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Review

# Yellow fever live attenuated vaccine: A very successful live attenuated vaccine but still we have problems controlling the disease

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ABSTRACT

Yellow fever (YF) is regarded as the original hemorrhagic fever and has been a major public health problem for at least 250 years. A very effective live attenuated vaccine, strain 17D, was developed in the 1930s and this has proved critical in the control of the disease. There is little doubt that without the vaccine, YF virus would be considered a biosafety level 4 pathogen. Significantly, YF is currently the only disease where an international vaccination certificate is required under the International Health Regulations. Despite having a very successful vaccine, there are occasional issues of supply and demand, such as that which occurred in Angola and Democratic Republic of Congo in 2016 when there was insufficient vaccine available. For the first time fractional dosing of the vaccine was approved on an emergency basis. Thus, continued vigilance and improvements in supply and demand are needed in the future.



**Contents**



## 1. Introduction

Yellow fever virus (YFV) is the prototype member of the genus Flavivirus, a group of predominantly arthropod-borne viruses that are capable of causing severe clinical disease. In the case of YFV, it is a mosquito-borne virus that is maintained in a transmission cycle involving mosquitoes and primates in sub-Saharan Africa and tropical South America with over 50 countries at risk. Ninety percent of YF cases are in Africa and the remaining 10% in South America. The jungle cycle involves tree-hole breeding mosquitoes and non-human primates with the exact species of mosquito and monkey varying by geographic location. The urban cycle involves *Aedes aegypti* and humans, and is the cycle of most concern from a public health perspective. Here the reproductive number ($R_o$) can be as high as 5–7, i.e. one infected individual can lead to infection of 7 other individuals [1]. The disease YF is biphasic with a

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short incubation period of less than a week following the bite of a virus-infected mosquito and a febrile illness consisting of muscle pain, fever and headache. Most patients recover from the febrile illness, but unfortunately some cases proceed to the toxic phase where there is infection of the liver, kidneys and heart with jaundice [2]. There is potentially high mortality for those individuals who proceed to the toxic phase (up to 50%) due to kidney failure, internal bleeding and circulatory collapse. From a transmission perspective, the primate is viremic during the incubation period (the first three days after infection with the virus) when the individual may not be aware they are infected and as such if a mosquito vector for YFV feeds on the infected individual there is the risk of continuing the mosquito-primate transmission cycle. There are no antivirals for any flavivirus disease so the only therapy is supportive. Clearly vaccination is a critical tool in preventing disease.

YF has been known for centuries and is often called the "original" hemorrhagic fever. History has described YF activity in not only Africa and the Americas but also European coastal ports where small outbreaks took place due to ships maintaining the mosquito-human cycle on trips from YF endemic areas [3]. There has also been a history of YF outbreaks in North American coastal areas with the last one in New Orleans in 1905 involving 5000 cases, including 1000 deaths. There is little doubt, if we did not have a vaccine, YFV would be regarded as the major biosafety level 4 (BSL-4) pathogen in the world. Indeed, despite having a very effective vaccine it is estimated that approximately 130,000 cases, including 78,000 deaths, still occur each year [4].

## 2. Development of live attenuated YF vaccines

Much effort was spent in the early parts of the 20th century developing a vaccine following successful isolation of the virus (strain Asibi) from a mild human case in Ghana ("Mr. Asibi") by Stokes et al. in 1927 [5]. Two approaches were taken to develop a live attenuated vaccine. French workers took the wildtype French viscerotropic virus (FVV; isolated from Francoise Mayali in Senegal in 1928) and passaged it in mouse brain to develop the French neurotropic vaccine (FNV) while wild-type strain Asibi was attenuated by passage in chicken tissue to develop the 17D vaccine.

## 3. French neurotropic vaccine

The first attenuated FNV variant was identified following 128 passages in mouse brain and subsequently vaccine was used at mouse brain passage 260. The vaccine virus had lost viscerotropism (infection of the liver) and mosquito competence. The latter is very important for a live attenuated vaccine strain based on a virus transmitted by mosquitoes as it is critical to prevent both transmission to humans and reversion to virulence in mosquitoes. FNV was used extensively in French-speaking areas of Africa and was responsible for a great reduction in numbers of YF cases, however, the passage in mouse brain increased the neurological potential of FNV such that it resulted in a high rate of post-vaccinal encephalitis in children. This resulted in the vaccine being contraindicated in children under the age of 14. Subsequently, the vaccine was discontinued in 1980 due to the success of the 17D vaccine. Nonetheless, FNV was a very effective vaccine at controlling YF in Africa during the 1940s and 1950s.

## 4. History of 17D vaccine

Development of the 17D vaccine involved taking wild-type strain Asibi and passaging 18 times in minced whole mouse embryo, followed by 58 passages in minced whole chicken embryo, and finally 128 passages in minced chicken embryo with the brain and spinal cord removed. Thus, vaccine strain 17D was identified after 176 passages in chicken tissue and had the "ideal" properties of a live attenuated vaccine of being both highly immunogenic and attenuated. In terms of attenuation, 17D had lost viscerotropism, neurotropism, and mosquito competence. The vaccine virus was also highly immunogenic with protective immunity induced by less than 1000 infectious units (see below). Theiler and Smith [6] reported the first human immunization studies and the vaccine was rapidly implemented as a vaccine grown in embryonated chicken eggs. Significantly, 17D vaccine was utilized by many sources and the passage level of the vaccine was greatly extended, sometimes up to passage 400. Unfortunately, the extended passage histories sometimes resulted in over-attenuation such that the vaccine had poor immunogenicity. This resulted in the establishment of a seed-lot system in 1945 involving "primary seed" viruses used to generate "secondary seed" viruses, and the secondary seed virus used to generate vaccine lots to immunize humans [7]. The seed-lot system has worked very well over the past 75 years and 13 manufacturers in total have produced approximately 650 million doses of vaccine.

The vaccine is a freeze dried product that is reconstituted as a single dose of 0.5 ml given either by the subcutaneous or intramuscular routes. Each dose must contain at least 1000 international units (IUs) and there is no maximum quantity of virus in a dose. Usually a dose contains anywhere from 4000 to one million IUs. Although studies indicate that 99% of adult vaccinees have seroprotective levels of neutralizing antibodies by 30 days post-immunization, recent evidence suggests that this figure is below 90% in children [8,9]. Until recently, booster doses were needed every 10 years, however, a review by the World Health Organization recommended that booster doses be eliminated for most groups due to evidence of long term, probably life-long protective immunity [10]. This change went into effect in July 2016 and grandfathers previously issued vaccine certificates. However, not all countries have followed this recommendation, e.g. Brazil, who continues 10-year boosters.

The 17D vaccine is often considered a "legacy" vaccine, i.e., the vaccine was developed in the 1930s before modern techniques were introduced for developing a vaccine. This does cause some complications in the 21st century. The vaccine was developed before cell culture and so for a long time virus was titrated in terms of 50% mouse intracerebral lethal dose ($MICLD_{50}$). Subsequently, the virus titration was modified to use plaques (pfu) in monkey kidney Vero cells. However, it became clear that virus plaque assays varied between producers as did the ratio of pfu to $MICLD_{50}$-s. This led to the use of IUs so that vaccines from different producers could be standardized. The other major issue is measuring protection. All vaccines need a correlate or surrogate of protection. In the case of 17D vaccine, this was derived by the ability of antibodies to mediate passive protection when given to non- human primates, and was found to be a neutralizing antibody titer of 0.7 by log neutralization index (LNI). This assay involves constant antibody and varying (10-fold) concentrations of virus so that a seroprotective titer is where the virus titer is reduced $10^{0.7}$. Today we use 50% plaque reduction neutralization tests ($PRNT_{50}$) where we vary the concentration of antibody and keep the concentration of virus constant (usually approximately 50 pfu). The LNI and $PRNT_{50}$ assays have never been directly compared in regulated tests, however, there are data from a hamster study to suggest that a $PRNT_{50}$ titer of 1 in 40 is seroprotective [11] but it is unknown if the hamster data will be translatable to humans. In reality, the current approach for approving a vaccine is by non-inferiority studies in clinical evaluation where a "new" vaccine is shown to be non-inferior to a licensed vaccine [12].

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