralization test in the identification of viruses has led to the proliferation of distinct virus names to populations of genetically similar organisms, especially
among viruses with segmented genomes. Speciation in their insect and vertebrate hosts accentuates the apparent divergence of virus populations. Classification of viruses by the diseases they cause has long been discarded and it is possible that classification by reference to protective immunity may eventually be superseded by comparative analyses of the genetic structure of virus populations.

2.2 Universal system of classification (5)

(a) Togaviridae: Alphavirus. The genus consists of 28 viruses in 6 complexes (6). The viruses have an RNA-associated, non-glycosylated capsid protein and at least two envelope glycoproteins. The envelope contains lipid and thus infectivity is destroyed by lipid solvents and detergents. Alphaviruses are 70 nm in diameter. The viruses replicate in the cytoplasm and mature by the budding of nucleocapsids through the plasma membrane. The genome is of messenger RNA (sense positive), producing 42–50 S and 26 S mRNAs during transcription. The 42–50 S mRNA translates precursor polyproteins, including nonstructural proteins, while the 26 S mRNA translates structural proteins. The proteins are elaborated by post-translational cleavage.

(b) Togaviridae: Flavivirus. The genus consists of 65 viruses in 22 complexes. The viruses have ssRNA, a non-glycosylated capsid protein, a small polypeptide, and one envelope glycoprotein. The envelope contains lipid, and thus infectivity is destroyed by lipid solvents and detergents. Flaviviruses are 40–50 nm in diameter. The viruses replicate in the cytoplasm and mature through intracytoplasmic membranes (mostly endoplasmic reticulum). The RNA is of positive sense; the message is 40–45 S. A precursor protein has not been identified and it has been proposed that multiple initiation sites account for the multiple proteins (7).

(c) Bunyaviridae. The viruses have 3-segmented ssRNA of negative sense. High-frequency reassortment of RNA occurs in closely related viruses. The viruses mature by budding into the Golgi cisternae, presumably acquiring their lipid envelope from Golgi membranes. The infectivity is destroyed by lipid solvents and detergents. There are two glycoproteins projecting through the surface envelope and a nucleocapsid protein. There is also a large protein, which presumably has transcriptase activity, and at least two non-structural proteins. Particles are 90–100 nm in diameter.
The small RNA codes for the nucleocapsid protein and a non-structural protein. The middle-sized RNA codes for the two glycoproteins and a non-structural protein. The large RNA is believed to code for the transcriptase. The glycoproteins determine the major virulence properties of the bunyaviruses.

The genera in the family Bunyaviridae differ in characteristic properties of RNA and proteins.

*Bunyavirus.* The genus was constructed from viruses formerly included in the Bunyamwera super-group, which had distinct serological relationships by the HI test. Many of these viruses have since been characterized in the universal system (8). There are 148 viruses in 16 serogroups, including 3 viruses unassigned to groups.

*Phlebovirus, Nairovirus,* and *Uukuvirus.* There are 36 phlebo-viruses in 7 complexes, including 12 with no complexes assigned; 22 nairoviruses in 6 serogroups; and 7 uukuviruses in a single serogroup. There are also 30 additional members of the Bunyaviridae family in 7 serogroups, including 11 with serogroup unassigned (9). These three genera and viruses with unassigned genus have the same morphology and morphogenesis as bunyaviruses and similar mechanisms of replication.

(d) Reoviridae: *Orbivirus.* There are 121 viruses in the genus, divided into 12 recognized serological groups (10) with 12 viruses not assigned to a serogroup. Revision of the genus is in progress, since some viruses within a single group may be reassigned to new groups and the viruses of the Colorado tick fever group may form part of a new genus. The orbivirus capsid consists of 2 protein shells with an outer diameter of 70 nm. There is no envelope, although infectivity is either sensitive to detergents (uncommon) or moderately resistant. The viruses contain 10 segments of dsRNA except Colorado tick fever virus, which contains 12 segments. There are at least 10 virus-specific polypeptides, of which 7 are found in the virion, and there are at least 2 non-structural proteins. Virions replicate in the cytoplasm and are associated with prominent massed nucleocapsid inclusions and tubular structures. Particles are released by cell lysis. High-frequency reassortment of RNA segments occurs with closely related viruses.

(e) Rhabdoviridae: *Lyssavirus, Vesiculovirus.* There are 53 rhabdoviruses, most of which are arboviruses. They belong to 7 serogroups and include 15 viruses with no group assigned. Rhabdoviruses are bullet-shaped or conical, negative-sense ssRNA viruses, which replicate and mature in the cytoplasm. Some
rhabdoviruses mature in or near distinctive inclusions of massed nucleocapsid material. There are 5 (or sometime 4) major polypeptides designated L, G, N, NS and M in the case of vesicular stomatitis virus. The virus contains transcriptase. A lipid envelope is present and accounts for destruction of infectivity by lipid solvents and detergents. The RNA is transcribed into several positive-strand mRNAs, which are translated in the cytoplasm. The nucleocapsids bud through plasma membrane at sites of G protein insertion, or particles are formed in the cytoplasm and bud through intracytoplasmic membranes. The nucleocapsid is helical.

(f) Arenaviridae. The arenaviruses consist of 11 viruses. These are rodent-borne or, in the case of Tacaribe virus, bat-borne. There are 2 serogroups. The virions are pleomorphic, about 120 nm in diameter, and may contain cell ribosomes acquired when budding through the plasma membrane. The lipid envelope is susceptible to destruction by solvents and detergents. There is a prominent inclusion body made up of ribosomes and nucleoprotein matrix. The ssRNA has two segments (large and small); reassortment occurs with closely related viruses. Viral RNA is transcribed into complementary mRNA, which is translated into a large glycoprotein; this is thought to be post-translationally cleaved. There are two envelope glycoproteins, a nucleocapsid protein, and a transcriptase.

(g) Orthomyxoviridae (genus not established). There are 3 viruses recognized to have 7 segments of negative-sense ssRNA. The viruses are tick-borne and haemagglutinate goose cells.

(h) Filoviridae. Marburg and Ebola viruses (two varieties) constitute this proposed family. They differ from rhabdoviruses in their longer particle length, unique proteins, and smaller axial channel. They are pleomorphic filamentous particles, which are usually 790–970 nm in length, but may be up to 14 000 nm, with a diameter of 80 nm. The particles obtain their lipid envelope by budding through cytoplasmic membranes. They are associated with intracytoplasmic nucleoprotein inclusions. They contain ssRNA of negative sense. There are at least 5 proteins, 1 of which is the envelope glycoprotein that makes up the surface projections, and 2 are nucleoproteins associated with the helical nucleocapsid (II).

(i) The single viruses in the families Iridoviridae, Picornaviridae, and Coronaviridae may represent unusual adaptations of viruses to arthropods or vertebrates. There are many viruses that have not been characterized fully and some may belong to new taxa.
3. GLOBAL AND REGIONAL PUBLIC HEALTH PROBLEMS

3.1 Global public health problems

Of almost 500 viruses registered in 1982 in the *International catalogue of arboviruses*, including certain other viruses of vertebrates, about 100 were recorded as causing either subclinical or clinical infections in man. Among them, 36 are prevalent in more than one of the great geographical regions and 31 of these may be associated with clinical syndromes, such as fevers, encephalitis, or haemorrhagic fever (Table 1). However, only a few of these viruses are of global or major regional public health importance, either in terms of morbidity and mortality or in terms of measures that are necessary for the prevention or containment of outbreaks. The most important are: yellow fever, dengue 1–4, Crimean–Congo haemorrhagic fever, Marburg, Lassa, Ebola, tick-borne encephalitis, Japanese encephalitis, Venezuelan equine encephalitis, St Louis encephalitis, eastern and western equine encephalomyelitis, West Nile, and Hantaan viruses. The spread of these viruses to several regions may cause international health problems.

3.1.1 Factors enhancing the interregional spread of these viruses

Arthropod-borne and rodent-borne viruses tend to have localized natural foci with specific receptive vertebrate and invertebrate hosts, which play an amplifying role in transmission cycles. Man can be implicated either as an incidental host in a dead-end cycle or as an active reservoir host causing other human infections, either by direct contact or through specific insect vectors. In the first case, the spread of the virus in human populations is linked to a geographical extension of the animal reservoir and of insect vectors, if they are involved in the transmission cycle. In the second case, man being an active reservoir can himself disseminate the virus outside the natural foci. Many factors that may disturb the natural equilibrium have been proved to be involved or incriminated in the geographical spread or transmission of these viruses. These include ecological changes, intrusion of man in the mosquito and rodent habitats, deforestation, irrigation, uncontrolled extensive urbanization resulting in the lack of piped water and storage of water at home,
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*These viruses are considered present to be of public health importance.
population movements, increase in travel facilities, animal trade, and long-distance bird migrations.

3.1.2 Present trends

The viruses enumerated above have already extended their areas of occurrence and will probably continue to do so in warm climates in cities and villages where mosquito vectors are present in increasing densities. Other viruses may follow the same trend, such as chikungunya virus, which is transmitted by *Aedes aegypti*. O'nyong-nyong virus might appear again where the vectors, *Anopheles funestus* and *Anopheles gambiae*, are extending their habitats.

The absence of yellow fever in Asia, India, and Oceania, areas where dengue is endemic, is a most intriguing problem. It has been demonstrated the *Aedes aegypti* specimens from different places in South-East Asia could be infected experimentally and could transmit the yellow fever virus to laboratory animals. Although one colonized strain of *Aedes aegypti* from Thailand had a relatively high infection threshold and a low percentage of mosquitoes infected, the hypothesis that vector competence accounts for the absence of yellow fever from Asia is neither proved nor disproved. Monkey species of South-East Asia are susceptible to the virus and could play the role of amplifier hosts. Two hypotheses attempt to explain an apparent barrier against yellow fever but are not satisfactory. In laboratory conditions, dengue-infected mosquitoes were poor vectors of yellow fever, but this mechanism would be effective in the field only if a majority of mosquitoes were infected by dengue virus at any possible place of entry of the yellow fever virus. Antibodies to dengue and some other flaviviruses, such as Wesselsbron and Kyasanur Forest disease viruses, decrease the level of viraemia when monkeys are challenged with yellow fever virus, but this mechanism would be effective in the field only if a majority of persons have been infected previously with cross-reacting viruses. More studies are necessary, particularly to elucidate whether a single infected mosquito or person may start an outbreak, either in a cross-immune or in a non-immune human population.

3.1.3 International public health measures

Yellow fever is the only arthropod-borne virus disease that is dealt with in the International Health Regulations (12). These
regulations provide that any suspected case should be notified urgently to the World Health Organization, travellers coming from infected zones and going to receptive zones where *Aedes aegypti* exists should be vaccinated, and aircraft and ships travelling between these zones should be disinfected. Misunderstanding of recommendations has led to excessive quarantine measures, and small boats, light aircraft, and road vehicles can easily evade the prescribed measures. The accent is now on improving national surveillance systems to detect imported communicable diseases and locally occurring cases so that containment measures can be started rapidly. It is critical that the alert should be given without any delay and that information should be exchanged with neighbouring countries and rapidly disseminated by WHO.

The efficacy of preventive or control measures relies on countries being prepared by contingency planning and adequate emergency services. Among the measures of preparedness, WHO has constituted stocks of insecticides and sprayers, protective clothing, and yellow fever vaccine, with a strategic reserve of seed-lot that will make it possible to meet with minimum delay a demand for several million doses should a very densely populated area be at risk.

3.2 Regional public health problems

3.2.1 Africa

The following arbovirus diseases are of concern in Africa south of the Sahara: yellow fever, Lassa fever, Marburg virus disease, Ebola virus disease, Rift Valley fever (RVF) and, to a lesser extent, Crimean–Congo haemorrhagic fever.

Outbreaks of yellow fever occur between the 15°N and 10°S parallels and more frequently in West Africa than in Central and East Africa. Some outbreaks have caused a considerable number of fatalities, e.g., in Ethiopia in 1960–62 with at least 100 000 cases and 30 000 deaths in a population of about one million persons. During the past decade, limited outbreaks have occurred in almost all countries of West Africa. Surprisingly, *Aedes aegypti*, although present in high densities in Africa, does not seem to have played a major role in these outbreaks. Instead, other mosquito species were incriminated in the transmission of the virus, such as *Aedes simpsoni*, *Aedes africanus*, and other species that have indifferently sylvatic or semi-urban habitats and transmitted the virus in rural areas and
small cities. These species were also responsible for sporadic cases of sylvatic yellow fever. Official statistics provided for outbreaks and sporadic cases are generally underestimated and epidemiological surveys frequently show a higher incidence rate than first disclosed. Ecological studies were carried out in the past in East Africa and demonstrated the existence of three cycles of transmission of yellow fever virus: sylvatic from monkey to monkey by *Aedes africanus*, rural from monkey to man by *Aedes simpsoni*, and urban from man to man by *Aedes aegypti*. Recent studies in West and Central Africa have shown the amplifying role of monkeys in the Congolese forest or riverine forests up to the Sudanese savannah belts, where epidemics may erupt. The virus might be maintained during the dry season through transovarian transmission in mosquitoes for two or three consecutive years in a series of moving secondary enzootic foci. Limited vector control and vaccination campaigns have been efficient tools to halt outbreaks, but consideration is now given to preventive mass vaccination of populations at risk in enzootic and epizootic zones.

The first recognized epidemic of Lassa fever occurred in Nigeria in 1969. Since then, seroepidemiological surveys have established that this virus is active in all West African countries between Senegal and Zaire. In Sierra Leone alone, the disease accounts for 10% of all febrile illnesses admitted to hospital and for 1.7% of the general death rate. Recent serological surveys have established Lassa virus antibodies in 5% of human sera and 25% of fever patients in hospitals in Liberia. Village surveys showed a 1–8% infection rate, and in a single village the infection rate was 14%. Surveys among hospital staff and patients in Liberia showed that this virus had infected people in all regions. *Mastomys natalensis* has been identified as the principal rodent reservoir host of Lassa virus.

*Marburg* (MBG) virus was first isolated in 1967 in the Federal Republic of Germany and in Yugoslavia from an outbreak of a highly fatal haemorrhagic disease among laboratory workers who had had contact with tissues of African green monkeys recently imported from Uganda. Since then the virus has been isolated in South Africa in 1975 from two travellers from Zimbabwe, in Kenya in 1980, and again in Zimbabwe in 1982. Serological investigations of the Zimbabwean case showed antibodies in a household dog and a rodent from the nearby bush. Seroepidemiological surveys have detected infection in human populations in the Central African Republic, Liberia, Kenya, and Zimbabwe. Antibodies to this virus,
as well as to Ebola (EBO) and Rift Valley fever (RVF) viruses, have recently been demonstrated in East African primates.

Ebola virus caused two epidemics of haemorrhagic disease with hundreds of notified cases and 326 deaths in the Sudan and Zaire in 1976. The disease reappeared in the Sudan in the same focus in 1979, producing 34 confirmed cases and 22 deaths. Serological surveys in humans have established the presence of antibodies to Ebola virus in Kenya, Liberia, the Sudan, and Zimbabwe.

In summary, serological surveys found Ebola virus seroactivity in rain forests in East, Central, and West Africa, and Marburg virus in East, Central, and southern Africa. The presence of antibodies in persons who gave no history of illness or contact with known cases suggests that these viruses usually cause mild or even asymptomatic infection in man. An alarming finding is that once they have become adapted to man through contact with reservoir hosts, they may become more contagious and highly transmissible from person to person.

Congo virus is enzootic in a wide area of Africa, from Senegal and Nigeria eastwards to Kenya and Ethiopia and southwards to South Africa. It has been shown to be identical to the agent of Crimean–Congo haemorrhagic fever. It occurs in limited foci in semi-deserts or in savannah zones with long dry seasons. Sporadic human cases have been confirmed by isolation of the virus from patients, but the incidence is certainly higher, as virological examinations cannot easily be carried out in the areas of prevalence. The disease in Africa seems to be milder than that in eastern Europe. No nosocomial cases have been described in Africa, but laboratory-acquired infections have been documented. A few serological surveys have demonstrated a very low prevalence in human populations and up to 8% antibody in cattle. The virus has been isolated from tick species belonging to several genera: *Hyalomma, Amblyomma, Boophilus*, and *Rhipicephalus*.

Dengue fever. Isolates of dengue type 1 and type 2 viruses have been obtained from humans in the following West African countries: Burkina Faso, Guinea, Ivory Coast, Nigeria, and Senegal. Similar isolates have been recovered from sylvatic mosquitoes and from monkeys, suggesting the existence of a jungle cycle in certain parts of West Africa. Dengue type 2 was also obtained offshore of East Africa in Seychelles between 1976 and 1979 and in Réunion. Dengue-like epidemics have occurred in East Africa in the past and dengue virus was isolated recently in Kenya.
Chikungunya virus caused in outbreak of febrile illness in the United Republic of Tanzania in the 1950s and has since then been isolated in East and West Africa. A closely related alphavirus, ONN virus, caused an epidemic of chikungunya-like disease that swept through eastern Africa between 1959 and 1960. The attack rate of ONN virus was over 90% but there were no fatalities directly attributed to this infection.

Rift Valley fever. Until recently RVF had not been an important human disease because outbreaks observed in sub-Saharan Africa for the past 50 years affected domestic animals and produced only mild disease in a few people working closely with these animals. However, the first recognition of several cases of fatal RVF in man during an outbreak was reported in South Africa in 1975 and in Zimbabwe in 1978. Zinga virus, formerly isolated in different countries of West and Central Africa and in Madagascar, has been shown to be identical to RVF. There seem to be at present two different epidemiological patterns for RVF: epizootics and epidemics in eastern and southern Africa, enzootic and endemic incidence in western Africa.

Association of arboviruses with fevers of undetermined origin. The role arboviruses play in fevers of undetermined origin is not well established in Africa because some of the symptoms produced by arboviruses are similar to those elicited by protozoal and bacterial infections, such as malaria and typhoid fever. Because of the prevalence of malaria and, to a lesser extent, of typhoid fever in tropical Africa, most fever patients are subjected to the trial use of antimalarial drugs and antibiotics; confirmatory laboratory tests are rarely performed owing to the lack of facilities or manpower. Viral infections are therefore first suspected as the cause of illness when fevers fail to respond to the trial treatment. Specimens taken at this time do not yield virus, hence the cause of the illness is unlikely to be recognized unless serological tests are conducted. There is therefore a need to determine the role of arboviruses in public health by virus isolation from acute-phase sera of fever patients and/or by seroconversion.

3.2.2 The Americas

3.2.2.1 Dengue and systemic febrile illnesses. All four serotypes of dengue virus occur in the Americas. Extensive outbreaks caused by dengue virus types 2 and 3 were reported in the Caribbean and
northern South America in the 1960s and 1970s. Conservative estimates indicate that at least 650,000 cases occurred in Colombia during the outbreaks caused by serotypes 2 and 3, in 1971–72 and 1977, respectively.

In 1977, a type 1 pandemic began in Jamaica and spread from there clockwise around the Caribbean causing epidemics in almost every island. By late 1977, epidemics occurred in the Guianas and Venezuela, and in 1978 in Colombia and Central America. The spread continued through Mexico in 1978–79 and in 1980 it reached Texas, where it caused the first recorded case in the USA since 1945. About 550,000 cases were registered in Cuba, and an estimate made in Colombia indicated that 770,000 cases had occurred. Only about 700,000 dengue cases were notified by countries to the Pan American Health Organization during 1977–80.

Two important events were registered in 1981: the introduction of dengue virus type 4 in the Americas and the first outbreak of dengue haemorrhagic fever in the hemisphere.

Dengue virus type 4 activity was documented in many islands of the Caribbean during 1981–82. Outbreaks were also recorded in Brazil, Colombia, and Suriname in 1982. Both dengue type 1 and dengue type 4 were isolated in the Brazilian outbreak, the first time that dengue viruses have been isolated in the country. Illness associated with dengue type 4 viral infection has been self-limited and generally mild.

From May to October 1981, Cuba experienced a widespread outbreak of dengue virus type 2 during which 344,203 cases were notified. In addition to the classic benign febrile syndrome, serious haemorrhagic and shock manifestations were present. A total of 116,143 cases were hospitalized, of which some 10,000 were cases of DHF/DSS. In all, 158 deaths were recorded, one-third of which were among persons over 15 years of age. An intensive *Aedes aegypti* eradication programme brought the outbreak under control. No more dengue cases have been identified in Cuba since the end of 1981.

**Oropouche virus disease.** During the period 1961–81, at least 250,000 persons were infected by Oropouche virus in the Amazon region of Brazil. Patients usually developed a febrile illness and sometimes aseptic meningitis. A rash was occasionally observed. The disease was not fatal and there was an absence of sequelae. The infection at times caused severe manifestations, including one or more episodes of recurrence of symptoms, and sometimes required
hospitalization. Some outbreaks were explosive and caused temporary disruption of community activities. Epidemics occurred in urban settings, where the virus is biologically transmitted from person to person through the bite of the midge Culicoides paraensis. Oropouche virus is the only arbovirus of human public health importance known to be transmitted by Culicoides. The mosquito Culex quinquefasciatus may act as a secondary vector.

Mayaro virus disease. Immunity to Mayaro virus is widely distributed in human populations of rural areas of tropical South America. In some localities up to 60% of the population have demonstrable antibodies to this alphavirus, but only a few outbreaks due to this agent have been described. No fatalities attributable to Mayaro infection have been reported, but patients may exhibit severe arthralgia, particularly of the extremities, which may cause temporary incapacitation.

3.2.2.2 Arboviral encephalitis. Most arboviral encephalitis present in the Americas is associated with six arboviruses.

St Louis encephalitis (SLE). Although SLE virus is widely distributed in the hemisphere, only in North America is the disease recognized as a public health problem (13). It is estimated that as many as 10,000 cases of encephalitis and about 1000 deaths have been recorded since the discovery of the virus in 1933. The great majority of them were registered in the USA. The largest epidemic in the history of the disease occurred in 1975, when 1815 cases were recorded in that country. Outside Canada and the USA, a single outbreak was recorded, in Mexico in 1974, when 51 cases were diagnosed. Seven cases of encephalitis with serological evidence of SLE virus infection were reported from Argentina, Jamaica, Suriname, and Trinidad and Tobago, between 1953 and 1965. The few cases of SLE virus infection confirmed by isolation of the agent in Central and South America have generally been characterized by relatively mild febrile illness. In the continental USA, all but six states have reported cases of SLE. Several important urban outbreaks have occurred in some of these states. The incidence of severe disease is 5-40 times higher in persons over 60 years of age than in the age group 0-9 years.

California group viral encephalitis. La Crosse virus is responsible for 30-160 cases annually in the USA (13). Isolated cases have been reported from Canada. The virus principally affects children under 12 years of age and has a case-fatality rate of less than 1%. The disease is highly focal in distribution, depending on the presence of
hardwood forests, tree-holes being one of the main breeding sites of its prime vector, Aedes triseriatus. In recent years, however, foci of infection have consistently been located around rubbish dumps and playgrounds, were the mosquito has been found breeding in discarded tyres. Three closely related viruses, those responsible for snowshoe hare, Jamestown Canyon, and California encephalitis, may also be implicated less frequently.

*Eastern equine encephalomyelitis (EEE)*. EEE is a rare but severe disease of man in the Americas. The overall case-fatality rate among clinical cases in North America approaches 70%. Outside the USA, one outbreak was recorded in the Dominican Republic in 1948–49, one in Jamaica in 1962, two cases from Trinidad and Tobago, and one from Brazil. Several outbreaks have been described among horses, and among exotic birds such as quails, pheasants, Peking ducks, and partridges. In the latter instance, attack rates of up to 50% have been recorded in North America, causing serious economic losses.

*Western equine encephalomyelitis (WEE)*. WEE in man is basically an exclusive problem of Canada and the USA. A total of 897 cases of WEE were recorded in the USA during the period 1955–76 (13). The highest incidence was observed in 1965, when 172 cases were recorded. The only case registered in Latin America and the Caribbean was diagnosed in Rio de Janeiro in 1961 on clinical and serological grounds. Epizootics among equines have been documented in Argentina, Brazil, Guyana, and the USA. Flocks of pheasants and partridges have also been stricken by the agent, but the outbreaks are not so devastating as those caused by EEE virus.

*Venezuelan equine encephalitis (VEE)*. VEE is endemic in Central America and northern South America as well as in Mexico, Trinidad and Tobago, and the State of Florida in the USA. It appears periodically as epizootics and epidemics, as observed during 1967–71, when the virus spread from South America through Central America and into the USA. Over 100 000 equine deaths and hundreds of thousands of human infections occurred during this period. About 1% of infected persons developed clinical encephalitis. In the absence of adequate medical care, case-fatality rates as high as 20–30% have been reported in children less than 5 years of age. Virus activity has been silent in recent years, probably owing to intensive vaccination control programmes in horses.

*Rocio virus disease*. Rocio virus disease is a focal disease that has occurred exclusively on the southern coast of São Paulo State,
Brazil. The virus apparently emerged for the first time in 1975 and for two consecutive years caused outbreaks during which about 1000 clinical cases were diagnosed. The overall case-fatality rate among hospitalized patients was about 13%. Approximately 20% of the survivors exhibited significant residual impairment of cerebral functions. Transmission was associated with forest contact and most cases were seen in persons over 15 years of age. No more clinical cases have been observed since 1976.

3.2.2.3 Haemorrhagic fevers

Yellow fever. Jungle yellow fever continues as a major threat in endemic areas of South America; ten countries reported a total of 1204 cases in the past decade — Bolivia, Brazil, Colombia, Ecuador, Panama, Paraguay, Peru, Suriname, Trinidad and Tobago, and Venezuela. A 63% increase in the number of reported cases was observed in the second half of the 1970s, as compared with the first half of the same decade. A total of 487 cases (provisional figures) were notified from 1980 to 1982, 80% of which occurred in Bolivia and Peru. Colonists and temporary agricultural workers from non-endemic areas, together with forestry workers from endemic zones, were the main target of the disease. The virus is enzootic in the tropical forests of South America, such as those of the Amazon region and the Orinoco and Magdalena valleys.

The periodic occurrence of YF outbreaks in central Brazil, at intervals of 5–9 years, may be due to virus excursions from the Amazon region. Although transovarian transmission of YF virus in Aedes aegypti and in Haemagogus has been documented in the laboratory, limited field studies do not support the occurrence of this phenomenon in nature. Notably, YF virus is able to reappear in areas after being silent for long periods of time. The outbreaks in the Tarra River and in Sierra Nevada, in Colombia, and in Trinidad and Tobago in 1978–79, after 19 or more years of silence, demonstrate the potential of resurgence of the virus. Similarly, the 1981 outbreak in Bolivia occurred after more than three decades of absence of the disease. Of special concern was the occurrence in some countries of cases in close proximity to areas infested with Aedes aegypti and the consequent risk of urbanization of YF. Most cases of YF continue to occur in the first half of the year, peaking in March–April. The great majority of cases in the Americas are in males over the age of 15 years, who are infected in the forest.
Junin haemorrhagic fever (JHF). Since the early 1950s, JHF has been recognized as a major public health problem in certain agricultural areas of Argentina. Over 18,000 cases were reported in that country from 1958 to 1980, with a mortality rate of 10–15% in untreated patients. A gradual increase in the endemic area of JHF has been observed since 1958. The infection occurs almost exclusively among maize- and wheatfield workers. The disease has a marked seasonal variation, with the highest incidence of cases in April, May, and June.

Machupo haemorrhagic fever (MHF). The first outbreak of MHF was identified in 1962 and subsequently several others were detected, all in the 1960s. The two main outbreaks occurred in the community of Orobayaya and in the town of San Joaquin, but hamlets and farms of the same department (Beni) experienced sizeable epidemics. Altogether, it is estimated that 2000–3000 persons were affected by the disease, with a case-fatality rate of about 20%. A small nosocomial outbreak involving 6 persons, 5 of whom died, was reported in Cochabamba in 1971.

An effective rodent control programme against infected Callomys calosus, the host of MHF virus, has been undertaken by the Bolivian authorities in the infected area and as a result no human cases of MHF have been registered since 1974.

Haemorrhagic fever with renal syndrome (HFRS). Antibodies to Hantaan virus, the cause of HFRS, have been found among urban rats collected in some cities of the USA and in human and urban rat populations of the Amazon region of Brazil.

3.2.3 Eastern Mediterranean

Rift Valley fever erupted as an epidemic in Egypt, for the first time in history, during the summer of 1977. The epidemic exhibited certain characteristics differing from those of previous outbreaks elsewhere. There was heavy mortality among sheep and lambs, extensive human disease (an estimated 20,000–200,000 cases), with substantial clinical complications and relatively high mortality.

RVF virus had previously been limited to sub-Saharan Africa; epizootics occurred in the Sudan in 1973 and 1976 and enzootic RVF is apparently widespread in that country. RVF virus was probably introduced from the Sudan into neighbouring southern Egypt in 1977 by domestic animals, possibly in sheep, which develop high
levels of viraemia. *Culex* mosquito transmission, inhalation of infectious aerosols, and direct contact with sick animals are all thought to have played a role in disseminating the virus from infected to susceptible humans in densely populated agricultural areas of Egypt.

In late June 1978, there was a second RVF outbreak in Egypt. About 400 human cases were officially recorded between June and December, but, as in 1977, morbidity was undoubtedly higher than the official records show. In 1979 and 1980, there was little evidence of RVF infection in humans and domestic animals. The virus may still circulate in Egypt but, if so, it goes undetected. The maintenance cycle of RVF is not understood and there is a considerable threat of its reappearance in Egypt and neighbouring countries. A high level of surveillance should be maintained.

From seroepidemiological surveys, West Nile, Sicilian phlebotomus fever, and Sindbis viruses are the three main arboviruses endemic in Egypt. A study on sera from rodents showed the establishment of certain viruses (Matariya, Matruh, Wanowrie, Bahig, Wad Medani, Thimiri, Chenuda, Crimean–Congo haemorrhagic fever, Nyamanini, and Kemerovo viruses); some of these were associated with migrating birds and ticks in their epidemiology (14).

Crimean–Congo haemorrhagic fever (CCHF) virus presents a threat to persons in rural, semi-desert areas heavily infested with *Hyalomma* ticks. This species bites humans more readily than other ticks and is thus more often implicated in human cases than are other species. *Hyalomma* populations may increase in climatic cycles or during environmental changes caused by war, floods, irrigation, and land reclamation. *Hyalomma* ticks have been implicated in epidemics and sporadic cases of CCHF. Nosocomial infections have been common where the disease was unexpected and undiagnosed: medical personnel, hospital attendants, and household members caring for infected haemorrhagic patients frequently became seriously ill and some died.

In Pakistan in 1976, a CCHF patient admitted with haematemesis infected five persons; two died in addition to the patient. In 1979, the first reported outbreak of the disease occurred in Iraq; two patients were infected through nosocomial spread from an index case and two others were infected elsewhere; the initial three patients died. Afterwards, numerous other Iraqi villagers suffered from it. Two months after the Iraq outbreak, the first CCHF outbreak was
reported in Dubai in November 1979. Five nosocomial infections occurred; the index case and two nurses died.

The recent cases and fatalities in Dubai, Iraq, and Pakistan, and another in South Africa, demonstrate the need for CCHF surveillance wherever Hyalomma ticks occur in Africa, Asia, and Europe, even where the virus has not yet been recognized.

CCHF virus prevalence in Egypt was investigated by CF tests in 1174 sera (433 from humans, 741 from domestic animals). Antibodies to the virus were detected in sera from sheep, camels, buffaloes, and rodents. Six of the 30 species of tick demonstrated to be reservoir-vectors of the virus are common in Egypt. The most important of these are Hyalomma (H.) anatolicum anatolicum and H. marginatum rufipes. Sporadic human cases of the disease are possibly more common in Egypt than is realized and might become more evident through nosocomial cases, such as those in Dubai, Iraq, and Pakistan.

The prevalence of arthropod-borne and rodent-borne viruses has recently been investigated in two other areas of the Eastern Mediterranean. Between 1969 and 1971, WHO, in collaboration with the University of Teheran Institute of Public Health Research, surveyed rodent-related diseases in the Islamic Republic of Iran. The results showed the presence of West Nile (WN), Far-Eastern tick-borne encephalitis, Sindbis, and Bhanja (BHA) viruses.

A serological survey of rodents collected from Pakistan in 1979 showed high prevalence rates for Wad Medani (21.6%), BHA (16.6%), Dera Ghazi Khan (10.2%) CCHF (9.5%), and phlebotomus fever (Naples) (9.5%) viruses. The highest prevalence rates in humans and domestic animals were for West Nile (10.2%) and BHA (8.8%) viruses.

3.2.4 Europe

3.2.4.1 Tick-borne European meningoencephalitis. Tick-borne encephalitis was first identified in 1932. It has two subtypes, Central European encephalitis and Far-Eastern encephalitis (also called Russian spring-summer meningoencephalitis), and is clinically the most important European arbovirus infection among 15 arboviruses proved to infect man. This flavivirus is transmitted in Europe by the common castor-bean tick Ixodes (I.) ricinus (in Asian USSR mainly by I. persulcatus) in small but numerous permanent natural foci in mixed deciduous forests, young forest, underbrush, and forest-fringe
habitats, where the ecological conditions, temperature, and vegetation are suitable for ticks and reservoir hosts. Small mammals with short generation times and high reproduction rates, such as field mice and the voles Clethrionomys glareolus and Apodemus flavicollis, are the primary vertebrate hosts. Transmission to man is through bites by all three stages of free-living ticks, which remain infected for life. Infection can, however, be transmitted also by raw milk from infected domestic animals, particularly cows, goats, and sheep, and by inhalation in laboratories. Those most often affected are forestry workers, hunters, tourists, and mushroom collectors. There is no transmission from person to person.

This disease occurs in two forms: a very severe form taiga encephalitis, frequently lethal, seen in the Soviet Far East; and a milder form, seen in Europe. In the following countries, TBE is of particular importance: Austria, Czechoslovakia, German Democratic Republic, Federal Republic of Germany, Hungary, Poland, Switzerland, the USSR, Yugoslavia; less frequently in Bulgaria, Finland, Romania, and Sweden; and very exceptionally in Denmark and France. Serological evidence for the disease was found in Albania, Greece, Italy, Norway and Turkey. The questions to be answered are as follows:

—Are all the geographical areas where TBE occurs defined? Are the many blank spots on the map correct?
—What is a typical biotope in ecological terms? Is it a focus composed of one or a number of different ecological biocenoses in which the virus circulates independently?
—Are the foci temporary or permanent?
—Are the foci linked with the two tick species only? (The transmission potential of Haemaphysalis concina has also been demonstrated.)

There is a need for the systematic mapping of tick distribution and for the delineation of the focal areas—clinically, serologically, and by isolation of the virus in man, tick, and animal. Such studies should then be followed by detailed zoological and virological studies to demonstrate the critical mammalian carrier host(s) responsible for the maintenance of the focus.

Among other arboviral diseases in Europe are the recently observed new Pogosta disease in Finland in 1974 and 1981, caused by an arbovirus similar to Sindbis virus, presumably carried by the Culex mosquito and perhaps imported by migrating birds; and
Ockelbo disease, first observed in 1960 and occurring annually in late summer. The latter is found in Sweden just north of the 60th parallel and is associated with a Sindbis-like virus (many cases occurred in the east-central part of the country in 1981). It is characterized by an epidemic polyarthritis with an exanthem. A similar disease has also been recorded in the USSR.

3.2.4.2 Haemorrhagic fever with renal syndrome (haemorrhagic nephrosonephritis). The disease is found in Europe in rural areas in early winter in two geographically distinct areas:

(1) In Finland, roughly north of the 60th parallel, it first occurred in military personnel in 1942–43 with over 6000 cases. There are now about 10–100 cases per year. It was first described in Sweden in 1934 as nephropathia epidemica (279 cases in Umeå). In Denmark there were seven cases in Svendborg. In Norway in 1949, there were about 46 cases in an area north of Oslo. There have been cases in adjoining parts of the USSR (Murmansk and the Baltic republics). The disease has been encountered since 1957 and 1962 with a periodicity of 3–4 years. The clinical picture is milder than in China, the Republic of Korea, and the far-eastern part of the Soviet Union.

(2) In Bulgaria in 1953–77 there were 389 cases, and in Yugoslavia in 1951–68 some 340 cases. The disease is known as epidemic nephritis and is to be distinguished from nephropathia balkanica in Bulgaria and Yugoslavia, which has a chronic character and is still of uncertain etiology. It is rare in Czechoslovakia, Hungary, and Romania, and in the European part of the USSR (where it was called Tula fever).

The disease was recently observed in urban populations in the above countries. About 500–2000 cases occur annually in the Russian Soviet Federal Socialist Republic and at least 300 in the far-eastern part of the Soviet Union. Fatality rates vary from 1% to 11%, but in the Yaroslavl epidemic the fatality rate was 30%. Larger epidemic outbreaks with at least 1500 cases occurred in 1964 in Bashkiria.

In Finland, the Puumala agent, which seems to be related to the Hantaan virus, has been detected in the lungs of the bank vole Clethrionomys glareolus. On the basis of clinical and serological differences, Finnish authors postulate the existence of 2–3 virus serotypes. Apodemus sylvaticus and Apodemus flavicollis in vegetable gardens invaded by voles, and Rattus norvegicus in urban and port areas, are also thought to be hosts. The disease is believed to be spreading slowly in Europe: in 1981, it was demonstrated
serologically in healthy persons and patients with glomerular disease in northern Greece adjoining Bulgaria and Yugoslavia. As, until recently, the disease presented diagnostic difficulties and was, moreover, not reportable except in the USSR, the exact numbers of cases occurring in Europe are unknown. Cautious assessment of published material gives the impression that they are higher than assumed, as probably only severe cases come to the attention of the medical services. An evaluation of the size of the problem and the distribution of the disease and a review of the, as yet, unanswered epidemiological questions in Europe are necessary, particularly with regard to the transmission, virulence, persistence, and pathogenesis of the disease in man. Special attention should be paid to the danger of spread of the disease to new areas.

3.2.4.3 Crimean–Congo haemorrhagic fever. Knowledge of this disease was acquired from epidemics that occurred in 1944–45 as a consequence of the environmental disturbances caused by the Second World War in the Crimea and of improvement work in the swampy areas of virgin steppes for collective agriculture in various parts of the USSR. Epizootics occurred depending on the density of the tick *Hyalomma marginatum* and other species infecting persons working in agriculture and rural industries, woodcutters, and persons collecting mushrooms or picnicking during the summer months. While there have been no recent cases in Moldavia, the disease has continued to occur sporadically in natural foci in the Crimea and Astrakhan areas. The virus was isolated in 1967 and found to be identical to the Congo virus of the Bunyaviridae family, which causes a similar disease in Africa, and related to a third virus, the tick-borne Hazara virus of Pakistan.

In Central European countries, serological evidence of the circulation of the virus has been obtained in a wide range of animals—cows, horses, hares, and birds, which are thought to be responsible for the geographical spread of the disease as observed in recent times. Person-to-person transmission occurs by close contact with a patient and through blood or blood-contaminated material. In Bulgaria, the disease was first observed in 1946 and is supposed to have been introduced by tick-infested horses or fodder during the Second World War.

The disease was intensively studied between 1952 and 1968. It has continued to occur sporadically in numerous foci in persons working in agriculture, animal husbandry, or forestry. Between 6 and 34 cases were registered yearly in the period 1970–77. Specific globulin for
treatment has been prepared and selective vaccination is carried out in laboratory workers and in the exposed population in the focal areas. In Bulgaria, the virus has been isolated from the ticks *Hyalomma plumbeum*, *Rhipicephalus sanguineus*, and *Boophilus calcaratus*. Antibodies were detected in all types of domestic animals as well as in numerous wild animals, lagomorphs, field mice, and other rodents. Foci have also been observed in Yugoslavia with sporadic cases in humans in the period 1954–67 and one epidemic in 1970. Serological evidence of the circulation of the virus has also been demonstrated in France, Greece, Hungary, and Turkey. The existence of other silent foci in a wide range of ecological areas is possible. No importation into other parts of Europe is on record.

3.2.4.4 *Omsk haemorrhagic fever* (similar to Kyasanur Forest fever) has shown very little activity in recent years. It is a flavivirus, transmitted by the ticks *Dermacentor pictus* and *D. marginatus*; these are probably not the main vectors in view of recent direct transmission from muskrat to man.

3.2.5 *South-East Asia*

The available knowledge on the impact of arthropod-borne and rodent-borne viral diseases in South-East Asia is mainly limited to two diseases: dengue and Japanese encephalitis. In addition, limited information is available on HFRS, West Nile fever, and Kyasanur Forest disease.

3.2.5.1 *Dengue haemorrhagic fever*. All four serotypes of dengue virus were identified in countries of South-East Asia where the disease prevails. Dengue haemorrhagic fever with shock syndrome (DHF/DSS) seems to be on the increase in this region.

Epidemiological studies were initiated in 1980–81 in endemic and silent areas of South-East Asia (Indonesia, Sri Lanka, and Thailand) to obtain an explanation of the rarity of DHF in some countries where dengue viruses are endemic and to clarify the immunopathology of the disease where DHF does occur.

In South-East Asia, DHF first occurred in Bangkok in 1958. Since then it has been reported continuously there and it has spread to other countries and to rural areas.

(a) *Thailand*. Large epidemics have been recorded about every two years; the highest number of cases was 43,382 in 1980, the area of highest morbidity being the North-Eastern region (117 cases/100,000). The case-fatality rate has declined from 3.4% to 0.8% in
1980. A shift to a higher age-specific attack rate has been observed. During 1977–80, about 50% of the total reported cases were in the 5–9 age group and the next age groups were 10–14 and 1–4, respectively. Of the total DHF patients, 2% were under 1 year of age, the female to male ratio being 1.5:1. There were 25,670 cases reported in 1981 and 21,350 in 1982.

(b) Burma. Burma first recorded DHF/DSS in 1968 and included it in the list of the principal notifiable diseases. The first major epidemic occurred in Rangoon in June 1970, and subsequently it has been reported every year from other divisions and states. From hospital studies it has been shown that dengue virus types 2 and 3 are commonly isolated from shock and non-shock patients.

(c) Sri Lanka. Endemic clinical dengue has been confirmed serologically since early 1960. A feature has been the relative absence of severe forms of the disease DHF/DSS so that it has been characterized as a “silent” area. Serological surveys in 1966 and 1976–78 have established that there is dengue activity in all major towns at an elevation of less than 1200 metres. The activity is significantly greater in the capital city of Colombo, where dengue is endemic.

(d) India. Dengue fever has become endemic in many areas although it is recognized mainly when it causes epidemics. Only a few cases of authenticated DHF have been recorded, although outbreaks were reported from Calcutta and Vishakhapatnam in India between 1963 and 1965; in these outbreaks, dengue and chikungunya viruses were isolated.

While dengue fever occurs in many parts of the country, as noted from serological and virological evidence, haemorrhagic manifestations have been seen in only a few cases. The all-India figures for dengue range from less than 1000 to about 5000 cases per year, and the case-fatality rate is less than 0.5%.

Dengue fever is included in the health statistics of communicable diseases in India. However, in the absence of any organized surveillance programme backed up by diagnostic laboratory facilities, the above information may be an underestimation of the situation.

(e) Indonesia. In 1969, the first DHF outbreak was noted in Jakarta. Since then local outbreaks have been reported from other provinces. Dengue infection is now widespread throughout the country, as are the vectors Aedes aegypti and Aedes albopictus with variations in densities observed in different areas.
There is no specific surveillance programme, owing to the low priority given to the disease and the lack of laboratory facilities. Special surveys on dengue infection were carried out in 1978–80 in several urban areas; all of them have shown high rates of infection (55.4–96.0%).

The number of cases increased sharply in 1975 and now there are around 5000–7000 cases each year. The case-fatality rates for DHF/DSS decreased from 41% in 1968 to 4% in 1981. Most of the cases were in children under 15 years of age.

3.2.5.2 Japanese encephalitis (JE). The countries of South-East Asia with JE activity are Bangladesh, Burma, India, Nepal, Sri Lanka, and Thailand. The disease has increased in importance during recent years and foci have been detected where it was previously rare or absent. It seems to be endemic throughout the year. However, peaks occur at the end of the rainy season or in summer/autumn, when mosquito populations are at their maximum.

Large-scale immunization is at present not feasible, owing to the high cost of the vaccine and other technical constraints.

(a) India. Japanese encephalitis has been known since 1952 and in 1956 serological studies showed that human infections were extensive in Tamil Nadu and Karnataka. From 1973 to 1978, outbreaks were reported in many parts of the country (Assam, West Bengal, Bihar, south India, Uttar Pradesh). All age groups seemed to be affected in north India, while in south India children aged under 15 were the risk group. On the average, 1–1.5 cases/village have been reported in south India, indicating a scattered incidence of the disease. JE cases have a heavy mortality (29–40%).

(b) Sri Lanka. The virus was first isolated in 1968 and since then cases have been reported from pig-breeding and large rice-growing areas. It accounts for 1030 hospital admissions per year (0.07/1000 population) with an average case-fatality rate of 38%. However, a virological diagnosis has been established in only 30 cases, owing to the paucity of specimens.

(c) Thailand. The first outbreak was observed in Chiang Mai in 1969. During the last decade, 3 major outbreaks have been noted, with 2432 cases in 1981, the highest incidence being in the northern region. Almost half were confirmed serologically as JE, the incidence being highest in the age group 5–9 years, followed by that of 10–14 years.

(d) Burma. The first outbreak was noted in 1974 in Shan State and cases have been reported every year since then in this area. In 1976,
JE became notifiable. In 1977, 84 confirmed cases were reported with 16 deaths, and for the first time the disease was notified outside Shan State.

(e) Bangladesh. In 1977, the first outbreak was reported in Tangail district.

(f) Nepal. Outbreaks of JE occurred in the southern part of Nepal in 1978, 1980, and 1982. In 1980, 622 cases occurred with 231 deaths. The age distribution showed less than 15% in preschool children and 41–78% in children aged 15 years and over.

3.5.2.3 Other arthropod-borne and rodent-borne viral diseases

(a) Kyasanur Forest disease. This tick-borne disease was first recognized in 1957 in the forests of Karnataka State, India, where it caused a fatal infection of monkeys. It was subsequently found to be also a serious disease of man. The disease is closely related to Omsk haemorrhagic fever of Siberia, and tick-borne encephalitis of eastern and central Europe. A tendency of the disease to spread locally has become more obvious in recent years. In the prevention and control of KFD, a formalin-killed viral vaccine has shown promise.

(b) Crimean–Congo haemorrhagic fever is a disease not commonly recognized in South-East Asia. It is acquired through a tick bite and may spread from person to person through contact with infected blood. Mild and intermediate forms of the disease are seen, and inapparent infections with the virus can also occur. Since serological studies have shown the activity of the virus in Tamil Nadu and Rajasthan States of India in both human and domestic animals and since the disease often resembles “typhoid-like” illness, the need for surveillance is indisputable.

(c) Other viruses. West Nile virus has so far been recognized in Bangladesh, Burma, and India, where it produces mild illness in man. Several isolations of the virus have been made from various Culex species and from a few human encephalitis cases in India. Chikungunya virus has appeared periodically in about six-yearly cycles. Occasionally Wanowrie virus is isolated from man, and Gajum (Nairobi sheep disease) virus infection of man has been confirmed serologically.
3.2.6 Western Pacific

(a) Overview. Arthropod-borne viral diseases remain a public health problem in the Western Pacific region and the most important of these are DHF and JE. The sudden appearance of Ross River virus in Fiji and other islands of the South Pacific presented a new problem.

HFRS has been found in China, Japan, and the Republic of Korea, and the isolation of Hantaan virus has enabled wider-scale serological studies to be carried out. Hantaan virus, or a virus similar to it, has spread widely in other countries among field rodents, urban rats, laboratory rats, and humans.

(b) Murray Valley encephalitis. Epidemics of Murray Valley encephalitis occur infrequently in the Murray Valley region of Australia. The last major outbreak (58 cases and 13 deaths) occurred in 1974. Sporadic cases caused by Australian encephalitis virus and the related Kunjin and Alfuy viruses occur mainly in Queensland and in the north-west of Western Australia.

(c) Haemorrhagic fever with renal syndrome (HFRS) has been endemic in China and the Republic of Korea for many years. In China, more than 30,000 cases with a case-fatality rate of 6.8% were reported in 1980. In the Republic of Korea, annual hospitalized cases number 400–500, with a case-fatality rate of 4–5%. In Japan, outbreaks with more than 114 cases of this disease and 2 deaths were observed in the 1960s among people living in Osaka city. Since 1975, outbreaks have been reported among animal handlers in many research institutes, and the source of infection was traced to laboratory rats. Hantaan virus antibodies have been detected among humans and among field and urban rodents of several species in many countries other than China, Japan, and the Republic of Korea, suggesting that Hantaan virus, or related viruses, has spread more widely in species of rodents than previously recognized. However, human disease has not been identified in countries of this region other than China, Japan, and the Republic of Korea.

(d) Ross River fever (epidemic polyarthritis). Until 1979, the virus was active only in Australia, West Indonesia, Papua New Guinea, and the Solomon Islands. In 1979, a big epidemic of polyarthritis with rash (RRV infection) was observed in Fiji with 30,000 clinical cases, and many cases were also found in American Samoa, spreading to other islands and countries in the South Pacific. It has been suggested that the epidemic in Fiji was caused by the
introduction of RRV by infected mosquitos or possibly by viraemic persons arriving by air. The subsequent spread to adjacent island groups could well have been due to viraemic people travelling from Fiji.

(e) **Dengue fever and dengue haemorrhagic fever (DF/DHF).** Dengue infection has occurred widely through the Western Pacific region except in areas climatically unsuited to the vectors. DHF has posed an important public health problem in Viet Nam and Malaysia. In Viet Nam, 49 318 DF/DHF cases with 462 deaths were reported in 1980, and in Malaysia, 2953 DF/DHF cases with 35 deaths were reported in 1982. Dengue virus types 1, 2, and 3 were isolated in Malaysia in 1982. The existence of a jungle cycle involving monkeys has also been demonstrated for dengue in Malaysia.

In the South Pacific countries and in Niue, with a population of 3000, 616 DF/DHF cases with 4 deaths were reported. In southern China, a large outbreak of DF/DHF by dengue virus type 4, with 22 122 cases and 14 deaths, occurred in 1978 after dengue had been absent from the area for 30 years.

In Northern Queensland, Australia, a DF epidemic with 375 confirmed cases was reported in 1981–82 after the area had been free of the disease for 25 years.

On the other hand, DF/DHF has decreased in importance in the Philippines since the 1970s, and with good vector control, also in Singapore.

(f) **Japanese encephalitis** has been well controlled in Japan by vaccination with inactivated mouse-brain vaccine and with extensive vector control. There has also been a considerable reduction in recent years in the incidence in China and in the Republic of Korea. However, in 1982 more than 20 000 cases were observed in China and 1500–2000 cases in the Republic of Korea.

4. EPIDEMIOLOGICAL CONCEPTS

The terms “arthropod-borne” and “rodent-borne” refer to major biological phenomena involved in the transmission cycles of certain viruses and do not correspond to the taxonomic classification of these agents. Most of the diseases caused by such viruses are zoonoses, that is, the viruses are maintained in nature by invertebrate and/or vertebrate hosts other than man. Although many of these agents can be transmitted by aerosols or by body
fluids, such as milk, urine, or blood, in most instances complex interactions among virus, arthropod vector, vertebrate host, and the environment determine both the intensity and the temporal pattern of virus transmission.

4.1 Arthropods

4.1.1 Criteria for role as biological vectors

For many arboviruses, a vector is defined as an arthropod that feeds on a viremic vertebrate, becomes infected and, after a variable incubation period, is able to transmit the agent to another vertebrate during a subsequent blood feeding. The concept denotes biological rather than mere mechanical transmission due to virus contamination of arthropod mouth parts. Although the latter mechanism may be important, especially during major epizootics or epidemics, the singular importance of biological transmission lies in the fact that the arthropod is usually infected for life and can transmit virus each time that it feeds.

From this definition, it follows that mere isolation of a virus from an arthropod does not indicate its status as a vector. It is known that a few species of non-biting arthropods can be parenterally infected with certain arboviruses with virus persistence but no mechanism for transfer to other hosts. There are also arthropods that may be infected by ingestion but are unable to transmit virus. Even if successful experimental transmission of an agent by a given arthropod proves its potential as a vector, epidemiological factors may mitigate its role as a true vector. Neither its abundance nor longevity may be adequate to allow transmission to occur. Likewise, its feeding habits may cause avoidance of particular vertebrates capable of expressing sufficient viraemia to sustain a transmission chain. Thus, in a given locality where two or more arthropod species are present and from which a given virus is recovered, it may be that several species are important vectors or that only one or two are responsible for effective biological transmission.

For these reasons, it is important to define criteria clearly for arthropod vector status. Suspected vectors are those from which a virus has been isolated in the absence of detectable vertebrate blood. Potential vectors are those that, in addition, have been demonstrated experimentally to be capable of biological virus transmission, while confirmed vectors are those that meet both these criteria as well as
criteria related to the important epidemiological parameters of virus transmission in time, place, and pertinent vertebrate hosts.

Arbovirus diseases affecting man are most often vectored by mosquitoes; other vectors, in order of importance, are ticks, phlebotomine sandflies, and culicoid flies.

4.1.2 Vector competence

Increasing attention is being given to the concept of vector competence, which basically poses the question in virus–vector relationships: “How good a vector of virus X is arthropod Y?” Many factors determine the answer to this question. Among them are the genetics of both virus and vector, effects of temperature, autogeny, and virus concentration (threshold effect) on infection, and several as yet incompletely answered problems involving the pathogenesis of infection in the vector, e.g., gut barrier and salivary gland barrier to infection.

From this work it is becoming evident that it is often possible to distinguish maintenance as opposed to amplification vectors. The former have low infection thresholds, minimum evidence of barriers to completely disseminated infection, and very high transmission efficiency by bite. Amplification vectors may be infected only by high concentrations of virus and often exhibit poor rates of bite transmission, phenomena often compensated for by extremely high seasonal population densities and a pattern of feeding on many vertebrate species. It is evident from the foregoing that it is important to conduct vector competence studies with virus strains that have undergone minimal laboratory manipulation and with vectors obtained directly from nature rather than from colonies.

4.1.3 Transovarian transmission

Ticks have long been known to be able to transmit certain arboviruses trans-stadially or even in their eggs, thereby lessening or perhaps eliminating the need for feeding upon viraemic vertebrates to maintain the virus. The convincing experimental demonstration of transovarian transmission of vesicular stomatitis virus by phlebotomine flies in 1972 reopened the search for this biologically significant mechanism among other arboviruses vectored by dipteran insects, particularly mosquitoes. Positive data were not long in coming. To date, members of the Bunyaviridae appear to be the
most prominent users of this strategy. Experimental data also show that certain flaviviruses and rhabdoviruses exhibit similar behaviour, although the importance of transovarian transmission in natural maintenance of these agents has not yet been clearly documented.

As for vector competence, there are a multitude of variables to consider when examining transovarian transmission. It has been shown when rearing immature stages of the insects that water temperature has a major effect on transmission rate, as does ovarian cycle following infection of the adult. Recent genetic work showing the possibility of selecting mosquito subpopulations that exhibited virtually 100% filial transmission rates (stabilized cytoplasmic infection) provides a potentially rich yet complex source of new understanding regarding the epidemiology of arboviruses. In such instances, invertebrates must be considered as both reservoir and vector of the virus. Together with the phenomenon of virus genetic reassortment (see below), they also provoke new thought regarding the basic evolution and dispersion of many of these agents.

4.2 Vertebrate hosts/reservoirs

For arboviruses, vertebrate hosts are usually mammals or birds that are susceptible to a virus and are able to circulate it in titres high enough to infect the vector. The concentration of virus ingested by the vector has an important bearing on the subsequent progress of the infection in the arthropod. This phenomenon, called infection threshold, determines in part the competence of a vector. The most effective vertebrate hosts are those that themselves remain healthy but circulate the virus in sufficient titres and duration to infect a very large number of the competent vectors. A large number of susceptible small vertebrates with a rapid turnover of populations, or a small number of large mammals, may produce a similar effect—for example, horses infected with VEE virus or sparrows with SLE virus. If, however, a significant proportion of animals suffer from severe illness leading to death, then they are less effective as maintenance hosts since this depletes the reservoir population. Vertebrate hosts that help in enhancing the number of infected vectors are considered as amplifying hosts (e.g., pigs in the JE virus cycle).

With few exceptions, large vertebrates are typically amplifying hosts, because they serve as feeding targets for many vectors in a short time and then either become immune or succumb to infection.
In certain cases (e.g., EEE), large animals are mere dead-end hosts, never or rarely able to infect vector species despite the fact that they may suffer fatal infection, which attracts the attention of owners and of animal and human health authorities.

The role of rodents in the maintenance and transmission of rodent-borne viruses is dual; they act as both reservoir and vector. The best-studied instances are marked by chronic infection of at least a fraction of the rodent population, with persistent urinary, salivary, and sometimes fecal excretion of virus into the environment. This chronic infection is characterized either by modified immune response to infection of one or very few rodent species (arenaviruses) or by virus persistence and excretion during non-cytolytic, immunocompetent infection of a variety of rodents (Hantaan and related viruses). The epidemiology of these agents is therefore subsumed by geographical and ecological distribution of the pertinent rodents, by their degree of spatial association with man, and by incompletely understood rodent genetic factors that influence the induction of chronic virus infection.

4.3 Viruses

In epidemiological terms, there are at least two major unresolved questions: virus virulence for man or important domestic animals, and virus detection and genetic heterogeneity. They are intertwined. We know, for example, that the ratios of infection to severe disease for a number of arboviruses affecting man are very high. Is the particular virus strain that causes acute illness more virulent than other strains? Are there clear differences in virulence among viruses of the same immunotype in distinct geographical–ecological settings? The answers are far from clear and the equation certainly must consider virus–vector–host relationships to be meaningful. The methods of attacking such questions are far from perfect. Oligonucleotide mapping of viral RNA can often reveal discrete differences among virus strains but it does not yet allow any conclusions regarding virulence. Animal models do show virulence differences among strains of a given virus but we cannot be sure that they predict virulence for man. Genetic reassortment has been achieved in the laboratory for certain bunyaviruses and the associated virulence changes were referable to genes coding for virion surface glycoproteins.
Virus detection is at times a similarly difficult problem with many unanswered questions of epidemiological importance. Although it is apparent that in the short term and using classical methods of detection, most arboviruses appear to be relatively stable genetically, careful search for persistent infection by recourse to techniques such as co-cultivation may disclose more virus than is now recognized in a given situation, as well as genetically different virus. It is important to recall that the history of arbovirus discovery has been marked by spurts of new detection following the introduction of new technology: from the suckling mouse, to the vertebrate cultured cell, to the invertebrate cell. Viruses that apparently replicate only in invertebrate cells are now being encountered with frequency. Should even one of these prove to share antigens with a previously known arbovirus, the epidemiological consequences might be far-reaching.

The considerations cited above for vectors, vertebrate hosts, and viruses bring home one message for all who are engaged in arbovirus field research: it is more than ever important to make a stock pool of each viral isolate at the very first laboratory passage. Virus strains manipulated as little as possible are vital to the eventual understanding of many of the issues posed here.

4.4 Environment and man

With certain significant exceptions, e.g., urban yellow fever, dengue, chikungunya, and Oropouche, human beings are generally not involved as vertebrate hosts in the maintenance and spread of arthropod-borne and rodent-borne viruses. However, human social and behavioural patterns have a great impact on the epidemiology of these viruses.

Rapid population growth in the past 40 years, the rise and dissemination of technology, and worldwide access to communication, bringing with it the desire for a different and better life, have all served to modify human behaviour and the ecosystems in which man lives in ways that have frequently led to major increases in disease due to known arboviruses, or to the discovery of new diseases caused by such agents.

Examples of these phenomena abound. Tin cans and tyres have supplemented the classical water containers as breeding sites for *Aedes aegypti*, the mosquito vector of dengue and urban yellow fever. Hydroelectric dams, with their resultant new lakes and frequent irrigation schemes, provide rich new habitats for certain
culicine mosquitos and the viruses that they transmit, such as Japanese, St Louis, and Western encephalitis.

Forest clearing results in human exposure to arboreal vectors of sylvan yellow fever, to direct exposure of forest workers to tick-borne agents, such as the virus of Kyasanur forest disease, and to the dissemination of such agents to nearby villagers, as was documented in India recently when forest adjacent to a known focus of KFD was cleared and nearby villagers noted increased numbers of monkeys and rodents prior to the onset of clinical illnesses in the village.

Junin haemorrhagic fever became an annual epidemic disease in the 1950s just a few years after the introduction of selective herbicides to the rich pampa maize fields. Grasses, providing harbourage and food for the reservoir rodent *Calomys musculinus*, came to dominate these cultivars and the disease became an occupational risk for persons harvesting maize. The recent introduction of soybeans as a rotational crop to maize appears to have caused a decline in AHF. Soybean cultivars spread between the rows, are free from weeds and grass, and support only about one tenth of the *Calomys* population supported by maize plantings.

Finally, it is becoming increasingly evident that man rather than nature is likely to be responsible for the direct or indirect dispersal of an arbovirus over great distances. Although by no means proved, it is distinctly possible that recent major and singular epizootic–epidemic outbreaks of Venezuelan equine encephalitis in Central and North America and of Rift Valley fever in Egypt were caused by human behaviour. In any event, it is now clear that most disease-producing arboviruses are not introduced into a known endemic region annually by migratory birds or other long-distance non-human travellers.

In the scheme of arboviral epidemiology, man is usually only a tangential host. In the long run, however, it is man’s behaviour, together with discrete environmental factors, that determines the activity and spread of these viruses.

5. THE DISEASES AND THEIR MANAGEMENT

Many arboviruses and rodent-borne viruses are pathogenic for man, causing acute fever and viraemia and nonspecific systemic symptoms. However, some agents also typically induce

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characteristic rash, arthralgia, or retinitis, and others have the capacity to invade major organs and produce the clinically severe syndromes of encephalitis and haemorrhagic fever. Expression of virulence is determined by many host and virus variables, and it is well known that the ratios of infection to clinical response vary widely. For example, haemorrhagic fever is an unusual complication of RVF infection, whereas this response is much more frequent with Marburg and Ebola viruses.

The following section is divided into systemic febrile illness, haemorrhagic fever, and encephalitis. It should be remembered that systemic febrile disease may be the predominant response to infection by agents that can also cause haemorrhagic fevers or encephalitis.

5.1 Systemic febrile illness

Included under this heading is a large group of diseases, all of which manifest fever. In some, rash, myalgia, arthralgia, and lymphadenopathy are also present; the duration of fever is usually from 3 to 7 days.

Dengue fever is caused by four types of antigenically related flaviviruses. The term “dengue-like fevers” became common after specific laboratory methods were developed to differentiate many febrile illnesses from dengue, particularly in tropical and subtropical areas where dengue had been recognized. Single cases have to be diagnosed etiologically by specific virological laboratory tests. The differential diagnosis includes many agents belonging to different viral groups. However, some of these diseases have sufficiently marked characteristics to permit clinical differentiation from classical dengue during an epidemic.

Typical dengue has an abrupt onset associated with malaise and severe prostration, severe headache, retro-orbital pain, backache, muscle and limb pain, arthralgia, diphasic fever curve and rash, lymphadenopathy, and leukopenia; it runs a course of about one week's duration followed by severe depression and weakness during convalescence; in some cases petechiae develop on the lower limbs. Dengue patients usually recover without sequelae. Dengue is often observed in epidemic form.

Arboviruses recognized as causing disease closely resembling this syndrome are listed in Table 2. West Nile virus may, in the adolescent or adult, cause an illness clinically indistinguishable from
Table 2. Some arboviruses causing systemic febrile illness

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<td></td>
<td></td>
<td>Venezuelan equine encephalomyelitis</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ross River</td>
<td>mosquito</td>
</tr>
<tr>
<td>Bunyaviridae</td>
<td>Phlebovirus</td>
<td>Sandfly fever (Sicilian)</td>
<td>sandfly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sandfly fever (Naples)</td>
<td>sandfly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rift Valley fever</td>
<td>mosquito</td>
</tr>
<tr>
<td>Bunyavirus</td>
<td></td>
<td>Apeu</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caraparu</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Itaquí</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Marituba</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Murutucu</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oríboca</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bunyamwera</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catu</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guama</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bwamba</td>
<td>mosquito</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oropouche</td>
<td>biting midges</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tataguine</td>
<td>mosquito</td>
</tr>
<tr>
<td>Reoviridae</td>
<td>Orbivirus</td>
<td>Colorado tick fever</td>
<td>tick</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orungo</td>
<td>mosquito</td>
</tr>
</tbody>
</table>

dengue. However, the inflammation of the lymph nodes is often more marked and sometimes a self-limited meningeal syndrome occurs. In the child, the infection is often inapparent; in elderly people, while the viraemic stage is often asymptomatic, severe and sometimes fatal encephalitis may occur later.

Chikungunya and some other alphavirus infections differ from dengue in that pains tend to be confined to the joints; the febrile period is shorter and not biphasic; many patients suffer residual joint pains after the acute episode.

The sandfly fever caused by the Naples or Sicilian strains is clinically the same. The onset is abrupt and in a few hours fever appears, accompanied by headaches, orbital pain with photophobia, sweating, lumbar pain, arthralgia, and stiffness of the neck and back. Digestive disorders—anorexia, nausea, and vomiting—are frequent.
Leukopenia is almost invariably found. A particularly frequent symptom, quite typical of sandfly fever, is congestion of the face and neck resembling erythema from sun exposure, which sometimes causes this disease to be confused with sunstroke. The illness usually lasts three or four days, but relapses are possible.

During the epidemic of RVF seen in Egypt, 95% of the human cases were uncomplicated; patients had fever, rigor, malaise, headache, retro-orbital pain, myalgias, anorexia, and they made a slow uneventful recovery. The complicated cases showed three courses: haemorrhagic, encephalitic, and ocular. The haemorrhagic and encephalitic complications are described elsewhere. The ocular complications occurred 7–20 days after fever by exudate formation in macular, paramacular, and extramacular sections of the retina; vasculitis and vascular occlusion were frequent and there was a high incidence of permanent loss of visual acuity (about 50%), whereas resolution of the lesion occurred within 6 months in the other patients.

In Oropouche virus disease, the acute phase is often followed by one or more episodes of recurrence of symptoms for a period of 1 or 2 weeks. The recurrences may be characterized by the return of all the manifestations of the acute phase or simply by fever, headache, and asthenia. Instances of meningitis associated with Oropouche infection have also been documented.

Colorado tick fever frequently follows a biphasic curve, sometimes with a maculopapular eruption or petechiae. Occasionally, in children under 10 years of age, it is accompanied by a haemorrhagic or encephalitic syndrome.

There is no specific treatment for any of the systemic febrile illnesses other than supportive measures. Rest in bed, plenty of fluids, antipyretics, and analgesics constitute the most important lines in the clinical management of patients.

5.2 Viral encephalitis

Encephalitis is one of the most severe manifestations caused by certain arboviruses. It is associated with neuronal damage and viral replication in nervous tissues. Neurological manifestations may also occur in the absence of inflammation and of virus in the brain. The latter condition is compatible with an encephalopathy, which is commonly observed among Junin and Machupo patients, and occasionally in dengue infection.
Although the clinical course and severity of the different arboviral encephalitides may exhibit clear variations, they are remarkably similar in the general pattern of neurological manifestations and are often indistinguishable.

5.2.1 Clinical manifestations

The incubation period varies from 3 to 15 days. There may be a prodromal phase, which lasts 1–4 days. It is characterized by pronounced malaise, extreme lassitude, chills, myalgias, and nausea, and is usually accompanied by fever and headache. More often, however, the disease begins abruptly with fever, headache, photophobia, generalized malaise, dizziness, nausea, vomiting, and often with nuchal rigidity. Gradually, signs and symptoms of central nervous system disease begin to appear. Among these are alterations of the sensorium, such as confusion, drowsiness, lethargy, and coma. Tremors, paresis, or paralysis may be present. Deep tendon reflexes may be hyperactive or hypoactive, and pathological reflexes, such as Babinski, Wartenberg, and Hoffman, may occur. Patients may also exhibit blurring or disturbance of vision and diplopia. Convulsions vary in frequency with the type of encephalitis, but are most commonly observed among children. Dysphagia may be present. Signs of thalamic, brainstem, and cerebellar dysfunction include diffuse myoclonic twitching, nystagmus, and ataxia. Physical signs of markedly raised intracranial pressure are unusual. Certain patients tend to remain immobile. Abnormalities of renal and bladder function include urinary retention, incontinence, microscopic haematuria, proteinuria, pyuria, and elevations of blood urea nitrogen and serum creatinine. In St Louis encephalitis, kidney injury may be related to virus replication in this organ, since the presence of SLE viral antigen in uroepithelial cells of four patients was detected by immunofluorescence and SLE virus-like particles were also found in the urine by electron microscopy. In addition, bacterial superinfections were encountered in some of these patients and may have played a role in urinary pathology.

Central European tick-borne encephalitis usually has a diphasic course, but in nearly half of the recognized cases, either the febrile or the encephalitic phase may be inapparent (15). The first viremic phase is typically influenza-like and persists for about a week, followed by general improvement for several days. The second phase appears abruptly, with a clinical course similar to, but commonly
milder than, the Far-Eastern TBE. In this type, epileptiform convulsions, paresis or paralysis, usually ascending, may develop.

The case-fatality rate varies considerably. It is lower than 1% in California encephalitis but may reach 30% or more in EEE, JE, and Rocio encephalitis.

The cerebrospinal fluid shows pleocytosis with cell counts that vary from 5 to more than 1000/mm³. A marked lymphocyte predominance is usually observed but polymorphonuclear cells may predominate on initial lumbar punctures. The protein content is elevated, but the glucose level is unchanged. White blood cell counts are generally characterized by a moderate neutrophilic leukocytosis.

5.2.2 Complications

A variety of complications unrelated to the primary meningoencephalitis process can markedly affect the clinical course and outcome of the disease. These include bronchopneumonia, necrotizing bronchopneumonitis, pulmonary embolism, gastrointestinal haemorrhage, and aspiration of gastric contents.

5.2.3 Sequelae

Although recovery may be uneventful, survivors may sometimes exhibit residual effects from the infection. The incidence and severity of sequelae varies according to the agent, but is also influenced by age. Sequelae are more pronounced in EEE, JE, TBE, and Murray Valley encephalitis. In JE, sequelae are observed in 30–40% of persons 5–40 years old, but in children under 4 years of age the occurrence is much higher (16); there may be long-term and psychiatric residual effects, with intellectual impairment, confusion, psychosis, and delusions. Sequelae are also more frequent in children than in adults infected with EEE virus. Children may develop variable degrees of mental retardation. In WEE, sequelae are rare in adults but frequent in young children; more than half of afflicted infants of less than a month old are left with recurring convulsions or motor or behavioural disorders. In SLE virus infections residual effects are more common in older persons. They occur in 30–50% of SLE patients, and are characterized by weakness, fatigue, nervousness, tremulousness, irritability, sleeplessness, depression, memory loss, difficulty in concentrating, and headaches, but they clear within 3 years in about 80% of the patients (17).
Table 3. Summary of clinical and epidemiological features of some important arboencephalitides*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Agent: Family/genus</th>
<th>Age-groups predominately affected</th>
<th>Case-fatality rate</th>
<th>Sequelae</th>
<th>Estimated ratio of inapparent to apparent infections</th>
<th>Environment</th>
<th>Disease distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern equine encephalomyelitis</td>
<td>Togaviridae: Alphavirus</td>
<td>Infants, elderly</td>
<td>50-70%</td>
<td>30-50% severe, especially in children. Mental and motor</td>
<td>10:1 (infants) 50:1 (middle-aged) 20:1 (elderly) 50:1 (under 5) 1000:1 (over 15)</td>
<td>Rural</td>
<td>North America, Jamaica, Dominican Republic, Trinidad</td>
</tr>
<tr>
<td>Western equine encephalomyelitis</td>
<td>Togaviridae: Alphavirus</td>
<td>Children</td>
<td>3-7%</td>
<td>Common and severe only in infants. Mental and motor</td>
<td>50:1 (under 5) 1000:1 (over 15)</td>
<td>Rural</td>
<td>North America</td>
</tr>
<tr>
<td>Venezuelan equine encephalitis</td>
<td>Togaviridae: Alphavirus</td>
<td>Children</td>
<td>20-30% in children aged &lt; 5 years, &lt; 10% in older persons</td>
<td>Moderate. Incidence unknown 25:1 (under 15) ≥ 1000:1 (over 15)</td>
<td>Rural</td>
<td>South and Central America, Texas</td>
<td></td>
</tr>
<tr>
<td>St Louis encephalitis</td>
<td>Togaviridae: Flavivirus</td>
<td>Elderly</td>
<td>5-10% (30% in persons above 65 years of age)</td>
<td>30-50% in adults. Emotional disturbance. Clear in 3 years in about 80% of persons</td>
<td>800:1 (&lt; 9 years) 400:1 (9-49 years) 85:1 (&gt; 60 years)</td>
<td>Urban/rural</td>
<td>North America, Jamaica</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>Togaviridae: Flavivirus</td>
<td>Children, elderly</td>
<td>30-70%</td>
<td>30-40% in persons &gt; 50 years old. Higher and more severe in children &lt; 4 years. Mental, motor</td>
<td>Rural/semi-urban</td>
<td>Pacific Islands, Asia</td>
<td></td>
</tr>
<tr>
<td>Tick-borne encephalitis (Far Eastern, Central European)</td>
<td>Togaviridae: Flavivirus</td>
<td>All ages</td>
<td>20-25% in Far East 1-2% in Europe</td>
<td>15-40% of persons. Motor (paralysis)</td>
<td>Inapparent infections are common</td>
<td>Rural</td>
<td>Soviet Union, Europe</td>
</tr>
<tr>
<td>Virus/Military Disease</td>
<td>Family</td>
<td>Age Group</td>
<td>Mortality</td>
<td>Infection Rate</td>
<td>Risk Factors</td>
<td>Country/Location</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
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<td>-----------</td>
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<td>-----------------------</td>
<td>------------------------------</td>
<td></td>
</tr>
<tr>
<td>Murray Valley encephalitis</td>
<td>Togaviridae</td>
<td>All ages</td>
<td>30-50%</td>
<td>Similar to JE</td>
<td>Between 500:1 and 1000:1</td>
<td>Rural Australia, New Guinea</td>
<td></td>
</tr>
<tr>
<td>Rocio virus disease</td>
<td>Togaviridae</td>
<td>Adult males</td>
<td>4.3-30% (average 13%)</td>
<td>20% of persons, Motor, equilibrium</td>
<td>Unknown Rural Brazil (São Paulo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>California encephalitis</td>
<td>Bunyaviridae</td>
<td>Children under 15</td>
<td>&lt;1%</td>
<td>Rare, Emotional disturbance</td>
<td>&quot;High&quot; Suburban, rural North America</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Other arboviruses less commonly associated with encephalitis in man include West Nile, Powassan, Illinios, Kunjin, Louping ill, Sindbis, Semiliki Forest disease, Bhanja, Bat salivary gland, Negishi, Rift Valley fever, Thogrov, Colorado tick fever.*
Approximately 20% of survivors of Rocio virus infection presented residual impairment of cerebral functions. Residual paralysis or paresis may be associated with TBE, particularly in the Far East. Recurrent seizures and depressed intellectual function are occasionally described as sequelae of La Crosse virus disease. Table 3 gives a summary of the clinical and epidemiological features of some important arbovirus encephalitides.

5.2.4 Treatment

There is no specific treatment other than good supportive care for patients. Expert nursing care of comatose patients is vital. Continuous close attention to skin care is essential to prevent decubitus ulcers. Protection of the eyes and good oral hygiene are necessary. Pulmonary care (including sterile suction, postural drainage, percussion, and supportive respirator), together with attention to indwelling urinary catheters, will help to prevent secondary septic complications. Close monitoring of fluid and electrolyte balance is required. Hyponatraemia secondary to the syndrome of inappropriate secretion of antidiuretic hormone observed in certain patients with SLE is managed by means of water restriction. Administration of antipyretics, antiemetics, and anticonvulsants may be necessary. Corticosteroid therapy has been advocated in severe cases of encephalitis, but its value is uncertain. Early physiotherapy is recommended for patients with motor sequelae. Adequate feeding, initially by the parenteral route, should be provided, particularly to undernourished children.

The importance of good medical care was illustrated in the outbreak of Rocio encephalitis in São Paulo, during which lethality was reduced from 30% to 4.3% after an emergency hospital with an intensive therapy unit was set up in the epidemic focus.

5.3 Viral haemorrhagic fevers

5.3.1 The viruses and their general ecology (23, 24)

Sixteen distinct viruses are credited with human virulence marked by an acute haemorrhagic diathesis and all contain RNA. These viruses are found in several continents and have distinct ecological properties that, together with patterns of human behaviour,
determine their transmission from the environment to man. In some geographical areas, only a single agent is the likely cause of acute haemorrhagic fever. In some regions of the world, where DHF has so far occurred, no other viral haemorrhagic fever is a significant possibility. In such places, the differential diagnosis of DHF is primarily from other medical non-zoonotic causes of haemorrhagic syndromes. A single etiology for haemorrhagic fever also exists for JHF and MHF, for YF in the Americas, for KFD, OHF, CCHF in Asia Minor, and for HFRS in northern East Asia.

In contrast, Africa is a special and complex case. At least six of the haemorrhagic fever viruses are present on this continent, and although there are apparent subcontinental patterns of distribution (Lassa virus in West Africa, RVF mainly in East and southern Africa), much remains to be learned concerning the true geographical ecology of these six, and perhaps other, as yet undiscovered, agents. Differential diagnosis thus requires virus-specific technology, which is at present limited in availability. No realistic appraisal of the public health significance of viral haemorrhagic fever in Africa is possible until this problem has been solved.

5.3.2 Clinical manifestations

Viral haemorrhagic fever is not a unimodal clinical syndrome. As depicted in Table 4, the incubation period is variable, and clinical onset may be either abrupt or insidious. In nearly all these diseases, however, patients early in illness are best described as toxic, with headache, severe myalgia, and sometimes bradycardia as prominent symptoms. There are usually one or more manifestations suggesting that blood capillaries are affected—flushing of the skin with blanching on pressure, rash, and pronounced conjunctivitis. Severe infections are relentlessly progressive and lead to haemorrhages from the oral cavity and the gastrointestinal and reproductive tracts. Blood loss per se is a significant problem only in CCHF and possibly RVF. Clinical, refractory shock, usually with evidence of haemoconcentration indicating functional hypovolaemia, represents the most common harbinger of death. Patients surviving such a crisis generally recover slowly but completely; clinically significant residua are unusual although persistent peripheral neuritis has been reported in CCHF from the Soviet Union.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Incubation (days)</th>
<th>Onset*</th>
<th>Haemorrhage</th>
<th>Hepatitis</th>
<th>Pneumonitis</th>
<th>Encephalitis</th>
<th>Nephritis</th>
<th>Estimated fatality rate %</th>
<th>Estimated ratio infection: death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow fever</td>
<td>3–7</td>
<td>A</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10–30</td>
<td>50:1</td>
</tr>
<tr>
<td>Crimean–Congo haemorrhagic fever</td>
<td>4–8</td>
<td>A</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
<td>5–20</td>
<td>25:1</td>
</tr>
<tr>
<td>Marburg virus disease</td>
<td>5–13</td>
<td>A or I</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
<td>20–30</td>
<td>unknown</td>
</tr>
<tr>
<td>Ebola virus disease</td>
<td>5–16</td>
<td>A or I</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
<td>60–85</td>
<td>unknown</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>3–6</td>
<td>A</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
<td>30–50</td>
<td>1000:1</td>
</tr>
<tr>
<td>Dengue</td>
<td>5–8</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>2–10</td>
<td>2000:1</td>
</tr>
<tr>
<td>Haemorrhagic fever</td>
<td>10–35</td>
<td>I</td>
<td>± to ++</td>
<td>±</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>5–10</td>
<td>50–5000:1</td>
</tr>
<tr>
<td>with renal syndrome  **</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Nephropathia epidemica&quot;</td>
<td>7–45</td>
<td>A</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&lt; 1</td>
<td>&gt;1000:1</td>
</tr>
<tr>
<td>Junin haemorrhagic fever</td>
<td>7–14</td>
<td>I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>1–15</td>
<td>10:1</td>
</tr>
<tr>
<td>Machupo haemorrhagic fever</td>
<td>7–14</td>
<td>I</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>15–35</td>
<td>6:1</td>
</tr>
<tr>
<td>Lassa fever</td>
<td>6–15</td>
<td>I</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10–25</td>
<td>100:1</td>
</tr>
<tr>
<td>Kaysanur Forest Disease</td>
<td>3–12</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2–10</td>
<td>unknown</td>
</tr>
<tr>
<td>Omak haemorrhagic fever</td>
<td>3–10</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2–5</td>
<td>unknown</td>
</tr>
</tbody>
</table>

*A = acute; † = insidious
*encephalopathy
Viral attack on parenchymal organ systems is quite variable. Indeed, only about half of the haemorrhagic fevers deserve classification as overtly (i.e., commonly) haemorrhagic. With few exceptions, the causative viruses, including those of CCHF, DHF, YF, MBG, EBO, and RVF, are also highly hepatotropic, although only YF and RVF induce clinical jaundice. Disseminated intravascular coagulation is known or postulated to occur in these diseases. In contrast, major capillary leakage may occur in HFRS without much haemorrhage and with little or no hepatitis; but there may be major renal tubular destruction, which is uncommon among viral haemorrhagic fevers. Some patients surviving the toxic, hepatic phase of YF also experience severe renal tubular necrosis. Indeed, the renal lesion is observed in the virtual absence of haemorrhage in illness due to Puumala virus (PUU) infection (nephropathia epidemica) seen in eastern Europe and Scandinavia. Capillary leakage with little direct parenchymal damage and limited haemorrhage is also characteristic of arenaviral JHF and MHF, although LF is sometimes marked by significant hepatitis.

Pneumonitis is a conspicuous feature of OHF, and is stated to be caused by direct viral replication. Pulmonary inflammation in many of the other diseases represents either viral damage or secondary bacterial infection or both. OHF and KFD are quite likely to cause clinical encephalitis with associated cellular changes in cerebrospinal fluid. Specific methods are thus required to identify a given case and to discriminate it from other viral causes of encephalitis.

5.3.3 Clinical management

In most viral haemorrhagic fevers the life-threatening event is hypovolaemic shock. The best management of this condition is preventive. Frequent haematocrit determinations, careful monitoring of blood pressure, daily examination of urine for protein content, and strict supervision of fluid intake and output are indicated. Volume expanders, such as human albumin, are indicated when arterial pressure narrows, but must be used cautiously during frank clinical shock because of the danger of inducing a refractory pulmonary oedema, termed shock lung. Dextran 70 or mannitol are preferred in this situation. Central venous pressure should be constantly monitored and appropriate fluids administered to keep it between 10 and 15 mmHg (1.33–2.00 kPa). Hypoxia should be corrected with oxygen, or even a mechanical respirator, and acidosis
must be corrected with sodium bicarbonate. Arrhythmias must also be corrected. Lidocaine or electroshock are indicated for sustained ventricular tachycardia, and digoxin is the drug of choice for atrial disturbances. Corticosteroids have been administered to patients with several of the viral haemorrhagic fevers without notable success. Further work on the causes and management of shock in viral haemorrhagic fever is needed.

Disseminated intravascular coagulation represents a special challenge to management of some of the diseases, in particular, MBG, EBO, RVF, CCHF, and in some instances LF. When an etiological diagnosis can be made with certainty or is highly presumptive in advance of haemorrhage, heparin (10–15 units per kilogram of body-weight per hour) may be given intravenously. Therapy is guided by coagulation parameters, particularly fibrinogen and fibrin degradation product levels. In patients with haemorrhagic signs, replacement of platelets and clotting factors (fresh plasma) is preferred.

5.3.4 Specific measures

Specific treatment of viral haemorrhagic fever is in its infancy, but it must be based on rapid, specific viral diagnosis of infection. In some instances, such as DHF, HFRS, KFD and OHF, where the critical pathogenesis is either very brief or involves parenchymal organs such as kidneys or brain, the prospects for eventual antiviral intervention are not bright. With many other diseases, however, where the disease process compromises principally circulatory, reticuloendothelial, and hepatic functions, greater optimism is called for. There is little doubt that the cardinal objective in management of Gram-negative bacterial sepsis with haemorrhage and shock is to eliminate the endotoxin-producing organisms as quickly as possible. That same goal would appear highly rational for many viral haemorrhagic fever syndromes. Two approaches are plausible; specific antiviral antibodies and antiviral chemicals.

Notable success has been achieved with the former approach in the case of JHF. Mortality was reduced from 16% to 1% using convalescent human plasma given within 8 days of disease onset. The immediate and permanent elimination of Junin virus from blood was achieved by this treatment. By analogy, it is likely that this method would work equally well in the case of the closely related Machupo virus that causes MHF.
Passive antibody has also been tried in CCHF, EBO, and LF syndromes. The EBO experience is so far limited to a single patient who survived. Antibodies were found not to be effective in a series of CCHF patients treated in the USSR, and such therapy has been judged a mixed success for LF. But much of the plasma therapy work to date has been highly unscientific. It is essential to know the quantities and kind of antibodies administered and patients must be monitored carefully for concentrations of virus in blood as well as for various clinical pathological parameters. Neutralizing antibodies are almost certainly the important immunoglobulins, and other "antiviral" tests may give discordant information. In the case of LF, it has been found that immunofluorescence titres do not correlate well with neutralization titres and that amounts of the latter antibodies found in recovered patients are rarely sufficient to treat monkeys that have been successfully infected experimentally. Concentration of such antibodies, however, permits life-saving clinical intervention and such materials should be evaluated in LF and perhaps other viral haemorrhagic fevers.

Antiviral treatment for viral haemorrhagic fever is also limited. There are few compounds that are active against membrane-bound RNA viruses and sufficiently non-toxic to be tested in man. One such compound is ribavirin. It is highly active in vitro and in animals against both Lassa and RVF viruses. This compound also causes anaemia, but is currently under test in the treatment of severe LF. Four grams are given intravenously in divided doses for 5 days and half that amount for another 5 days. Combined antibody-ribavirin therapy is also under evaluation. The future of specific therapy for viral haemorrhagic fever awaits new antiviral drugs, new ways of administering them, such as linking them to specific monoclonal antibodies, as well as further controlled prospective studies of passively administered antibodies. An organized antiviral screening programme employing the actual viruses or closely related avirulent members of the same species is needed urgently. Such work should include an evaluation of highly purified interferons, which may now be considered to represent potential antiviral chemicals.
6. METHODS FOR DETECTION OF ARTHROPOD- AND RODENT-BORNE VIRUS INFECTIONS

6.1 Conventional reference methods

Many techniques developed for yellow fever studies have formed the starting point for extended investigations on other arboviruses. These include virus isolation and identification. Virus isolation is conducted by inoculating specimens into mice, arthropods, arthropod cell lines, e.g., *Aedes albopictus* (C6/36) or *Aedes pseudoscutopteralis* (AP-61), and vertebrate cell lines, e.g., Vero, CV1.

The following techniques are commonly used: neutralization (N) test, complement-fixation (CF) test and haemagglutination-inhibition (HI) test. These tests have been widely used and can therefore be referred to as reference methods in arbovirology.

The isolated virus is identified using various techniques: for example, its ability to pass through filters, electron microscopic appearance, and susceptibility to treatment with lipid solvents (e.g., ether or chloroform).

The neutralization test is considered to be the most specific of the serological tests in use. It is therefore employed as a confirmatory test in the identification of virus isolates. However, some cross-reactions occur in this test. Consequently, the neutralization of a virus by a typing serum without quantitative comparison with the homologous system is not adequate for identification. This test is commonly conducted in newborn mice as a mouse protection test and also in tissue culture using the plaque-assay technique.

The CF test can be used to place viral isolates in antigenic groups. The test is not commonly applied as a confirmatory test because of cross-reactions observed among viruses belonging to the same antigenic group. These cross-reactions can be reduced by the use of specific monoclonal antibodies. For some groups of viruses, such as alphaviruses, where minimum cross-reactions are demonstrable, the CF test is used for specific indentification. The presence of nonspecific reactions, and also the finding that some sera and viral antigens are anticomplementary, cause difficulties in interpreting CF results. These unfavourable reactions can, however, be minimized by treating antigens and sera. The CF test is also used as an aid to the serological diagnosis of infectious diseases because it can easily detect seroconversion.
The HI test is used for placing virus strains in appropriate antigenic groups but not for specific identification because of marked cross-reactions demonstrable among viruses belonging to the same antigenic group. It is also used extensively in epidemiological surveys of virus infections because HI antibodies are relatively long-lasting. Its application is, however, limited to haemagglutinating viruses. Passive haemagglutination-inhibition tests can be used for those viruses that do not haemagglutinate under conventional conditions. Nonspecific inhibitors and natural haemagglutinins that cause problems in interpreting the results can be removed by the treatment of sera.

In addition to the conventional techniques described above, other techniques have also been introduced in reference laboratories. These include:

— the detection of viral antigens by direct and indirect immunofluorescence techniques;
— the application of monoclonal antibodies for the identification of new viral isolates;
— the use of enzyme immunoassay (EIA) and radioimmunoassay (RIA);
— the application of biochemical analysis of viral proteins and nucleic acids, e.g., polyacrylamide gel electrophoresis and oligonucleotide mapping.

6.2 Rapid diagnostic methods

The use of rapid methods in the diagnosis of viral infections has become increasingly important in recent years (18). Rapid diagnosis is particularly important with arboviruses and rodent-borne viruses in view of the many life-threatening diseases in this category (e.g., yellow fever, dengue haemorrhagic fever, the encephalitides, and Lassa fever) and the often rapid spread of the agents by appropriate vector(s).

Several general approaches seem applicable, including those described below.

6.2.1 Detection of antigen

(a) Direct detection of antigen or virus is possible under certain circumstances. If virus is plasma-associated and reaches a sufficiently high level it may be possible to detect the virus in the
serum during the viraemic stage directly by EIA or RIA. Other specimens may also be used, e.g., urine, cerebrospinal fluid, and throat washings. However, this technique is successful in only a few diseases, e.g., West Nile fever, CCHF (18). The principal requirement for the success of this test is the presence of sufficient virus in the specimen. In many arboviral infections, viraemia is often transient and does not reach very high levels. In some situations (e.g., dengue), the presence of antibodies and antigen-antibody complexes causes further complications. Direct visualization by electron microscopy appears to have only limited application with these viruses. In situations where the virus is cell-associated, viral antigens on circulating leukocytes or erythrocytes could be detected by the fluorescent antibody technique (FAT).

The detection of antigen in mosquitoes collected in the field is also possible (e.g., by EIA or FAT). In addition, with more specific reagents (e.g., monoclonal antibodies) it may be possible with EIA to detect antigen in the absence of demonstrable viraemia. Another possible new approach is the detection of viral RNA in clinical specimens by in situ DNA–RNA hybridization with biotin-labelled DNA (19), and RNA–RNA hybridization using 3'-end-labelled RNA and nitrocellulose blotting. However, further evaluation and development of this approach is necessary.

(b) If insufficient virus is present to be detected directly, specimens can be inoculated into cell cultures or mosquitoes, incubated for 2–3 days and then detected by FAT (direct or indirect method). The sensitivity of this method may also be improved significantly by the recent development of more sensitive cell lines (especially those of mosquito origin), which may permit earlier detection of viral antigens. Monoclonal antibodies can be used for specific antigen detection. Other methods, such as reverse passive haemagglutination (RPHA), may also be used to detect viruses in culture fluids.

Further cloning and selection of cell lines sensitive to infection by these viruses need to be pursued. In this regard, there is a special need for lines sensitive to tick-borne and phlebotomine-borne viruses. Monoclonal antibodies for subtyping certain viruses (e.g., VEE, TBE) need to be developed.

6.2.2 Detection of antibodies

(a) Serological diagnosis still relies largely on the use of conventional tests, such as CF, HI, and neutralization. Single radial
haemolysis (SRH) is useful in the diagnosis of dengue, TBE, YF, West Nile fever, and VEE.

(b) Immunoglobulin M (IgM) is the first antibody class produced following infection and its presence is usually diagnostic of current infection. The efficiency of detection of IgM is influenced by the type of infecting virus, the course of illness, and the method used for detection. Two problems are often encountered. The presence of large amounts of antiviral immunoglobulin G (IgG) may block the binding of IgM to antigen, which may result in false negatives. Also, the presence of rheumatoid factors could result in binding to IgG and yield false positive reactions.

A large number of different techniques have been used in the detection of IgM (e.g., FAT, EIA, RIA, HI) and virtually all require preliminary removal of IgG and rheumatoid factors except the IgM “antibody-capture” enzyme-linked immunosorbent assay, which uses an anti-μ chain globulin on a solid phase as the first layer. This technique has been used successfully in the rapid detection of specific IgM in the cerebrospinal fluid of patients with JE within 12 hours of hospital admission (20) and also in epidemic polyarthritis, in infections with La Crosse, YF, and TBE viruses. Several factors related to the method need to be considered. The specificity of the IgM antibody in a particular virus infection will need to be determined carefully.

Cross-reactions may occur, especially with the flaviviruses, which may result in false positives. Also, although it is thought that specific IgM arises as early as the second or third day after the onset of illness in most virus infections, the time of appearance needs to be established carefully for each virus. In primary dengue infection, for example, specific IgM appears in the serum between the fourth and sixth days after the onset of illness. In secondary dengue infections, which constitute the majority of dengue cases in South-East Asia, the IgM response is markedly suppressed, often to undetectable levels. These observations relate to the use of the HI test and it is possible that more sensitive methods (e.g., EIA) may be able to detect IgM earlier in the course of illness. Lastly, it is crucial that high-quality reagents should be used in the IgM antibody-capture EIA technique; there is a need for the development of reagents that are stable under field conditions.
6.3 Techniques in rapid diagnosis

Details relating to the technical characteristics, advantages and disadvantages, reagent standards, and quality control and applications of various rapid diagnostic techniques have been described in detail elsewhere (18).

On the basis of disease severity, number of people affected, and recent disease activity, several arbovirus and rodent-borne viral diseases would appear to justify a high priority with regard to the application of rapid diagnostic methods (Table 5). The various methods that could be used in the laboratory diagnosis of these diseases are discussed below.

Table 5. Priority viral infections for application of rapid diagnostic methods

<table>
<thead>
<tr>
<th>Group</th>
<th>Disease</th>
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<tr>
<td>Arthropod-borne viruses</td>
<td>Dengue fever/dengue haemorrhagic fever</td>
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<td></td>
<td>Yellow fever</td>
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<td></td>
<td>Rift Valley fever</td>
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<td></td>
<td>Epidemic polyarthritis</td>
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<td>Japanese encephalitis</td>
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<td>Venezuelan encephalitis</td>
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<td>Crimean-Congo haemorrhagic fever</td>
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<td>St Louis encephalitis</td>
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<td>California encephalitis</td>
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<td></td>
<td>Tick-borne encephalitis</td>
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<tr>
<td>Rodent-borne viruses</td>
<td>Machupo haemorrhagic fever</td>
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<td></td>
<td>Junin haemorrhagic fever</td>
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<td></td>
<td>Haemorrhagic fever with renal syndrome</td>
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<td></td>
<td>Lassa fever</td>
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<tr>
<td>Viruses with unknown mode of</td>
<td>Marburg virus disease</td>
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<tr>
<td>transmission</td>
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<td></td>
<td>Ebola virus disease</td>
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6.3.1 *Arthropod-borne viruses*

(a) *Dengue*. The viruses can be isolated in C6/36 (*Aedes albopictus*), AP-61 (*Aedes pseudoscutellaris*), or TRA-284 (*Toxorhynchites amboinensis*) cell lines and the antigen detected by FAT using fluorescein isothiocyanate (FITC)-conjugated pooled convalescent sera. The isolates can also be typed by FAT using type-specific monoclonal antibodies. The most sensitive cell lines appear to be AP-61 and TRA-284. Intrathoracic inoculation of mosquitos could also be used but is more time-consuming. EIA methods to
detect antibody have been developed and IgM antibody-capture for
dengue-specific IgM may prove useful. However, further evaluation
is required. Intracerebral inoculation of mosquitoes, rather than
inoculation by the conventional intrathoracic route, permitted the
detection of dengue-2 antigens by direct FAT as early as 4 days after
inoculation, compared with the 10–14 days following intrathoracic
inoculation (2f). CV-1 cells (of monkey origin) provided a sensitive
system, permitting the detection of dengue viruses in 48 hours and
5 days after inoculation by FAT and electron microscopy
respectively. It should be possible to detect anti-dengue antibodies
synthesized by peripheral blood lymphocytes from dengue patients
following a period of in vitro culture.

(b) Yellow fever. The virus can be isolated in cell cultures
(e.g., C6/36, AP-61, Vero) and in mosquitoes and detected by
FAT using polyclonal and monoclonal antibodies. Rapid serological
diagnosis can be carried out by means of the detection of specific
IgM.

(c) Rift Valley fever. When viraemia is high enough, the presence
of antigen in acute-phase human serum may be detected using agar
gel immunodiffusion. Virus isolations can be made from cell cultures
(e.g., Vero) or sucking mice and isolated virus identified by HI, CF,
or FAT. EIA methods for the rapid detection of antigen and
antibody have also been developed. RPHA can also be used to detect
antibodies. RVF virus can be detected by FAT and EM 22 hours
after the inoculation of CV-1 cells.

(d) Ross River disease. The recent spread of Ross River virus in
the Pacific and, potentially at least, in South-East Asia emphasizes
the need for a rapid diagnostic method. Ross River virus is isolated
using Vero cells or sucking mice and identified by the neutralization
test. Rapid methods for detecting specific IgM by EIA and IgM
antibody-capture ELISA (enzyme-linked immunosorbent assay)
have been developed but require further testing.

(e) Japanese encephalitis. IgM antibody-capture ELISA will
detect specific IgM in the acute-phase serum and cerebrospinal fluid
(20). The test should be repeated in 5–7 days if acute specimens are
negative.

(f) Venezuelan encephalitis. Virus can be isolated in Vero cells and
suckling mice and identified by HI, CF, EIA, or neutralization.

(g) Crimean–Congo haemorrhagic fever. Suckling mice are used
for virus isolation and antibody detected by FAT on infected cells.
An EIA and a solid-phase RIA test have been developed.
(h) St Louis encephalitis, California encephalitis, and tick-borne encephalitis. Antibody-capture ELISA is used to detect specific IgM in the cerebrospinal fluid.

6.3.2 Rodent-borne viruses

(a) Haemorrhagic fever with renal syndrome. Antibodies to Hantaan virus are detected by FAT in the lungs of virus-infected Apodemus mice or A-549 cells. Antigen can also be detected by EIA in rodent tissues and human serum. EIA and RIA methods may be more sensitive and reproducible than FAT.

(b) Junin haemorrhagic fever and Machupo haemorrhagic fever. A FAT test on urinary sediment round cells has been used to diagnose JHF as early as 24–48 hours after the onset of symptoms. Otherwise, isolations of virus are made from blood in Vero cells (JHF) and antibodies detected by FAT or standard tests in convalescent JHF and MHF. It is important to use reagents from virus strains from the geographical region in question because sufficient antigenic variation is found in the FAT to give false-negative results if specimens and reagents are mismatched in this regard.

(c) Lassa fever, Marburg virus disease and Ebola virus disease. Specimens from suspected cases should be handled only by maximum security reference laboratories. Viruses isolated from human sera in Vero cells are identified by FAT using mouse monoclonal reference antisera. Electron microscopy may be used to detect virus in autopsy material, particularly in liver specimens. Antibodies to these viruses may be detected by indirect FAT using slides with spots prepared from virus-infected cells.

6.4 WHO Collaborating Centres

Several institutions active in arbovirology have been designated WHO Collaborating Centres to assist the WHO Virus Diseases Programme in developing international collaboration towards the understanding, prevention, and control of virus diseases (Annex 2). The terms of reference of a collaborating centre are:

1. To provide assistance in identifying arbovirus strains isolated in its area of coverage. Submission of strains to regional reference centres for further identification facilitates the recognition of new viruses.

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2. To prepare reference sera and antigens from viruses isolated in its region and to provide small amounts of these sera and inactivated antigens to laboratories working with these viruses.

3. To maintain and provide prototype and reference strains.

4. To provide consultant advice when requested by WHO to national and other laboratories dealing with arthropod-borne viruses.

5. To collect and disseminate epidemiological and technical information on arthropod-borne viruses.

6. To receive fellows for training purposes.

7. To keep WHO fully informed of newly isolated strains and of epidemiological developments, and to report annually on the work performed.

8. To evaluate new or updated diagnostic techniques.

9. To assist in the quality control of laboratory reagents.

10. Upon request, to give assistance or advice during epidemics.

Close cooperation between these centres is vital to the WHO Virus Diseases Programme.

7. PATHOGENESIS

7.1 Pathogenesis of systemic febrile disease

Although there are examples of human arboviral infections in which viraemia without fever has been demonstrated, and many instances where clinically silent infections result in specific antibody formation (viraemia unknown), the basic clinical response to infection by arboviruses and rodent-borne viruses is fever and viraemia. Very little is known about the pathogenesis of febrile disease. What little we know stems primarily from animal experiments with a few viruses, such as VEE and dengue. The following description is speculative but may be useful, especially to indicate areas for future investigation.

Following the bite of an infected arthropod, the virus enters the microvascular circulation directly or replicates in the dermal tissue at the site of the bite. From there the virus is transported by afferent lymphatics to the regional lymph nodes. Viraemia follows virus replication in lymphoid tissue and in some cases possibly in mononuclear leukocytes, vascular endothelium, or immature cells of
the erythrocyte series. In most arbovirus infections of man, the viraemic phase is inapparent, with no associated clinical response. Presumably, in these silent infections, the virus does not infect target organs such as the liver or brain or, if it does, there is no functional incapacitation. In the vast majority also, the immune response results in rapid clearance of the virus from the circulation.

Systemic signs and symptoms are associated with, or follow the onset of, viraemia. These are typically fever, headache, myalgia, and photophobia. Thrombocytopenia, leukopenia, or mild leukocytosis with relative lymphopenia are observed. Conjunctivitis, lymphadenopathy, skin rash, and arthralgia may be seen in diseases produced by some of the arboviruses. The fever pattern may be diphasic and it has been postulated but not proved that the second febrile episode is the result of viral, antigen-antibody complex formation. The initial events in infection with viruses that have the capacity to produce the syndromes of haemorrhagic fever and/or encephalitis are thought to be fundamentally similar, although other virus properties must exist to account for the virulence of these agents.

A number of the alphaviruses are notable causes of acute febrile illness marked by significant polyarthritis, which may persist or recur long beyond the acute primary infection. This joint syndrome is not histologically compatible with acute antigen-complex disease. It is seen much more often in adults than in children infected with RR virus and has been postulated to be related to activation of natural killer cells of the lymphocytic immune system. Investigation of this problem, possibly in an animal model, but most likely in patients, is highly desirable. Furthermore, comparative pathogenic studies, whether in vitro or in vivo, between related viruses producing only self-limited systemic fever and more serious symptoms, are likely to advance understanding of the pathogenesis of the viruses.

7.2 Encephalitides

Encephalitis is a primary manifestation in man of the alphaviruses: EEE, WEE, VEE, and Semliki Forest; the mosquito-borne flaviviruses: SLE, JE, Murray Valley encephalitis (MVE), WNE, Ilheus, Rocio, and Ntaya; the tick-borne flaviviruses: Far Eastern, Central European, louping ill, Powassan, and Negishi; the flavivirus of unknown vector: Rio Bravo; the bunyaviruses: La Crosse, California encephalitis, snowshoe hare, Jamestown Canyon,
and RFV; the orthomyxovirus: Thogoto; and the unclassified tick-borne virus: Bhanja. Encephalitis may be a rare manifestation of other arbovirus infections, including post-vaccination with the 17D and French neurotropic strains of yellow fever. Central nervous system (CNS) disease occurs in some cases of arenavirus infections, such as Lassa fever (LF) and post-immunotherapy AHF.

7.2.1 Pathogenesis in man

The pathogenesis in man is poorly understood. The pathology indicates that arboviral encephalitides are acute viral infections of the brain with varying degrees of associated meningitis. Destruction of neurons and other elements in the brain is believed to result from direct invasion of the virus and not primarily from immunopathology. Following introduction of the virus by the bite of the arthropod, there may be local replication either in the skin, the lymphatics, or the endothelium of the blood vessels. Viraemia occurs. Entry into the nervous system is either by capillary seeding through the endothelium into the meninges and the brain, or through infection of nerve endings, such as the neuromuscular junctions and the olfactory receptors, with subsequent axoplasmic transport to neurons.

It is not at all clear that arboviral encephalitis invariably involves the actual infection and destruction of neurons. Since viraemia is a prominent precursor of CNS infection, it is possible that arteritis rather than neuronitis accounts in some infections for the clinical signs and for the histopathological findings, such as perivascular cuffing. Studies are needed of human encephalitis to localize accurately within the brain parenchyma the sites of antigen formation and the specific cell types involved.

The human encephalitis associated with RVF may or may not be a result of immunopathology. Unlike other arboviral encephalitides, the CNS manifestations occur late (5–15 days after the febrile phase). JE in some populations and SLE have peak incidence in persons over 60 years of age. The explanation is not understood, but it may relate to aging of the immune system or, in the case of JE, to the waning of protective antibodies. An animal model should be developed to study this age-dependent virulence and the mechanism should be explored.

Because encephalitis during arboviral infections follows the viraemic phase, most patients at the time of presentation for medical
care already have an antibody response, both peripherally and in the CNS. CNS antibody is produced by immunocytes in the brain; antibody titres in the cerebrospinal fluid are sometimes higher than in serum, and antibody-producing lymphocytes have been recovered from cerebrospinal fluid during acute encephalitis. Rapid and early diagnosis by EIA for IgM in serum and cerebrospinal fluid is practical in many of these cases.

7.2.2 Pathogenesis in animal models

Most of the information on pathogenesis of arboviral and arenaviral encephalitis derives from animal experiments.

Alphaviruses and flaviviruses in the family Togaviridae are nearly all pathogenic for mice (neuroadapted) if inoculated into the brain. Several, including Semliki Forest, Venezuelan equine encephalitis, Western equine encephalomyelitis, Eastern equine encephalomyelitis, Rocio, Far-Eastern tick-borne encephalitis, and Banzi viruses also produce encephalitis in adult mice following peripheral inoculation. The resulting infection consists of a viraemic phase followed by entry into, and infection of, the CNS similar to that observed in human infections with some of these viruses. Death presumably results from neuronal damage as a direct effect of the virus.

Early studies with alphaviruses showed that the expression of neurovirulence depended on the ability of the mouse to mount an immune response more rapidly than the virus-induced cell injury could occur. These experiments involved Western equine encephalomyelitis viruses that replicated at different rates. Virus strains that replicated rapidly killed mice, while those that replicated more slowly did not kill.

A wide variety of studies with flaviviruses and alphaviruses, mostly in mice, have been reviewed by Nathanson (22). Although results varied according to sex, virus, dose, host, age, route of inoculation, and temperature, some conclusions can be drawn.

Following subcutaneous inoculation, virus travelled by afferent lymphatics to regional nodes. Virus replicated at times in the subcutaneous tissue and commonly in the regional node. Lymph was a major source of viraemia, although vascular endothelium may also have contributed. The plasma contained most of the virus. Clearance of alphaviruses from the circulation was associated with a low
charge on the virus particles and the turnover rate of flavivirus in the plasma was about 30 minutes. The probability of CNS invasion correlated with the level of viraemia. Viraemia terminated when serum antibody appeared, although new virus-antibody complexes continued to be formed. The mode of entry into the neuroparenchyma is not known. Infection spread rapidly once neurons were infected. Different parts of the brain differed in their susceptibility, which varied from virus to virus.

The CNS lesions involved destruction of neurons, invasion of mononuclear cells, and perivascular cuffing. Inflammation was often proportional to survival time after infection of the CNS. Both antibody and killer T cells were formed. The antibody was produced in the CNS. Flaviviruses induced interferon and were sensitive to it. Passively transferred antibody was protective even after virus had invaded the CNS; however, infected animals often died, even in the presence of an excellent immune response. An immunopathological response in the CNS was reported with TBE, but could not be demonstrated in viruses of the SLE/WN/JE subgroup.

*Effect of host and viral genetics on pathogenesis.* Genetic properties of the host affected virulence. Genetically resistant inbred mice supported infection with a flavivirus as well as did genetically susceptible mice. If immunosuppressed, the resistant mice died, and it was shown that resistance was related to cell-mediated immunity. The genetic mechanism of control is not clear.

Genetic properties of the virus may also determine virulence. La Crosse virus was neurotropic in young adult mice following peripheral inoculation, while the related bunyavirus, Tahyna, was not. The neurotropism segregated in reassortment experiments with the middle-sized RNA segment, which also coded for the viral surface glycoproteins involved in attachment. The ability of the virus to enter the CNS and infect neurons appeared to be an attachment-related phenomenon. La Crosse virus, inoculated subcutaneously into the hind limb of a mouse, produced viraemia over a period of 40 hours, then at 48 hours was detected as fluorescing antigen, first in spinal cord motor neurons and in nerve tracts innervating hind limb muscles; Tahyna virus did not enter the nervous system. These findings were consistent with the hypothesis that virus entered the nervous system via the neuromuscular junction rather than by viremic seeding of the brain. Immunosuppression with cyclophosphamide of mice infected with La Crosse virus had no
observed effect on the outcome of the encephalitis, indicating that in mice the La Crosse encephalitis is primarily a direct effect of the virus and not an immunopathological phenomenon.

In a study of the pathogenesis of RVF encephalitis in the inbred rat, rats of susceptible lines died rapidly with liver necrosis, while resistant animals developed encephalitis 1–3 weeks after peripheral inoculation of virus. When encephalitis developed, the viraemic and hepatic phases had already terminated, as is the case in man. There were high titres of virus in the brain and focal acute necrotic lesions with perivascular cuffing, which may have been a response to direct viral cytopathology or to antibody and complement-inducing chemotaxis.

Induction of encephalopathy. The rhesus monkey has been used to study the pathogenesis of the arenaviruses. A generalized arteritis occurred in monkeys recovering during specific antibody therapy. The arteritis was accompanied by CNS signs. The encephalitic signs seen could well represent an encephalopathy rather than encephalitis. It has been postulated that the delayed CNS disease that sometimes follows varicella infection is an encephalopathy rather than encephalitis. Encephalopathy is of unknown pathogenesis but is not a result of direct virus infection in the brain. Presumably it is a CNS toxic manifestation of viral products or cell damage in other parts of the body.

7.3 Viral haemorrhagic fevers

7.3.1 General considerations

The haemorrhagic fever syndrome associated with both arboviruses and rodent-borne viruses may have some common features in terms of clinical and pathological expression, yet each displays certain distinct characteristics. In general, the pathogenesis of most of these viral diseases with haemorrhagic fever syndrome is unclear and much more research is needed to elucidate some of the issues involved. As a general principle, the major effector pathogenic pathways appear to be haemorrhage and an increase in vascular permeability. Haemorrhage is brought on by thrombocytopenia, coagulation defects, activation of the intrinsic coagulation system, which leads to varying degrees of disseminated intravascular
coagulation (DIC), and vasculopathy. Some of these factors are more important in certain diseases than in others. The increase in vascular permeability leads to the leakage of plasma protein, water, and electrolytes into the extravascular compartment and hence to hypovolaemic shock and haemoconcentration. Again, the outcome varies among different haemorrhagic fevers, but DHF/DSS is a good example of this pathophysiology. Since, in most instances, definable pathology of the microvascular system (capillaries and venules) is not apparent, it is believed that in some of these diseases mediators that cause an increase in vascular permeability are released or activated by some as yet unknown mechanism. Activation of the complement system appears to be an interesting feature in a number of these haemorrhagic fever syndromes. The major organs involved in these diseases include liver, kidneys, lung, skin, brain, and the reticuloendothelial system. The last of these organs appears to be the major site, i.e., in the lymphoid tissue, of viral replication prior to viraemia. The degree of involvement of organs again varies from disease to disease. The liver, which is a reticuloendothelial organ as well as a parenchymal one, can vary its pathological response from outright icteric hepatitis with massive liver cell failure (YF) to anicteric hepatitis, but still with severe parenchymal cell damage (LF), or to major involvement of the Kupffer cells with mild to moderate liver cell involvement (DHF).

In some of the diseases, direct cell injury by the virus appears to be a major pathological event, while in others this is less certain and immunologically mediated injury has been implicated. The exact mechanism remains to be elucidated. While in most virus haemorrhagic fevers, the clinical syndrome can be manifested during the primary infection, in DHF/DSS the majority of cases occur during the secondary infection with a different subtype of the virus. Animal models are available for some of the viral haemorrhagic fevers, but in many other diseases study of the patients appears to be the only way to probe the intricacy of the pathogenetic mechanisms.

Despite these limitations, much has been learned about some of the viral haemorrhagic fevers and this information has already had a major impact on the therapy of at least one disease, DHF. Aspects that seem likely to influence the outcome of infection leading to a haemorrhagic fever syndrome and that require further careful investigation include:
(a) Genetic variation in both human host and infecting virus leading to the induction of haemorrhagic fever. The expression of such genetic factors may vary widely.

(b) Virus-reticuloendothelial cell interactions. Virus may infect and replicate in macrophages and/or lymphoblasts, leading to suppression of the immune response, thus permitting direct attack on parenchymal organs with subsequent activation of the coagulation system. Alternatively, virus infection of such cells, possibly aided by small amounts of preformed or newly induced antibody, may lead to sudden destruction of such cells with release of mediators, which cause vascular injury and shock. Interactions between virus-infected cells and the host immune system may result in the killing of infected cells (e.g., by cytotoxic T cells) with the resultant release of mediators. It is also conceivable that mediators (lymphokines) are released from T cells following antigenic stimulation. Virus-platelet interactions may be important, and up to now only a few studies have been done. The role of interferons in limiting viral infection is far from understood. Host genetics may play a crucial role in determining individual responses to these mediators as well as a role in the mechanisms of immunopathogenesis already cited.

7.3.2 **Junin haemorrhagic fever**

About 20 years ago it was postulated that both MHF and JHF were immunosuppressive diseases. This suggestion rested on the relatively long interval between the termination of acute illness and the development of neutralizing antiviral antibodies (10–20 days) and on the high incidence of secondary bacterial pneumonia that characterized both syndromes.

It has now been amply documented in both human beings and guinea-pigs that Junin virus produces both humoral and cell-mediated immunodepression. The latter is both virus-specific and nonspecific. Other studies have shown that there is complement activation but no apparent cascade of clotting defects, and there is little evidence to support the occurrence of DIC. Although the pathogenetic trigger for haemorrhage and shock remains elusive, these new data suggest that fatal outcome in JHF may correlate with inability to initiate a cell-mediated immune response. If this were so, and no significant variation in virus virulence exists, then it would
seem that about 15% of patients have a functional deficiency in their ability to respond immunologically to Junin virus.

Even more interesting, and certainly more important, is the fact that virus-specific antibodies administered to patients during the first week of illness are able to clear the virus rapidly from the blood and to prevent death. Thus, virus in circulation or kinins released by infection of certain as yet unknown cells may be the trigger(s) for haemorrhage and shock. One must conclude also that, were it not for the humoral immunosuppression regularly induced by Junin (and the related Machupo) virus, the infection in man would cause no more than a brief nonspecific illness.

7.3.3. Lassa fever

This disease cogently illustrates that host responses to members of a given virus family (in this case arenaviruses) are not identical. Little has yet been done with respect to cell-mediated immunity in Lassa fever. In a small number of fatal cases, skin test anergy and clear suppression of circulating T-helper lymphocytes has been demonstrated. It is known that Lassa virus replicates in vitro in macrophages and in vivo in lymphoblasts of infected rodents. Failure of normal host immune response to infection in patients with severe disease is further indicated by the long febrile course of illness, with viraemia lasting as long as 3 weeks. Indeed, the amount of virus in blood, together with the concentration of circulating serum aspartate aminotransferase (AST) (E.C. 2.6.1.1)\(^1\), indicative of severity of viral-induced liver damage, reliably predicts death or survival in LF. It is estimated that about 1% of Lassa virus human infections result in this severe pattern of uncontrolled virus replication.

Humoral antibody response to Lassa virus infection is unique among viral haemorrhagic fevers. Antibodies are formed early in clinical disease, so that about 40% of patients are positive on admission to hospital. Both IgM and IgG are present and neither the time of appearance nor the titre of these proteins appears to differ in fatal as compared with nonfatal disease. These antibodies do not neutralize the virus. Virus and antibody are detectable for several days in a given specimen without resort to methods for the

\(^1\) Previously known as glutamic oxaloacetic transaminase.
dissociation of virus-antibody complexes. The virus is precipitated from serum by anti-IgM but not anti-IgG antibodies, indicating that it is coated with non-neutralizing and possibly non-opsinizing IgM antibodies. Neutralizing antibodies begin to appear about one month after the onset of the disease, but quantitative development is a slow process. In contrast to immunofluorescent non-neutralizing antibodies, which reach high titre by 30–60 days after disease onset and decline thereafter, neutralizing antibodies frequently take 6 months or more to achieve their highest titres. In addition, such antibodies are poor neutralizers of the virus in vitro. The constant serum-virus dilution method yields best results and indices of 10^2 are reached by only a minority of LF patients.

A basically similar pattern is observed following infection of rhesus monkeys or guinea-pigs, but those animals that survive acute infection achieve neutralizing antibody titres of about 10^5 at 6 months after inoculation. In these models, reasonable doses of such antibodies were found capable of preventing death in cynomolgous monkeys and strain 13 inbred guinea-pigs, all of which otherwise succumb. Treatment was successful when started 4 days after infection. Titration of these antibodies revealed that an index of 10^2.3 was required for effectiveness. Human plasmas do not contain that much antibody, but concentration of such material permitted the establishment of a similar therapeutic index in guinea-pigs.

These experimental data are compatible with the results of controlled trials of human plasma in the treatment of LF in Sierra Leone. The fatal course of illness in patients admitted with high viraemia and major elevation of serum AST was unaffected by such treatment, and viraemia was not reduced. In contrast, patients with less viraemia and hepatitis frequently terminated viraemia immediately after receiving plasma, although the average hospital stay was not reduced. It seems possible that such patients might have survived in any case.

Taken together, these observations perhaps explain why passive antibody therapy for LF has had mixed results. There are clearly two sets of hospitalized patients and there are now objective criteria for their recognition. Available antibodies are insufficient to treat the fatal set. Another lesson to be relearned, and it may have specific value for other haemorrhagic fevers, is that the aim is to administer a known amount of virus-neutralizing antibodies, not simply “plasma”.

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7.3.4 Rift Valley fever

Three complications of otherwise benign febrile RVF have now been recognized: vascular retinitis, encephalitis, and haemorrhagic fever. The pathogenesis of these phenomena remains unclear. The true incidence of each syndrome is also unknown, although less than 5% of infections produce retinitis and probably less than 1 in 1000 infections lead to the more severe complications. Interestingly, no patients have been observed to have more than one of this triad of troubles.

The incubation period of RVF is 3–6 days, and infection may occur by arthropod bite, through direct contact with blood or the carcasses of animals, or by aerosol, although nosocomial infections are not recorded. High viraemia ($10^4$–$10^6$/ml) is the rule, but it persists only 3–5 days in most cases. Retinitis becomes symptomatic 1–2 weeks later, as a rule, and there is also a "latent" period without viraemia prior to the onset of clinical encephalitis. High titres of virus-specific antibodies in the cerebrospinal fluid of such patients bear witness to virus replication in the brain, but shed no light on how and when the virus reaches this organ. In contrast, the haemorrhagic fever complication usually occurs towards the end of the febrile-viraemia phase of illness. There is severe hepatitis with clinical jaundice and persistent viraemia, sometimes in the presence of antibodies. Haemorrhagic phenomena are grave signs and mortality in this syndrome is extremely high. Whatever the pathogenic mechanisms may be, the very low frequency of occurrence of complications suggests that human genetic factors are most likely the principal determinants of severe illness.

Such a pattern has now been demonstrated for experimental RVF infection using inbred rats. Wistar-Furth rats are highly sensitive to the virus and die with fulminant hepatitis within 5 days of receiving as little as 3 plaque-forming units (PFU). Lewis rats, in contrast, are clinically resistant to more than $10^6$ PFU, while Maxx strain rats exhibit a pattern of silent or late encephalitic infection. Resistance to lethal hepatic necrotizing infection was found to segregate as a single autosomal dominant genetic trait. Furthermore, it has been learned that macrophages from resistant Lewis rats are highly sensitive to interferon, with the result that virus replication in these cells is restricted as compared with macrophages from Wistar-Furth rats. Because the haemorrhagic form of RVF occurs after only a brief episode of fever and appears to result from direct attack by
virus replicating at a very high rate, it is reasonable to speculate that macrophage function, rather than immune response, may represent the critical event in determining the outcome of infection. Human population studies to determine patterns of response of circulating monocytes to interferon and RVF virus might prove rewarding.

7.3.5 Haemorrhagic fever with renal syndrome and "nephropathia epidemica" (NE)

The pathogenesis of these diseases is far from understood. However, the recent isolation of their immunologically related causative viruses and the development of assays for virus-specific antibodies now permit work to begin on this problem. Acute renal interstitial nephritis, sometimes with complete functional shutdown, is a major and relatively late manifestation of these diseases. Is the lesion induced by antigen–antibody complexes? As yet there is no answer, but it seems possible. Complement activation has recently been demonstrated. Immunofluorescent and neutralizing antibodies are found in sera of most patients early in the febrile phase of illness and increase steadily for about 30 days. Indeed, it has proved very difficult to recover virus from acute blood specimens when patients enter hospital. Antibodies also persist for decades after infection, sometimes in surprisingly high titres, and some patients show significant increases in titres between 2 and 12 months after illness. Finally, circulating antigen–antibody complexes of uncertain specificity have been found in NE patients and they persist well beyond the phase of acute illness.

Progress in understanding these fascinating pathogens will be made if a suitable animal model can be found. Subhuman primates are currently under investigation.

7.3.6 Yellow fever

Despite the venerable status of the yellow fever virus as the oldest described haemorrhagic fever agent, much remains to be learned concerning the pathogenesis of this disease. It has long been known that death due to YF virus infection is largely attributable either to an early fulminating haemorrhagic fever syndrome with hepatitis and clinical jaundice or to a later renal tubular lesion with renal insufficiency reminiscent of that seen in HFRS.
Recent studies in rhesus monkeys demonstrate that high viraemia and major hepatitis somehow lead rapidly to severe acidosis, hyperkalaemia, renal insufficiency, and hypotension, the so-called hepatorenal syndrome. The time course of rhesus monkey infection is compressed as compared with that observed in human YF, thus casting some doubt as to whether this model is valid. For example, there are virtually no data on the quantitative development of viraemia in YF. In one fatal human case, daily titrations done in cell cultures revealed about $10^7$ infectious units/ml until the day of death, when there was an abrupt drop to $10^2$. Simultaneous measurement in mosquitos, however, showed that all specimens contained approximately $10^7$ infectious units. This is taken as evidence that antigen–antibody complexes were formed during the last day of life, and it was further found that the patient's serum had small amounts of neutralizing antibody at the time of death.

Histological lesions in the liver of the YF-infected monkey were not observed until the day of death. Could it be that necrosis was massively induced by complement-dependent immune cytolysis of infected cells having virus-specific membrane antigens at the time of initial antibody response? And would such an event lead to renal tubular disease if the liver insult were somehow survived? These are worthwhile questions because, despite the existence of YF vaccine and the new physiological knowledge gained from experimental work, people still die from YF. What is important to know is whether or not passive antibodies might be effective in the treatment of the disease. Work done half a century ago gave a negative verdict, but examination of those reports reveals that only small amounts of antibody were administered. In the light of developments with other haemorrhagic fevers, this problem should be re-examined. Search for an animal model of a virus strain–host combination that simulates the course of human infection is indicated.

7.3.7 Dengue haemorrhagic fever

The pathogenesis of DHF could be considered in relation to direct cellular injury by dengue viruses, or to immunologically mediated injury, or to a combination of both mechanisms (25). The observations that there are at least two kinds of clinical syndrome associated with dengue infection, i.e., classical DF and DHF, and that a large number of subjects who manifest DHF/DSS syndrome have secondary infection, led to the idea that immunopathological
injury may be important in the pathogenesis of DHF/DSS. Whatever this mechanism may be, there seem to be two important effector pathways. One leads to haemorrhage manifested through thrombocytopenia, coagulation defects, and some degree of intravascular clotting, and the other leads to an increase in vascular permeability with leakage of water, protein, and electrolytes from the vessels, and hypovolaemic shock. Activation of the complement system is a constant feature of severe DHF, both primary and secondary. Some unique features of DHF/DSS deserve consideration. There is no animal model for DHF/DSS so that the study of patients is the only way to understand the disease. The fact that several controversial issues are to be found in the literature may be related partly to inconsistency in clinical definition and to lack of precision in grading the severity of the disease, despite repeated attempts by WHO to standardize these by the publication of its technical guides. Other problems relate to the rapidity with which the DSS syndrome evolves from preshock to shock and to recovery or death. Since this usually occurs in a matter of hours, the molecular and cellular events that lead to the pathophysiology and the homeostatic adjustment attempts of the body could be difficult to define in a sequential systematic manner.

_Dengue virus/cell interaction._ All four types of dengue virus have been isolated from the blood of patients with DHF/DSS during the febrile stage. The viruses have also been recovered from tissue such as the liver, lymph nodes, bone marrow, and lungs of fatal cases. Certain types of dengue virus may be more important in the morbidity and mortality related to DHF in some countries, such as type 3 in Indonesia and type 2 in Thailand, but it is well accepted that all 4 types appear to be pathogenic for DHF/DSS. It may be possible to relate some inherent properties of different strains of dengue virus to the morbidity of the infection.

Peripheral blood monocytes support dengue virus replication, and replication is enhanced in the presence of subneutralizing levels of antibodies to dengue and some other flaviviruses. The viruses grow in human B lymphocytes, monocytes, and endothelial cells. Dengue viral antigen has been localized in peripheral blood monocytes from both primary and secondary cases of DHF. The proportion of dengue-antigen-carrying monocytes in the peripheral blood is rather small (0.05–0.1%). Dengue virions have been observed in those monocytes by transmission and scanning electron
microscopy. Dengue viral antigen was localized at autopsy in the tissue of fatal cases of DHF. The viral antigen was found by FAT in the Kupffer cells of the liver, in the phagocytic cells lining the sinuses of the lymph nodes and spleen, in the subcortical portion of the thymus glands, and in the alveolar macrophages in the lung. More antigen was observed in autopsy material from infants (aged less than one year) who died from DHF associated with primary infection than in material from older children with secondary dengue infection. This was true even after repeated attempts to elute the antibody that may cover the antigenic site of the virus in the cells. The observations in infants led to speculation that an in vivo enhancement phenomenon occurred caused by passage of cytphilic IgG through the placenta. Circulating monocytes and fixed phagocytic cells of the reticuloendothelial cells were capable of supporting dengue virus replication and the enhancement phenomenon may amplify the process. It has been suggested that monocytes may release vascular permeability factors and substances that could activate the clotting system and the complement system. Monocyte–virus interaction may be central to the major events in DHF. Several studies have demonstrated dengue virus antigen–antibody complexes on the surface of a small percentage of circulating B lymphocytes, in the glomeruli of some patients who manifested transient glomerulonephritis, and in the terminal microvascular beds in the dermal papillae of the skin rash in patients. Dengue viral antigen alone, or with immunoglobulin and complement, was found on the surface of platelets of DHF patients. This suggests that dengue virus or virus–antibody complexes interacting through some receptor sites on plasma membranes may be an effector mechanism leading to thrombocytopenia.

Shortening of the half-life survival of platelets of some DHF patients has been observed. The half-life returned to normal after the illness. Attempts to localize dengue antigen on the endothelial cells in vivo have not been successful, except that the presence of some antigen has been demonstrated in the flat sinusoidal lining cells of the liver, which could differentiate into Kupffer cells.

Dengue virions could not be found in the endothelial cells of the skin vasculature by transmission electron microscopy. Dengue antigen and complement (C3) were detected by FAT in the skin biopsy of DHF rash, mainly on the vessel walls and in monocyte-like cells around the microvasculature. The rash lesions looked like an antibody-dependent Arthus reaction, but there was no
necrotizing vasculitis. Some perivascular infiltration by monocytes and lymphocytes was observed, suggesting that cell-mediated immunopathological injury may account for the rash. The levels of circulating dengue virus–antibody complexes were not significantly high compared with the levels found in an immune complex disease such as systemic lupus erythematosus. Dengue antigen fixed in the tissue was not related to important pathological lesions, except in the case of skin rash.

An *in vivo* study of cultured human B lymphocytes has shown that these cells can support dengue virus replication. Dengue viral antigen was found by the FAT in a small number of medium-sized lymphocytes in a study of peripheral blood leukocytes obtained from DHF patients. Thus, dengue virus replication in lymphocytes or lymphoblasts cannot be excluded. Activated lymphocytes may also release several types of mediator. The large number of blast-transformed lymphocytes in secondary DHF may have a role, as yet undefined, in the central mechanism of pathogenesis in DHF.

*Reticuloendothelial system response in DHF.* Autopsy findings made several years ago demonstrated significant reticuloendothelial responses to dengue infection in DHF cases. There was evidence of marked lymphocytolysis and phagocytosis of lymphocytes in the spleen and lymphoid tissue, which indicates a high turnover of lymphocytes. A more recent study of autopsy material concluded that the depletion of lymphocytes occurs in the T-lymphocyte-dependent zones of the lymphocytic tissue.

In an earlier autopsy study, the proliferation of large lymphocytes or lymphoblasts and young plasma cells had been observed in the lymphoid tissue of DHF cases. One study of the buffy coat showed that in DHF associated with secondary dengue infection there may be some 20–40% of lymphoblasts in the peripheral blood (D.S. Burke, personal communication). Other studies have demonstrated a mild decrease in the number of T lymphocytes, with relative or absolute increase in the number of B lymphocytes. Some of the increased lymphocytes may be neither B nor T cells (null cells). There seems to be general agreement that in these DHF cases there is a decrease in the number of T lymphocytes and a corresponding expansion of both young and immature B lymphocytes. Whether the alteration in T lymphocytes is associated with the immune elimination of the virus in a general way or in a dengue-specific, or even a DHF-specific, manner remains unclear.

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A significant increase in absolute levels of serum IgE has been demonstrated in the sera of patients with DHF/DSS from Burma and Thailand as compared with control sera. In view of the high levels normally found among the healthy children in these areas, this finding needs further confirmation. It has been suggested that it may be related to the depletion of the T suppressor cell population.

**Complement activation in DHF.** It has been shown that in the acute stage of DHF, the serum levels of C1q, C3, C4, C5–8, and C3 proactivator are depressed and more so in the severe grades of DHF. 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 alternative pathway as well. This may result in the formation of C3a and C5a, which are potent anaphylotoxins and which may lead to a massive increase in vascular permeability, either directly or via the histamine system. Severe complement activation could also be demonstrated in DHF associated with primary dengue infection. Despite these observations, normal levels of complement have been found in some cases of DHF/DSS.

There are other possible mediators of vascular permeability in DHF. The kinin system was found not to be affected. An autopsy study in the Philippines suggested that DIC could be found more often in older children and adolescents. An increase in the consumption of fibrinogen was shown in non-shock cases, but without any other clotting changes. It is generally agreed that, while some degree of mild to moderate intravascular clotting may be found, this does not act as a primary pathogenic event in DHF/DSS. Fibrinopeptide as a possible vascular permeability factor did not appear to be implicated.

Since anaphylotoxins (C3a and C5a) were postulated to have been formed as a consequence of the massive complement activation, these could act on the walls of venules to increase the permeability directly or via the mast cells, with exocytosis of histamine. Histamine will further act on the receptors on the wall of the blood vessels leading to an increase in vascular permeability as well. A study of the histamine content in the blood and urine was conducted with equivocal results. Antihistamine therapy has been tried, but without any appreciable effect. The problems lie perhaps in improper timing of the therapy, or in the use of antihistamines.
that were unsuitable for the specific receptors involved in this pathophysiology.

*Histocompatibility antigens and DHF.* Earlier studies to find genetic markers that may represent risk factors in DHF, such as blood groups and G6PD deficiency, had not been successful. Eighty-seven unrelated patients with DHF/DSS were screened for histocompatibility antigens (A and B) and compared with 138 non-dengue control subjects; a positive DSS association was found for the antigens HLA-A2 and HLA-blank and a negative relationship for HLA-B13 were found.

*Clinical epidemiology of DHF.* Problems remain to be solved relating to the precise serological definition of primary and secondary infection and ways of determining the serotype of the virus in the secondary infection. Knowledge about risk factors, which could be obtained from an epidemiological study, could be extremely useful, both for a better understanding of the pathogenic mechanism and for intervention measures, such as vaccination.

8. BIOLOGICAL HAZARDS ASSOCIATED WITH CERTAIN VIRUSES

In recent decades, two phenomena have occurred that have caused renewed concern that microbiological practices and facilities need extensive review and strengthening. These are the perceived potential for increased virulence inherent in studies involving recombinant DNA and the discovery of new arboviruses and rodent-borne viruses that are not only highly virulent for man, but are also capable of being transmitted from person to person. They include Lassa, Marburg, and Ebola viruses. The classification of microbial agents in risk categories is the responsibility of national governments.

A variety of problems, including the collection and transport of specimens, the safe management of patients suspected of having such infections, disease diagnosis and safe manipulation of potentially infected material for clinical-pathological determinations, and basic investigation of the viruses *per se*, have been discussed in WHO in a *Laboratory biosafety manual* (34). Table 6 summarizes the classifications of agents by degree of hazard recommended in the United Kingdom, in the USA, and by WHO, together with the
Table 6. Some examples of classifications of agents on the basis of risk

<table>
<thead>
<tr>
<th>United Kingdom</th>
<th>United States of America</th>
<th>World Health Organization</th>
</tr>
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<tbody>
<tr>
<td>Category C</td>
<td>Class P1</td>
<td>Class P2</td>
</tr>
<tr>
<td></td>
<td>No or minimal hazard under ordinary conditions of handling</td>
<td>Ordinary potential hazard to laboratory workers from accidental inoculation or injection</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>Category B</td>
<td>Class P3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Special hazard to laboratory workers. Requires special conditions for containment</td>
<td></td>
</tr>
<tr>
<td>Category A</td>
<td>Class P4</td>
<td>Extremely hazardous to laboratory workers or may cause epidemic disease. Requires most stringent conditions for containment</td>
</tr>
<tr>
<td></td>
<td>Extremely hazardous to laboratory workers or may cause epidemic disease. Requires conditions of maximum containment</td>
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appropriate levels of containment. In the Laboratory biosafety manual, emphasis is placed on levels of containment less than maximum (P4 or Category A), which is rightly felt to represent a small but very important number of special cases involving highly infectious pathogens. Problems, specimens, and consultations should be referred direct to the centres for special pathogens listed in Annex 2. Information regarding questions that relate to other levels of containment, and that are relevant to most arboviruses, will be found in the WHO Laboratory biosafety manual or can be obtained from the WHO Special Programme on Safety Measures in Microbiology.

9. SURVEILLANCE, PREVENTION, AND CONTROL

Many countries lack the resources to achieve ideal levels of surveillance, prevention, and control. The measures recommended in this section therefore relate to desirable technical requirements and objectives.
9.1 Surveillance

Basically, surveillance should comprise three main areas: (1) clinico-virological surveillance; (2) serological and histological surveillance; and (3) arthropod and rodent vector surveillance.

9.1.1 Clinico-virological surveillance

Fundamental to clinical surveillance is the recording of sick persons admitted to hospital or attending outpatient and private clinics. This is achieved by encouraging regular reports from doctors in hospitals and other health service establishments; these data can give a satisfactory approximation of the attack rates. In some countries, important arboviral diseases are made notifiable, e.g., DHF in Indonesia, the Philippines, and Thailand, for the purpose of increasing the completeness and accuracy of reporting. Centralized reporting at the state or national level serves as a mechanism for alerting the public and physicians in order to promote recognition of disease and to extend the system to a broader geographical area. However, it must be borne in mind that data collected from a hospital study or by clinical surveillance merely represent the tip of the iceberg, as only the very sick are admitted to hospital and, moreover, hospitals recruit patients in a limited area. There may also be obvious discrepancies in the collection of data based on clinical symptoms unless these are very well defined, e.g., shock in DHF/DSS. There will therefore be a danger of over- or under-reporting unless laboratory confirmation is available.

Serotypes and strains should be monitored in each area and correlated with the severity of disease (e.g., dengue). To achieve this, one needs to use the most rapid and sensitive host system for the isolation of the viruses concerned. The recent development of the mosquito inoculation technique and the use of more sensitive cell cultures has provided a highly sensitive method for the isolation and assay of dengue viruses. Using the mosquito inoculation technique, a DHF surveillance system was developed in Indonesia that allowed for the monitoring of dengue viruses from cases as well as from mosquitoes. The geographical distribution of some viruses, e.g., those of haemorrhagic fevers in Africa and the Hantaan virus of HFRS, remains to be established, and an active clinico-virological surveillance in appropriate laboratories is highly desirable. In addition, there must be vigilance to detect any variation in the
clinical manifestations of an established syndrome or an arboviral infection.

9.1.2 **Serological and histological surveillance**

It is important to implement a baseline study and to maintain serological surveillance among humans and among domestic and other animals in order to determine the immunity status in respect of arboviruses in any geographical region. Such surveillance contributes to the knowledge of virus transmission in a defined population and identifies groups of people at risk and possible reservoirs of the virus.

Serological surveillance can be designed in such a way that it can provide answers to specific problems. The following are some possibilities:

1. **Prospective design** can provide insight into the etiology of complex infectious processes (e.g., DHF) and can be used to develop predictive models useful in cost-benefit analysis of particular problems; it can also generate baseline parameters critical to the evaluation of measures designed for disease prevention and control (i.e., vaccine trials and vector control).

2. For **YF surveillance**, viscerotomy of the liver of patients who died with icteric hepatitis syndrome has been found useful and should be encouraged. However, problems may arise because other types of haemorrhagic fever, such as DHF and LF, may show liver changes similar to those of YF.

3. In some countries, **sentinel animals**, such as horses, pigs, chickens, and monkeys, are used to monitor virus activities.

9.1.3 **Arthropod vector and rodent surveillance**

Outbreaks of vector-borne viral diseases are usually associated with the recent or concurrent presence of vectors in high numbers. Early detection and monitoring of vectors through efficient surveillance are therefore important requisites in the prevention and control of arboviral and rodent-borne diseases. These measures include:

1. Identification of local vectors and hosts. Appropriate keys and a reference collection of specimens should be available.
(2) Longitudinal assessments of the densities of arthropod vectors and rodents in their principal habitats, using established sampling methods.

(3) Determination of ecological and geographical distribution, including production of detailed maps showing distribution of vectors, e.g., *Aedes aegypti*.

(4) Collection and pooling of arthropod material for isolation and identification of arboviruses and their prevalence.

(5) Use of bait animals, e.g., chickens or pigs, for the collection of infected mosquito vectors.

(6) Determination at intervals (normally about six months) of the susceptibility of possible arthropod vectors to the principal insecticides (26) and of rodent vectors to rodenticides.

(7) Continuous surveys for detection of additional and new types of breeding sites.

(8) Since outbreaks of arboviral diseases are often linked with unusual weather conditions, longitudinal meteorological observations on temperature, humidity, rainfall, and wind (speed and direction) should be maintained.

(9) Application of the *International Health Regulations* in respect of surveillance measures to be applied for control of vectors in international transport, e.g., vector surveillance at airports and seaports, and in aircraft and ships. In some areas, surveillance is also required for international land transport.

### 9.2 Preventive measures

Measures for personal protection and for preventing the build-up of arboviral and rodent vector populations overlap with measures used for controlling vectors during outbreaks of disease. Nevertheless, there are certain measures usually associated with individual or community action, as distinct from measures taken by specialist vector control units, which are essentially preventive. These include the use of vaccines.

#### 9.2.1 Preventive measures against arthropod and rodent vectors

##### 9.2.1.1 Mosquito arboviral vectors

(1) Use of well-maintained bed-nets and mesh-screens fitted to doors and windows.
(2) Removal, followed by burial, destruction, or recycling of a great variety of small man-made containers that are prolific sources of important vectors, such as Aedes aegypti and Aedes albopictus. Particular attention should be given to the prevention of breeding in discarded tyres, which should be adequately pierced if they cannot be removed.

(3) Provision of piped water to houses or to standpipes near houses. The need to store drinking-water in pots, in the absence of piped water, contributes to the high prevalence of Aedes aegypti in many tropical countries, particularly in periurban conditions.

(4) Management of liquid wastes through the construction and maintenance of drains to reduce vector breeding.

(5) Management of irrigated crops to reduce vector breeding—for example, a controlled water supply is effective against Culex tritaeniorhynchus.

9.2.1.2 Tick arboviral vectors
(1) Personal protection through the use of protective clothing impregnated with a suitable repellent, e.g., deet, indalone.

(2) Routine dipping of cattle and sheep, and treatment of dogs with acaricides (see Table 10, p. 97).

(3) Routine treatment of areas such as camp sites with acaricides.

9.2.1.3 Culicoides and phlebotomine vectors of arbovirus diseases
(1) Personal protection through use of bed-nets of appropriate mesh.

(2) Personal protection through use of appropriate repellents applied to the skin, e.g., deet, dimethyl phthalate.

9.2.1.4 Rodent vectors. Preventive measures that may be taken against commensal rodents include:

(1) Rodent proofing of premises as far as is practicable, with particular attention to food-storage areas.

(2) Disposal of domestic and public waste that is a source of food or a harbourage for rodents.

(3) Use of permanent anticoagulant baiting stations to keep down rodent vector populations in high-risk areas.

Preventive measures for arthropod and rodent vectors of viral diseases also include those outlined in the International Health Regulations for the control of mosquitoes and rodents in airports and ports and disinsection of aircraft and shipping. Another preventive measure, of general application, involves health education of
communities by formal teaching in schools, publicity campaigns,
and the regular use of the press, posters, radio, and television.

9.2.2 Prevention by use of vaccines

With increasing difficulty in the control of vectors because of
acquired resistance to pesticides and the inaccessibility of certain
vectors to control measures, vaccines, when they exist, become an
important line of defence against arbovirus infections.

Effective control of certain arbovirus diseases can be achieved by
the vaccination of persons at risk. Human beings can, however, also
be protected from infection by certain zoonotic viruses through
immunization of the vertebrate reservoir or amplifying hosts.

Infection of man with arboviruses and rodent-borne viruses
usually occurs in focal outbreaks or in geographically isolated
endemic situations. Only isolated populations may be at risk and the
limited market provides little incentive for the development of
vaccines. There are some notable exceptions, such as yellow fever
and dengue viruses, which infect large population groups
throughout much of the tropics and which have the potential for
seasonal epidemic spread into temperature zones. For these,
widespread vaccination is appropriate. In addition, geographically
localized infections, such as Lassa fever, Japanese encephalitis, and
St Louis encephalitis, are regional problems of such clinical severity
and magnitude that development of vaccines becomes a rational
approach to prophylaxis, especially in vaccination of specific age
groups at highest risk.

Animal experiments indicate that protection against challenge
can be induced by live and inactivated vaccines for nearly every
known arbovirus and rodent-borne virus. Possible exceptions are
reported failures to immunize with inactivated dengue viruses, but
with newer methods of inactivation and application with adjuvants,
these vaccines may also be successful.

9.2.2.1 Present vaccines. The available arbovirus vaccines are of
two general types, live attenuated and inactivated. Inactivated
vaccines for WEE and EEE have been employed to vaccinate horses
for many years. Similar vaccines are used for the immunization of
laboratory workers but are not licensed for general use. A
description of the licensed or widely available arboviral vaccines
follows.

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(a) Yellow fever. The 17D yellow fever vaccine is a live attenuated product made in chicken embryos. Leukosis-free vaccines have been developed but are not in general use. Vaccine that is not leukosis-free is still in general use in much of the world because it is less expensive and there is no evidence that cancer or other side-effects are associated with its use. WHO recommends revaccination after 10 years for international travel. However, antibody persists for 40 years in at least 62% of vaccinees and experience during epidemics indicates an even higher percentage of persistence.

(b) Tick-borne encephalitis. Several effective vaccines against TBE are in use in the USSR and other European countries. Separate subtypes in the far-eastern Soviet Union and Central Europe require the use of separate vaccines. An inactivated vaccine for the Central European subtype prepared by continuous-flow zonal ultracentrifugation provides excellent antibody response with few side-effects as compared with less purified products (27).

(c) Japanese encephalitis. An inactivated adult mouse brain JE vaccine, partially purified by physical means, has been widely used in Japan. It is generally credited, along with changed agricultural practices, with almost complete control of the human disease in Japan over the past 25 years. Although the technology for producing this vaccine can be transferred to other countries, the expense may make large-scale production impracticable in some endemic areas.

In China, inactivated human JE vaccine is produced in primary hamster kidney cells and is used in children throughout much of the country; 2 or 3 booster injections are given. Although this vaccine presumably protects many children, the disease continues to occur with over 10,000 cases reported per year.

Attenuated JE vaccines have been developed for pigs and employed successfully in China and Japan. Attenuated JE vaccines developed in China by several passages of the SA-14 parent virus in primary hamster kidney cells have been used successfully in horses and with some success in humans (28). Other attenuated strains are under development.

(d) Rift Valley fever. A human formalin-inactivated RVF vaccine was developed in primary green monkey cells for use in veterinarians, laboratory workers, military personnel, and others at risk. When given in three doses it was immunogenic in over 95% of recipients (29). No cases of RVF were reported in vaccinated persons. Starting in 1976, new vaccine lots were made in diploid fetal rhesus lung cells. This product has proved equally effective.
Control of human RVF can presumably be achieved through immunization of sheep and cattle. An inactivated tissue culture vaccine was developed in South Africa (30). It is effective but requires boosting. A similar inactivated veterinary vaccine is now produced in Egypt. The Smithburn live attenuated vaccine is inexpensive and easy to produce in large quantities. It causes abortion in a small number of recipient animals, has been demonstrated in the laboratory (but not in field studies) to revert to virulence, and is not completely effective in some animal species. It is not recommended for use in non-RVF enzootic zones.

(e) *Venezuelan equine encephalomyelitis*. The TC83 attenuated VEE vaccine was developed for use in laboratory workers and military personnel. It is effective although it still produces febrile disease in a minority of human recipients. It is also used widely as an equine vaccine. The public health value of vaccinating equines was demonstrated in 1971 when the northward progress of a VEE epizootic in horses was stopped, apparently by vector control and by vaccination.

An experimental formalin-inactivated vaccine made from TC83 virus was highly effective in human vaccinees and produced only minimal side-effects (31).

9.2.2.2 *Research needs for conventional vaccines*. Future vaccines may be developed through long-term research programmes embodying recombinant DNA, reassortant viruses, and polypeptide synthesis technologies, but there are ample opportunities for relatively short-term gains through the improvement of existing vaccines and new development using present-generation technology. Specific examples of research needs and opportunities follow.

(a) *Dengue*. No vaccine against dengue is yet available, but progress is being made in the development of live attenuated vaccines.

Since a population exposed to dengue virus infections in endemic areas usually becomes immune and is not at risk for DHF/DSS thereafter, it should be possible to immunize young children in high-risk areas with a dengue vaccine.

Many successful virus vaccines have been developed by attenuation of the virus by passage in a non-adaptive host. Primary dog kidney (PDK) cells support replication of all four dengue serotypes. WHO is sponsoring a project in Thailand that aims to
produce candidate vaccine strains by serial passage of the viruses in PDK cells.

An alternative approach is to clone naturally occurring or mutagen-induced variants with appropriate attenuated characteristics. Such a dengue type 2 vaccine has been tested in about 150 persons. The candidate vaccine is an apparently genetically stable small plaque variant that is temperature-sensitive and produces little or no lethality when inoculated into suckling mice.

(b) Yellow fever. The performance of neurovirulence tests in the rhesus monkey is a WHO requirement for obtaining approval of 17D seed lots for vaccine preparation. This has become an expensive procedure and in some cases a difficult requirement to fulfill because of the paucity of commercially available rhesus monkeys. Studies are needed to develop a substitute for the monkey test.

It should be possible, after developmental research, to produce an improved 17D vaccine in chick embryo cell cultures (free of avian leukosis virus), which could be ready in a few years. The goal should be a vaccine that is not only cheaper but also more thermostable than the present product.

(c) Japanese encephalitis. Two live attenuated vaccines have been developed in China and used in 8000 and 400 000 children respectively without serious side-effects. Both vaccines are clearly attenuated for man but the serological response varied from 50% to 93% depending on the vaccine and the population group immunized. Thermostability of the vaccines is a primary problem since they are stored as liquid at 4 °C and virus titres decrease over time at this temperature.

(d) Junin haemorrhagic fever. Junin virus was attenuated for guinea-pigs by serial passage in mouse brain and used experimentally in about 600 persons. Further passage and pseudocloning in fetal rhesus lung fibroblasts resulted in a new vaccine, attenuated for mice and guinea-pigs, but yet to be tried in monkeys or humans. This vaccine should be developed further.

(e) Lassa fever. The Mozambique strain of Lassa virus is a naturally occurring strain that protected monkeys against lethal challenge. It is a candidate vaccine and its further development through trials in animals and eventually in man is needed.

9.2.2.3 Future generation vaccines. Several new approaches are at present available for producing live attenuated and specific subunit vaccines. It is now feasible to determine the entire nucleotide sequence of RNA viruses. This has been done for type 1 poliovirus
and its attenuated Sabin vaccine strain 1 (32). Using the same approach, it should be possible to determine the sequence of 17D yellow fever vaccine virus and its parent Asibi strain. Likewise, examination of experimental attenuated JE virus and its parent virus may locate the functional region of the genome and may identify altered nucleotide sequences responsible for attenuation. It can be anticipated that if deleted or altered regions are found, such lesions could be induced in these or other flaviviruses to construct new vaccines. This approach is rational but would necessarily require extensive time and resources.

Segmental genome viruses, such as RVF, are amenable to reassortment with closely related agents. The middle-sized RNA segment of RVF virus codes for the G1 and G2 proteins, which in turn account for the major virulence characteristics and induce neutralizing antibodies. It may be possible to construct an attenuated strain by inducing mutation in one or more RNA segments and reassorting them with other homologous RNA segments of RVF or with RNA segments of a closely related phlebovirus.

Another future approach utilizes gene-splicing techniques in which complementary DNA is produced with reverse transcriptase and the DNA inserted through the use of restriction endonucleases into a yeast or bacterial plasmid. Alternatively, the DNA can be inserted into another virus and used directly in people as a vaccine. The organism then replicates the DNA, which in turn expresses large quantities of specific immunizing antigen. There are technical problems in locating the proper sequence of DNA and in glycosylation of the protein. This technique has not yet been successfully applied to arboviruses or rodent-borne viruses and such vaccines are not likely to become available without considerable future experimentation.

9.3 Vector control

Vector control measures may be considered under two headings: (1) emergency control measures; and (2) long-term control measures.

9.3.1 Emergency control measures

In an emergency situation, arising from an outbreak of an arboviral disease, there is a need for active public health education
relating to the outbreak, rapid mobilization of resources, and implementation of control measures.

(a) Health education. Community participation in the affected area should be stimulated through public notices, the media, and government officials, in order to assist personnel carrying out preliminary geographical reconnaissance and spraying operations. The local population should also be encouraged to carry out communal or individual activities to reduce vector breeding and disease transmission, e.g., removal or destruction of certain breeding sites and use of mosquito nets.

(b) Mobilization of resources. Rapid response to an arboviral outbreak requires:

— An administrative infrastructure capable of rapidly mobilizing funding, manpower, insecticides/rodenticides, spraying equipment, and transport.

— Rapid assessment of the resources and methods required to control the vectors. The necessary logistical data for these purposes would be obtained by quick geographical reconnaissance of the affected area and consideration of epidemiological information available from ongoing vector surveillance.

The quantities of suitable insecticides required for dealing with arboviral epidemics cannot always be supplied at short notice by pesticide marketing companies or manufacturers. Consequently, emergency stocks of insecticides to which the vectors are susceptible should be held in a dry, relatively cool storeroom, in which they would be protected from deterioration and from which they could be rapidly transported to areas liable to epidemics. Spraying equipment, suitable for vector control in an emergency, should be at hand or available at short notice. Particular attention should be given to the regular servicing and maintenance of spraying equipment so that it can be ready for immediate use in the event of an emergency. There should also be a readily available stock of protective clothing for spraymen.

Adequate and appropriate transport is vital for successful emergency vector-control operations and rapid interdepartmental procedures should exist for transference of vehicles from one department to another in emergencies.

(c) Control measures. Chemical insecticides are used in emergency measures to control arboviral vectors of epidemic viral diseases.
They can rapidly reduce the density of the adult vector population and thus stop, or drastically reduce, transmission. A quick-acting formulation, applied over a short period to cover the epidemic area, is required. Space-sprays (Table 7) are ideal for use against flying vectors because the insecticide droplets are suspended in the air and the flying insects may acquire a lethal dose by colliding with the droplets. This method quickly interrupts transmission after two or three applications with a few days’ interval between them and can therefore halt an epidemic. If the area is large, aerial spraying might be required. Space-spraying is best carried out under conditions of minimal wind velocity and of atmospheric thermal inversion. With aerial spraying, particular attention is required to avoid spraying people and causing unwanted environmental effects. In smaller or highly built-up areas, where roads are adequate, back-carried or vehicle-mounted space-spraying equipment might be appropriate. Residual contact insecticides (Table 8) and larvicides (Table 9) may also have a part to play in emergency situations, the former for use

Table 7. Insecticides suitable as cold aerosol-sprays and for thermal fogs* for mosquito control

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Chemical type</th>
<th>Dosage of active ingredient (g/ha)</th>
<th>Toxicity* (oral LD₅₀ for rats: mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cold-sprays</td>
<td>Thermal fogs</td>
</tr>
<tr>
<td>biocresmethrin</td>
<td>PY</td>
<td>5–10</td>
<td>20–30</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>OP</td>
<td>10–40</td>
<td>150–200</td>
</tr>
<tr>
<td>deltamethrin</td>
<td>PY</td>
<td>0.5–1.0</td>
<td>–</td>
</tr>
<tr>
<td>dichlorvos</td>
<td>OP</td>
<td>56–280</td>
<td>200–300</td>
</tr>
<tr>
<td>fenitrothion</td>
<td>OP</td>
<td>250–300</td>
<td>270–300</td>
</tr>
<tr>
<td>fenitrothion</td>
<td>OP</td>
<td>112</td>
<td>–</td>
</tr>
<tr>
<td>iodofenphos</td>
<td>OP</td>
<td>100–200</td>
<td>–</td>
</tr>
<tr>
<td>malathion</td>
<td>OP</td>
<td>112–693</td>
<td>500–600</td>
</tr>
<tr>
<td>naled</td>
<td>OP</td>
<td>56–280</td>
<td>–</td>
</tr>
<tr>
<td>permethrin'</td>
<td>PY</td>
<td>5–10</td>
<td>–</td>
</tr>
<tr>
<td>pirimiphos-methyl</td>
<td>OP</td>
<td>230–330</td>
<td>180–200</td>
</tr>
<tr>
<td>propoxur</td>
<td>C</td>
<td>53–75</td>
<td>–</td>
</tr>
<tr>
<td>resmethrin</td>
<td>PY</td>
<td>7–16</td>
<td>–</td>
</tr>
</tbody>
</table>

*The strength of the finished formulation applied differs widely according to the performance of the spraying equipment used.

*PY = synthetic pyrethroid; OP = organophosphate; C = carbamate.

*Toxicity and hazard are not necessarily parallel; LD₅₀ refers to active ingredient.

*Dermal toxicity.

*Because of their low dermal toxicity, and on the basis of experience with their use, these products have been classified in the WHO Hazard Classification in Class III, Table 5 (products unlikely to present acute hazards in normal use).

*Also used in mixtures with knock-down agents or synergists.

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Table 8. Insecticides suitable as residual spray applications against mosquito vectors

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Chemical type</th>
<th>Dosage of active ingredient (g/m²)</th>
<th>Duration of effective action (months)</th>
<th>Insecticidal action</th>
<th>Toxicity* (oral LD₅₀ for rats: mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bendiocarb</td>
<td>C</td>
<td>0.4</td>
<td>2–3</td>
<td>contact+ airborne</td>
<td>55</td>
</tr>
<tr>
<td>chlorphoxim</td>
<td>OP</td>
<td>2</td>
<td>1–3</td>
<td>contact</td>
<td>500₆</td>
</tr>
<tr>
<td>permethrin</td>
<td>PY</td>
<td>0.5</td>
<td>4 or more</td>
<td>contact</td>
<td>360</td>
</tr>
<tr>
<td>DDT</td>
<td>OC</td>
<td>1–2</td>
<td>6 or more</td>
<td>contact</td>
<td>113</td>
</tr>
<tr>
<td>deltamethrin</td>
<td>PY</td>
<td>0.05</td>
<td>2–3</td>
<td>contact</td>
<td>2940₄</td>
</tr>
<tr>
<td>fenitrothion</td>
<td>OP</td>
<td>1–2</td>
<td>3 or more</td>
<td>contact+ airborne</td>
<td>500</td>
</tr>
<tr>
<td>lindane</td>
<td>OC</td>
<td>0.2–0.5</td>
<td>3 or more</td>
<td>contact+ airborne</td>
<td>100</td>
</tr>
<tr>
<td>(gamma HCH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>malathion</td>
<td>OP</td>
<td>1–2</td>
<td>2–3</td>
<td>contact</td>
<td>2100</td>
</tr>
<tr>
<td>permethrin</td>
<td>PY</td>
<td>0.5</td>
<td>2–3</td>
<td>contact</td>
<td>4000₄</td>
</tr>
<tr>
<td>propoxur</td>
<td>C</td>
<td>1–2</td>
<td>2–3</td>
<td>contact+ airborne</td>
<td>95</td>
</tr>
</tbody>
</table>

*OC = organochlorine; OP = organophosphate; C = carbamate; PY = synthetic pyrethroid.

*Toxicity and hazard are not necessarily parallel; LD₅₀ refers to active ingredient.

*Dermal toxicity.

*Because of their low dermal toxicity, and on the basis of experience with their use, these products have been classified in the WHO Hazard Classification in Class III, Table 5 (products unlikely to present acute hazards in normal use).

in forming a cordon sanitaire around the foci of an outbreak, if the vector is sufficiently endophilic. Larvicides may be used as a complementary measure to space-spraying where vector breeding occurs in large swamps or irrigated areas.

Emergency control of tick-borne arboviral diseases is achieved through treatment of the vegetation in which the ticks naturally occur, and by residual contact insecticides applied by power-operated sprays. Details of spraying equipment for vector control have been published by WHO (33).

9.3.2 Long-term control measures

In the long-term, a combination of vector control methods, selected and integrated to optimize results, is more likely to confer long-lasting benefits in terms of cost-effectiveness and less environmental contamination by pesticides. The measures for possible integration into long-term strategies for vector control are described below.
<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Chemical type*</th>
<th>Dosage of active ingredient (g/ha)</th>
<th>Formulation*</th>
<th>Duration of effective action (weeks)</th>
<th>Toxicity* (oral LD₅₀ for rats: mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorpyrifos</td>
<td>OP</td>
<td>100</td>
<td>EC, GR, WP</td>
<td>2–7</td>
<td>500*</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>PY</td>
<td>1–10</td>
<td>EC, GR, WP</td>
<td>3–4</td>
<td>136</td>
</tr>
<tr>
<td>deltamethrin</td>
<td>IGR</td>
<td>25–100</td>
<td>GR, WP</td>
<td>1–3</td>
<td>2940*</td>
</tr>
<tr>
<td>diflubenzuron</td>
<td>OP</td>
<td>100–100</td>
<td>EC, GR</td>
<td>1–2</td>
<td>460</td>
</tr>
<tr>
<td>fenitrothion</td>
<td>OP</td>
<td>100–100</td>
<td>EC, GR</td>
<td>1–2</td>
<td>592</td>
</tr>
<tr>
<td>fenothion</td>
<td>OP</td>
<td>100–100</td>
<td>EC, GR</td>
<td>2–11</td>
<td>330*</td>
</tr>
<tr>
<td>fenthion</td>
<td>OP</td>
<td>100–100</td>
<td>EC, GR</td>
<td>1–2</td>
<td>negligibl</td>
</tr>
<tr>
<td>fuel oil</td>
<td>OP</td>
<td>50–100</td>
<td>EC, GR</td>
<td>1–7</td>
<td>2100</td>
</tr>
<tr>
<td>iodoaphosphos</td>
<td>OP</td>
<td>2×10</td>
<td>Soln</td>
<td>1–2</td>
<td>negligibl</td>
</tr>
<tr>
<td>larvicid(oil</td>
<td>–</td>
<td>224–1000</td>
<td>Soln</td>
<td>4–8</td>
<td>3400</td>
</tr>
<tr>
<td>methoprene</td>
<td>IGR</td>
<td>100–1000</td>
<td>SRS</td>
<td>4–8</td>
<td>3400</td>
</tr>
<tr>
<td>Paris green</td>
<td>CAC</td>
<td>840–1000</td>
<td>Dust, Soln</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>permethrin</td>
<td>PY</td>
<td>5–10†</td>
<td>EC, GR</td>
<td>5–10</td>
<td>430</td>
</tr>
<tr>
<td>phoxim</td>
<td>OP</td>
<td>100</td>
<td>EC, GR</td>
<td>1–6</td>
<td>1000</td>
</tr>
<tr>
<td>pirimiphos-methyl</td>
<td>OP</td>
<td>50–400</td>
<td>EC</td>
<td>1–11</td>
<td>2018</td>
</tr>
<tr>
<td>temephos</td>
<td>OP</td>
<td>56–112</td>
<td>EC, GR</td>
<td>2–4</td>
<td>8600</td>
</tr>
</tbody>
</table>

*OP = organophosphate; PY = synthetic pyrethroid; IGR = insect growth regulator; CAC = copper-arsenic complex.

*EC = emulsion concentrate; GR = granular formulation; WDP = wettable powder; SRS = slow release suspension; Soln = solution.

*Toxicity and hazard are not necessarily parallel; LD₅₀ refers to active ingredient.

*Because of their low dermal toxicity, and on the basis of experience with their use, these products have been classified in the WHO Hazard Classification in Class III, Table 5 (products unlikely to present acute hazards in normal use).

*The lowest levels are recommended in fish-bearing waters.

+ + = Apply at 1/45–1/90 l/ha, or 19–47 l/ha if spreading agent added.

+ = Apply at 19–47 l/ha.

9.3.2.1 For mosquito vectors of arboviral diseases

(a) Elimination or reduction of breeding sites, e.g., by installation of piped water to houses, improved surface and subsurface drainage, evapotranspiration beds, shoreline maintenance, properly covered latrines or improved latrine designs, effective soakage-pits, filling in of small surface depressions and burrow-pits, and frequent removal of domestic, industrial and agricultural small-container waste.

(b) Siting of new houses away from major vector-breeding areas, minor modifications to houses, e.g., screens on windows and doors.

(c) Management of farm animal reservoirs through reduction of their association with man and with principal vector-breeding sites.

(d) Use of chemical insecticides: space-spraying, larvicides, and residual spraying as appropriate. Suitable compounds and dosages are indicated in Tables 7, 8, and 9.
(e) Use of biological control agents, e.g., fish, Bacillus thuringiensis H-14.

(f) Personal protection, including use of bed-nets where applicable.

Intersectoral coordination of integrated vector control measures is required and is of particular importance between the agricultural and public health sectors, since environmental management of irrigated crops and coordinated pesticide usage can reduce vector mosquito breeding and delay the onset of resistance. Direct community participation in simple measures, such as waste-container disposal and use of bed-nets, may play an important role in sustaining vector mosquito control over a long period.

9.3.2.2 For Culicoides vectors of arboviral diseases. Buttonwillow virus of California, the Sango and Shuni viruses of Nigeria, and Oropouche virus of Brazil have been isolated from Culicoides. Since Culicoides spp. breed mainly in mud and moist sand, engineering methods of control, such as water-level management, draining, and irrigation, can provide effective control. Chemical larviciding can also be effective for periods of about two months, provided that the larval habitats are accurately located and the applications are correctly timed in relation to seasonal abundance and, where there is intertidal breeding, also in relation to tidal flow. Effective larvicides are malathion solution at 1120–1400 g of active ingredient per ha or diazinon at 336 g of active ingredient per ha. Temephos can be applied at 56–112 g of active ingredient per ha. If the area is extensive, aerial application is preferred to ground applications. Space-spraying with insecticides such as those indicated in Table 7 can provide temporary relief. Where there is intertidal breeding, space-spraying operations should coincide with any pattern of adult emergence related to tide cycles. In areas where the preservation of fish or wildlife is of concern, insecticides should be used with caution.

9.3.2.3 For phlebotomine vectors of arboviral diseases. Sandfly fever, prevalent in Africa, Central and South America, and the Mediterranean countries, and the Changuinola groups of virus of Central and South America, are transmitted by phlebotomine sandflies. The most widely used and effective method of control is residual spraying of the interior wall surfaces of dwellings, nearby animal shelters, stone walls, and other resting sites around houses. DDT suspension is the insecticide of choice, as development of resistance to this compound has been demonstrated only in
*Phlebotomus papatasi* in Bihar State, India. HCH or malathion may also be used where resistance to these compounds does not occur. DDT or malathion are applied at 1 or 2 g/m² and HCH is applied at 0.5 g/m². Special attention should be given to ensuring that the spray penetrates into cracks and crevices where the adult *Phlebotomus* rest. Diazinon (4%) can be used for the treatment of refuse.

Environmental management around residential areas, aimed at reducing the number of resting places of sandflies in the vicinity of man, should be carried out as far as is practicable, e.g., by the removal of rubble and garbage, by the filling in of crevices in walls, and by the destruction of animal burrows.

Space-sprays are sometimes used for *Phlebotomus* control in public meeting places, e.g., outdoor markets, streets, or areas where sandflies are prevalent. Ground applications are made of iodofenphos (2%) applied once or twice a week as a thermal fog or as a cold aerosol at the rate of 0.5 litres of ULV concentrate per ha. Resmehrin can also be applied as a ULV formulation at 0.5 litres/ha. Entire townships have also been treated from the air for *Phlebotomus* control, using a ULV formulation of iodofenphos plus dichlorvos applied 16–24 times per annum at the rate of 2 litres/ha.

9.3.2.4 For tick vectors of arboviral diseases. The hard tick vectors of arboviral diseases are largely controlled by chemical methods. The floor, baseboard, and wall crevices, as well as dogs and their quarters, should be treated. For surface treatment, a solution or emulsion of DDT (5%) or lindane (0.5%) can be used. If the ticks are resistant to this group of chemicals, bendiocarb (0.25–0.48%), carbaryl (5%), chlordane (0.5%), diazinon (0.5%), malathion (2%), or pirimiphos-methyl (1%) gives effective control. Dust formulations are also suitable. Hand sprayers or pressurized container sprayers are used. DDT, lindane, and malathion may be applied extensively indoors, but the other pesticides should be employed only as spot treatments. Various forms of treatment for dogs are effective (e.g., dip, wash, shampoo, dust, and spray) and suitable compounds and dosages are indicated in Table 10.

For the control of tick vectors of other arboviral diseases of man, e.g., Colorado tick fever, CCHF, TBE, and KFD, treatments are directed to outdoor areas where ticks are prevalent. Acaricides, mentioned above for interior treatments, may also be used for exterior treatments. Applications can be made by hand or power
### Table 10. Pesticides used for animal treatments

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Chemical type*</th>
<th>Formulation</th>
<th>Concentration (%)</th>
<th>Toxicity* (oral LD₅₀ to rats: mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>amitraz</td>
<td>C</td>
<td>wash</td>
<td>0.025-0.05</td>
<td>800</td>
</tr>
<tr>
<td>carbaryl</td>
<td></td>
<td>dip or wash</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>deltamethrin</td>
<td>PY</td>
<td>spray or shampoo</td>
<td>0.0025</td>
<td>2940*</td>
</tr>
<tr>
<td>iodofenphos</td>
<td>OP</td>
<td>dip</td>
<td>0.5</td>
<td>2100</td>
</tr>
<tr>
<td>lindane⁺</td>
<td>OC</td>
<td>dust</td>
<td>2.0-5.0⁺</td>
<td></td>
</tr>
<tr>
<td>malathion</td>
<td>OP</td>
<td>dip</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dust</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>spray</td>
<td>0.5</td>
<td>2000</td>
</tr>
<tr>
<td>natural pyrethrins</td>
<td></td>
<td>dust, spray</td>
<td>0.2 + 2.0</td>
<td>200-2600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or shampoo</td>
<td>synergest</td>
<td></td>
</tr>
<tr>
<td>permethrin</td>
<td>PY</td>
<td>dust</td>
<td>1.0</td>
<td>4000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>spray or shampoo</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wash</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>propetamphos</td>
<td>OP</td>
<td>collar</td>
<td>10.0</td>
<td>75</td>
</tr>
<tr>
<td>propoxur</td>
<td>C</td>
<td>spray</td>
<td>1.0</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dust</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>rotenone</td>
<td>ED</td>
<td>dust</td>
<td>1.0</td>
<td>132-1500</td>
</tr>
</tbody>
</table>

*OC = organochlorine; OP = organophosphate; C = carbamate; PY = synthetic pyrethroid; ED = extract of derris root.

⁺Toxicity and hazard are not necessarily parallel. LD₅₀ refers to active ingredient.

*do not use on cats under 4 weeks of age.

*do not use on dogs under 4 months of age or on cats.

*Dermal toxicity. Because of their low dermal toxicity, and on the basis of experience with their use, these products have been classified in the WHO Hazard Classification in Class III, Table 5 (products unlikely to present acute hazards in normal use).

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equipment or by aircraft-mounted equipment to dispense carbaryl, fenthion, naled, and propoxur at 2.24 kg of active ingredient per ha. Pirimiphos-methyl has been found effective at 0.1–11.0 g of active ingredient per ha, depending on the time of year, and deltamethrin gives good control at 3.0–50.0 g of active ingredient per ha, according to species, site, and stage of development.

These applications generally prevent reinestation for a month or longer. Care must be taken to avoid the contamination of watercourses and adjacent areas and to prevent a hazard to non-target organisms. Large-scale chemical control of tick vectors is impracticable in most situations, owing to high operational costs.

#### 9.3.2.5 For rodent vectors of viral diseases

In viral zoonoses, where rodents act as reservoirs and transmission to man is through arthropod vectors, disease control is generally achieved through control of the arthropod vector. The control of commensal and wild rodent vectors of viral diseases is, however, of particular importance where transmission occurs directly between rodent and man, e.g., in
HFRS, lymphocytic choriomeningitis, LF, JHF, and MHF. Control methods may include environmental sanitation, rodent exclusion, poisoning, fumigation, and trapping. In general, the control of non-commensal rodents is much less effective than the control of commensal rodents.

(a) Environmental sanitation. For the control of commensal rodents, food and food wastes in residential and commercial premises should be stored in covered, rodent-proof containers or rooms. On farms, the improper disposal of food wastes and refuse can encourage rodent infestations, but of even more concern is the often poor storage of farm crops and animal forage, which should be kept in rodent-proof buildings. Access to water should also be prevented by ensuring proper drainage, attending to leaky taps and plumbing, and emptying all sinks and other sources of unprotected water.

The control of non-commensal rodents may be achieved through the discriminative clearance of scrub and other vegetation in rodent-infested areas frequented by man.

(b) Rodent exclusion. Rodent exclusion largely involves the use of mechanical or chemical barriers to keep rodents from penetrating an area or moving from one place to another. The rat-proof construction of ships has gone a long way in preventing the spread of rodent vectors by shipping, but better rodent control is now required in international ports because rat-infested containerized cargoes have been found in recent years. Chemical barriers, i.e., repellents, have been developed for incorporation into packaging materials to inhibit rodent attack. Commonly used repellents are thiram, cyclohexamide, R-55, and Rotran.

(c) Trapping. Traps are the preferred method of killing or capturing rodents in situations where the use of rodenticides is considered undesirable, e.g., where poisoned animals dying in inaccessible areas could cause unpleasant odours. In campaigns against rats, traps should be placed near runs and at other locations where there are clear signs of rat activity. Trapping success can be improved by leaving the traps baited but unset for a few days. For economic or other reasons, traps are of little value in controlling large infestations of mice. Before starting a trapping programme, particularly for mice, as many sources of accessible food as possible should be protected or eliminated.

(d) Fumigants. Fumigants are used to kill rodents and their ectoparasites living in inaccessible areas in buildings, ships, or
burrows in the soil. Fumigants are quite dangerous, both to the persons using them and to other humans or animals in the immediate area, and should be applied only by qualified and experienced operators. Fumigants most commonly used against rodents are calcium cyanide (to produce hydrogen cyanide), methyl bromide, chloropicrin, and aluminium phosphide (to produce phosphine). More rarely used are carbon dioxide, carbon monoxide, and sulfur dioxide.

(e) Rodenticides. Most measures to control commensal rodents depend on the application of rodenticides incorporated in bait, powder, or water.

The rodenticides of first choice against commensal rodents, in most control operations, are the anticoagulant poisons since these are slow-acting compounds. Ten anticoagulants in current use are shown in Table 11.

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Rattus norvegicus</th>
<th>Rattus rattus</th>
<th>Mus musculus</th>
</tr>
</thead>
<tbody>
<tr>
<td>brodifacoum</td>
<td>0.001</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>bromadiolone</td>
<td>0.005</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>chlorophacinone</td>
<td>0.005–0.01</td>
<td>0.005–0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>coumatetralil</td>
<td>0.03–0.05</td>
<td>0.03–0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>difenacoum</td>
<td>0.005</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>diphenicoum</td>
<td>0.005–0.01</td>
<td>0.005–0.01</td>
<td>0.0125–0.025</td>
</tr>
<tr>
<td>fumarin</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025–0.05</td>
</tr>
<tr>
<td>pivalone</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025–0.05</td>
</tr>
<tr>
<td>warfarin</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025–0.05</td>
</tr>
</tbody>
</table>

In contrast to the slow-acting (multiple-dose) anticoagulant poisons, acute (single-dose, quick-acting) rodenticides are employed mainly in situations demanding a rapid reduction in high-density rodent populations. The acute rodenticides at present in use are shown in Table 12. Application of acute rodenticides is hazardous to man and should be carried out only by qualified and experienced operators.

9.3.3 Research needs for vector control

9.3.3.1 Yellow fever, dengue, and DHF vectors. In the Americas, the policy regarding Aedes aegypti is still based on eradication of the vector. The following are some of the research needs:
<table>
<thead>
<tr>
<th>Rodenticide</th>
<th>Lethal dose (mg/kg)*</th>
<th>Percentage used in baits</th>
<th>Species effective against</th>
<th>Acceptance in baits</th>
<th>Solubility: water/oil</th>
<th>Hazard to man</th>
<th>Antidote</th>
<th>Restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>alphachloralose</td>
<td>300</td>
<td>4.0</td>
<td>x</td>
<td>fair</td>
<td>neither</td>
<td>moderate</td>
<td></td>
<td>not recommended</td>
</tr>
<tr>
<td>Antu</td>
<td>6-8</td>
<td>1.5</td>
<td>x</td>
<td>fair</td>
<td>neither</td>
<td>moderate</td>
<td></td>
<td>not recommended</td>
</tr>
<tr>
<td>arsenic trioxide (micronized)</td>
<td>13–25</td>
<td>1.5</td>
<td>x x x</td>
<td>fair</td>
<td>water</td>
<td>extreme</td>
<td></td>
<td>porcine calcitonin</td>
</tr>
<tr>
<td>calciferol</td>
<td>40</td>
<td>0.1</td>
<td>x x x</td>
<td>good</td>
<td>oil</td>
<td>moderate</td>
<td>porcine calcitonin</td>
<td></td>
</tr>
<tr>
<td>crimidine</td>
<td>1–5</td>
<td>0.5</td>
<td>x x x</td>
<td>poor</td>
<td>oil</td>
<td>extreme</td>
<td>sodium</td>
<td>pentobarbital</td>
</tr>
<tr>
<td>fluoroacetamide</td>
<td>13–16</td>
<td>2.0</td>
<td>x x x</td>
<td>good</td>
<td>water</td>
<td>extreme</td>
<td></td>
<td>not recommended</td>
</tr>
<tr>
<td>norbormide</td>
<td>12</td>
<td>1.0</td>
<td>x</td>
<td>poor</td>
<td>oil</td>
<td>low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>red squill</td>
<td>50</td>
<td>10.0</td>
<td>x</td>
<td>fair</td>
<td>water/oil</td>
<td>low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>scilliroxide</td>
<td>0.42</td>
<td>0.015</td>
<td>x</td>
<td>fair</td>
<td>water/oil</td>
<td>moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>silatrame</td>
<td>1–4</td>
<td>0.5</td>
<td>x x x</td>
<td>fair</td>
<td>oil</td>
<td>extreme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sodium fluoroacetate</td>
<td>5–10</td>
<td>0.25</td>
<td>x x x</td>
<td>good</td>
<td>water</td>
<td>extreme</td>
<td></td>
<td>not recommended</td>
</tr>
<tr>
<td>strychnine</td>
<td>0–8</td>
<td>0.8</td>
<td>x</td>
<td>poor</td>
<td>water</td>
<td>extreme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>thallium sulfate</td>
<td>25</td>
<td>1.5</td>
<td>x x x</td>
<td>good</td>
<td>water</td>
<td>extreme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>zinc phosphate</td>
<td>40</td>
<td>1.0</td>
<td>x x x</td>
<td>fair</td>
<td>oil</td>
<td>moderate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LD₅₀ for Rattus norvegicus.
*Rn = Rattus norvegicus; Rr = Rattus rattus; Mm = Mus musculus.
(a) Study of the spread of *Aedes aegypti* into rural areas.
(b) Development of better surveillance measures to prevent the reintroduction and establishment of *Aedes aegypti* in countries from which it has been eliminated.
(c) Routine testing of the susceptibility of *Aedes aegypti* to new insecticides.
(d) Sociological research on community motivation, participation, and discipline in community-based antil larval campaigns, whether these take the form of source reduction or chemical larvicide (e.g., with temephos).
(e) Studies on the transmission of yellow fever by alternative vectors, especially in the Caribbean region.
(f) More research on the reservoirs/vectors of jungle yellow fever. This disease has recently been reported from many countries where the usual vectors and reservoirs have not been found. For example, it would be useful to define just how much movement there is of *Haemagogus* into *Aedes aegypti* breeding areas—or vice versa.
(g) Research on refinements in vector surveillance techniques to determine the degree of control required to prevent transmission of virus. Suitable epidemiological criteria (serological or otherwise) should be found that can help in the interpretation of the vector density/longevity data. In Asia, dengue and DHF are transmitted by a number of vectors, primarily *Aedes aegypti*, but also *Aedes albopictus* and members of the *Aedes scutellaris* complex. In many situations, eradication of these species is impracticable since all are well established.
(h) Continuous surveillance in Asia of changes in the breeding habits of *Aedes aegypti*, e.g., treehole and well breeding in some parts of India. Continuous surveillance is important since adoption of natural breeding sites by this species can cause additional problems of control.
(i) Exploration and field testing of vector control methods based on self-help and community participation, and parallel health education activities.
(j) Investigation of the spatial structure of jungle yellow fever and identification of epidemiological zones offering optimum conditions for yellow fever virus infection of man. More work on the potential vectors and survival of the vector during critical climatic seasons may be needed.
9.3.3.2 Specific control measures

(a) Development of appropriate covers for water storage containers traditionally used in Asia. The feasibility of using larval/pupal traps to "clean" water containers should be tested in some specific situations. The impact of insecticide-impregnated materials placed in the locations where mosquitoes may rest in a house, e.g., lower walls or surfaces near breeding habitats, should also be assessed.

(b) Testing and evaluation of new formulations, products, and equipment for larviciding (including biological control agents) and adulticiding from ground or air.

(c) Assessment of the suitability of biological control agents in certain situations where the use of insecticides is impracticable, e.g., against *Aedes polynesiensis* breeding in crab-holes.

(d) Refinement of surveillance and vector control techniques. Yellow fever can be efficiently controlled in human populations by vaccination; however, vector control may have a role to play during epidemics, particularly in urban and periurban areas.

9.3.3.3 Japanese encephalitis vectors. Since the land masses involved are generally very large, a programme of vector surveillance is required to delimit the zones at risk so that vector control measures can be cost-effective. In the event of an epidemic, emergency vector control measures can be applied from the ground or the air.

Research items required include:

(a) A study of different agricultural practices for rice cultivation and animal husbandry that might have a bearing on the density of mosquito vectors and transmission of the virus. Ecological studies might indicate factors leading to lowered transmission through modified irrigation techniques or animal husbandry.

(b) Studies on vector competence, including the role of transovarian transmission of JE virus.

(c) Assessment of the use of insecticides in animal shelters for the control of JE vector mosquitoes. In some areas, the spraying of animals with low-toxicity pesticides for the control of these vector mosquitoes should be studied.

(d) Use of mosquito nets (simple or impregnated with insecticides) at the community level through community participation.
9.3.3.4 Rift Valley fever vectors

(a) Research is required on the identity of vectors involved in the persistence of virus during the interepizootic phase of RVF in different settings of East, West, and South Africa. Probable vectors and hosts should be identified.

(b) The potential of transovarian transmission of the virus needs investigation.

(c) The role of Culicoides and phlebotomines in the transmission of RVF also requires investigation, with particular reference to the generation of epizootics.

(d) The efficacy of mosquito repellents applied to sheep and cattle during epizootics should be field-tested.

9.3.3.5 Ross River virus vectors

(a) Recent laboratory investigations have suggested that *Aedes polynesiensis* and some strains of *Aedes aegypti* are efficient vectors; if true, this poses a serious threat of the spread of Ross River virus to several countries. Hence, studies are required on vector competence in transmitting this virus.

(b) Research on water management practices is required to reduce the breeding potential of *Culex annulirostris*, *Aedes vigilax*, and *Aedes vexans*.

9.3.3.6 Tick-borne encephalitis vectors

(a) Multidisciplinary studies of the foci are required taking into consideration both epidemiological and ecological factors. Information is required on the exact definition of a typical biotype in ecological terms. The nature of the foci, e.g., temporary or permanent, should also be investigated.

(b) Investigations are required to determine whether TBE is spreading. Information is needed on the effect of human activities on the spatial distribution of foci, e.g., reduction of forests, transformation of landscapes, changes in the population of small mammals, and changes in the type and distribution of housing.

(c) There is a need for systematic mapping of tick distribution and for the delineation of focal areas by clinical and serological methods and by isolation of the virus from human cases.
9.3.3.7 Rodent reservoirs.

There is a need for a more thorough investigation, using cytotaxonomic techniques, of the identity of rodents. More intensive serological surveys are also required to determine the extent of infection of rodents with viruses in both endemic and non-endemic areas.

10. PREPAREDNESS FOR EMERGENCIES CAUSED BY ARTHROPOD-BORNE AND RODENT-BORNE VIRAL DISEASES

Arthropod-borne and rodent-borne viral diseases may occur as sporadic cases or may have low or high endemicity and give rise to sudden epidemics. There have been several examples in the past of epidemics that took public health services by surprise and caused heavy loss of life among people and domestic animals, and placed a severe burden on the economy. These disasters may be avoided by the institution in advance of appropriate administrative and technical structures with a view to bringing an immediate and appropriate response to a threatening epidemic. Preparedness and coordinated emergency operations are key factors in mounting such a response, as indicated in Fig. 1.

10.1 Preparedness

This is assured by organizing an administrative mechanism to link the existing ministerial structures and, on the technical side, by building a reliable early warning system for the detection of threatening epidemics.

10.1.1 Administrative organization

A section in the health services should be given responsibility for emergencies. It should have its own budgetary allocation and a responsible officer should be appointed with the functions of coordinator. His main duty will be to elaborate a contingency plan for the mobilization of resources and to establish an early warning system. A committee for emergencies should be designated with the participation of key persons from different ministries, public services, and private organizations who might have to intervene during emergencies.
Fig. 1. Flow chart of operations for emergencies caused by arthropod-borne and rodent-borne viral diseases

**PREPAREDNESS**

- Administrative
  - Emergency health service
  - Contingency planning

- Technical
  - Early warning system

**COORDINATED EMERGENCY OPERATIONS**

- Ascertainment of epidemic situation
- Plan of operations
- Mobilization of resources

- Epidemiological investigations
- Control measures

- Follow-up
10.1.2 Contingency planning

Planning for an emergency should include the preparation of an up-to-date inventory of resources that may be found in the country or that could be made available with external assistance. This inventory should concern mainly:

—investigative and control teams;
—laboratory support for etiological diagnosis and serological surveys;
—hospital treatment and management of cases;
—“fire-fighting” vaccination operations, when feasible;
—vector (arthropod and rodent) control operations;
—environmental sanitation operations.

A directory of personnel competent in these activities is important. These technicians, or designated alternatives, should be available at any time.

The coordinator should prepare schemes for intervention in the event of an outbreak of any of the arthropod-borne and rodent-borne diseases that are likely to occur or to be imported into the region.

Training of auxiliary personnel and trial runs are needed to assess the efficiency of the system.

10.1.3 Early warning system

A network of sentinel hospitals and of “spotter” physicians and primary health care workers should be organized to report without delay any “excess” disease. The feedback of information is an indispensable incentive to the vigilance of personnel.

In countries where arbovirus and rodent-borne diseases are a permanent threat to public health, permanent and active surveillance programmes should be set up with the objectives indicated in section 9.1. To ensure early warning, surveillance must be extended to the transmission chain that is likely to precede the occurrence of human cases, and which includes domestic or wild animal reservoirs and vectors.

The mass media are an important source of rapid information, and keeping in close contact with those responsible for them is beneficial and may avoid dissemination of inconsistent rumours.

WHO provides an international information service on epidemics in the Weekly epidemiological record, which is sent to the health
authorities of its Member States. Whenever the epidemiological situation justifies it, urgent information is available through the automatic telex service. Reciprocally, countries are urged to communicate to WHO any epidemiological information that may be of interest to the international community.  

10.2 Emergency operations

Although an epidemic may give rise to some panic and political pressure, the mastering of events requires a methodical approach that includes first the ascertainment of the reality of an epidemic and, once this has been done, the mobilization of appropriate resources to act within a coordinated plan of action.

10.2.1 Ascertainment of the reality of an epidemic

A preliminary investigation is carried out to establish the reality of an epidemic using the following criteria:

—clinical diagnosis, based on signs and symptoms, and possibly a laboratory diagnosis in order to constitute the case definition;
— the genuine character of the situation: either a clustering of cases beyond what can be expected from the seasonal variation of an endemic disease (excess sickness) or the appearance of a previously unrecognized disease;
— the intervention of a transmission factor that may influence further spread of the disease;
— the existence of a risk segment of the population.

Certain arbovirus and rodent-borne diseases may cause explosive outbreaks that mimic diseases transmitted from person to person. A careful analysis of their spread usually shows that there is in fact, a common source of transmission, even in households. Whenever a disease is observed to spread over long distances, it has to be determined whether the incriminated vector and/or reservoir is present in those places where the disease is occurring. Direct person-to-person transmission may occur from primary cases, as happens with the haemorrhagic fevers.

1 Directions for obtaining or furnishing information are given in each issue of the Weekly epidemiological record.
A knowledge of the ecology of the suspected virus, its vectors, and its reservoirs should make it possible to predict the limits to the potential spread of the disease.

According to circumstances, the outcome of the preliminary investigation may be either an almost certain diagnosis or a range of possible diagnoses requiring further etiological examination. One should always keep an open mind on differential diagnosis at this stage.

10.2.2 Mobilization of resources

The results of the preliminary investigations should be presented to the emergency committee and each key member should mobilize the resources under his jurisdiction as required by the coordinator and approved by the committee. An official plan of operations identifying each person’s responsibility is the basis for achieving the indispensable coordination of measures.

WHO and other international organizations have cooperated in many instances with countries facing an outbreak requiring resources beyond those locally available. In brief, WHO can provide:

—short-term consultants in different disciplines (epidemiologists, virologists, entomologists, mammalogists, sanitary engineers, etc.) from a panel of volunteers available at very short notice (less than 24 hours);
—labatory support of the WHO Collaborating Centres for virological and vector investigations, and particularly of the Centres for special pathogens (see Annex 1);
—supplies, including vaccines, jet injectors, insecticides, spraying equipment, protective clothing, and material for collecting laboratory specimens.

10.2.3 Epidemiological investigations

Investigative teams should be constituted in sufficient numbers to cover all the suspected infected area and its surroundings in a very few days. They may consist of epidemiologists, physicians, microbiologists, entomologists, and veterinarians, as necessary.

They are provided with a case definition appropriate to the suspected disease, to be used for the establishment of the overt disease attack rate, the infection rate, and the case-fatality ratio.
They should identify the source of infection, the mode of transmission, and the sequence of contact cases if any. Cases are recorded as "possible", "probable", or "confirmed"; and laboratory specimens are taken to confirm the diagnosis.

Investigative teams must be given strict instructions on safety procedures and provided with the necessary equipment.

An analysis of epidemiological investigations is presented to the emergency committee as often as desirable, sometimes daily. The committee may need to modify instructions for further investigations and control measures. It should also seek to obtain the cooperation of mass media and the community.

10.2.4 Control measures

The analysis of investigations should indicate the appropriate treatment of patients, which may be purely symptomatic or may involve specific therapy (see section 5).

Seasonal preventive measures may be instituted once the vectors and reservoirs have been identified. At present, mass vaccination is used mainly for yellow fever, although the protection takes effect only after seven days. Personal vaccination must be instituted for persons at risk in investigative teams or laboratories.

Intensive vector control should be instituted as soon as possible after possible resistance of the incriminated vectors to certain insecticides and rodenticides has been investigated. Community measures aimed at reducing breeding sources for arthropods or rodents and their contact with man and susceptible domestic animals should be considered as a basic method for vector control.

10.3 The aftermath of epidemics

Long-term measures are generally instituted to prevent a subsequent outbreak of the disease. This may require further research on the ecosystem that enabled the epidemic to develop in the first place.

The experience that has been accumulated during an outbreak is of great value to other countries at similar risk and is generally published. This is often the beginning of a fruitful international cooperation in which WHO may be instrumental.
11. RECOMMENDATIONS

A. General

1. Ecological studies should be encouraged in natural foci of arthropod-borne and rodent-borne viruses in endemic regions to determine the role of reservoirs and vectors, and the risk of spread to human populations with a view to establishing appropriate preventive and containment measures. The presence of virus diseases of international importance with possible person-to-person transmission, especially in Africa, dictates an urgent need to determine the geographical distribution, reservoirs, amplifying hosts, and modes of transmission in endemic countries in order to prevent the spread of these diseases to other countries.

2. Research on epidemiology, including the prevalence and relevant clinical aspects of arbovirus and rodent-borne diseases, should be promoted and implemented. Such studies will result in a better understanding of the complex relationships among the clinical, ecogeographical, and other epidemiological aspects of the diseases.

3. Special studies should be directed towards a better understanding of the risk of introducing yellow fever into countries within the areas of the WHO South-East Asia and Western Pacific Regions, where susceptible vectors are present.

4. Countries should provide special training for health staff involved in case-finding, treatment, epidemiological surveillance, rapid laboratory diagnostic techniques, and vector control activities.

5. Each country in an endemic zone should establish at least one laboratory for etiological diagnosis of arthropod-borne and rodent-borne viruses.

6. Because of possibility of rapid international spread of several arthropod-borne and rodent-borne diseases, each country should participate actively in the surveillance and control of these diseases and provide neighbouring countries and WHO with the necessary information.

7. In view of the public health and economic importance of DHF/DSS, emphasis should be given to finding cost-effective methods of control.
B. Surveillance, prevention, and control

1. Surveillance procedures for arthropod and rodent vectors should be implemented or strengthened in high-risk areas. Surveillance should include, wherever possible and applicable, monitoring of virus circulation in vector and host populations. Passive and active surveillance is needed in countries where the ecosystem is changing rapidly.

2. WHO should play a role in encouraging research aimed at developing vaccines for DHF and JE and at applying cell culture techniques to the development of a thermostable vaccine for YF.

3. Efforts should continue to develop veterinary and human vaccines for certain other viral agents, including RVF and Lassa viruses.

4. Community participation should form an integral component of surveillance, prevention, and control.

C. Laboratory services for diagnosis

1. WHO should continue to organize laboratory training workshops in the use of modern methods in virus isolation, rapid diagnosis, and seroepidemiological surveys.

2. WHO should encourage the development, evaluation, and field testing of newer diagnostic techniques (e.g., IgM capture ELISA, in situ DNA-RNA/RNA-RNA hybridization). This aim could be achieved through collaborative projects between various laboratories.

3. WHO should consider implementing a reagents programme and conducting training workshops in the production and quality control of reagents according to standard protocols. Emphasis should also be given to the standardization of techniques used in various laboratories.

4. WHO should encourage the wider application of microdiagnostic techniques, including the collection of finger-prick blood specimens on filter papers, in disease surveillance and seroepidemiological surveys.

D. Research and development

1. WHO should encourage research into flavivirus replication and genetics.
2. WHO should encourage the collection of virus strains and the integration of the results of molecular epidemiological studies and seroepidemiology in a computer-based retrieval system.

3. WHO should encourage and support research into the pathogenesis and epidemiology of systemic febrile illnesses and haemorrhagic fever syndromes.

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