Yellow fever

Handbook

Rapid field entomological assessment during yellow fever outbreaks in Africa

Methodological field approaches for scientists with a basic background in entomology

World Health Organization

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Pandemic and epidemic diseases publications are available online at www.who.int/csr/resources/publications.

Acronyms and abbreviations

BG sentinel	Biogents sentinel
CDC	Centers for Disease Control and Prevention
COMBI	Communication for Behavioural Impact
DDT	dichlorodiphenyl-trichloroethane
IgM	immunoglobulin M
PCR	polymerase chain reaction
PAHO	Pan American Health Organization
PBS	Phosphate-buffered saline
QIAmp®	Viral kit for RNA extraction protocol
RNA	ribonucleic acid
WHO	World Health Organization
YF	yellow fever
YFV	yellow fever virus

Introduction

Yellow fever (YF) is one of the most important mosquito-borne viral haemorrhagic diseases transmitted to humans. It is mostly vectored by the genus Aedes in Africa, and it is a serious public health problem in Africa, as well as in the South and Central Americas. In the past few decades, despite a highly effective vaccine, YF is re-emerging in Africa (Reiter et al., 1993; Thonnon et al. 1998; Onyango et al., 2004a, 2004b; Ellis & Barett, 2008; Gould et al., 2008), with an increase in reported urban outbreaks in Africa. The increase in virus circulation among the non-immune human population – along with its geographical spread – was highly disconcerting, as multiple outbreaks occurred simultaneously in several places.

Surveillance remains the best approach for early detection of outbreaks. The current surveillance system implemented in almost all African countries includes only a human component, which is based on clinical detection of jaundice, blood sample collection, and immunoglobulin M (IgM) detection in national laboratories and their confirmation in a regional laboratory. This approach, while strongly recommended, is limited, because disease transmission is often only alerted when it is already established in the community. This may be too late for a disease like YF, which is characterized by a large proportion of asymptomatic cases.

So that outbreaks in urban and rural areas can be promptly responded to, an investigation that includes entomological survey is essential, to assess accurately the best interventions, and then guide decision-makers about the relevance and urgency to undertake a mass vaccination and/or vector control campaign.

Survey actions include previous entomological information on YF vectors through the identification of susceptible vector population species, and assessment of vector densities and behaviour, to identify type of transmission cycle and mosquito virus infection. Such a study requires some expertise and methodology in entomology, which are not always available in some parts of Africa. Sometimes, entomologists know vector-borne diseases very well, but are specialized in other diseases such as malaria, trypanosomiasis or onchocerciasis. The entomological assessment of these diseases is quite different; it does not require a vector density index, and the species involved are better known and characterized than they are for YF.

This handbook has been written in a practical context. It provides some field methodological approaches that can be used in peripheral or central level, and by any scientist with some basic background on entomology, to prepare and implement a YF entomological assessment during outbreaks.

1 Overview of yellow fever

1.1 Yellow fever vectors in Africa

From an epidemiological point of view, the mosquitoes involved in yellow fever virus (YFV) transmission can be classified as follows:

- Those found associated with YFV in nature, but without experimental proof of their competence.
- Those in which the vector competence is proven, but whose association with YFV has never been obtained in nature.
- Those found associated with YFV in nature and from which the ability to transmit the virus is proven experimentally.

Table 1 shows the African mosquito vector species of YFV found to have natural infection and/or proven laboratory competence (Reed, 1901; Bauer, 1928; Philip, 1930; Lewis et al., 1942). Nearly all mosquito species implicated in YFV transmission belong to the genus *Aedes*, and especially to the subgenera *Stegomyia* and *Diceromyia*. *Ae. vittatus* is the only *Aedes* sp. incriminated in YFV transmission that belongs to the subgenus *Aedimorphus*. Among the *Aedes genus*, only eight species can be considered as proven vectors of yellow fever (YF). During YF outbreaks, *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. bromeliae*, *Ae. furcifer* and *Ae. taylori* have been shown to be the main vectors. *Ae. vittatus*, *Ae. metallicus*, *Ae. opock* and *Ae. neoafricanus* tend to play a secondary role, while the other species of the subgenus *Aedimorphus* and the genus *Eretmapodites* play a subsidiary role. The role of the species belonging to the genera *Culex*, *Mansonia* and *Coquilletidia* has not been confirmed.

Table 1 African mosquito vector species of yellow fever virus

Species	Natural infection (in vivo)	Laboratory competence (in vitro)
Aedes (Stegomyia) aegypti	Yes	Yes
Aedes (Stegomyia) africanus	Yes	Yes
Aedes (Diceromyia) taylori	Yes	Yes
Aedes (Diceromyia) furcifer	Yes	Yes
Aedes (Stegomyia) luteocephalus	Yes	Yes
Aedes (Stegomyia) simpsoni	Yes	Yes
Aedes (Aedimorphus) vittatus	Yes	Yes
Aedes (Stegomyia) metallicus	Yes	Yes
Aedes (Stegomyia) opock	Yes	NP
Aedes (Stegomyia) neoafricanus	Yes	NP
Aedes (Stegomyia) pseudoafricanus	NP	Yes
Aedes (Stegomyia) keniensis	Yes	NP
Aedes (Stegomyia) bromeliae	Yes	NP
Coquillettidia fuscopennata	Yes	NP
Aedes (Aedimorphus) dentatus	Yes	NP
Aedes (Aedimorphus) stokesi	Yes	NP
Aedes (Stegomyia) gr. *tarsalis*	Yes	NP
Eretmapodites inornatus	Yes	NP
Eretmapodites gr. *chrysogaster*	Yes	Yes
Eretmapodites quinquevittatus	NP	Yes
Culex (Culex) thalassius	NP	Yes
Culex (Culex) pipiens	NP	Yes
Mansonia (Mansonioides) africana	NP	Yes

NP, not proven.

1.2 Bioecology and geographical distribution of vectors

Bioecological studies have been conducted for each of the species listed. The major YFV vectors are discussed in the following sections.

1.2.1 *Aedes africanus* group

Aedes africanus group, which is actually a complex of four sibling species: *Ae. africanus, Ae. neoafricanus, Ae. opok* and *Ae. pseudoafricanus*, which were previously confounded (Huang, 1990).

Ae. africanus is found in forest areas, but can also be found in forest galleries of savannah regions. The species is abundant in east and central Africa, but it has a more localized distribution in west Africa. It is primatophilic and very aggressive to humans. It bites preferentially in the forest canopy, but can also bite at the ground level. It has crepuscular activity, yet also becomes active during the day if a human enters its habitat – an "intrusion effect". Its role in the jungle transmission of YF has been described in Uganda in 1948 (Smithburn et al., 1949) and confirmed in several geographical contexts. It has the distinction of being confined to the forest environment and is seen infrequently in the domestic area. Such a pattern would limit the involvement of *Ae. africanus* in the production of YF sporadic cases.

The other species of the group have similar bioecological patterns to *Ae. africanus*. However, *Ae. opok* could be considered a major YF vector in central Africa because of its high densities, which sometimes exceed that of *Ae. africanus* (Herve et al., 1977). *Ae. pseudoafricanus* and *Ae. neoafricanus*, though involved in the wild cycle of transmission, tend to play a minor role (Smithburn et al., 1949) because of their rarity.

1.2.2 *Aedes luteocephalus*

Ae. luteocephalus is another species of the subgenus *Stegomyia* morphologically similar to *Ae. africanus* group species. It is simioanthropophilic, has a crepuscular behaviour and takes blood meals at the canopy, but occasionally bites on the ground. The species are abundant in forest habitat and may be present in very rare situations in the villages surrounding the forest. It is one of the major vectors of YF in West Africa and has been confirmed in various outbreaks (Lee & Moore, 1972).

1.2.3 *Aedes simpsoni* group

For many years, it was assumed that the *Ae. simpsoni* group had a wide geographical distribution in Africa. However, recent studies have shown behavioural divergences in diverse geographical contexts, indicating the existence of a morphologically similar group (Huang, 1986a). These studies split the simpsoni group into three sibling species: *Ae. simpsoni, Ae. bromeliae* and *Ae. lilii*. The respective roles of these sibling species in YFV transmission have also been clarified. These species are abundant in forest, and in transition areas of forest and savannah (ecotone) with heavy rainfalls. In forest areas, they are present throughout the year, while in pre-forestry areas their activity is seasonal.

For a long time, *Ae. simpsoni* has been considered as a major YF vector, particularly in east Africa as described in the Haddow et al. (1968) scheme. Today, it has been established that *Ae. bromeliae* was mistaken for *Ae. simpsoni*. Actually, *Ae. simpsoni* seems to be present in South Africa and Zimbabwe. It does not play a role in YFV transmission to humans because of its zoophilic tendency. *Ae. simpsoni* breeds in sylvatic sites (plant leaves and tree holes).

1.2.4 *Aedes bromeliae*

Ae. bromeliae, the only species of the group involved in YF transmission, is present in several African countries, but has important behavioural variations that might affect its vector status depending on geographical context. It is the major YF vector in east Africa; there is evidence of association with YFV in nature and the vector is abundant with an anthropophilic feeding pattern. In central Africa, the species is suspected of YFV transmission because of its close interaction with humans. In this part of the continent, the mosquito is especially aggressive during the afternoon, but it may also bite at dusk and early in the morning. In west Africa, although its presence is reported, it does not seem to play a role in YF transmission to human because of its zoophilic tendencies. The species shares the same natural breeding sites as *Ae. simpsoni* (plant leaves, tree holes, fruit husks, etc.), but also uses containers in east Africa in the domestic and peridomestic environment.

These new elements help to clarify the Haddow's cycle, which deserves some adjustments – particularly the need to substitute *Ae. bromeliae* for *Ae. simpsoni* in east Africa.

1.2.5 *Aedes lilii*

Ae. lilii appears to have a geographical distribution limited to east Africa. The species does not seem to be involved in the YFV transmission in this part of the continent because it does not bite humans. Its breeding sites are sylvatic.

1.2.6 *Aedes aegypti*

Ae. aegypti has two subspecies that are distinguished by ecological and behavioural characteristics. *Ae. aegypti aegypti*, the domestic and peri-domestic forms found in tropical urban areas, is believed to have evolved from *Ae. aegypti formosus*, the ancestral African tree-hole form. Previous studies have shown that *Ae. aegypti aegypti* exists in Asia and the New World, while in Africa both subspecies exist, but with a limited distribution of *Ae. aegypti aegypti* on Africa's east coast (Tabachnick & Powell, 1979 ; Powell et al., 1980).

The presence of *Ae. aegypti aegypti* in west and central Africa is still debated – mainly because of the lack of reliable methods to distinguish the different subspecies. Available morphological keys are not sufficiently accurate to distinguish *Ae. aegypti aegypti* from *Ae. aegypti* formosus or intermediary forms.

In spite of the taxonomic debate, we can assume that, in all of Africa, there are at least two different *Ae. aegypti* populations based on bionomics:

- A domestic, anthropophilic subpopulation that bites during the daytime – especially in the late afternoon – and uses artificial containers (water storage containers, cans, old tires, abandoned containers, etc.) as breeding sites. Due to the artificial nature of these breeding sites, this *Ae. aegypti* subpopulation is present throughout the year.
- A wild, zoophilic subpopulation breeding in natural habitat (rock holes, tree holes, fruit husks, etc.).

1.2.7 *Aedes (Diceromyia) furcifer/ taylori* group

The *Aedes (Diceromyia)* furcifer/ taylori group was initially considered to be composed of two species: *Ae. taylori* and *Ae. furcifer* (Ferrara et al., 1984). *Ae. furcifer* has been split into *Ae. furcifer ss.* and *Ae. cordellieri* (Huang, 1986b; Jupp, 1998) recently. The three species are found in west, central and south Africa, but only *Ae. furcifer* and *Ae. taylori* seem to be well established in west Africa and central Africa. *Ae. cordellieri* is the only species of the group present in east Africa.

These species share several bioecological characteristics. They are absent in forest areas and well represented in the savannah areas in open spaces. Their breeding sites are natural and include mainly tree holes, bamboo, fruit and so on. They are simioanthropophilic with a crepuscular peak in biting activity.

Ae. furcifer is the only species of the group that has activity in villages bordering the forest environment. Such behaviour confers to this species a very important role in domestic transmission cycles. *Ae. taylori* and *Ae. cordellieri* remain confined to the wild habitat.

1.2.8 *Aedes (Aedimorphus) vittatus*

Aedes (Aedimorphus) vittatus is the only species of the subgenus *Aedimorphus* involved in YFV transmission. *Ae. vittatus* has been found associated with YFV in nature and its ability to transmit was proven experimentally (Lewis et al. 1947). Nevertheless, it is still considered to be an accessory vector, since very little information has been obtained on its involvement during YF epidemics. It was only incriminated in the epidemic that occurred in Sudan (Kirk, 1941) in 1940 and was suspected to have played an accessory role during outbreaks that occurred in the Jos Plateau, Nigeria, in 1959 and 1969 (Lee & Moore, 1972). The species has pronounced seasonal dynamics, with an early peak of density at the beginning of the rainy season, which suggests a limited role in sylvatic transmission because the virus tends to emerge towards the end of the rainy season. *Ae. vittatus* is a savannah species that is abundant in rocky areas. It is especially prevalent in west African forest galleries, but it is also common in villages near forest. Females have daily and nocturnal activity with a significant crepuscular peak. They bite a wide range of vertebrate hosts, with a strong anthropophilic trend in specific locations.

1.3 Transmission cycles of yellow fever

Until 1928, YF epidemiology was based exclusively on a single transmission cycle involving *Ae. aegypti* and humans as the only vertebrate host. But these conceptions have changed when it was demonstrated that mosquitoes other than *Ae. aegypti* could intervene in the transmission cycle, and that monkeys were likewise susceptible to infection (Bauer, 1928; Stokes et al., 1928). Two observations made in South America have supported the existence of such a sylvatic cycle: (1) an abnormal mortality of monkey populations during outbreaks of YF has been documented; and (2) rural outbreaks of YF have occurred in the absence of *Ae. aegypti* in Brazil in 1933 (Soper et al., 1933). Moreover, investigations conducted during several years in the forest have demonstrated a natural association of YFV with mosquito species like *Ae. simpsoni* and *Ae. africanus* in east Africa, and *Ae. aegypti* in west Africa during and in between epidemics.

The first documented YFV transmission cycle in Africa was established in east Africa (Haddow, 1968). This scheme includes a sylvatic cycle, involving *Ae. africanus* group and monkeys, and a rural cycle, involving *Ae. aegypti* in villages on forest margins.

With the discovery of new vectors and bioecological data generated after several years of study, some adjustments have been introduced, which have differentiated the one cycle into three transmission cycles: a sylvatic cycle, a rural or intermediate cycle, and an urban cycle (Germain et al., 1981; Cordellier, 1991).

The sylvatic cycle exclusively involves wild mosquitoes transmitting the virus between wild vertebrate populations like monkeys. Humans can be infected when they impinge on the forest environment as well as open spaces (ecotone) near this forest environment. The characteristics of this cycle include the sylvatic pattern of vectors using natural breeding sites (tree holes, bamboos, fruits), and the feeding and biting behaviour of the vectors on simian and human populations, both on the ground and in the forest canopy.

From an ecogeographical point of view, this cycle is associated to forest environment, where YF is endemic. In these areas, the virus continually circulates silently between mosquitoes and monkeys. YFV can circulate all year round because weather conditions remain conducive to mosquito activity. The reservoir here is the mosquito that maintains the virus by vertical transmission (Cornet et al., 1979; Fontenille et al., 1997). Human infection is quite rare in the sylvatic cycle.

A rural cycle, known as an “epidemic of intermediate type”, involves *Ae. aegypti* plus sylvatic vectors active in domestic and peridomestic settings of rural village and settlements. Species such as *Ae. furcifer, Ae. vittatus, Ae. bromeliae* and *Ae. keniensis* travel between village and forest, sometimes biting humans.

Species such as *Ae. luteocephalus* and *Ae. metallicus* play only an occasional role due to their scarcity.

A rural cycle outbreak is associated with a geographical area called the “zone of emergence”, including the limits of forest, mosaic forest, savannah, forest galleries, wet savannahs and, to a certain extent, the drier savannahs. These areas are characterized by significant climate variations in alternating dry and wet seasons. Species such as *Ae. luteocephalus, Ae. furcifer, Ae. taylori* and *Ae. bromeliae* are very abundant. They bite in the canopy and on the ground, and can disperse over long distances along the forest galleries. Monkeys such as baboons, red monkeys and African green monkeys are abundant in certain areas – and can move over long distances – thus playing a key role in spreading the virus. They can also introduce the virus to human environments during their incursion in agriculture areas.

An urban cycle or “urban epidemic” occurs due to a single vector – domestic and peridomestic *Ae. aegypti*. In the urban cycle, sylvatic vectors are absent. The geographical or ecologic zones associated with this type of epidemic include dry savannahs and the Sahel. Humans infected from the zone of emergence are generally responsible for virus introduction. The scarcity of water and difficulties for water access in these areas cause a tendency for water storage for long periods of time – consequently, *Ae. aegypti* breeding sites are common in such areas.

Table 2 summaries the mosquitoes involved in each of these three transmission cycles.

Table 2 Vectors involved in the three transmission cycles

Cycle	Vectors involved	Vectors that have an occasional role
Sylvatic	*Ae. africanus, Ae. furcifer, Ae. taylori, Ae. luteocephalus, Ae. opock*	*Ae. neoafricanus, Ae. simpsoni, Ae. vittatus, Ae. Metallicus*
Rural	*Ae. aegypti, Ae. furcifer, Ae. vittatus, Ae. bromeliae, Ae. keniensis*	*Ae. luteocephalus, Ae. metallicus*
Urban	*Ae. aegypti*	

1.4 Epidemiological implication of the transmission cycles

The establishment of a transmission cycle facilitates management of a YF outbreak by providing suitable control strategies for each context. The flowchart below summarizes the different cycles and actions.

1.4.1 The sylvatic cycle

In the sylvatic cycle, several sylvatic vectors are involved. Vector control, as such, is not feasible. Monkeys are the primary vertebrate hosts involved. Humans can be accidentally infected, but only as sporadic cases. Like the rural cycle, the spread of the virus is not controllable during the sylvatic cycle, but epidemics are rare.

The sylvatic vectors have breeding sites that are difficult to access and thus render vector control unfeasible at times. The domestic vectors, on the other hand, can be controlled by targeting artificial breeding sites.

1.4.2 The rural cycle

Several vectors have been incriminated in rural cycle transmission, including the domestic mosquito *Ae. aegypti* and various sylvatic vectors.

In the rural cycle, it is difficult to control entirely the spread of the virus since monkeys involved in the cycle can move over a wide area. Mass immunization is the best option for preventing virus transmission.

1.4.3 The urban cycle

A single vector, *Ae. aegypti*, is involved in transmission in the urban cycle. Breeding sites are all artificial and are therefore controllable via mechanical destruction in domestic and peridomestic settings. Environmental management measures and public awareness of YF risk and the benefits of water conservation at home are crucial to preventing YFV transmission.

Humans are the only vertebrate host involved in the urban cycle. As such, the establishment of sanitary measures by the International Health Regulations – such as barriers at the entrances and exits of cities, and vaccination for travellers – could help control the urban cycle of YF.

Flowchart describing the different transmission cycles, their characteristics and epidemiological implications

Cycle	Characteristic of the cycle components					Action	
	Mosquito types	Breeding habitat	Area of activity	Vertebrate Host	Impact on Human	Vector Control	Vaccination
Sylvatic	Wild type	Natural	Forest	Monkeys Human	Sporadic cases	Unrealistic	Only method of control
	West Africa: *Ae. furcifer, Ae. taylori, Ae. luteocephalus* Central Africa : *Ae. africanus, Ae. opock* East Africa : *Ae. africanus*						
Rural	Wild type Domestic	Natural & Artificial	Forest Peridomestic Domestic	Monkeys Human	Several Human cases	Possible on artificial breeding sites Unrealistic for natural breeding sites	Mass campaign in large scale since virus Dissemination not under control
	West Africa: *Ae. furcifer, Ae. aegypti* Central Africa : *Ae. africanus, Ae. aegypti* East Africa : *Ae. bromeliae, Ae. keniensis*						
Urban	Domestic	Artificial	Peridomestic Domestic	Human	Huge no. of human cases	Destruction of Breeding sites by Environmental Management and education of people	Mass campaign in affected City Sanitary barrier at entrance and Exit of city to avoid expansion
	West Africa: *Ae. aegypti* Central Africa : *Ae. aegypti* East Africa : *Ae. aegypti*						

Ae., Aedes; no., number.

1.5 Prevention and control of yellow fever

There is currently no specific treatment for YF. The only option is the specific treatment of symptoms, which is often difficult to implement, particularly during epidemics when the cases may be numerous.

Due to the lack of treatment, measures for public health disease control rely on two prevention techniques – vaccination and vector control.

Fortunately, there is a safe and effective vaccine, which is a very useful tool to provide protection to a population and prevent the emergence or spread of an epidemic. Nevertheless, mass vaccination is often difficult because:

- sufficient quantities of vaccine are not available (vaccine production has not met population needs and it is thus distributed on the basis of risk);
- operational costs of mass vaccination campaigns might be onerous in endemic countries;
- the vaccine should not be given to pregnant women and children under 6 months of age.

It is impossible to fight wild vectors using vector control. In some contexts, however, practical steps can be taken to control vector populations living in the domestic and peridomestic environments, as can be done during urban transmission.

Based on perifocal treatment of *Ae. aegypti* breeding sites using dichlorodiphenyl-trichloroethane (DDT), the Pan American Health Organization began a campaign to eradicate *Ae. aegypti* from the Western Hemisphere in the mid-1950s. Spectacular success was recorded in the early 1960s when 22 countries were declared free of the vector (Kerr et al., 1964; Soper, 1965; Camargo, 1967). Now that DDT has been abandoned in the past 30 years, the World Health Organization and many public health authorities have promoted Communication for Behavioural Impact (COMBI) as a key method to control *Ae. aegypti*. The aim is to use education campaigns to encourage the elimination of infested containers by the community. Several novel approaches to control *Ae. aegypti* are under active investigation:

- using parasitic bacteria (*Wolbachia*) to alter the survival rate of the vectors
- releasing males sterilized by irradiation
- releasing of transgenic males with a lethal gene
- dispersing insect growth regulator by ovipositing females.

These ideas, although scientifically appealing, are complex and high tech; they may prove difficult to apply in the field, particularly on a large scale in low-income societies.

The technical details for vector control are various and all of them cannot be listed here. Some of the main possible actions are described in the next few subsections.

1.5.1 Attacking the immature stages

- Remove all potential breeding sites, including but not limited to, discarded containers, old tires, old vehicles and cans.
- Advise people to protect water storage containers and renew the water periodically after a quick rinse.
- Use biological or chemical larvicides (such as temephos or *Bacillus thuringensis*) that are non-toxic for consumption at recommended doses.

1.5.2 Attacking the adult stage

- Bed nets (treated or not treated), although proven successful against the malaria vector, are not effective in protecting people against YFV carried by mosquitoes that mainly bite during the daytime. The use of bed nets would be therefore limited, and recommended to patients suspected or confirmed of YF infection.
- Use individual protection measures against mosquito bites (using repellent, wearing long clothes, etc.)
- Use insecticides for indoor residual spraying, perifocal treatment (around the breeding sites) and fumigation inside the affected locality.

Unfortunately, although these YF vector control tools are attractive, each of them presents some shortcomings. Whatever the techniques considered, the key for success is a good knowledge of potential vectors, including their identity, distribution, abundance, ecology, behaviour, biology and developmental stages.

2 Overview of an entomological investigation

2.1 Finality and application

Like every response to a crisis, the main objective of the entomological investigation during outbreaks is to measure the epidemic risk, and assist and support the selection of the most appropriate control methods to interrupt the transmission. In the case of yellow fever (YF), epidemics can be prevented through vaccination of vulnerable populations and/or vector control. The entomological findings can be used for these different approaches to control YF.

2.1.1 Mass immunization

An entomological investigation may help guide decision-makers about the relevance and urgency to undertake a mass vaccination campaign. Given the numerous requests submitted simultaneously by different countries, decision-makers need a strategy to categorize countries, or regions within a country, according to their risk level. To accurately categorize countries, data from an entomological investigation are of great interest. The requested information takes into account the identity of the vectors, their density, the epidemic risk indices, as well as the climate profile from the affected areas. Thus, countries or regions within a country with low epidemic risk indices may not be prioritized. Likewise, countries or regions presenting vectors adapted to the rainy season while this season is ending will not be of primary concern. Entomological investigation can also help vaccination planning by identifying areas and periods of risk.

2.1.2 Vector control

When vector control is necessary, there are a number of factors to consider:

- the species and their stages to eliminate;
- the insecticide or larvicide to use;
- the formulation and application method;
- the site for application (which requires a good map of the breeding habitats and areas at risk, plus a detailed knowledge of the vectors behaviour);
- the season, duration and frequency of treatment (requiring precise knowledge on the dynamics of species to be destroyed).

Thus, only a well-conducted entomological investigation can help to characterize the vector and accurately assess the best interventions to undertake.

2.2 Preparation and organization

Preparation is the key to ensure a good entomological investigation, and actions such as the following should be included in the organizational phase:

- establish the terms of reference (ToR) of the investigation in partnership with the Ministry of Health authorities, and make sure the context, specific objectives and time schedules are clear;
- collect available data (published and non-published) on YF epidemiology and vectors in the area of interest to establish a consistent database;
- establish an investigation protocol, including the context, objectives, study areas, activities to be conducted, human resources, materials and logistics, chronogram of activities, budget, and expected results;
- discuss the ToR of the entomological investigation with the members of the local committee in charge of the outbreak response, to ensure the feasibility of activities;
- discuss the modalities of debriefing of the results with the committee, and submit the protocol to the ethical committee for their agreement;
- identify, in collaboration with the members of the committee, the members of the entomological team, which includes a scientist, technical assistant and a guide. The guide and one technical assistant are recruited locally in the community;
- present to the entomological team the protocol of the studies and organize a pilot study for their field training.

2.3 Specific objectives

The objectives of the entomological investigation should at least include:

- identifying the vector and delimiting their areas of activity
- estimating the abundance of vectors in the affected area
- determining the mode of transmission
- estimating the current risk and risk of spread of the epidemic into other geographical zones
- assisting the authorities to make decisions for better control
- training the local personnel.

3 Implementation of an entomological investigation

There are several factors to consider when implementing an entomological investigation, including study site selection, how and where to prospect, and laboratory methods. This section explains these elements in detail.

3.1 Key parameters when selecting study sites

The choice of places to be investigated should be based on epidemiological and virological documentation of clinical cases of yellow fever (YF), and must therefore be done in collaboration with teams in these disciplines.

3.1.1 Epidemiology

When selecting locations to be included in the entomological investigation, epidemiological factors are relevant, such as:

- the existence of YF cases – the localities where YF cases are reported will be prioritized for entomological investigation;
- the herd immunity of the population – the localities where YF vaccination coverage or/and seroprevalence for YF antibodies (if available) are low will be of priority;
- understanding patient displacement can provide information on a possible origin of contamination or likely locations of introduction. Identifying and visiting the localities frequented by YF patients for their work activities is also helpful – for example, where forest contact may have occurred, such as agricultural activities, cattle farming, hunting, gathering and harvesting wood for crafts, or collecting honey or fruits. This is especially important in the localities where the population could have been exposed to a YF cycle. Similarly, cities where cases have been endemic should also be strongly considered as a potential source of infection;
- communities and cities in the vicinities should be included to identify possible areas of YF expansion.

3.1.2 Virological aspects

The dynamics of virus replication and development of antibodies in human cases will be taken into account when selecting the study sites.

Locations will be prioritized using available information on the acute infection; that is, sites that have patients or non-human primates with positive PCR and/or virus isolation (indicating a viraemic period and a current transmission in the area) will increase the chances of finding infected mosquitoes. Such an investigation could facilitate the identification of species involved in transmission during the epidemic.

3.2 Prospecting immature stages

Immature mosquito life-cycle stages include eggs, larvae and pupae. They are usually called “aquatic stages” even if the eggs of YF mosquito vectors are not always laid directly on water surface. Instead, eggs are usually deposited on the edges of water bodies. These eggs have an ability to withstand desiccation, and remain viable year round – sometimes even in extremely dry environments.

Thus, particular attention should be paid during an epidemic investigation to the aquatic environments that harbour larvae and pupae, but also all other locations that may constitute potential niches for mosquito eggs. Any collection techniques could be used during these stages.

3.2.1 Aquatic stages: larvae and pupae

Purpose

- Determine the vectors and estimate indirectly adult density.
- Determine the types of breeding sites associated with these vectors.
- Use data to estimate the risk of epidemic.
- Use data to give recommendations on the best strategies for disease control.

Basic equipment

Flashlights and accessories (e.g. batteries and light), larvae dippers, pipette, aspirator, nets, larvae trays, nesting plastic bowl, mosquito breeders, waterproof notepad, cardboards or cages, stereo zoom microscope, flask and forceps.

Operational approach

Where to prospect

All localities selected for the entomological investigation based on criteria described in Section 3.1 should be prospected. In each locality, domestic and peridomestic environments should be investigated.

Surveys should be done in a randomly selected household. This requires an initial estimate of the number of concessions to visit in each locality to be statistically significant (i.e. a confidence interval of 95%). This estimation is based on geographical data that can give a precise estimate of the total number of households in each community. If these data do not exist, an estimation of the total number of households can be done based on demographics data – specifically, population size and density (i.e. number of persons per household). The number of households will be determined by dividing the population size of the community by the number of people living in a house.

Once a sample size (number of household to be visited) is determined for each affected locality, the number will be divided and equally allocated to the number of households where a YF case has been recorded (household index). Each index household will be a starting-point. After visiting this index household, the next household to be visited will be selected using a systematic sampling procedure. The sampling step will be calculated using the ratio of the number of households in the community to the number of households to be sampled.

In each household, prospection will be done inside human dwellings and outdoors (in the courtyard of concessions). For any activities that require access to individuals' private homes, community guides can be especially important for community acceptance of the entomological survey.

How to prospect

First, request informed oral consent from all local personnel involved in the mosquito survey and collection, as well as the one of the heads of each household in which larval and adult prospection will be undertaken. In each concession to be prospected, brief the head of household or their legal representative(s) about the rational of the investigative mission, the approach that will be followed in carrying out the activities, how the results will be used and how the findings may be able to help the community to control YF.

All artificial containers containing water, inside households and outside in the courtyard, will be inspected using a flashlight. All natural breeding sites containing water in the domestic and peridomestic environment will also be inspected. Figures 1 and 2 show some examples of the types of breeding sites that may be associated with YF vectors. Note that breeding sites are very different to those of other diseases vectors like malaria, or those usually reported by people and authorities because of their pest effect.

Figure 1: *Aedes aegypti* breeding sites in peridomestic environment

Discarded containers










Figure 2: *Aedes aegypti* breading sites in domestic environment

Containers used for wastage storage












When a container is found harbouring at least one larvae or pupae, it is considered positive. In this case, a sample of larvae and pupae is taken and kept alive in a bottle containing water from the breeding site. The bottle is carefully labelled with information concerning the date of collection, the house number, the type of breeding site and so on. Information about the number of habitation units should be collected from the legal representative of the household. A habitation unit is defined as the number of rooms where at least one individual is sleeping.

All information from the surveys should be recorded. See Annex 1 for a sample form.

To guarantee success of prospecting activities, it is important to avoid causing panic among the community. The interpretation made about the presence of positive breeding sites in one's household can affect social coexistence and even create stigma. Providing adequate knowledge to the community authorities and population about the bionomic aspects leading to the attraction of mosquitoes to certain kinds of storage containers is essential to prevent stigmatization.

How to handle and conserve samples

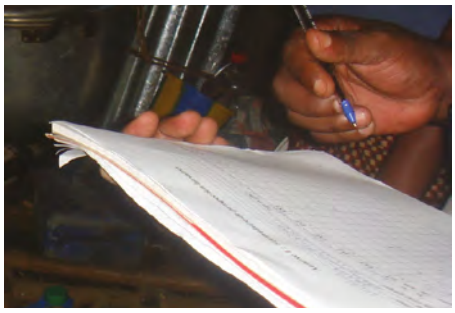
Once larvae and pupae are collected, two approaches can be used to handle and conserve the samples:

1. Larvae and pupae collected in the field are transferred in small containers to an insectary (Figure 3.2). They will be reared in mosquito cages or in the collections containers covered with a net. The cages or breeders are inspected daily to verify the presence of emerging adults. The emerging adults will be collected by aspiration and killed by freezing. They will then be identified and pooled by species, and according to their geographic origin and sex. All information should be recorded. See annexes 2–3 for sample forms.
2. When transportation is risky and/or suitable facilities are unavailable, larvae and pupae collected in the field are stored in alcohol for further identification. Identification may be done directly on larvae and pupae samples after mounting on slides. This approach, although very accurate for the species diagnosis, has some concerns – it is time consuming, because slide preparation is quite long, which is not convenient during an outbreak investigation, and the samples are unsuitable for virus research.

Figure 3: Different steps for immature stage prospecting and insectary

Step 1. Field prospecting of immature stages

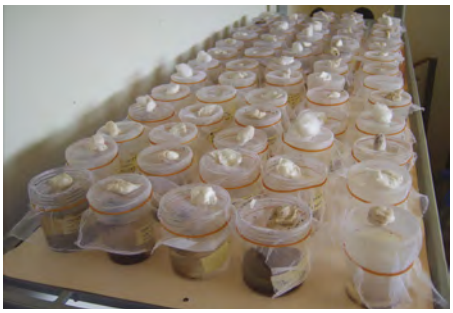
Inspecting container



Filling out form

Larvae samples transfer

Step 2. Larvae and pupae rearing in insectary



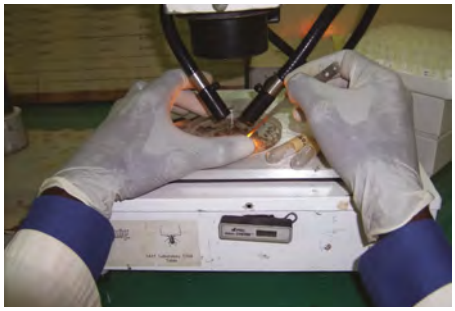
Sample covered with net

Cage containing larvae samples

Cardbord containing larvae samples

Step 3. Killing in freezer & Aspirating emerging adults

Step 4. Morphological identification of the emerging



Analysis of results

This analysis should address at least two fundamental aspects or the investigation: epidemic risk indices and abundance curves.

Epidemic risk indices

Several vector indices have been developed for estimating disease outbreak risk, but for YF, the Breteau, container and house indices are most commonly used. Breteau index is defined as the number of containers found positive for larvae and pupae per 100 houses surveyed. The container index is the percentage of containers that were found positive. The house index is the percentage of houses found positive for larvae or pupae (the calculation of this index depends on the configuration of the house, which are difficult to accurately classify in some African contexts).

According to the World Health Organization (WHO, 1986), there is an epidemic risk when these indices are more than the thresholds of 5% for the Breteau, 3% for container and 4% for the house index.

This risk assessment must be done in each affected locality to localize and delimitate precisely the area at risk.

Abundance curves

The second level of analysis is to determine the typology of breeding sites and to draw a curve of their abundance in each locality. This analysis will be performed to estimate the abundance of the breeding sites containing water and then determine the number found positive. In each case, first classify breeding sites according to their localization in the domestic or peridomestic environment. Next, look to see which specific types of containers are mostly infested in a given area.

Figures 4 and 5 illustrate the type of results that can be obtained from a typology analysis. Figure 4 shows the case of a locality where most of the containers were of domestic nature. Nearly all the discarded containers and other devices, such as old tires, and natural breeding sites did not contain water. The highest levels of infestation were also obtained from domestic containers. Figure 5 shows the opposite situation.

Figure 4: Typology of flooded and positive container

(domestic containers)

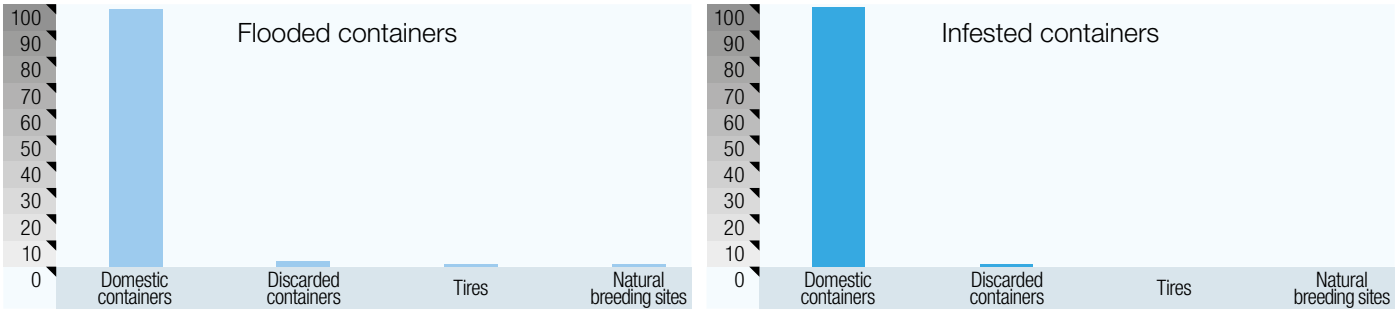
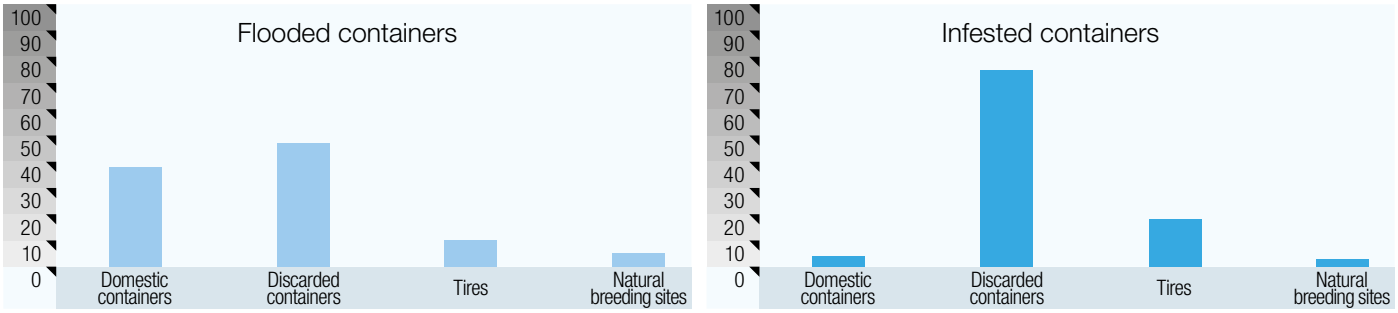


Figure 5: Typology of flooded and positive container

(discarded containers)



An analysis of the data shows that, in the domestic environment, the types of breeding sites containing water and/or infested are not the same. The figures provide a perfect illustration of these observations. In Figure 6 (locality 2), it appears that while the clay jars are major containers found containing water in the domestic environment, they have not been found infested. Metallic drums, flower pots, tanks, and other devices were mostly infested. In Figure 7 (locality 1), the containers found containing water were more diversified, but clay jars were still the most common container found. However, a smaller range of containers were infested and tanks seem to be responsible for this infestation.

Such analyses may allow specific targeting of the mosquito sources.

Figure 6: Typology of flooded and positive containers

(locality 2)

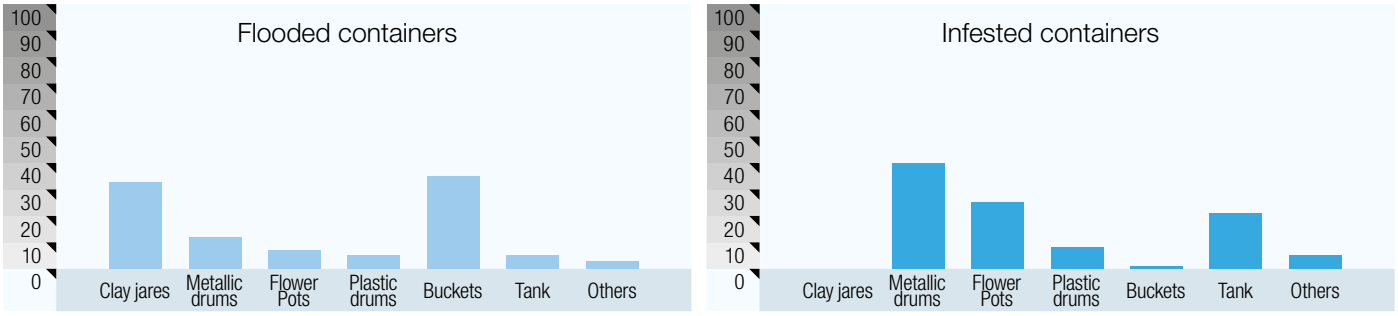
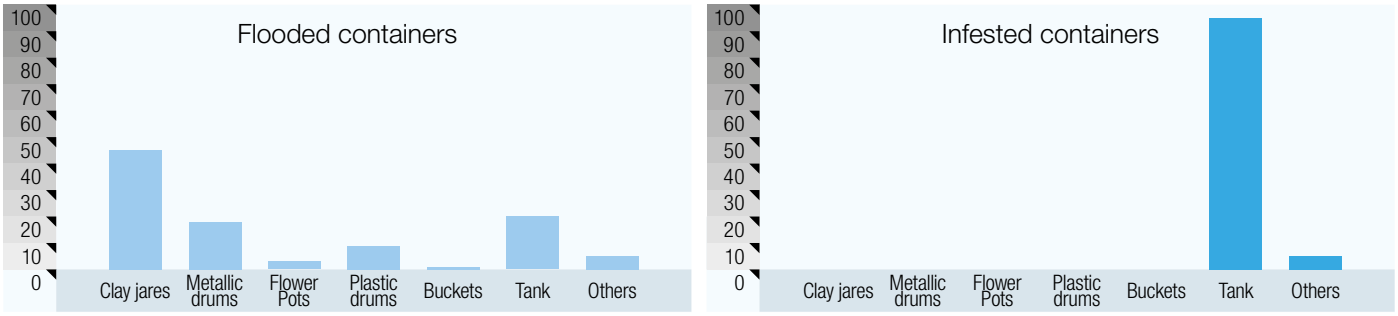


Figure 7: Typology of flooded and positive containers

(locality 1)



Comments and interpretation of results

Identification of breeding sites for better control strategies against aquatic stages

Unlike adult mosquitoes, which are free in their movement, larval stages are confined to a given space and are thus easier to eliminate. Specific actions should be taken according to the large number of vector control tools available, diversity of vectors, and different epidemiological and socioeconomic contexts. A well-conducted entomological investigation can help to identify breeding sites and choose the most appropriate vector control method.

The entomological investigation can be used to advise workers if vector control activities are appropriate for the particular situation. Then, the investigation should inform the most suitable tools/approaches for vector elimination, if applicable. In cases of sylvatic breeding sites, no such vector control is practical. In contrast, when breeding sites identified are artificial, several actions can be undertaken based on context and typology of the breeding sites, such as using larvicides, and social mobilization and education campaigns.

Larvicides, such as *Bacillus thuringiensis* and/or temephos, can be used. The efficiency has been proven in some contexts, but the acceptable use in drinking-water is questionable by communities.

Temephos is a water-soluble organophosphate insecticide that has been widely used for mosquito larvae control and other aquatic invertebrates for more than 30 years, and is approved for use in drinking-water. It is designed to last at least 3 weeks in treated water, depending on the vector's susceptibility in each geographical context. However, its effectiveness is debated for several reasons.

Temephos is a larvicide used for dengue vectors control. It can be used in discarded containers as well as containers used for water storage, and also for drinking-water. The approach is to put temephos (mainly under granules) in each container that holds water to reach a final concentration of 1%. The quantity of temephos used in each container will then depend to the volume of water inside the container. Depending to the geographical context, the product can last several weeks or months after a container is treated. Evaluation of the vector susceptibility to temephos should be done every 2–3 years. However, in some unaware communities, conserving temephos in treated containers is usually compromised by people who frequently rinse their containers because of debris and clay particles in the container, and the temephos taste in drinking-water.

Social mobilization and education campaigns can encourage the elimination of infested containers by the community. Actions will include periodic clearing and draining of domestic vector breeding sites, the use of screw-cap containers for water storage, and the periodic renewal of storage water, removal of discarded containers.

Vaccine planning

The second advantage of the aquatic stages investigation is to determine the risk of disease outbreaks and to delimitate the areas at risk; this will lead to a good planning and a rational management of vaccination. Indeed, with the different thresholds of the epidemic risk indices, it will be possible to classify areas of interest according to their risk level and thus to identify priority areas for mass vaccination.

Three scenarios are possible:

- All indices have values above the risk threshold; then the epidemic risk is established.
- One index has a value over the risk threshold, while others are below their threshold; risk is thus established in the area(s) based on the values over the risk threshold.
- None of the indices has a value greater than the risk threshold. In this case, the area is considered safe or without risk of YF epidemic.

3.2.2 Aquatic stages: eggs

Purpose

The main objective is to identify the mosquito species present and estimate their density.

Larval surveys remain a relevant method for monitoring vectors. However, their success depends on detecting all present breeding sites. Thus, ovitraps – a passive approach for mosquito sampling – may help detect the presence of adult mosquitoes in the selected study area, particularly during low vector activity.

Basic equipment

Ovitraps (these may be made locally with remarkably simple materials, such as discarded cans and black paint, or any black cylindrical container), filter-paper, cotton and thin pieces of wood.

Operational approach

Where and how to prospect

The ovitrap, equipped with filter-paper or a piece of wood, are 60% filled with water, and set up in the domestic environment (in the courtyard of concessions) or in the forest. They are set up either on the ground or at height, hanging on trees or wall at the early beginning of the investigation and left in the field for a sufficient period of time (at least one week).

They are inspected regularly every 5 days (Lourenço-de-Oliveira et al., 2008) so that eggs will not hatch. If egg hatching does occur unexpectedly, this frequency of visits would avoid adult emergences. Each time a postive ovitrap is found, the eggs on the filter-paper and the water is collected. It is again filled with water and equipped with a cover plate or, on the edge, with new filter-paper.

How to treat samples

To handle samples, filter-paper, cotton or a wooden disk containing collected eggs will be put in plastic bags in the field. In the laboratory, they are put in a pan containing water to allow the eggs to hatch.

The emerging adult stages are cold-anaesthetized and identified on a chill table. Adults are pooled according to their geographical origin, gender and date of collection for yellow fever virus (YFV) detection tests.

Analysis and interpretation of results

The initial analysis will show the inventory of mosquito species at certain sites, which will help understand the type of transmission cycle present. The abundance of mosquitoes is estimated indirectly by using the number of eggs per ovitrap or the ovitrap index (number of ovitraps that were *Aedes* positive per total ovitraps set up in a specific area). This index can be expressed as weekly or monthly.

3.2.3 Tree holes and other natural breeding sites

Purpose

This approach is especially recommended for an entomological investigation being done during the dry season when the vectors with a dynamic dependent on the rain (which is the case for YF sylvatic vectors) are generally absent. The main objective here will be to reconstruct the scenario that would have prevailed during the rainy season and thus determine the presence or absence of wild vectors in the selected location.

Operational approach

Where and how to prospect

This activity is carried out in the nearby forest or ecotone (transition zone between humid gallery forest and savannah). To prospect using this method, scrape tree holes with spoons, knives or other devices (Figure 8) to collect *Aedes* eggs, which are known to be resistant to desiccation. Then all content of tree holes, as well as the content of other potential natural breeding sites (e.g. bamboo, fruit husks, rock holes) found dried, are of interest.

Figure 8: Prospecting tree holes

How to treat samples

As described in Section 3.2.2, samples from tree holes and other breeding sites will be stored in plastic boxes in the field and flooded in the laboratory to hatch the eggs. Several immersions in water are sometimes necessary to stimulate hatching. That is, if the first flooding fails to provide conclusive results, the samples must be dried and put back into water after few days.

The emerging larvae will be reared to the adult stage, then frozen, identified and pooled by species and gender for virus detection.

Analysis and interpretation results

The analysis will be based upon the repertoire of vectors present in the location, and therefore the transmission cycle that prevailed during the epidemic or in the area of interest. The data can be used to estimate mosquito prevalence and to estimate indirectly their density in area of interest.

3.3 Prospecting adult stages

3.3.1 Purpose

The objective of prospecting the adult stage is to identify mosquitoes present in the study area, particularly the YF vectors, to estimate their density, to study their biological and ecological behaviour (e.g. aggressiveness and biting behaviour indoors and outdoors, and resting behaviour indoors and outdoors) and their association with YFV in nature.

3.3.2 Basic equipment and facilities

Insectary, storage container (e.g. nitrogen liquid container), equipment for mosquito rearing (pan, pipettes, forceps, cages, vials, cardboard, cotton, Petri dish, humidifier), stereo zoom microscope.

3.3.3 Operational approach

Sampling active adult mosquitoes with human volunteers

Objectives

This objectives of this approach are to:

- identify mosquito species biting humans;
- census the known YF vectors in the region. The information on the species composition is particularly useful to define the patterns of transmission which, as noted elsewhere, have an epidemiological implication and can therefore provide guidance on the most appropriate approaches for control strategy and implementation;
- measure the degree of involvement of each species in the epidemic transmission through measurable parameters such as
  - the aggressiveness rate, estimated by the number of bites that one person could receive during a unit of time in an area
  - the inoculation rate, estimated by the number of infective bites per person per unit of time;
- assess the involvement of each species in the epidemic transmission by studying the ecological and biological behaviour to delimitate its areas of activities and implement better vector control strategies.

Why and where to prospect

In the domestic environment, the mosquito capture will be conducted indoors and outdoors to measure the exophagic or endophagic trends of potential vectors.

Certain YF vectors are sylvatic (forest), but may have domestic activity. However, in the domestic environment, there are few species that bite inside human dwellings. This is the case, for example, in west Africa, *Ae. furcifer* does not bite inside human dwellings but is very active in the courtyards. This is also true for *Ae. bromeliae* in east Africa and *Ae. aegypti*, which is known for both endophagic and exophagic behaviour. In such cases, focusing on only one of these sites of collection may bias the results, underestimating the abundance of the vectors or considering them altogether absent.

Based on vector behaviour, interventions could be implemented. For example, for indoor residual spraying, this would only be done if the vectors have an endophagic trend.

In the forest environment, it is important to carry out sampling in forests since the YFV initially comes from the jungle environment. The disease in the domestic environment is sometimes preceded by circulation in the forest.

Since most YF vectors are known for their activity in the canopy, captures will be conducted both at height and on the ground.

When to perform the surveys

We often assume all mosquito activity is at night. This is true for certain diseases vectors, such as malaria vectors, but is unfounded for YF vectors. Many YF vectors are active at dusk, some are diurnal (e.g. *Ae. aegypti*) and some are active in the early morning.

An ideal investigation would capture mosquitoes for a 24-hour period to identify accurately the peak activity for each species. As a result, subsequent collections could be based upon peak activity.

As most of the YF vectors are daytime biters, it is recommended to choose at least three periods of 2–3 hours collections during daytime and at twilight (mainly at dusk). For instance, the times 8:00–11:00, 12:00–15:00 and 17:00–20:00 could be used. If no day-long survey is implemented, crepuscular and early-morning captures could be adequate.

How to capture mosquitoes

The volunteer is essentially the bait and two approaches are used:

- Mosquitoes landing on his/her uncovered legs are seen using a flashlight and then individually trapped using a glass tube. The tube is then sealed with a cap or with cotton.
- Mosquitoes attracted by his/her uncovered legs are collected, even before landing, using a mouth aspirator or a small net and then transferred into a cardboard (Figure 9).

Figure 9: Human landing collection

Basic equipment

Glass tubes, cotton, small bags, flashlights, aspirator and small nets.

How to handle and conserve samples

After each capture session, mosquitoes are identified immediately in the field after killing them at low temperature, or while they are kept alive in tubes. If the field conditions are not adequate for mosquito identification, the mosquitoes are killed and transferred into tubes before being frozen in nitrogen liquid and transported to the laboratory.

In the laboratory, adult mosquitoes are identified using a chill table microscope and morphological keys (Edwards, 1941; Ferrara et al., 1984; Huang, 1986a, 1986b; Jupp, 1997). Care should be taken to identify mosquitoes separately according to their geographical and ecological origin (city, village, forest, domestic, peridomestic, indoor, outdoor, typology of the breeding sites, canopy, ground level, etc.).

Males are separated from females, and engorged separated from non-fed females, and pooled with a maximum of 10 individuals per species, by gender, and geographical and ecological origins. The samples should be classified according to their geographical origin (household by household), their breeding sites and collection date.

Each sample should be documented by filing out a form (see Annex 2 for a sample form) and providing information on at least the following aspects: date of collection, location, habitat of origin, gender and method of collection. Monospecific pools of mosquitoes should be classified according to gender, origin, feeding status (separate fed and unfed females) and collection date, and this information should be recorded. See annexes 2–3 for sample forms.

Mosquito pools are then stored at –70 °C or –180 °C until tested for virus by real-time PCR and/or viral isolation. Vertical transmission is confirmed if the virus is detected the with real-time PCR or virus isolation (see technique in Section 5.4). A positive result means that the virus could be maintained in the area and generate a new transmission cycle if the conditions are favourable.

Ethical considerations

It is important mentioning the ethical considerations of this method of collection, which could expose the workers to mosquito bites. However, it remains the only technique for sampling certain mosquito populations, particularly the YF sylvatic vectors. There is no alternative method available to collect these vectors. For YF and dengue fever, several alternative techniques were developed or are under evaluation in the field (see following subsection), but none of them competes with the human landing collection. Most of these traps have provided interesting data with the epidemic vectors (*Ae. aegypti, Ae. albopictus* or *Ae. polynesiensis*) of YF, and dengue, and Chikungunya fevers; there are no data indicating their performance with the subgenus *Stegomyia* and *Diceromyia*. We thus recommend the human landing collection method, after training field workers, immunizing against YF and prescribing malaria prophylaxis.

Due to the level of education and limited time for the investigation, oral consent could be requested from all participants in the field. The study protocol could be carefully explained to the legal representatives of each community visited to obtain their consent. Informed oral consent could also be obtained from all team members, local personnel involved in the mosquito survey and collection (including volunteers for human landing collection), and the heads of each household in which immature and adult mosquitoes will be investigated.

Other methods for collecting active adult mosquitoes

Alternative methods to collect YF vectors are numerous, such as the BG Sentinel Trap, Gravid Trap, CDC Light Trap, sticky trap and other odour-baited traps that use different chemical or natural lures (Figure 10). They all have the limitation of not being efficient for catching sylvatic vectors of YF; their performance is far below that of the human landing collection.

Figure 10: Variety of mosquitoes sampling devices

Like the human catches, traps can be set up in the same locations (indoor and outdoor domestic environment, and on the ground level and at the canopy in forests). They should remain in place all night or even for 24 hours.

The Gravid Traps have the advantage of collecting females searching for a breeding site (i.e. females that have taken a blood-meal), which increases the chance of virus isolation.

The sticky trap functions the same way as the ovitrap. However, the sticky trap has a glue that collects and kills the female mosquitoes that get inside for oviposition. The main issue is that captured mosquitoes are difficult to identify due to the glue. The glue may interfere with virus detection, which should be considered. Since some mosquitoes are going to be found dead several hours after being captured by the trap (therefore, they cannot be used for virus isolation) and, depending on the temperature and humidity, they may not be useful for real-time PCR.

Collection of adult mosquitoes resting in a domestic environment

Purpose

There are two objectives for collecting adult mosquitoes in a domestic environment:

- to identify the species present in the study area;
- to study the behaviour of resting mosquitoes and, therefore, locate the natural or artificial ecological niches for mosquitoes.

Where to prospect in human dwellings randomly selected

Prospect for mosquitoes in humid or dark places, which may be the resting places of mosquitoes in the houses and courtyards. Forest vegetation, and the peridomestic and domestic environment should all be checked.

When to prospect

Prospecting may be done during the daytime, but it is preferable to do it in the morning.

How to sample resting mosquitoes

This sampling can be achieved in two ways:

- Indoor residual spraying with pyrethroid, according to the same principles used during malaria vector collection with spray catches (Figure 11). Lay down white sheets over the entire floor of a room. Then two individuals may spray insecticide simultaneously – one indoors on the room walls and roof, and the other outdoors on the possible mosquito exits (doors and windows through which mosquitoes could possibly escape). After 10 minutes of waiting, the sheets are then carefully removed and dead mosquitoes on the sheets are collected in cups bearing the house number and the room. This technique is effective, but has the disadvantage of using a chemical that may be toxic to the virus.
- Aspiration of resting adult mosquitoes in rooms and surrounding vegetation. This technique has the advantage of possible use of mosquitoes for viral detection attempts, but its performance will depend very much on the collection effort (Figure 12).

Figure 11: Indoor spraying for collection mosquitoes

Figure 12: Aspiration of resting adult mosquitoes

Basic equipment

White sheets, insecticides, masks, vials, forceps, stereo zoom microscopes, a backpack aspirator and accessories.

How to handle and conserve samples

Mosquitoes collected will be killed by freezing and then identified. They will be separated into monospecific pools for virological detection attempts.

All information from the surveys should be recorded. See annexes 1–3 for sample forms.

Analysis and interpretation of results

Using this approach, the vector density (number of mosquitoes collected per room) can be estimated. The presence of significant numbers of vectors resting inside the rooms is synonymous with mosquito biting or transmission taking place all day long. In such situations, residual spraying of insecticide would be very valuable for vector control.

3.4 Detection of yellow fever virus in mosquito vectors by real-time PCR

The real-time PCR is an advanced version of the classical PCR. It is based on the binding of a fluorescent signal to the accumulation of amplification products in the reaction. The accumulation of the signal is measured and displayed as a graph over time.

3.4.1 Grinding mosquito samples

Pools of mosquitoes, containing a maximum of 10 individuals, are triturated using a piston in Eagle's culture media, such as minimal essential medium or Leitbovitz culture medium supplemented with 10% fetal bovine serum, nonessential amino acids and antibiotics. For biosafety reasons and conservation, all the process is done in biosafety level 2 containment and ice since RNA viruses are sensitive to temperature gradients. The homogenate is then centrifuged at 4 °C and the supernatant collected and stored at –70 °C.

3.4.2 RNA extraction

RNA is extracted from sera using the QIAamp® RNA Viral Kit (Qiagen GmbH, Heiden, Germany) according to the manufacturer's recommendations. The extract is resuspended in 60 µl of Buffer AVE and stored at –80 °C before real-time PCR is carried out.

3.4.3 Yellow fever real-time PCR assay

Real-time PCR is performed on all of the vector samples. The fluorescence is analysed at the end of the amplification.

3.5 Vector competence studies

The main objective of the vector competence measure is to assign a vectorial rank (probable vector, proven vector, possible vector) to each species. This aspect is recommended because populations of the same mosquito species may exhibit different competence to the virus, and susceptibility to virus is genetically determined. It also determines if mosquitoes from different geographical areas, but belonging to the same species, have distinct levels of vector competence.

The most common technical approaches used for vector competence studies are presented in the following subsections.

3.5.1 Mosquito strains selection

- The mosquitoes to be used should be collected from the localities where YFV is circulating.
- The species found associated with YFV during the epidemic should be prioritized for testing to confirm their role in the epidemic transmission.
- Only mosquitoes from non-infected parent females are used for the infection tests.
- Mosquitoes that are 2–5 days old from the less advanced laboratory generation (F1 or F2) are used.
- Mosquitoes should be characterized in terms of host and geographical origin, biotope and climate condition of rearing – food, environment (temperature and relative humidity), photoperiodicity and physiological status.
- When available, a laboratory control strain of mosquito whose virus competence is well determined (e.g. *Ae. aegypti*), should be considered.

3.5.2 Virus strain selection and stock preparation

- The virus strains isolated and identified during the epidemic or from the area of interest are relevant for the vector competent studies.
- If no strain was isolated during the epidemic, it is recommended to use a strain isolated in the same geographical context and/or phylogenetically similar to the virus strains circulating during the epidemic.
- The strains to be used are characterized according to their geographical origin, host origin (vertebrate or arthropod) and passage number. Depending on the sensitivity and characteristics of the virus, the stock could be prepared using several experimental approaches:
  - intracerebral inoculation to suckling mice and collection of the supernatant of the brains triturated in a medium several days postinoculation;
  - intrathoracic inoculation to mosquitoes of the genus *Toxorhynchites* and collection of the supernatant of the individuals triturated in a medium 14 days postinoculation;
  - inoculation into cell cultures and collecting of the supernatant after 7–14 days of incubation.

3.5.3 Experimental procedure

- Only erythrocytes are used, to avoid potential presence of antibodies against YFV in any vertebrate sera collected.
- The blood is preferentially collected 24–48 hours before the infection in a tube containing a 2% heparin solution.
- The blood is then centrifuged at 4 °C at 1200 rpm/min for 5 minutes.
- After centrifuging, the supernatant (serum) is removed and the erythrocytes mixed with 1x PBS and centrifuged again. This step is repeated three times.
- The viraemic blood will include the rinsed erythrocytes, the viral suspension (with a high titre) and ATP at a final concentration of 5 × 10–3 M.
- The volume of each component of this viraemic blood could vary according to the titre of the viral stock. The most common proportion used is 2/3 washed erythrocytes and 1/3 viral suspension, or 1/2 washed erythrocytes and 1/2 viral suspension.
- According to the species and their feeding habits, a component such as 10% sucrose, and a pinch of sodium bicarbonate and other chemicals could be added to the viraemic blood-meal as a phagostimulant or an attractant, respectively.
- The viraemic blood-meal is administered in glass membrane feeders using mouse, chicken skins or parafilm as membranes.
- The viraemic blood-meal is exposed to mosquitoes for 30 minutes to allow them to feed.
- At the end of the feeding, a sample of the blood-meal is collected and stored at –80 °C for titration.
- The mosquitoes are cold-anaesthetized and engorged specimens are sorted and incubated at 28 °C and a relative humidity of 70–80%, and provided 10% sucrose for 14 days.
- After this incubation period, the mosquitoes are permitted to bite a non-infected vertebrate (e.g. mice) or to salivate in a capillary tube containing a solution (fetal bovine serum, a medium or an immersion oil can be used) (Aitken et al., 1977).
- When the mice are used to assess virus transmission, they are monitored for several days after exposure to mosquito bites. Signs of paralysis, deafness, detection of YFV or antibodies against YFV indicate transmission.
- When the capillary tubes are used, after the incubation, mosquitoes are cold-anaesthetized, their legs and wings are removed and their proboscis inserted into the capillary tube for 20–30 minutes.
- At the end of each exposure, mosquito legs and wings are put together in the same vial, while bodies and the saliva collected from the capillary tube are tested separately using PCR for YFV detection or virus isolation.

3.5.4 Data analysis

- Mosquitoes are tested individually, and a sample is considered positive if virus is detected with real-time PCR.
- Detection of YFV in the mosquito body without infection of the legs, which contain haemolymph, indicates a non-disseminated infection limited to the midgut.
- The presence of YFV in both the mosquito body and legs/wings indicates an infection disseminated into the haemocoel.
- The presence of the virus in saliva or exposed vertebrate (mice) indicates a possible transmission of YFV.
- The infection rates are calculated as the number of infected mosquito bodies divided by the total number of mosquitoes tested.
- The dissemination rates are estimated as the number of mosquitoes with infected wings and legs divided by the total number of infected mosquitoes.
- The transmission rates are estimated as the number of mosquitoes with infected saliva divided by the number of mosquitoes with positive legs and wings.

Remarks

- The dissemination can be directly estimated by doing an immunofluorescence assay on the mosquito head squashes after the extrinsic incubation period.
- Transmission can be indirectly estimated by the dissemination rate, since a mosquito capable to disseminate an infection is usually capable to transmit.
- This vector competence experiment can be used to assess the vertical transmission of the virus and, therefore, estimate the possibility for virus maintenance in the affected area.

3.6 Testing insecticide susceptibility of the vectors

- When vector control is of concern, activities will include insecticide susceptibility tests over local populations. Therefore, several classes of commonly used, internationally approved and environmentally friendly insecticides will be used to identify those that kill the highest proportion of YF vectors collected from the different study sites. Mosquito populations are reared in the same conditions as for vector competence studies. Adults emerging from larvae/pupae collected in the field or the progeny of females collected in the field can be used. Adult mosquitoes are maintained with a 10% sucrose solution.
- Bioassays are carried out using World Health Organization (WHO) test kits for adult mosquitoes (WHO, 1998).
- Impregnated papers are provided by the Vector Control Research Unit, School of Biological Sciences (Universiti Sains Malaysia), a WHO Collaborating Centre.
- The tests are carried out using a 2–5-day-old unfed females.
- Pools of 20–25 females are exposed to impregnated papers for 30 minutes to 4% DDT and 1 hour to the other tested insecticides.
- The number of knockdown mosquitoes is recorded every 10 minutes during exposure. The knockdown times for 50% (KDT50) and 95% (KDT95) of tested mosquitoes are calculated using a log-probit software (StatPlus version 2009) according to Finney (1971).
- The mortality rate is recorded after 24 hours.
- Tests with untreated papers are systematically run as controls. When control mortality is between 5% and 20%, mortality rate in tested samples is corrected using Abbott formula (Abbott, 1925).
- The WHO criteria for determining resistance or susceptibility is applied (WHO, 1998).

4 Budget planning for the investigation

Tables 3.1–3.4 are tables to help plan a budget for an entomological investigation.

Table 3.1 Personnel budget

Personnel	No.	No. of days	Total	Status	Rate (US$)	
					Per day	Total
Scientist				Entomologist		
Trained technician						
Local technician						
Guide						
Field workers						
Subtotal						

no., number.

Table 3.2 Supplies budget

Items	Price/unit (US$)	Number of units	Total
Flashlight & accessories			
Larvae dippers			
Pint ladle			
Pipettes			
Larvae trays			
Cardboards or cages			
Vials			
Forceps			
Mechanical aspirators			
Mouth aspirators			
Nesting bowls			
Mosquito breeders			
Cotton			
Petri dishes			
Humidifier			
Glass tubes			
Masks			
Cryotubes			
Cardboards			
Cages			
Dry ice			
Liquid nitrogen			
Ladder			
White sheets			
Insecticide sprays			
Maps			
Subtotal			

Items	Price/unit (US$)	Number of units	Total
Small equipment			
Backpack aspirator			
CDC Light Trap			
Sticky trap			
Gravid Trap			
BG Sentinel Trap			
Stereo zoom microscope			
Chill table			
Nitrogen liquid container			
Filter-paper			
Subtotal			

Table 3.3 Transportation budget

Items	Price/unit (US$)	Number of units	Total
Vehicle			
Fuel			
Subtotal			

Table 3.4 Communications budget

Items	Price/unit (US$)	Number of units	Total
Mobile modem			
Phone			
Subtotal			

Project total: US$ _____

References

Abbott WS (1925). A method of computing the effectiveness of an insecticide. *Journal Economic Entomology*, 18: 265–267.

Aitken THG (1977). An in vitro feeding technique for artificially demonstrating virus transmission by mosquitoes. *Mosquito News*, 37(1):130–133.

Bauer JH (1928). The transmission of yellow fever by mosquitoes other than *Aedes aegypti. American Journal of Tropical Medicine*, 8(4):261–283.

Camargo S (1967). History of *Aedes aegypti* eradication in the Americas. *Bulletin of the World Health Organization*, 36:602–603.

Cordellier R (1991). L'épidémiologie de la fièvre jaune en Afrique de l'Ouest. *Bulletin de l'Organisation Mondiale de la Santé*, 69(1):73–84.

Cornet M, Robin Y, Heme G, Adam C, Renaudet J, Valade M, Eyraud M (1979). Une poussée épizootique de fièvre jaune selvatique au Sénégaloriental. Isolement du virus de lots de moustiques adultes mâles et femelles. *Médecine et Maladies Infectieuses*, 9:63–66.

Edwards FW (1941). *Mosquitoes of the Ethiopian region: III culicine adults and pupae*. London, British Museum (Natural History).

Ellis B, Barrett A (2008). The enigma of yellow fever in east Africa. *Reviews in Medical Virology*, 18:331–346.

Ferrara L, Germain M, Hervy JP (1984). *Aedes (Diceromyia) furcifer* (Edwards, 1913) et *Aedes (Diceromyia) taylori* (Edwards, 1936): le point sur la différentiation des adultes. *Cahier ORSTOM Série Entomologie Médicale et Parasitologie*, 22:95–98.

Finney DJ (1971). *Probit analysis*. Cambridge, Cambridge University Press.

Fontenille D, Diallo M, Mondo M, Ndiaye M, Thonnon J (1997). First evidence of natural vertical transmission of yellow fever virus in *Aedes aegypti*, its epidemic vector. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, 91:533–535.

Germain M, Cornet M, Mouchet J, Herve JP, Robert V, Camicas JL, Cordellier R, Hervy JP, Digoutte JP, Monath TP, Salaun JJ, Deubel V, Robin Y, Coz J, Taufflieb R, Saluzzo JF, Gonzalez JP (1981). La fièvre jaune selvatique en Afrique : Données récentes et conception actuelles. *Médecine Tropicale*, 41(1):33–43.

Gould LH, Osman MS, Farnon EC, Griffith KS, Godsey MS, Karch S, Mulenda B, El Kholy A, Grandesso F, de Radiguès X, Brair ME, Briand S, El Tayeb el SM, Hayes EB, Zeller H, Perea W (2008). An outbreak of YF with concurrent Chikungunya virus transmission in South Kordofan, Sudan, 2005. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102(12):1247–1254.

Haddow AJ (1968). The natural history of yellow fever in Africa. *Proceedings of the Royal Society of Edinburgh*, 70:191–227.

Herve JP, Germain M, Geoffroy B (1977). Bioecologie compare d'*Aedes (Stegomyia)* opok Corbet et Van Someren et *A. (S.) africanus* (Theobald) dans une galerie forestiere du sud de l'Empire Centrafricain II. Cycles saisonniers d'abondance. *Cahier ORSTOM Série Entomologie Médicale et Parasitologie*, 15: 271–281.

Huang YM (1986a). *Aedes (Stegomyia) bromeliae* (Diptera: Culicidae), the yellow fever virus vector in East Africa. *Journal of Medical Entomology*, 23:196–200.

Huang YM (1986b). Notes on the *Aedes (Diceromyia) furcifer* group, with a description of a new species (Diptera: Culicidae). *Proceedings of the Entomological Society of Washington*, 88(4):634–649.

Huang YM (1990). The subgenus *Stegomyia* of *Aedes* in the Afrotropical region, I. The Africanus group of species (Diptera: Culicidae). *Contributions of the American Entomological Institute*, 26:1–90.

Jupp PG (1997). *Mosquitoes of southern Africa: Culicinae and Toxorhynchitinae*. Hartebeespoort, South Africa, Ekogilde cc Publishers.

Jupp PG (1998). *Aedes (Diceromyia) furcifer* (Edwards) and *Aedes (Diceromyia) cordellieri* Huang in southern Africa: distribution and morphological differentiation. *Journal of the American Mosquito Control Association*, 14(3):273–276.

Kerr JA, Decamargo S, Abedi ZH (1964). Eradication of *Aedes aegypti* in Latin America. *Mosquito News*, 24(3):276–282.

Kirk R (1941). An epidemic of yellow fever in the Nuba Mountains, Anglo–Egyptian Sudan. *Annals of Tropical Medicine and Parasitology*, 35:67–108.

Lee VH, Moore DL (1972). Vectors of the 1969 yellow fever epidemic on the Jos Plateau, Nigeria. *Bulletin of the World Health Organization*, 46(5):669–673.

Lewis DJ, Hughes TP, Mahaffy AF (1942). Experimental transmission of yellow fever by three common species of mosquitoes from the Anglo–Egyptian Sudan. *Annals of Tropical Medicine and Parasitology*, 36:34–38.

Lourenço-de-Oliveira R et al. (2008). Comparison of different uses of adult traps and ovitraps for assessing dengue vector infestation in endemic areas. *Journal of the American Mosquito Control Association*, 24(3):387–392.

Onyango CO, Grobbelaar AA, Gibson GV, Sang RC, Sow A, Swaneopel R, Burt FJ. (2004a). Yellow Fever outbreak, southern Sudan, 2003. *Emerging Infectious Diseases*, 10(9):1668–1670.

Onyango CO, Ofula VO, Sang RC, Konongoi SL, Sow A, De Cock KM, Tukei PM, Okoth FA, Swanepoel R, Burt FJ, Waters NC, Coldren RL. (2004b). Yellow fever outbreak, Imatong, southern Sudan. *Emerging Infectious Diseases*, 10:1063–1068.

Phillip CB (1930). Studies on the transmission of experimental yellow fever by mosquitoes other than *Aedes. American Journal of Tropical Medicine and Hygiene*, 10:1–16.

Powell JR, Tabachnick WJ, Arnold J (1980). Genetics and the origin of a vector population: *Aedes aegypti*, a case study. Science, 208:1385–1387.

Reed, W (1901). Propagation of yellow fever: observation based on recent researches. *Medical Record*, 60:201–209.

Reiter P, Cordellier R, Ouma JO, Cropp CB, Savage HM, Sanders EJ, Marfin AA, Tukei PM, Agata NN, Gitau LG, Rapuoda BA, Gubler DJ (1998). First recorded outbreak of YF in Kenya, 1992–1993. II. Entomologic investigations. *American Journal of Tropical Medicine and Hygiene*, 59:650–656.

Smithburn KC, Haddow AJ, Lumsden WHR (1949). An outbreak of sylvan yellow fever in Uganda with *Aedes (Stegomyia) africanus* Theobald as principal vector and insect host of the virus. *Annals of Tropical Medicine and Parasitology*, 43:74–89.

Soper FL (1965). The 1964 status of *Aedes aegypti* eradication and yellow fever in the United States. *American Journal of Tropical Medicine and Hygiene*, 14(6):887–891.

Soper FL et al. (1933). Yellow fever without *Aedes aegypti*: study of rural epidemic in Valle do Chanaan, Espirito Santo, Brasil. *American Journal of Tropical Medicine and Hygiene*, 18:555–587.

Stokes, Bauer JH, Hudson NP (1928). Experimental transmission of yellow fever to laboratory animals. *American Journal of Tropical Medicine*, 8:103–164.

Tabachnick WJ, Powell JR (1979). A world-wide survey of genetic variation in the yellow fever mosquito, *Ae. aegypti. Genetics Research*, 34:215–219.

Thonnon J et al. (1998). Re-emergence of yellow fever in Senegal in 1995. *American Journal of Tropical Medicine and Hygiene*, 59:108–114.

WHO (World Health Organization) (1986). *Prevention and control of yellow fever in Africa*. Geneva, WHO.

WHO (World Health Organization) (1998). *Tests procedures for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces*. Report of the WHO Informal Consultation (WHO/MAL/98), Geneva, WHO.

Annex 1 Form to report prototyping aquatic stages

Date:

District: Parish:

Subcounty: Village:

Geographical position:

House no.	No. of habitation units	No. of personnel	Type of breeding sites																		GPS coordinate
			Plastic tank				Metallic barrel				Clay jar				Tires		Flowerpot		Well		Others		
			Indoor		Outdoor		Indoor		Outdoor		Indoor		Outdoor										
			+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	

GPS, global positioning system; no., number.

Annex 2 Form to summarize the mosquito population investigation

Date(s): _____

General information	
District:	Parish:
Subcounty:	Village:
Name of the person who file out the form:	
Summary – Investigation in mosquito population	
Person contacted in the site (name/titre):	
Name and titre of the local technician:	
Name and titre of the team guide:	
Number of households planned to be sampled:	
Number of household visited:	
Number of habitation unit:	
Total number of habitants in the household:	
Total number of containers inspected:	
Total number of positive containers:	
Number of samples (vial with larvae/pupae):	
Number of tubes of adults stored:	

Annex 3 Form for recording pooled mosquitoes

Geographical origin	Method of collection	Localization[a]	Date	Species	Sex	Feeding status	Number specimens	ID number

ID, identification

[a] Forest, domestic, peridomestic environment. Please indicate if any other is used.

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2014, Geneva, Switzerland