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Preface

Yellow fever is prevalent in the tropical zones of Africa and the Americas. Although the same virus—with minor antigenic differences—is responsible, the epidemiology and the public health impact of the disease are very different in these two regions.

Until the 1940s, yellow fever was known in Africa only as an epidemic, devastating disease. At that time, workers at the East African Virus Research Institute, Entebbe, Uganda, described a sylvatic cycle of transmission, involving monkeys and the mosquito *Aedes africanus* in the forest canopy; in banana plantations close to the forest, another mosquito, *A. simpsoni*, acted as the vector between monkeys and man.

During the last decade, entomologists and virologists in West and Central Africa have found other transmission cycles between monkeys, mosquitos and man. It is now possible, therefore, to present a complete picture of the transmission of yellow fever in Africa.

This publication provides practical guidance for diagnosis, surveillance, management of cases and epidemics, and prevention of the disease. The information presented represents the consensus of a group of experienced workers in this field who met in Dakar, Senegal, from 30 May to 3 June 1983 (see Annex 1 for a list of participants).
1. Historical Review

Outbreaks of yellow fever have occurred at intervals in Africa for many years. In 1925, a major investigation into the disease was begun in West Africa, and in 1927 the causative agent was isolated and confirmed to be a virus.

Serological surveys carried out in practically all parts of Africa since 1932 have delineated with reasonable accuracy the boundaries of the area in which the disease has occurred. The endemic zone in Africa lies between parallels of latitude 15°N and 10°S (1), extending from the southern borders of the Sahara to Angola, and from the west to the east coast. Fig. 1 shows the yellow fever endemic zone, determined in the 1940s by immunity surveys for the purpose of the International Sanitary Regulations. These limits may be considered to be still valid.

Yellow fever has occurred in Africa either as sporadic cases of jungle yellow fever, mainly in the forest area, or as outbreaks, mainly in savanna areas. It has been found that, in addition to *Aedes aegypti*, at least 13 species of mosquito are able to transmit yellow fever and some of these potential vectors have been captured in large numbers during epidemics (2). Yellow fever in tropical Africa is enzootic in monkeys in forested areas. Monkeys may not be the actual reservoir, but they are at least responsible for enhancing the circulation of the virus (3). The mosquito vectors responsible for transmission vary in different regions of Africa.

Before mass immunization campaigns were started in Africa, typical urban outbreaks occurred in Lagos, Nigeria, in 1925–1926, in Accra, Ghana, in 1926–1927 and again in 1937, and in Banjul (Bathurst), the Gambia, in 1934–1935. A severe epidemic occurred in Sudan in 1940, when 15,641 cases and 1,627 deaths were reported among 230,000 inhabitants. Estimations made on the basis of serological evidence produced the figure of approximately 40,000 infections and a death rate of about 10% (4).

In 1940, a mass immunization campaign was initiated in French-speaking countries in West Africa (Benin, Burkina Faso (Upper Volta), Côte d’Ivoire (Ivory Coast), Guinea, Senegal, Togo) and Equatorial Africa (Cameroon, Chad, Congo, Gabon), where 25 million people were immunized about every 4 years. As a consequence, yellow fever disappeared gradually in these countries, while epidemic and endemic activity continued in countries without immunization programmes (5). The decreasing number of cases
Fig. 1. Yellow fever endemic zone in Africa

NOTE: Although the yellow fever endemic zones are no longer included in the International Health Regulations, a number of countries consider these zones as infective areas and require an international certificate of vaccination against yellow fever from travellers arriving from those areas.

resulted in a lack of interest in yellow fever, and surveillance and immunization were progressively neglected in the early 1960s.

In 1958, there was a period of virus activity in Central and East Africa with an outbreak in what are now the Equateur and Haut-Zaïre Provinces of Zaire. The following year the virus appeared in Sudan. The most severe outbreak occurred in Ethiopia in 1960–62, when a dramatic epidemic affected the south-west of the country and 3000 deaths were notified. It was estimated that as many as 100 000
cases and 30,000 deaths occurred in this area where the population numbered one million (6). According to previous serological surveys, yellow fever had never penetrated this area before, which explains the large number of victims.

During the past 25 years several outbreaks of the disease have occurred. The most important of these are listed in Table 1.

Table 1. Yellow fever outbreaks in Africa, 1958–82 showing numbers of cases and deaths, and suspected principal vectors

<table>
<thead>
<tr>
<th>Country</th>
<th>Date</th>
<th>No. of cases</th>
<th>No. of deaths or % fatality rate</th>
<th>Suspected principal vectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaire</td>
<td>1958</td>
<td>60</td>
<td>23</td>
<td>?</td>
</tr>
<tr>
<td>Sudan</td>
<td>1959</td>
<td>120</td>
<td>88</td>
<td>A. vittatus, A. falcifera-taylori</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1960–62</td>
<td>100,000 (estimated)</td>
<td>30,000 (estimated)</td>
<td>A. siminsoni, A. africanus</td>
</tr>
<tr>
<td>Guinea</td>
<td>1964</td>
<td>6</td>
<td>6</td>
<td>?</td>
</tr>
<tr>
<td>Senegal</td>
<td>1965</td>
<td>2,000 to 20,000 (estimated)</td>
<td>up to 44%</td>
<td>A. aegypti</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1966</td>
<td>?</td>
<td>350 (estimated)</td>
<td>?</td>
</tr>
<tr>
<td>Ghana</td>
<td>1969</td>
<td>250 (estimated)</td>
<td>73 (estimated)</td>
<td>multiple vectors</td>
</tr>
<tr>
<td>Mali</td>
<td>1969</td>
<td>21</td>
<td>12 (estimated)</td>
<td>multiple vectors</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>1969</td>
<td>3,000 (estimated)</td>
<td>100 (estimated)</td>
<td>multiple vectors</td>
</tr>
<tr>
<td>Nigeria</td>
<td>1969</td>
<td>100,000 (estimated)</td>
<td>up to 40%</td>
<td>A. luteoccephalus</td>
</tr>
<tr>
<td>Nigeria</td>
<td>1970</td>
<td>786 (estimated)</td>
<td>15–40 (estimated)</td>
<td>A. africanus</td>
</tr>
<tr>
<td>Angola</td>
<td>1971</td>
<td>65</td>
<td>42 (estimated)</td>
<td>A. aegypti</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>1975</td>
<td>130</td>
<td>36 (estimated)</td>
<td>A. aegypti</td>
</tr>
<tr>
<td>Gambia</td>
<td>1978–79</td>
<td>8,400 (estimated)</td>
<td>1,600 (estimated)</td>
<td>A. falcifera-taylori, A. aegypti, A. falcifera-taylori, A. aegypti</td>
</tr>
<tr>
<td>Senegal</td>
<td>1981</td>
<td>2</td>
<td>0 (estimated)</td>
<td>A. aegypti</td>
</tr>
<tr>
<td>Côte d’Ivoire</td>
<td>1982</td>
<td>25</td>
<td>25 (estimated)</td>
<td>A. aegypti</td>
</tr>
</tbody>
</table>
2. The Public Health Problem

Yellow fever continues to be a major threat in endemic zones of Africa where the virus reappears even after long periods of quiescence. Undoubtedly, from the historical perspective, yellow fever potentially has serious consequences in relation to morbidity and mortality. In some severe epidemics, it has been estimated that hundreds of thousands of people have been affected, with thousands of deaths (6).

The exact prevalence and incidence of yellow fever in Africa are not known since many cases are not recognized and thus are not reported. The main reasons for this include: (a) occurrence of the disease in relatively remote areas with few medical services; (b) unfamiliarity of medical personnel with the disease and a "low index of suspicion"; (c) confusion with other endemic diseases, e.g., viral hepatitis and malaria, especially early in the outbreak when the high death rate is not yet apparent; (d) lack of access to specific diagnostic laboratory tests and histopathological services; (e) inefficient disease reporting and difficult communications; (f) popular beliefs that discount the ability of western medicine to treat jaundice, with the result that severely ill patients are often removed from hospital.

Although the reporting of yellow fever cases is obligatory for WHO Member States, the statistical data available largely underestimate the true incidence of the disease. Only a small percentage of cases that actually occur in Africa are seen or recognized and it is only when immunity surveys are carried out that the true extent and distribution of the disease is realized. In Table 2 a comparison is made between the number of cases officially reported during various epidemics and the number of cases estimated from epidemiological investigations; these data indicate that epidemic morbidity and mortality are underestimated by a factor of 10–1000 times. Perhaps more important than the problem of underreporting is the late recognition and investigation of outbreaks, since this delays the initiation of control efforts. A delay of 2 months or more has often occurred between the onset of an epidemic and its recognition (Table 3).

No study has been made of the economic cost of yellow fever epidemics. This aspect deserves attention in the future, since cost-benefit considerations would be extremely useful to health authorities in planning prevention and control strategies. The 1969 outbreaks, which involved many countries in West Africa, must have been extremely expensive, in terms of both direct costs of vaccine, vaccine
Table 2. Discrepancies between the numbers of officially notified yellow fever cases and deaths, and estimates of morbidity and mortality rates from direct investigations of epidemics.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year(s)</th>
<th>Number of cases (deaths in parentheses)</th>
<th>Officially notified</th>
<th>Determined by epidemiological investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>1960-1962</td>
<td>100,000 (30,000)</td>
<td>3,000 (3,000)</td>
<td></td>
</tr>
<tr>
<td>Senegal</td>
<td>1965</td>
<td>243 (216)</td>
<td>100,000 (20,000)</td>
<td>(200-2,000)</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>1969</td>
<td>87 (44)</td>
<td>3,000 (1,000)</td>
<td></td>
</tr>
<tr>
<td>(Upper Volta)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>1969</td>
<td>206 (60)</td>
<td>100,000 (1,000)</td>
<td>786 (15-40)</td>
</tr>
<tr>
<td>Gambia</td>
<td>1978-1979</td>
<td>4 (1)</td>
<td>5,000-8,000</td>
<td>(1,000-1,700)</td>
</tr>
<tr>
<td>Senegal</td>
<td>1981</td>
<td>2 (0)</td>
<td>3,000 (1,000)</td>
<td>(several hundreds)</td>
</tr>
</tbody>
</table>


Table 3. Interval between onset of yellow fever epidemics and date of first recognition.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>Epidemic onset</th>
<th>First recognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudan</td>
<td>1959</td>
<td>August</td>
<td>Late October</td>
</tr>
<tr>
<td>Senegal</td>
<td>1965</td>
<td>July</td>
<td>October</td>
</tr>
<tr>
<td>Nigeria</td>
<td>1969</td>
<td>July</td>
<td>September</td>
</tr>
<tr>
<td>Gambia</td>
<td>1978</td>
<td>August</td>
<td>November</td>
</tr>
<tr>
<td>Senegal</td>
<td>1981</td>
<td>August</td>
<td>September</td>
</tr>
</tbody>
</table>

delivery, and medical services, and indirect costs, such as the impact on economic production and the losses of human life. Such epidemics put strains on the resources of African countries. Another factor that must be considered is the harmful impact of epidemics on tourism.

Although no urban yellow fever transmitted by *Aedes aegypti* has occurred during recent decades in large African towns, such an outbreak is always possible.
3. The Virus

The causative agent of yellow fever is an arthropod-borne virus from the Flavivirus genus of the family Flaviviridae. This virus shares group-specific antigens with other members of the genus (former group B viruses—e.g., in Africa: Zika, West Nile, Wesselsbron, dengue, Spondweni, Banzi).

Yellow fever and other flaviviruses possess a single-stranded, positive-polarity RNA genome. Viral particles are 43 nm in size; they are made up of a ribonucleoprotein core and a lipoprotein envelope. The virus is inactivated by deoxycholate, ether, proteases, and lipases. The envelope contains a single glycoprotein with type- and group-specific antigens.

The morphogenesis of yellow fever virus is similar to that observed for other flaviviruses, i.e., viral synthesis and maturation appear to occur predominantly in the rough endoplasmic reticulum of the host cell. The site of formation of the surrounding envelope of the virion remains unclear. Mature virus particles accumulate within the cisternae of membranous organelles and are released from the cell by exocytosis or by plasma membrane rupture.

The virus is pathogenic in adult mice following intracerebral inoculation, and in suckling mice following intracerebral, subcutaneous, or intraperitoneal inoculation. The rhesus monkey is highly susceptible to yellow fever virus and this animal may be used as a model for the study of the pathogenesis of the disease.

Yellow fever virus replicates in cell cultures of different origin, but these cultures vary in their sensitivity. Cell lines of mosquito, monkey kidney, and hamster kidney are useful for propagation and assay. Wild strains of yellow fever virus vary in their pathogenicity for a host, but the molecular basis for virulence is poorly understood and host factors, including genetic and immunological parameters, must also be considered.

Yellow fever virus replication appears to be analogous to that of other flaviviruses, such as West Nile, Japanese encephalitis, and dengue viruses. Host-cell macromolecular synthesis is not seriously affected by the yellow fever virus infection. Treatment of cells with actinomycin D inhibits host-cell RNA synthesis but does not affect viral RNA synthesis. Peak viral RNA synthesis occurs when the virus titre in the supernatant reaches a maximum. Three types of viral RNA are observed: the genomic-size RNA with a sedimentation coefficient of 40S; the RNase-resistant RNA identified as the
replicative intermediate, which is soluble in 2 mol/litre HCl and sediments at about 20S; and the partially RNase-resistant RNA, sedimenting at about 28S, which is presumed to be the replicative form.

The RNA genome is 10,862 nucleotides in length and has a relative molecular mass of $3.75 \times 10^6$. It encodes for three structural proteins and up to 12 nonstructural proteins which are synthesized in infected cells (64). The translation of the nucleotide sequence initiates with the capsid protein C (relative molecular mass, 13,500), which forms complexes with the RNA, followed by the small membrane nonglycosylated protein M (relative molecular mass, 8,500), and the glycosylated or nonglycosylated E protein of the envelope (relative molecular mass 51,000). These structural proteins of the yellow fever virus are virtually indistinguishable from the other mosquito-borne flaviviruses by polyacrylamide gel electrophoresis and differ only slightly from tick-borne flaviviruses in the migration of the small membrane protein. Among the nonstructural proteins, NS 1, which is glycosylated and present in the membrane of infected cells, can induce immunity in mice and monkeys without stimulating antibodies to virion components. Monoclonal antibodies against NS 1 can mediate the complement-dependent lysis of infected cells. These findings could lead to the development of new vaccines and possibly the use of the 17D strain as a vector of other arboviral genes (65).
4. Diagnosis of Yellow Fever

4.1 Clinical Diagnosis

Yellow fever is an acute infectious disease. Typically, the disease is characterized by a sudden onset with a two-phase development, the phases being separated by a short period of remission. Viraemia occurs during the first phase, which is clinically undifferentiated, while the second phase is characterized by hepatorenal dysfunction and haemorrhage.

So-called "classical" yellow fever is usually recognized during epidemics, when the disease is observed in many individuals and is relatively easy to diagnose clinically. The incubation period is generally 3–6 days after the bite of an infected mosquito, but may be longer. The disease onset is sudden and marked by a temperature of 39–40°C, chills, intense headache, lumbosacral and generalized muscular pains, nausea and vomiting, conjunctival injection, and a flushed face. The urine at this stage is dark in colour and may not contain albumin. Faget's sign (slow pulse in relation to the fever) is typical.

Generally, on the third or fourth day after onset, a remission occurs, which is characterized by a fall in temperature, disappearance of the headache, and an improvement in the general condition of the patient. However, this remission is short-lived, lasting only a few hours, and is followed by the period of intoxication, the hepatorenal phase, which is characterized by a rise in temperature, the reappearance of generalized symptoms, jaundice, vomiting—the vomitus may contain digested blood (black vomitus)—other haemorrhagic signs (bleeding of the gums, ecchymoses, menorrhagia, and haematuria), albuminuria, and oliguria. Progressive tachycardia, shock, and intractable hiccups are considered ominous signs. The case-fatality rate of severe yellow fever is 50%. Death usually occurs between the seventh and tenth day after onset. The chronology of clinical events in the patient with yellow fever is given in Fig. 2.

Yellow fever infection may vary in intensity from a preliminary illness unaccompanied by any classical signs and symptoms with death following in 2–3 days, to very mild or subclinical forms.

The clinical diagnosis of yellow fever may be difficult even during epidemics, and is often impossible in mild or atypical cases. The disease can be definitely diagnosed only by serology or virus isolation. Malaria, which is endemic in Africa, usually shows clinical symptoms
almost identical with those of the early stages of yellow fever: sudden onset, headache, generalized aches, and vomiting. Other diseases resembling anicteric yellow fever include: typhoid fever, rickettsial infections, other arboviral fevers, and influenza. It is particularly important to differentiate between yellow fever and other diseases with hepatorenal dysfunction and/or haemorrhagic manifestations, such as viral hepatitis, malaria, viral haemorrhagic fevers (Lassa fever, Marburg and Ebola virus diseases, Crimean-Congo haemorrhagic fever, Rift Valley fever), leptospirosis, infectious mononucleosis with jaundice, and surgical or toxic causes of jaundice.

4.2. Laboratory Diagnosis

4.2.1 General considerations

Laboratory and hospital staff must have been immunized at least 10 days before handling possibly infected necropsy tissue or other pathological, virological, haematological, or biochemical specimens.
The laboratory diagnosis of yellow fever requires special reagents and techniques as well as expertise in the interpretation of test results. Individual histopathologists, especially those with some experience of yellow fever, are often called upon for the diagnosis, although a number of national laboratories in Africa can provide specific serological diagnostic services; some of these can also perform virus isolation.

A list of WHO Collaborating Centres that provide reference services and reagents is given in Annex 2. These centres are a back-up resource for laboratories in the region.

Specimens for the laboratory diagnosis of yellow fever should be accompanied by certain information, and the use of an information form when submitting specimens is helpful. The following information is essential: name, age, sex, residence, date of onset of symptoms, and date of collection of specimens. The following information should be provided if available: occupation, data on presumed location where the infection was acquired, history of recent travel, history of immunization, symptomatology, and results of any clinical laboratory tests (e.g., leukocyte count, albuminuria, liver enzymes).

The virus may be isolated from, or antigen detected in, serum specimens taken as early as possible in the illness as well as from liver specimens taken at autopsy, and stored in the cold to preserve the viability of the virus. For serological diagnosis of yellow fever, appropriately timed paired samples are required. The acute-phase serum should be collected as early as possible in the illness, and if the patient survives, a convalescent sample should be taken during the second or third week after onset.

To facilitate the rapid processing of specimens from suspected cases of yellow fever, it is advisable, whenever possible, to inform the laboratory before sending diagnostic samples. The period of time required for laboratory diagnosis depends on the tests used (summarized in Table 4).

4.2.2 Histopathological diagnosis

Liver biopsy is absolutely contraindicated in the living patient, because of the danger of haemorrhage.

Liver samples may be obtained from fatal cases by abdominal incision or by the use of a viscerotome (I) or a large-calibre biopsy needle. As large a specimen as possible should be obtained to facilitate histopathological interpretation. It is often possible to isolate yellow fever virus from the liver, if the patient died before the tenth or twelfth day after onset of the disease. Liver specimens should
<table>
<thead>
<tr>
<th>Technique</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Specimen</th>
<th>Type</th>
<th>Phase of illness</th>
<th>Time needed to perform test (days)</th>
<th>Applicable to field situation</th>
<th>Relative complexity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Virus isolation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1 Suckling mice</td>
<td>moderate</td>
<td>moderate/high</td>
<td>serum, liver*</td>
<td>acute</td>
<td></td>
<td>7-14</td>
<td>no</td>
<td>moderate</td>
</tr>
<tr>
<td>1.2 Intrathoracic inoculation of mosquitos</td>
<td>high</td>
<td>moderate/high</td>
<td>serum, liver*</td>
<td>acute</td>
<td></td>
<td>10-15</td>
<td>no</td>
<td>high</td>
</tr>
<tr>
<td>1.3 Cell cultures</td>
<td>high</td>
<td>moderate/high</td>
<td>serum, liver*</td>
<td>acute</td>
<td></td>
<td>3-4</td>
<td>yes</td>
<td>high</td>
</tr>
<tr>
<td>2. Direct detection of antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2.1 ELISA</td>
<td>moderate/ high</td>
<td>moderate/high</td>
<td>serum, liver*</td>
<td>acute</td>
<td></td>
<td>&lt;1</td>
<td>yes</td>
<td>low/moderate</td>
</tr>
<tr>
<td>3. Serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1 Haemagglutination-inhibition</td>
<td>high</td>
<td>low</td>
<td>serum</td>
<td>convalescent</td>
<td>1-2</td>
<td>no</td>
<td>moderate/high</td>
<td></td>
</tr>
<tr>
<td>3.2 Complement-fixation</td>
<td>moderate</td>
<td>high</td>
<td>serum</td>
<td>convalescent</td>
<td>1-2</td>
<td>possibly</td>
<td>moderate/high</td>
<td></td>
</tr>
<tr>
<td>3.3 Neutralization</td>
<td>high</td>
<td>moderate/high</td>
<td>serum</td>
<td>convalescent</td>
<td>7-14</td>
<td>no</td>
<td>moderate/high</td>
<td></td>
</tr>
<tr>
<td>3.4 Indirect immunofluorescence</td>
<td>moderate</td>
<td>low/moderate (IgG), high (IgM)</td>
<td>serum</td>
<td>convalescent (IgG)</td>
<td>&lt;1</td>
<td>yes</td>
<td>moderate</td>
<td></td>
</tr>
<tr>
<td>3.5 ELISA</td>
<td>high</td>
<td>low/moderate (IgG), moderate/high (IgM)</td>
<td>serum</td>
<td>convalescent (IgG)</td>
<td>&lt;1</td>
<td>yes</td>
<td>moderate</td>
<td></td>
</tr>
</tbody>
</table>

* Post-mortem liver specimens only.
be divided into separate portions for virus isolation and for fixation and histopathological examination. For virus isolation, the sample should be obtained and maintained aseptically and refrigerated or frozen for transfer to the laboratory. If this is not possible, the specimen may be placed in 50% glycerol and transported at ambient temperature by the fastest route.

Specimens for histopathology should be fixed in Bouin’s solution or 10% formalin. The examination of frozen sections of liver stained with Sudan III or haematoxylin-eosin may allow a rapid presumptive diagnosis to be made. The examination of paraffin sections obtainable after 48 hours gives a more accurate diagnosis.

Interpretation of the observations is sometimes difficult and can be made only by an experienced histopathologist. In typical cases, the histological diagnosis is based on an eosinophilic degeneration of the hepatocytes leading to the formation of Councilman bodies, predominant midzonal necrosis, and microvacuolar steatosis. However, the histopathology of other infections, such as Lassa fever, Marburg and Ebola virus diseases, Crimean-Congo haemorrhagic fever, viral hepatitis, and leptospirosis, may be confused with that of yellow fever. A guide to the histopathological, differential diagnosis of yellow fever is available from WHO and includes photomicrographs.1

4.2.3 Virus isolation

The viraemia, which can appear 24 hours before the onset of symptoms, reaches a peak between the second and fourth day, and the success of virus isolation techniques rapidly decreases thereafter. Occasionally, the virus has been isolated as late as the fourteenth day (J). Specimens for virus isolation should be transported frozen or at a temperature between +4°C and +10°C, without being subjected to repeated freezing and thawing. If possible, the serum should be separated from the clot using an aseptic technique. The methods available for maintaining specimens at a low temperature during transportation include: wet ice, ice packs (“cold dogs”), dry ice, and liquid nitrogen.

The diagnosis of yellow fever is made by virus isolation followed by specific identification. Several methods are available, including the inoculation of suckling mice, the intrathoracic inoculation of mosquitoes, or inoculation of cell cultures. A flow chart of techniques available for diagnosis by virus isolation is given in Fig. 3.

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1 Copies of this guide are available from Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland.
Fig. 3. Flow chart for yellow fever virus isolation

LIVING PATIENT

MATERIAL FOR INOCULATION
Serum, plasma or whole blood (undiluted and 1:10 dilution)

TEST SYSTEM

1. 0.02 ml of serum or tissue suspension inoculated intracerebrally into 1-3-day-old mice.
2. Inoculate *Toxorhynchites* spp. or *A. aegypti* mosquitoes intrathoracically.
3. Inoculate cell cultures (mosquito or monkey kidney).

FATAL CASE

Histopathology, fluorescent antibody examination

OBSERVATION OR INCUBATION

1. A) Illness or death within 7-14 days
   B) Presence of specific antigen by immunofluorescence or complement-fixation test
   C) Survival of mice inoculated with specimen in presence of yellow fever hyperimmune ascitic fluid

2. A) Presence of immunofluorescent antigen in salivary glands and brains after 10-17 days' incubation at 30°C
   B) Use of mosquito tissues as antigen in complement-fixation test

3. A) Cytopathic effect or plaques in some cell cultures
   B) Immunofluorescent or complement-fixation antigen detected after 3-4 days' incubation

PASSAGE

10% W/V brain suspension passed to suckling mice

CONFIRMATION

Immunoﬂuorescence tests with type-speciﬁc monoclonal antibodies

Homogenized mosquito tissues passed to suckling mice

Neutralization test

Supernatant fluid or cells passed to suckling mice
(1) Isolation in mice. The classical method of virus isolation involves the inoculation of the sample into 1- or 2-day-old mice intracerebrally. It is also possible to use whole blood collected with an anticoagulant (heparin) or using a heparinized capillary tube. It is preferable to inoculate both the undiluted serum or blood and a 1:10 dilution. Newborn mice can also be used for the isolation of virus from mosquitoes. Identification of isolates is done by a neutralization test with specific antisera. Older (e.g., weanling) mice inoculated intracerebrally may be used if suckling mice are not available, but they are less susceptible than sucklings.

(2) Intrathoracic inoculation of mosquitoes. This simple and sensitive technique, first described for the isolation of dengue virus, may be used for the detection of yellow fever virus from clinical specimens (7, 8). Since mosquitoes do not develop signs of infection, the virus must be detected by an indirect method. The Toxorhynchites spp. mosquitoes of both sexes may be used, their advantage being the ease of inoculation (because of their large size) and safety (since they do not feed on blood). Large quantities of virus are obtained and used as the antigen in complement-fixation and immunofluorescence tests for identification. Aedes aegypti may also be used, but only male mosquitoes should be inoculated because of the potential danger should any inoculated females escape from the laboratory. The apparatus and techniques for intrathoracic inoculation are described by Rosen & Gubler (7).

Approximately 0.5 µl of human serum (diluted 1:20–1:50) is inoculated, and the mosquitoes are kept at 30 °C for 10–17 days. Head squashes of the mosquitoes are then examined by the immunofluorescence technique for the detection of viral antigen and the abdomen may be used as an antigen in the complement fixation test (8). Alternatively, the inoculated mosquitoes may be used as an amplification step with subpassage to mice or cell cultures for definitive isolation and identification.

The main advantage of this technique is its great sensitivity. Viral strains are recovered without passage through a vertebrate host. Serum specimens with some bacterial contamination can be used, since bacteria are not generally harmful to mosquitoes (whereas they will kill cells in culture). A disadvantage is the relatively long incubation time required before the virus can be detected.

(3) Inoculation of cell cultures. For the primary isolation of wild strains of yellow fever virus, both mosquito cells and continuous cell lines of monkey kidney (Vero, LLC-MK2) may be used. However, mosquito cell cultures are more sensitive than mammalian cultures for this purpose (9). Several comparative studies have been made on the use of continuous cell lines of mosquito cells (10, 11). The most frequently used cells are those of A. aegypti (clone C17 or C20), A. albopictus (clone C6-36) or A. pseudoscuteilaris (AP 61) grown in
4. DIAGNOSIS

flasks or tubes. The serum used for inoculation of the culture must be
diluted 1:50 or 1:100 to avoid toxic effects. *A. pseudoscutellaris* cells
show signs of a cytopathic effect (CPE), whereas other cell types must
be examined for viral antigen in the absence of CPE. After incubation
of the cultures at 28°C for 3–6 days, the cells are examined for virus
by immunofluorescence. The cells are suspended by scraping, washed,
and used to prepare spot slides for immunofluorescent staining.

There are several advantages in the use of culture techniques for
the isolation of virus: (a) cultures can be transported to and used in
the field; (b) for the primary isolation of yellow fever virus, cell
cultures are more sensitive than suckling mice and equally as sensitive
as the intrathoracic inoculation of mosquito; (c) isolation and virus
identification can be accomplished in 3 or 4 days; (d) the technique
can be used to isolate virus from pooled or individual mosquitoes, as
well as from human specimens.

Antisera for use in the indirect fluorescent antibody test are
prepared as hyperimmune mouse ascitic fluids; however, recently,
yellow fever type-specific monoclonal antibodies have been developed
that may be used in the indirect immunofluorescence test for specific
virus identification (12).

4.2.4 Direct detection of antigen

An antigen-capture enzyme-linked immunosorbent assay (ELISA)
has been described recently and can be used for the rapid detection of
virus in serum, blood, and liver (Annex 3). In preliminary tests on
human specimens, the technique was as sensitive as virus isolation in
suckling mice. The technique can be performed in less than 4 hours
and can be used in the field. Other techniques that may be useful
include immunofluorescence (see Annex 3) and reverse passive
haemagglutination.

4.2.5 Serological diagnosis

Blood is collected by venepuncture into a sterile syringe or vacuum
tube, allowed to clot, and the serum aseptically separated into a
sterile vial or tube. A minimum of 1 ml of serum should be obtained.
Specimens should be clearly labelled with the patient's name and the
date of collection. They should be kept in a refrigerator or freezer
until transferred to the virus laboratory, preferably by the same
methods previously described (see section 4.2.3 on virus isolation).
Under normal conditions, however, specimens may be transported at
ambient temperature if they have been handled aseptically. If
refrigerated storage prior to dispatch is not possible or if the transit
time is 3 days or longer, it is advisable to add a preservative to the
sample. Instructions for transportation should be requested from the
laboratory and responsible health authorities. Preservatives that might be used include sodium azide, thiomersal, and various antibiotics.

An alternative method for obtaining serological specimens is the use of fingerprick blood collected on to filter paper. The disadvantages of this technique are the loss of IgM antibodies and the development of anticomplementary reactivity. Capillary tubes may be used for blood collection; samples are diluted 1:5 in phosphate-buffered saline, centrifuged, and handled as for serum specimens.

Different methods may be used for serological diagnosis (13). The haemagglutination-inhibition (HI) test detects antibodies that usually appear early (within the first week after onset). In cases of primary yellow fever infection, a specific diagnosis is sometimes possible using this method. However, the HI-antibody response broadens with time, and cross-reactions with other flaviviruses may make interpretation difficult. In patients with prior exposure to heterologous flaviviruses, the HI-antibody response is broadly cross-reactive and a specific diagnosis is not possible.

The complement-fixation (CF) test is more specific than the HI test. Antibodies appear later (during the second week after onset) and may decline relatively rapidly to low levels 6–12 months after infection. Thus, the presence of CF antibodies usually indicates a recent infection. However, in some studies, CF antibodies have been shown to persist at moderate to high titres for prolonged periods (at least 2 years). The use of yellow fever 17D vaccine does not result in the formation of CF antibodies in individuals not previously exposed to a flavivirus. However, this vaccine may induce a CF-antibody response in individuals with background heterologous immunity (14).

The neutralization test is the most specific. Antibodies appear early (during the first week after onset) and last for many years (probably for life). Various techniques for the measurement of N antibody may be used, including plaque-reduction assays in cell culture and tests in mice (15).

Indirect immunofluorescence reactions (Annex 3) on cells infected with yellow-fever virus make it possible to detect both IgG and IgM antibodies in serum specimens. In the case of a primary infection, the IgG antibodies are regularly found and the specificity is comparable to that obtained in the CF and neutralization tests. In the case of superinfection, broad cross-reaction with the other flaviviruses occurs. IgM antibodies are highly specific but not always present (16).

The enzyme-linked immunosorbent assay (ELISA) (Annex 3) for IgG antibodies, using a viral protein specific to the yellow fever virus, is more sensitive and more specific than the CF and HI reactions and gives results equivalent to those obtained in seroneutralization (17). Recently, an antibody-capture IgM ELISA (or radioimmunoassay) has been shown to be extremely useful for the diagnosis of yellow fever (18).
4. DIAGNOSIS

Radial haemolysis-in-gel has been used for the detection of yellow fever IgG antibodies; it is as sensitive as the HI test with the additional advantage that nonspecific inhibitors do not have to be removed (19).

Interpretation of the serological diagnosis results must take into account both the interval between onset of illness and collection of serum and the specificity of the reaction used. A fourfold increase (or decrease) in IgG antibody titre is considered confirmatory. Primary infections with yellow fever virus present few complications in interpretation because of their relative specificity. In persons with a previous flavivirus infection, yellow fever superinfection induces a rapid and broad serological response. Occasionally, antibody titres to the previous infecting virus may exceed those to the current infection, i.e., yellow fever antigen ("original antigenic sin"). It is essential to determine the specificity of serological tests by using parallel tests with viruses antigenically close to yellow fever virus, such as Uganda S, Zika, Spondweni, Bani, Wesselsbron, and Bonboni. The presence of broadly reactive heterologous reactions is interpreted as a flavivirus superinfection.

A presumptive diagnosis of recent yellow fever infection may be made when IgM antibodies (e.g., by ELISA or indirect FA test) are detected or when there is a high, specific complement-fixation titre in a single sample of serum taken during convalescence.
5. Epidemiology

The epidemiology of yellow fever is explained by different transmission cycles of the virus between man, mosquitoes, and monkeys. The vector mosquito, which may belong to one of several species, becomes infected by feeding on a viraemic host (man or monkey) and then transmits the virus to another susceptible host.

5.1 Virus Reservoir

Monkeys and man have classically been considered as the "reservoirs" of yellow fever virus; in fact, these hosts have only short-lived viraemias.

In man, virus is present in the blood at levels high enough to infect mosquitoes from just before onset of the infection until the third day after onset; the levels decline from the fourth day after onset, when specific antibodies begin to appear.

In Africa, most species of monkey also develop an effective viraemia lasting several days or more. African monkeys rarely die from yellow fever infection and most of the time they produce protective antibody after the viraemic period. Other vertebrates have been suspected of being effective viraemic hosts, mainly species of the genus Galago (bush-babies), but their role, if any, in the transmission of the virus requires further study.

Ecological changes in Africa, including deforestation, have significantly influenced the number and distribution of monkey species. Not even all susceptible monkey species can be considered as the fundamental reservoir of yellow fever because viraemia is transient. The true reservoir is the susceptible mosquito species that remains infected throughout life and can even transmit the virus transovarially to a certain proportion of descendants through infected eggs. Man and monkey play the temporary role of amplifiers of the amount of virus available for the infection of mosquitoes.

5.2 Transmission by Mosquitoes

For about 30 years following Reed's discovery in Cuba that yellow fever is transmitted to man by Aedes aegypti, the epidemiology of yellow fever was based on the belief that man was the only
susceptible vertebrate host and that the mosquito *A. aegypti* was the sole vector. Many urban epidemics were eliminated by the control of the vector. However, later outbreaks of the disease were observed in *A. aegypti*-free areas. These outbreaks were always associated with forested areas and in many cases they occurred in places where the human population was scanty. It became evident that yellow fever can persist as a zoonosis in tropical areas of Africa and America, with nonhuman primates responsible for maintaining the infection. Furthermore, it was found that various species of forest mosquito play an important role as vectors and as reservoirs of the disease.

5.2.1 **Summary of transmission patterns**

From the epidemiological point of view the cycles of transmission involved may be illustrated as in Fig. 4. The schemes outlined in this figure are useful for understanding outbreaks, but most outbreaks have certain local features in transmission that do not necessarily fall within these generalizations.

5.2.2 **Epidemiological zones**

The following epidemiological zones have been identified in Africa; they are characterized by their climate, vegetation (Fig. 5), and the type of transmission cycle that occurs.

(1) **Endemic zones.**

(a) The rain forest of West and Central Africa, where sylvatic transmission is predominant.

(b) The emergence zone, which includes the humid and semihumid savanna (also called derived savanna, Southern Guinea savanna) surrounding the rain forest. Different cycles may take place (i.e., monkey-to-monkey, monkey-to-man) on an enzootic basis with periodic epizootics; these constitute the "intermediate" type of yellow fever transmission cycle.

(2) **Epidemic zone.** In the drier savanna (which includes Northern Guinea savanna, Sudan savanna, and Sahel savanna) man-to-man transmission by *A. aegypti* occurs in epidemics.

5.2.3 **Mosquito vectors involved in transmission cycles in different zones**

In each zone, transmission cycles involve different mosquito species. Since each species exhibits a different behaviour and has its own ecological characteristics, the patterns of transmission differ in each zone.

(1) **Rain forest.** The principal vectors are members of the *Aedes africanus* group, which transmit the virus among monkeys in the
Fig. 4. Cycles of transmission of yellow fever virus

RAIN FOREST

A. simpsoni
A. africanus

Monkey

Transovarial transmission

Man

EMERGENCE ZONE

A. africanus - A. luteocephalus
A. simpsoni - A. furcifer-taylori

Amblyomma variegatum
by transovarial transmission

EPIDEMIC ZONE

A. aegypti

Introduction of virus
from emergence zone

Man

A. furcifer-taylori and other species

Known part of cycles
Speculative part of cycles
canopy of the rain forest. In addition, in Cameroon and eastwards, anthropophilic species of the Aedes simpsoni group may, in certain places, transmit the virus from monkey to monkey and monkey to man. Circulation of the virus within the rain forest is generally at a low level throughout the year. Except in unusual situations, such as deforestation, man has little contact with the forest cycle. When contact takes place, sporadic cases in man can be identified. Epidemics may occur with the immigration and settlement of non-immune persons.

(2) Emergence zone. Different patterns of transmission may occur depending on the dominant mosquito vector species inhabiting the different types of savanna and on the density of the monkey population. Close to the rain forest the vectors are members of the A. africanaus group. Further from the forest this group of vectors is replaced by A. luteocephalus or other species, such as the A. simpsoni group or A. furcifer-taylori, which may be abundant in certain places and play an essential role in transmission. A. vittatus, A. metallicus, and A. aegypti (wild form), and possibly other unidentified species, may also play a secondary role in local transmission.

Cyclical epizootics, at intervals of several years, may be superimposed on a background of enzootic, low-level virus transmission. Epizootics occur in the late rainy season and at the beginning of the dry season, when transmission is at its highest level. Epizootics may recur during the rainy seasons in two or three consecutive years. The maintenance of the virus during the intervening dry seasons can best be explained by transovarial transmission of the virus (20). The same mechanism is perhaps involved in the maintenance of the virus between epizootics at intervals of several years. The virus has recently been isolated from naturally infected ticks, Amblyomma variegatum (21). These ticks have been shown experimentally to transmit the virus to their progeny, which can then infect monkeys; therefore, ticks may represent an alternative reservoir and their role should be studied further.

(3) Epidemic zone. Outside the rain forest and the emergence zones, the virus can invade the dry savanna in explosive epidemics wherever a receptive human population and domestic vectors exist, up to the limits of the borders of the endemic zones as defined in Chapter 1 (15° N, 10° S). Although A. aegypti is the main domestic vector, other species such as A. furcifer may contribute to man-to-man transmission, especially at the beginning of epidemics.

The virus may be introduced into this area in two ways. The monkey-to-monkey cycle, which takes place in the emergence zone, may be extended along the riverine forests during epizootics and penetrate the dry savanna area reaching villages where man-to-man transmission can occur. Individuals infected in the emergence zone
may be transported into villages where the domestic vector exists. Transmission of the virus in this zone is only of the epidemic type. Usually the outbreak spreads from village to village following the lines of communication used by man. When the epidemic has started, the virus can be transported to distant places either by infected persons or by infected mosquitos.

5.2.4 Ecology of vectors

Relatively few mosquito species (mainly Aedes spp.) are able to transmit the virus. Even among vectors, the ability to transmit the virus is influenced by many factors that are as yet incompletely understood. The level of viraemia required to infect is different for each vector species. After ingestion of the viraemic blood meal, the virus replicates in the midgut epithelium and finally reaches and multiplies in the salivary glands. This requires a delay (the so-called "extrinsic" incubation period) after which the mosquito becomes infective. The extrinsic incubation period is shorter at higher temperatures (ranging from 12 days at 18°C to 2 days at 30°C; very little transmission occurs below 18°C).

The mosquito remains infected throughout its life (up to 6 months). However, not all infected mosquitos are capable of transmitting the virus. Infection with yellow fever virus does not appear to produce any adverse effects in the mosquito vectors. Transovarian transmission, which has been observed to occur in some mosquito vectors, enhances the role of mosquitos as reservoirs of yellow fever virus.

(1) Aedes aegypti. There are two forms of A. aegypti—domestic and wild (22). The domestic form is adapted to the human habitat and primarily uses artificial breeding places, such as water-storage pots, tyres, and other man-made containers. It is highly anthropophilic and is present throughout the year with cyclical variations depending on climatic conditions. Its flight range is limited—largely within a distance of about 400 m—but it can be artificially transported over great distances. The wild form is more widely distributed and breeds in natural habitats, such as rock pools, tree holes, and fruit-shells. It feeds primarily on animals other than monkeys and man and is present only during the rainy season. Both forms of A. aegypti are daytime biters, mainly active in the afternoon.

(2) Aedes simpsoni group. This group is now understood to comprise a complex of at least three known species (A. lili, A. bromeliae, and A. simpsoni). Although the A. simpsoni group has been studied extensively, little is known about the biology of the separate species. Breeding places are plant axils and tree holes. In West Africa, these mosquitos do not bite man, whereas in certain
areas of Central and East Africa they are highly anthropophilic. They are weak fliers and mostly bite during the daytime.

(3) *Aedes africanus* group. Four species (*A. opok*, *A. neoaficanus*, *A. pseudoafricanus*, and *A. africanus*) have been characterized to date. They breed in tree holes. Host preference differs with each species; *A. africanus* is highly primatophilic. While the peak of biting activity is generally at dusk, vigorous biting occurs at any time when man intrudes into its habitat (intrusion effect). *A. africanus* bites mainly at the canopy level of the rain forests, but it also bites at ground level at the edge of the rain forests, especially during the daytime. In riverine forest, it bites both at canopy and at ground level with the same frequency. With *A. neoaficanus* and *A. opok* there is no intrusion effect. *A. africanus* is found in forest and wet savannas. *A. opok* and *A. neoaficanus* are found only in the humid and semihumid savanna areas. *A. pseudoafricanus* is known only from coastal mangrove areas in West and Central Africa.

(4) *Aedes luteocephalus*. This species is found in drier areas than those inhabited by the *A. africanus* group. It breeds in tree holes and is abundant in the canopy of the forests within the epidemic and emergence zones. *A. luteocephalus* is highly primatophilic, with some variation in its host preference across its range of distribution. The biting peak is at dusk and there is no intrusion effect. It is generally rare in villages except when they are situated close to a forest. However, in the Benue Plateau area of Nigeria, where prolific breeding occurs in holes of cut *Euphorbia* cactus used as hedging around villages, this vector was responsible for intense interhuman transmission during the 1969 epidemic (23).

(5) *Aedes furcifer* and *A. taylori*. Until recently the females of these two species were not easily separable on the basis of morphological characters and they were treated as a single species (referred to as *A. furcifer-taylori*). They occur in all types of savanna area. Their breeding places are mainly tree holes and occasionally fruit-shells. In West Africa they are strictly primatophilic but in southern Africa they also feed on other mammals. They bite at dusk and during the night, but never during the day. Both species are prevalent in the canopy of riverine forests in the epidemic and emergence zones, and they also frequently bite outside the forest in open savanna and in villages, even entering houses. In contrast to other vectors, they usually take two blood meals during each gonotrophic cycle, thus enhancing their role as vectors.

The eggs of all *Aedes* species withstand prolonged desiccation and this enables them to survive from one rainy season to another. The eggs of *A. aegypti* usually hatch at the first flooding, whereas the eggs of the other *Aedes* species generally require several floodings prior to hatching. Virus may be conserved in the egg during the dry season.
5. Epidemiology

5.3 Epidemiological Patterns in Man

5.3.1 Sylvatic yellow fever (forest yellow fever)

In the equatorial rain forest zone of Africa, where yellow fever virus is enzootically maintained by year-round transmission between monkeys and *A. africanus* mosquitos, human infections are sporadic and relatively rare. This is due principally to the great dilution of vectors and hosts in the uniform forest environment. Human infections follow exposure to the enzootic cycle, during activities such as clearing forest and cutting wood, but small outbreaks may occur in nearby village populations, especially where the forest has been recently disturbed. Immunity prevalence rates in human populations in the forest zone are generally low.

5.3.2 Intermediate yellow fever

In the savanna-mosaic surrounding the forest zone and extending to the Guinea and Sudan savanna zones (the so-called zone of emergence), yellow fever is endemic. Human infections may occur at varying frequency as vector populations expand during the rainy season. Antibody prevalence rates are high, and they increase with age. In some surveys, it has been possible to estimate an annual incidence of infection of at least 1% so that, by adulthood, immunity rates of 50% or more are not unusual (24). Clinical cases occur in an endemic pattern but are rarely recognized in the context of current surveillance activities in Africa. Man may be infected outside the village, e.g., by the *A. africanus* group or *A. simpsoni*, or inside the village where the virus may be introduced by species such as *A. furcifer-taylori*. If human and vector populations are large enough, and if the level of virus transmission increases sufficiently in the emergence zone, epidemics may occur, involving both monkey–man and interhuman virus transmission by sylvatic vectors. In some epidemics, sylvatic vectors were involved in intense interhuman spread, e.g., in Ethiopia in 1960–62 (*A. africanus*, *A. simpsoni*), and in Nigeria in 1969 (*A. luteocephalus*) and 1970 (*A. africanus*).

When the domestic form of *A. aegypti* is present in villages and can transmit the virus from man to man, epidemics may be observed involving both this vector and wild mosquitos. These are called “intermediate epidemics” because they are a mixture of man-to-man (formerly called urban transmission) and monkey-to-man transmission (sylvatic transmission). “Intermediate” epidemics are often characterized by focal outbreaks separated by areas without human cases.

5.3.3 Interhuman (A. aegypti-transmitted) yellow fever

The introduction of yellow fever virus from the emergence zone into the drier savanna area, where yellow fever is not endemic and the
human population has a low background of immunity, has caused explosive outbreaks in the past. In these situations, the virus is transmitted in a man–mosquito–man cycle by *A. aegypti*. Such epidemics have resulted in an incidence of infection of 40% or more (25). Case-fatality rates have varied from 20 to 50% (occasionally higher) depending upon the accuracy of case recognition. The age distribution of cases depends entirely on the background of immunity in the affected population. In the outbreaks in Senegal (1965), Gambia (1978–79), and northern Ghana (1977–79), children were principally affected because they were susceptible, whereas a high background of vaccine-induced or natural immunity was found in older persons.

Other characteristics of outbreaks. Some outbreaks have been characterized by an excess of cases in adult males. This may be related to an increased exposure of adult males to infected mosquito vectors. Differences in the susceptibility of races to yellow fever have not been clearly defined. On a theoretical basis, children of immune mothers may be protected by maternal antibodies up to about 3–4 months of age. In regions where infection with heterologous flaviviruses (e.g., Zika, Wesselsbron, and Uganda S viruses) are frequent, individuals older than 10–15 years may also be protected to some extent against severe yellow fever by an acquired cross-immunity. The ratio of inapparent to apparent infection has been estimated to be normally approximately 12:1 and the available data suggest that prior exposure to heterologous flaviviruses provides some protection against clinical yellow fever, since the inapparent:apparent infection ratio has been found to be higher in persons with superinfection than with primary serological responses (26). Experimental studies in monkeys have also shown that prior infection with Wesselsbron or Zika viruses reduced viraemia and disease in animals challenged with yellow fever virus (27).

The seasonal incidence of yellow fever corresponds to the late rainy season and early dry season, when infected vector populations and vector longevity are high.

From the public health point of view, it should be noted that the terms “jungle” yellow fever outbreak and “urban” yellow fever outbreak are no longer precise enough given the present knowledge of transmission cycles in Africa. Classically, jungle yellow fever was used to describe the monkey-to-man cycle with sylvatic vectors, and urban yellow fever the man-to-man cycle with *A. aegypti*. However, transmission from person to person of this “urban” type may occur in villages, mediated either by a sylvatic mosquito species or by *A. aegypti*. It is therefore proposed that a distinction be made between the three possible routes of human infection based on the relative role played by vectors and vertebrates as indicated in Table 5 and Fig. 4. Human cases that occur in the endemic area, which includes the
forest and different types of humid savanna, and that are transmitted by sylvatic vectors are better described by the term “sylvatic” yellow fever outbreaks. Human cases, occurring in villages or towns, that are transmitted by *A. aegypti* should be called “*Aedes aegypti*-transmitted” yellow fever outbreaks. Wherever a cluster of cases results from an intermingling of the two above-mentioned cycles, the outbreak should be defined as an “intermediate” yellow fever outbreak.

Table 5. Relative role of mosquito vectors and vertebrates in yellow fever transmission cycles

<table>
<thead>
<tr>
<th>Transmission cycle</th>
<th>Vectors</th>
<th>Vertebrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. aegypti</em></td>
<td>Sylvatic</td>
</tr>
<tr>
<td>Interhuman</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Intermediate*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sylvatic</td>
<td>–</td>
<td>+++</td>
</tr>
</tbody>
</table>

*Wherever a cluster of cases result from an intermingling of the two other cycles, the transmission cycle is defined as “intermediate”.*
6. Epidemiological Surveillance

6.1 Detection of Human Cases

Surveillance of human yellow fever infections is essential so that endemic transmission and impending epidemics can be recognized. Effective surveillance depends upon both the clinical and the laboratory diagnosis of cases, as well as on an efficient reporting system.

Some epidemics of yellow fever remain completely undetected, as do most cases of sporadic disease occurring in the endemic area as defined by Germain et al. (28). This fact has become clear only as a result of the operation of special programmes for yellow fever surveillance. For example, in 1970 a previously unknown outbreak of yellow fever was detected in Nigeria, when an epidemiologist assigned to a specific yellow fever project followed up an anecdotal report of an unusual level of mortality in Okwoga District (29). Surveillance activities in Nigeria (30), Côte d'Ivoire, and elsewhere have illustrated that such efforts can detect the occurrence of sporadic cases of yellow fever. The occurrence of such cases may reflect either endemic transmission or the early phase of an epidemic.

Surveillance of yellow fever is based on clinical diagnosis, but other diseases prevalent in Africa may resemble yellow fever. An important aspect of the clinical diagnosis is therefore case-definition. For effective surveillance the broadest possible case-definition should be used, i.e., febrile illness with jaundice. In some circumstances, the use of a more specific case-definition that helps to incriminate yellow fever in the etiology of jaundice may be recommended—for example, jaundice with albuminuria or jaundice with haemorrhagic manifestations.

Surveillance systems may be passive or active. Passive surveillance implies reliance on routine reports of suspect cases from dispensaries, hospitals, or other health delivery systems. Active surveillance implies a specific, directed effort to detect increased virus transmission. Both methods require laboratory support.

6.1.1 Passive surveillance

Most infections with yellow fever virus do not result in jaundice; however, this feature of the disease is useful as a basis for
surveillance, since the more common clinical signs are nonspecific. Most countries have a system for reporting cases of hepatitis, which can be adapted to provide information about the occurrence of yellow fever. The monthly incidence of hepatitis and the hepatitis death rate should be examined by geographical region or administrative area. Data indicating the possibility of an outbreak of yellow fever include an unusual case-fatality rate; in general, the rate for viral hepatitis and other causes of jaundice that may be confused with viral hepatitis is less than 1%, whereas the fatality rate of hospitalized yellow fever cases is high, usually greater than 30%. Comparisons of incidence and mortality data from different hospitals or geographical regions also provide important information, since yellow fever outbreaks may be quite localized.

The effectiveness of passive surveillance of this sort depends upon how quickly the disease incidence reports reach the country's epidemiologists and the speed with which the data are analysed.

Reports from health delivery systems or from the public of an unusual incidence of jaundice, especially when it is associated with haemorrhage or deaths, suggest a possible outbreak of yellow fever and must be investigated.

The applicability of surveillance methods depends largely on the interest and enthusiasm of individuals at the level of both the ministry of health and the various health delivery systems. The process of surveillance will be assisted by the identification of key individuals at reporting sites (hospitals, dispensaries, clinics, health centres, etc.), frequent communications, and the provision of information about the clinical features of yellow fever to medical personnel.

6.1.2 Active surveillance

In certain areas, particularly in the zone of emergence, active surveillance of hospitalized cases may be justified. The methods to be used include periodic contact (by telephone, telegraph, or site visit) to determine the incidence of cases meeting the yellow fever case definition. The collection of appropriate diagnostic laboratory specimens is critical to the interpretation of these data. Examples of the usefulness of this approach have been given elsewhere (30). Annual serological surveys of young children may also provide important information on the circulation of yellow fever virus. Surveys are most useful when conducted in the mid-dry season, so that any increase in seropositivity during the previous rainy season is detected. A danger of further amplification and epidemic spread in the following year may be predicted and used to formulate preventive measures.

Active surveillance may be extended to testing wild monkeys for antibodies, use of sentinel monkeys (tethered on platforms), and
surveys of vector mosquitoes. If increased transmission is detected or cases of yellow fever appear, other active surveillance techniques must be applied, as described in the next section.

6.1.3 Laboratory support

Both the collection and the testing of diagnostic samples from suspect cases present many difficulties in Africa; the solutions to these problems depend upon specific knowledge of local conditions and resources, and dedication of the staff assigned to the project. Both the ability to establish a specific diagnosis of yellow fever and facilities for the application of laboratory tests are important to routine surveillance. The main requirements include: (a) laboratory facilities and trained laboratory personnel; (b) selection and routine use of reliable and practical laboratory tests (discussed in Chapter 4); (c) identification of a few "key" medical facilities and selection of enthusiastic staff members to oversee the programme; (d) establishment of a system for collection of specimens (on the basis of the case definition) and provision of collection vials, shipping containers, and other necessary materials; (e) development of a rapid, routine, inexpensive, and simple means of transporting samples to the laboratory; (f) feedback of test results and interpretation to the medical facilities and frequent communication with the medical personnel involved.

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

6.2 Mosquito Surveillance

Entomological components of surveillance systems for the control of emergencies caused by yellow fever in Africa will have a great deal in common throughout the region. Certain approaches to surveillance may vary locally because of the presence of different principal vector species.

Outbreaks of yellow fever are usually associated with: (a) a low level of immunity in the human population; (b) the presence of vectors, especially domestic *A. aegypti*, at a high level; (c) the emergence zone (see section 5.2.2).
Prior identification of yellow fever emergence zones as areas at risk and early detection and monitoring of vectors through efficient surveillance measures are important in the prevention and control of emergencies.

6.2.1 Delimitation of areas at risk through assessment of densities of the domestic vector, A. aegypti

There are numerous indices for the assessment of the larval density of A. aegypti; the ideal index would be one that takes into account both the number of mosquitoes and the number of human inhabitants. In practice, however, only three indices are currently in use, and these do not require human census data:

—the house index, i.e., the number of houses containing breeding places per 100 houses visited. This index may vary significantly in relation to the type of dwelling; a precise definition of the term “house” should be given, since this represents the human population in the calculation of the index. Any structure in which at least one person customarily sleeps is defined as a “house”.

—the container index, i.e., the number of containers in which larval A. aegypti are found per 100 containers with water; this index is more precise than the previous one but does not take into account the human population.

—the Breteau index, i.e., the total number of containers with larvae of A. aegypti per 100 houses.

To determine these indices, all containers holding water are examined for larvae, both within dwellings (water storage pots, medicine jars, etc.) and outside (drinking troughs, old tyres, broken pots, tin cans, holes in trees, etc.).

Adult A. aegypti may be caught with hand nets in dwellings or by immunized insect collectors operating in the late afternoon inside and outside dwellings. A calculation is made of the average number of mosquitoes per dwelling or per collector, and this gives some idea of the density of the adult population. Improved methods for collecting adult female Aedes mosquitoes are under investigation. Adult mosquitoes may be preserved for virus isolation studies; such investigations require facilities for the cold transport of properly collected mosquitoes to a laboratory.

The morphology of adult insects caught also provides information on the anthropophilic preferences. As previously discussed, there are two forms of A. aegypti in Africa: (a) a domestic form adapted to the human habitat, which is highly anthropophilic and has an abdominal ornamentation in which white scales are plentiful; and (b) a more widely distributed wild form, which is not very anthropophilic and has darker markings. McClelland (22) has developed a method for calculating a pattern index to evaluate the extent of white areas on
the abdominal tergites; a low index suggests a wild population that is not very anthropophilic, while a high index suggests a more anthropophilic, domestic population.

The use of ovitraps also provides important information on the density of females and their rate of reproduction. The most frequently used model is that recommended by WHO, a smoked-glass jar half filled with water, containing a strip of cardboard upon which the mosquitoes lay their eggs. Ordinary tin cans with the inside lined with filter paper may be substituted. Ovitraps are placed inside and outside dwellings, and eggs are collected at regular intervals.

In situations where sylvatic vectors are involved in transmission, *A. aegypti* larval indices will not accurately reflect epidemic risk. Many other difficulties surround the interpretation of *A. aegypti* indices in relation to the potential for yellow fever virus transmission. For example, situations can arise where only a few containers are positive, but these may be producing very large numbers of larvae; in this instance, the index may be low, but a high density of adult vectors may result, with an increased risk of transmission. It may thus be appropriate to use a range of larval indices to reflect epidemic risk. Ranges that have been associated with outbreaks include a house index of 4–35 or above, a container index of 3–20 or above, and a Breteau index of 5–50 or above.

6.2.2. Delimitation of areas at risk with sylvatic and intermediate transmission

This operation requires the collection and identification of wild *Aedes* species. Appropriate keys and a reference collection of specimens are needed. An important component in surveillance is the demonstration of virus. Collection of mosquitoes, preparation of individual pools for each species, and processing for virus isolation allow an estimation of minimum infection rates.

The only effective technique for sylvatic vector surveillance is to collect the mosquitoes that land on immunized volunteers; this is carried out around dusk (from 3 hours before to 2 hours after). Collections may be made also inside the village, but the emphasis should be on collections outside if there are favourable zones (banana plantations, wooded areas). If wooded areas are found, some catches should be made high up in the trees, since this is the preferred area of activity of several wild vectors.

The surveillance activities provide an opportunity for field trials of equipment, insecticides, and the efficacy of emergency control measures (see Chapter 8).

6.2.3 Meteorological observations

Records of rainfall, temperature, humidity, and wind are useful additions to yellow fever surveillance; excessive rainfall has been
6. EPIDEMIOLOGICAL SURVEILLANCE

associated with the movement of virus from the emergence zone into the epidemic zone (31).

6.2.4 Implications for international health

*A. aegypti* surveillance is included in the International Health Regulations (see Annex 4), which provide measures applicable to surveillance and control in international airports and seaports, on board vessels, and in other situations involving international traffic (see Chapter 10).

Under the International Health Regulations, it is important that health services of countries fulfil their obligations with regard to maintaining ports and airports free of insect vectors, with particular reference to Article 19, which states that "Every port and the area within the perimeter of every airport shall be kept free from *Aedes aegypti* in its immature and adult stages and the mosquito vectors of malaria and other diseases of epidemiological significance in international traffic. For this purpose active anti-mosquito measures shall be maintained within a protective area extending for a distance of at least 400 metres around the perimeter". A density survey of *A. aegypti* is therefore an essential component of effective surveillance when implementing the International Health Regulations.

6.3 Monkey Surveillance

If there are wild nonhuman primates in the region it may be useful to obtain sera from a small sample for serological examination. Animals may be trapped alive or shot; however, surveillance programmes involving the killing of monkeys should be conducted only after consultation with informed national or international wildlife authorities to ensure that rare or endangered species are protected.

In some areas, sentinel monkeys (tethered on platforms) have been used to assess virus activity by serological conversions.
7. Outbreak Investigation

7.1 Case-finding

7.1.1 General indications

The following events require immediate investigation to determine whether an epidemic is in progress: the recognition of an increased incidence of fatal hepatitis (from hospital reports); the diagnosis of even a single case of yellow fever (by histopathology, serology, or virus isolation); and any reports of suspected cases of yellow fever. If initial information reveals that there is an outbreak, a thorough investigation should be undertaken to define the extent of the problem in terms of geography and case incidence and to determine the chronology of the outbreak and the vectors involved in transmission of the virus.

Direct contact with medical facilities in the affected and surrounding areas should be established by means of telephone or telegraph, where available, or periodic visits by ministry of health personnel. The frequency of such contact can be daily, twice-weekly, weekly, etc., depending upon available resources and the urgency of the situation. At hospitals, dispensaries, and clinics, one person should be designated as the principal contact to provide information about suspected cases. Information should be obtained on new cases, including identifying data, age, sex, presumed locality of infection, date of onset, and outcome, etc. These data are used by the epidemiologist to follow the progress of the outbreak.

Frequent analyses of data are required to allow up-to-date interpretation of the trends. Maps showing the location of suspected cases and epidemic curves based on presumed dates of onset should be updated at frequent intervals.

Special mobile teams may be needed to determine the incidence of non-hospitalized cases in the affected area and to survey more remote areas without easy access to medical services. These teams should consist of one (or more) health inspectors and include an individual with knowledge of the local area and its inhabitants. The specific functions of the team may include: (a) interviews with village chiefs and others to determine whether suspect cases and deaths have occurred; (b) examination of persons with suspect illnesses; (c) collection of blood specimens from suspect cases; (d) giving
advice on the management of sick persons, protection from mosquito bites, and reduction of A. aegypti breeding. During the course of the outbreak, selected villages should be revisited to monitor the progress of the epidemic.

If interviews indicate that a locality is experiencing an outbreak, it may be useful for the mobile team to conduct a more thorough investigation. In a small village, a house-to-house survey to find suspected cases is feasible. In larger villages and towns, random sampling can be used, or fever clinics can be organized. These survey techniques are most useful when combined with the collection of blood samples for virus isolation attempts, a procedure that requires a cold-chain to a virus laboratory. In any case, a detailed search for suspected cases as described will allow a more accurate assessment to be made of the impact of the epidemic in a locality or region.

A problem that must again be addressed in this context is case definition. The mobile team will have very limited facilities for distinguishing yellow fever from other infections under field conditions. In the retrospective surveys for suspected cases (e.g., interviews with chiefs), a broad case definition (e.g., jaundice, nonfatal or fatal) must be used, while recognizing that other causes, especially viral hepatitis, may confuse the interpretation. If more thorough, active case searches are undertaken, with the result that very recently or currently ill persons are interviewed or examined, it may be possible to narrow the case definition.

Teams should make use of established forms to record investigation data, thus allowing a uniform base that is more practical for statistical analyses.

It has been advantageous in some circumstances to combine epidemiological investigations and emergency control (immunization) activities.

7.1.2 Population surveys

In this context, "population survey" is meant to be a survey conducted during an epidemic or at an appropriate time after it in order to determine the geographical distribution of cases, morbidity, mortality, and incidence of infection with yellow fever. Both prospective and retrospective approaches can be used, and analyses can be designed to answer questions about risk on the basis of age, sex, exposure, immunological background, pregnancy, and other factors.

The main purposes of human population surveys are to: (a) extend data obtained during surveillance activities; (b) obtain a more accurate estimate of case incidence and fatality rates; (c) obtain serological evidence of infection rate; (d) confirm that the outbreak has ended. Surveillance efforts conducted during a period of emergency will necessarily be limited since the available resources will
be needed for control and other activities. Specific surveys may thus provide a more complete picture of the impact of the epidemic and an opportunity to answer questions of epidemiological importance.

It is often useful for a medical epidemiologist familiar with the clinical aspects of yellow fever to undertake a systematic review of hospital records. This can be done at all hospitals or at a representative selection of hospitals both within and outside the epidemic zone. Records for the corresponding period of time during the previous year should be reviewed and used as a comparison. In countries with large hospitals or with many smaller ones, and in instances where yellow fever attack rates are high, a sampling method (preferably randomized) can be applied—e.g., review of 20% of hospital records.

Since hospital records are frequently quite sketchy, and in any case will vary from hospital to hospital, it is difficult to use a uniform and objective approach to case definition and retrospective case-finding. The experience of the epidemiologist is thus important, and a judgement is required to separate cases according to clinical features and hospital course into categories such as possible yellow fever, probable yellow fever, and not yellow fever. A survey form should be used indicating the identity, date of admission, date of illness onset, admitting diagnosis, discharge diagnosis, outcome, and clinical features (fever, jaundice, bradycardia, albuminuria, renal failure, and haemorrhage).

These data may be used to draw an epidemic curve and to calculate the case-fatality rate for hospitalized patients. The use of hospital statistics to calculate incidence rates is usually not appropriate, since the population at risk cannot be determined, and since many cases of yellow fever are not hospitalized.

It is usually possible to trace some surviving discharged patients and to obtain blood samples for serological testing, although this is a labour-intensive process.

7.1.3 Seroepidemiological surveys

(1) Population at risk. In some parts of Africa, demographic (census) data are inaccurate or unavailable; this should be considered when calculating rates and designing sampling methods. It is often worth while to conduct a rough census during the course of seroepidemiological surveys.

(2) Sampling methods. Sampling design must take into account available population census data, geographical regions affected, accessibility, and expected cooperation of the population. Sample size should be statistically valid but feasible, given available resources. An important consideration in sample selection is immunization history, since serological analyses will be complicated by vaccine-induced
immunity. Theoretically, prospective surveys yield the most valuable and accurate information about incidence of disease and infection. In practice, they are difficult to perform, both for logistic-technical reasons, and because it is unethical to withhold immunization from population groups at risk.

(3) Retrospective surveys. For practical reasons, most surveys describing yellow fever epidemics have been conducted shortly after the outbreak has ended. Survey techniques are straightforward and include questioning about disease and deaths and collection of serological specimens. The nondescript nature of nonicteric yellow fever, the occurrence of many other febrile diseases, the hyperendemicity of viral hepatitis, and the inherent inaccuracy of relatively remote historical information limit the usefulness of disease incidence data. The usefulness of such data can be increased, in part, by the inclusion of appropriate control groups from outside the epidemic area; however, it may be difficult to match controls by demographic and cultural factors that can influence survey results.

Serological data are useful objective measurements of yellow fever infection, but three difficulties complicate their interpretation: sampling errors; antibody cross-reactivity due to prior exposure to heterologous flaviviruses; and confusion as the result of yellow fever immunization. Sampling problems can be overcome using appropriate sample size, randomization, geographical stratification, and other techniques. The use of relatively specific serological tests (complement-fixation, IgM ELISA) assists in the serological interpretation. However, these techniques may not help to distinguish the immune response due to natural yellow fever infection from that following immunization in persons previously exposed to a heterologous flavivirus (14). Thus, it is important that, whenever possible, surveys be completed before mass yellow fever immunization takes place.

To date, no case-control study of yellow fever has been conducted; this approach would be useful to improve the understanding of the factors underlying exposure and illness.

7.1.4 Collection of laboratory specimens

Instructions for collecting, processing, and transporting specimens should be provided to appropriate medical personnel. If possible, diagnostic specimens should be collected at frequent intervals by health authorities rather than relying on alternative means of transport to the laboratory. A cold-chain for diagnostic specimens is useful, particularly for acute-phase specimens that are suitable for virus isolation attempts. The cold-chain necessary for the transport and storage of 17D vaccine may be used for handling diagnostic specimens. A simple form completed by medical personnel should accompany all specimens, giving identifying data, locations, date of
onset, date of specimen, clinical features, history of yellow fever immunization, and other information that might be useful to the laboratory.

If the country has no laboratory capable of performing diagnostic tests, the government health authority should seek outside assistance and may use the WHO collaborating laboratories by making a request to the WHO Regional Office for Africa. Laboratory testing is important to establish the etiology of early cases, to delineate the geographical extent of the outbreak, and to establish an accurate assessment of case incidence.

7.1.5 Questions about yellow fever that can be answered by epidemiological research during epidemics

The principal goal of epidemiological investigations is to describe the outbreak as completely as possible; this information will be essential in designing and justifying future strategies for surveillance and prevention.

However, investigators of an epidemic should not lose sight of the many unresolved questions about yellow fever epidemiology and disease expression; in a sense, outbreaks may be viewed as natural experiments, and every effort should be made to obtain as much new information as possible. The unresolved questions that require further study include:

1. What is the influence of various host factors on disease expression and case-fatality rate, in particular: age, previous immunity to heterologous flaviviruses, pregnancy, underlying liver disease (especially chronic viral hepatitis), nutritional status, genetic factors (32), histocompatibility type?
2. What is the influence of medical treatment on the case-fatality rate?
3. What is the basis of the excess of cases in adult males in outbreaks with transmission by sylvatic vectors (33)?
4. Does the phenomenon of late death presumably due to cardiac or renal complications (during convalescence) really occur in yellow fever, and what is its incidence, its etiology?
5. Does yellow fever cause abortion or congenital infection?
6. Can new methods for rapid and early laboratory diagnosis be applied to surveillance and epidemiological investigation during emergencies?

7.2 Entomological Investigations

7.2.1 General indications

When there are suspected cases of yellow fever, an entomological inquiry is essential to determine the specific vector species involved and to formulate appropriate control methods.
The selection of sites for the collection of mosquitoes will be dictated by the occurrence of suspected human cases; villages are selected in which the disease incidence appears to be greatest and, within these villages, collections are made from in and around the dwellings of yellow fever patients.

The preservation and dispatch of the material collected raise some logistic problems that must be solved beforehand. Apart from mosquito-collecting equipment, which can be kept permanently at the ready, a method for the cold preservation of specimens is required, such as liquid nitrogen or dry ice. Transfer of the material to the laboratory for examination is often difficult if the affected locality is at some distance from the laboratory; the assistance of transport companies, especially air transport, must therefore be guaranteed. Air transport company regulations for the transport of liquid nitrogen and dry ice must be ascertained in advance.

The most effective means of obtaining mosquitoes is by man-biting collection using previously immunized volunteers. The biology of the usual domestic vector, *A. aegypti*, will determine the catching procedure; operations will be carried out mainly in the late afternoon, within, or in the immediate proximity of, dwellings where suspected cases have been reported. The largest possible number of mosquitoes must be collected in the minimum time, two days at the most.

These mosquitoes, preserved in liquid nitrogen or on dry ice, are sent to the laboratory. The virus may be detected by using several techniques (see Chapter 4) that differ in the rapidity with which results are obtained and in their sensitivity.

The most rapid technique is detection of the virus by an immunofluorescence (fluorescent antibody) test carried out on squash preparations of the heads of captured mosquitoes; the result is obtained in less than 24 hours, but the technique is not very sensitive, since positive results are obtained only on mosquitoes that have been infected for some days. Nevertheless, in view of its rapidity, it will be employed whenever possible. Since mosquitoes must be tested individually, this technique is applicable only to testing relatively small numbers of specimens.

Should the results of immunofluorescence tests be negative, or if specimens must be pooled for testing, infectivity or antigen detection assays should be available.

7.2.2 Determination of the mode of transmission

In the event of a suspected outbreak, the techniques of investigation to be employed are the same as those used for surveillance. As a rule, there is only time for the most essential investigations to determine the mode of transmission and, consequently, the initial steps that need to be taken to control the disease. However, in certain circumstances, such as where control
equipment is not immediately available, it may be possible to carry out more detailed investigations. The choice of localities to be visited will not be made at random but in relation to reported suspected cases. The entomological investigation will be conducted in coordination with the team investigating human cases. The essential entomological investigations to be made include the following studies on domestic and wild vectors.

(1) Sylvatic vectors. Rapid appraisal of the possible role of wild vectors in the outbreak may be obtained through examination of existing epidemiological and entomological surveillance data and their verification through additional catches made at dusk inside and outside dwellings to evaluate more accurately the extent of contact of wild vectors with their human hosts. Determination of the minimum virus infection rate (e.g., expressed as the number of virus-positive mosquitoes (pools) per 1000 mosquitoes tested) provides valuable information on the level of virus activity, and also may be used to ascertain whether vector control activities have broken the transmission chain.

(2) Domestic vectors. The role of *A. aegypti* in the outbreak may be rapidly assessed by the determination of the larval (e.g., Breteau) index. If there is time, adult catches will provide valuable information on adult densities and anthropophilism; mosquitoes obtained may also be tested for virus.

(3) Interpretation of the results. The results of these surveys may be used to identify the type of transmission involved and to indicate the most appropriate means for vector control.

*Situation (a).* The *A. aegypti* larval indices, the landing rates, and McClelland’s index are high; wild vectors are absent or present at low density; epidemic spread is of the interhuman type, in which transmission occurs predominantly between domestic vectors and man. If sylvatic vectors are present, monkeys are absent, and transmission does not occur outside the village, vector control will rapidly interrupt transmission. Since this type of epidemic generally fans out from an initial focus, control operations should begin around the periphery of the focus to limit its extension.

*Situation (b).* The *A. aegypti* larval indices are moderate, sometimes high; wild vectors are present and monkeys are involved in transmission; the epidemic is of the intermediate type with the involvement of both domestic and wild vectors, man, and wild vertebrates; the foci are in general multiple, leaving some areas unaffected. Vector control directed at *A. aegypti* can merely lower the rate of transmission without halting it.

*Situation (c).* The *A. aegypti* larval indices are zero; wild vectors are plentiful; and monkeys may play a large part in the cycle. This is sylvatic yellow fever; human cases are generally isolated or in small
groups. Transmission is from monkey to man via sylvatic vectors that may bite inside villages and in the forest. Occasionally there may be more extensive epidemics, due to interhuman spread by wild vectors. Immunization is the usual means of control; if vector control efforts are contemplated, they must be specifically designed to reduce sylvatic vector populations.

The above means of control are to be considered as a guide, since it is appreciated that the control methods implemented will, in practice, be largely influenced by the resources available.