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Live Attenuated Yellow Fever 17D Vaccine: A Legacy Vaccine Still Controlling Outbreaks In Modern Day

Natalie D. Collins[1] · **Alan D. T. Barrett**[2]

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Abstract
Purpose of Review Live attenuated 17D vaccine is considered one of the safest and efficacious vaccines developed to date. This review highlights what is known and the gaps in knowledge of vaccine-induced protective immunity.
Recent Findings Recently, the World Health Organization modifying its guidance from 10-year booster doses to one dose gives lifelong protection in most populations. Nonetheless, there are some data suggesting immunity, though protective, may wane over time in certain populations and more research is needed to address this question. Despite having an effective vaccine to control yellow fever, vaccine shortages were identified during outbreaks in 2016, eventuating the use of a fractional-dosing campaign in the Democratic Republic of the Congo.
Summary Limited studies hinder identification of the underlying mechanism(s) of vaccine longevity; however, concurrent outbreaks during 2016 provide an opportunity to evaluate vaccine immunity following fractional dosing and insights into vaccine longevity in populations where there is limited information.

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✉ Alan D. T. Barrett
abarrett@utmb.edu

Natalie D. Collins
ndcollin@UTMB.EDU

[1] Departments of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX 77555-0436, USA

[2] Department of Microbiology & Immunology, Department of Pathology Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555-0436, USA

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Introduction

Flaviviruses are a group of insect-borne viruses that can cause a range of clinical symptoms, including hemorrhagic and neurological manifestations. The *Flavivirus* genus (named after *flavus*, the Latin for yellow) contains a number of pathogens of public health importance, including mosquito-borne yellow fever (YF), dengue (DEN), Japanese encephalitis (JE), West Nile (WN) and Zika (ZIK) viruses, and tick-borne encephalitis (TBE) virus. YF virus (YFV), the prototype flavivirus, targets the liver causing severe liver damage and jaundice, hence the "yellow" in YFV. Once in the liver, YFV spreads to the kidneys and heart, causing severe and, sometimes, fatal visceral disease [1]. In comparison, JE, TBE, and WN viruses are associated with neurologic disease while DEN and ZIK viruses cause a febrile infection with a rash, although both can lead to severe disease with hemorrhagic fever and congenital Zika syndrome, respectively. Although there is a safe and effective vaccine that has been available since the late 1930s, YFV remains a public health problem in South America and sub-Saharan Africa, where it causes an estimated 130, 000 cases, including 78,000 deaths annually [2••]. More recently, the 2016 outbreak in Angola and Democratic Republic of Congo (DRC) that began in December of 2015 and continued through September 2016 (see below), fueled concern of the potential for outbreaks outside of endemic areas to areas primed for YFV transmission due to the presence of appropriate mosquito vectors. Given the lethality of YF disease and the numerous countries that harbor the vectors, YFV is by far the most important hemorrhagic flavivirus.

Ecology and Epidemiology

YFV is a mosquito-borne virus, transmitted by *Aedes* spp. in Africa and *Haemagogus* and *Sabethes* spp. in South America. The only vertebrate hosts for YFV in nature are primates where the virus causes an acute disease with high viremia, followed by death or recovery. The virus is maintained in nature by a transmission cycle between mosquitoes and non-human primates in what is referred to as the "jungle" or sylvatic cycle. The particular non-human primate species vary by geographic location. Rarely, humans either become infected after entering jungle areas where there are YFV-infected mosquitoes ("intermediate cycle") or the "urban" cycle. In the urban cycle, the transmission cycle is between humans and *Aedes aegypti*, and non-human primates are not involved.

Given the widespread distribution of *Ae. aegypti* in tropical climates, it is unclear why YFV has not spread to Asia [3]. *Ae. aegypti* mosquitoes in Asia transmit other related flaviviruses, such as dengue [4], and laboratory studies have shown them to be a competent vector for YFV. Since YFV originated in Africa and was introduced to the Americas during the slave trade [5, 6], concerns of spread outside the current endemic areas are warranted.

Genetic studies have shown that there are at least seven genotypes of YFV. Five are found in Africa (two in West Africa, one in East Africa, one in East/Central Africa, and one in Angola only) and two in South America (South America I and II). Interestingly, the 2016 Angola outbreak was caused by a strain indistinguishable genetically from the 1971 Angola outbreak, and these two outbreaks have been the only times when this strain has been identified. This raises important questions about the ecology and epidemiology of YFV, namely where did the Angola strain come from and where is the virus maintained over time? Our knowledge of the molecular epidemiology of YFV is limited and is an area where additional research would likely improve our abilities to understand the epidemiology and control of YF.

Until 2016, the number of clinical cases of YFV was on the decline most likely due to improved vaccine coverage in Africa and South America. Based on data from African countries in 2013, there is a burden of 84,000–170, 000 severe cases and 29,000–60,000 deaths due to YF [7]. In contrast, there were an estimated 73,000–530,000 severe cases and 27,000–250,000 deaths in 2005 [2••]. The vast majority of reported cases and deaths (>90%) occur in sub-Saharan Africa.

Vaccination

There are no antivirals to treat YF infections and the only course is supportive therapy. However, there is an excellent vaccine, which has been critical for public health measures to control YF disease. The live attenuated 17D vaccine was developed in the 1930s and currently, there are three 17D substrains in production; 17DD manufactured in Brazil, 17D-213 manufactured in Russia, and 17D-204 manufactured in China, France, Senegal, and the USA. Four of the vaccines (Brazil, France, Russia, and Senegal) are prequalified and used internationally for WHO/UNICEF vaccination campaigns. Since its establishment in 2000, the GAVI vaccine alliance, in collaboration with WHO and UNICEF, has supported vaccination in African countries at risk from YF. Over 150 million of the approximate 700 million individuals in 17 GAVI-eligible endemic countries have received vaccine. Notably, routine immunization has been introduced into 17 African countries and vaccine campaigns undertaken in 14 countries. In addition, there is an ambitious plan to immunize a further 1.3 billion individuals in the next 5 years as part of the "EYE" (eliminating yellow fever epidemics) plan [8]. This program has been very successful at preventing YF in West Africa with reduction of YF cases each year such that no outbreaks of YF were reported in West African countries during 2015.

Molecular Basis of Attenuation of 17D Vaccine

The 17D vaccine strain is considered to be one of the most effective and safe, live attenuated viruses developed to date. However, despite 17D vaccine being derived in the 1930s from wild-type strain Asibi by passage in chicken and mouse tissue, the mechanism of attenuation is poorly understood. With the advent of advance sequencing technology, some light has been shed on the mechanism of attenuation. Like most RNA viruses, wild-type YFV replication is error-prone due to the lack of proof-reading by the virus-encoded RNA-dependent RNA polymerase. These errors give rise to a subpopulation of genetically related viruses that contribute to immunogenicity and virulence. However, there is evidence that replication of 17D is not as error-prone as wild-type RNA viruses [9]. Recent studies have used next-generation sequencing to compare the RNA populations in wild-type Asibi and 17D vaccine virus. Wild-type Asibi virus was found to have the typical heterogeneous population of an RNA virus while the 17D vaccine population was relatively homogeneous and there is limited intra- and intervariability of 17D vaccines [10–12]. It is hypothesized that the limited genetic diversity of the 17D vaccine virus attributes to vaccine attenuation and safety.

Immune Response to Vaccination and Long-term Immunity

A single dose of 17D vaccine confers protection in greater than 95% of recipients within 30 days following vaccination

[13, 14]. The effectiveness of the 17D vaccine strain is attributed to induction of both innate and adaptive immunity that leads to the induction of neutralizing antibodies directed predominately against the envelope protein [15–18]. Moreover, innate immune cells secrete mixed and balanced anti-inflammatory and pro-inflammatory cytokines that modulate other immune cells and elicit a broad adaptive response. As with natural infection with wild-type YF, immunization with 17D is thought to give protective immunity for at least 10 years, and probably induce lifelong immunity. High levels of neutralizing antibodies and memory T cells have been detected in vaccinees 10–60 years following vaccination [19]. The exact mechanism for this long-term immunity is still being elucidated. However, there is evidence that suggests that in conjunction with humoral immunity, YFV specific $CD4^+$ T cells are preferentially activated following vaccination [20, 21•]; these activated $CD4^+$ T cells recognize both structural and non-structural proteins and are detectable years later [22]. Conversely, YFV specific $CD8^+$ T cells are also detectable decades after vaccination [23, 24] and correlate to initial viral loads following vaccination [25]. T cells may contribute to long-term immunity; however, it is secondary to the induction of neutralizing antibodies.

Until recently, booster doses of 17D vaccine were given every 10 years. In 2013, the WHO Strategic Advisory Group of Experts on Immunization recommended an amendment to the International Health Regulations to reflect the long-term protection from YF after a single vaccination with 17D vaccine, namely booster doses are not needed except for special populations such as pregnant women, HIV infected individuals, hematopoietic stem cell transplant recipients, and persons in higher-risk setting for exposure to YF virus; this change took effect in June 2016 [26, 27].This was supported, in part, by very limited evidence for vaccine failures [19]. However, some countries, such as Brazil, have elected to retain the 10-year booster based on several studies from Brazil that suggest significant drops in immunity over time [27–29]. This has not been observed in other countries but this could be due to a lack of studies. The United States Army Medical Research Institute screened over 1029 laboratory personnel for neutralizing antibodies by 80% plaque reduction neutralization test ($PRNT_{80}$)(a more stringent assay than the traditional $PRNT_{50}$ used for international tests) to evaluate the immune activation post 17D booster and determined titers dropped below 1:40 after 3 years, suggesting that a 10-year booster or earlier may be required to maintain high levels of neutralizing antibodies in high-risk groups [30] and would exceed the neutralization titer needed for protective immunity.

Additional studies have also suggested waning immunity over time. de Melo and colleagues [29] found that 35% of vaccinees had neutralizing antibody titers below 1:100 (although this exceed the seroprotective neutralizing antibody titer) at 10 years following vaccination with 17DD vaccine, while others showed that cellular and humoral immunity decreased by 4 years post immunization and only 85% of vaccinees were seropositive 12 years post vaccination [27–29]. A number of hypotheses have been proposed to explain these results, including variation in immune response to specific vaccine strains, pre-existing immunity and immune stimulation in endemic areas compared to non-endemic areas [31]. A meta-analysis study of 17D vaccination efficacy, totaling 4,686 vaccinations, found that vaccine efficacy was lower in vaccinees from endemic areas, supporting immune activation differs in endemic and non-endemic vaccinees [32]. Conversely, there is evidence of waning immunity in vaccinees in non-endemic areas, independent of exposure to related flaviviruses [27]. Additionally, it has been suggested that vaccinees who clear the virus without prolonged presentation of antigen to T cells and B cells may not mount a strong immune response, thereby failing to induce lifelong immunity [33].

Lessons learned from Concurrent Outbreaks in Africa: Fractional Dosing in Emergency Scenarios

Immunization strategies involve delivery of 17D vaccine in endemic settings via routine immunization and as a "travel" vaccine for those who visit endemic areas. Mass vaccination campaigns are used in endemic areas to catch-up on immunization of unvaccinated cohorts not eligible for routine immunization during outbreaks. Despite the successes of vaccination strategies in Africa, particularly West Africa, it became evident in 2016 that strategies to immunize individuals in countries where YF outbreaks are sporadic, often decades apart, need to be re-evaluated.

The Angola outbreak began in December of 2015 and peaked during February 2016, but cases continued until June 23, 2016. All 18 provinces in Angola reported cases and deaths due to YF. After multiple vaccination campaigns, requiring 20 million doses of 17D vaccine, the outbreak in Angola was under control [34] and was the largest outbreak in Angola since 1971 [35], with 4,347 suspected cases and 377 deaths [34]. There have been examples of introduction of YFV to other locales by virus-infected humans during outbreaks; this became very evident during the Angola outbreak. Unvaccinated travelers led to cases in DRC (2800), Mauritania (1), Kenya (2), and China (11) ([34, 36•, 37, 38]. Of the 2800 suspected cases in DRC, at least 57 were imported Angola and 13 were autochthonous, requiring control of the outbreak with 9.4 million doses of the 17D vaccine [34]. The cases in China were the most worrying. There were over 250,000 Chinese workers in Angola during the outbreak in 2016 and their YF vaccination status is unclear. Eleven clinical cases of YF were reported in different areas of China in travelers returning from Angola, representing the first cases of YF in Asia. This outbreak has been a wake-up call for the

potential spread of YFV to Asia, presumably by flights taken by non-vaccinated humans infected in Angola. There have been no reports of YF transmission in China but the question of pre-emptive vaccination of individuals in Asia or stockpiling vaccine for the potential control of YFV in Asia have been discussed. However, enforcement of the International Health Regulations, such that all individuals traveling to YF endemic areas are vaccinated and/or evidence of those returning from YF endemic areas have been vaccinated, are more practical measures given the huge population in *Ae. aegypti* infested areas of Asia.

Concurrently, outbreaks or sporadic cases not related to the ongoing outbreak in Angola were reported in Uganda, Brazil, Colombia, Chad, Ghana, and Peru [39]and approximately 800,000 doses of 17D vaccine were distributed in Uganda [40] Thus, there is a need for continual vigilance of potential YF activity and the need for vaccine to be available during emergencies.

UNICEF maintains a reserve stockpile of 6 million doses of vaccine for control of outbreaks. The outbreak in Angola, DRC, and Uganda diminished the stockpile of 17D vaccine, and in Angola specifically, the reserve was exhausted twice in 2016. There are only four WHO prequalified manufacturers who can produce 80 million doses annually between them [41]. By the end of August 2016, the projected doses needed to control the outbreak would exceeded the supply. Discussions turned to alternative approaches that could be used in emergency scenarios in which demand outstrips supply, such as administering the vaccine as a "fractional dose."

Although the minimum amount of virus is 1000 international units (IU) per dose, all producers manufacture vaccine containing an excess of virus, often 10,000 IU or higher; there are no regulations regarding the maximum amount of virus in a dose [36•]. Consequently, there was the possibility to give vaccinees less than one dose and still administer at least 1,000 IU. Each dose of vaccine is reconstituted in a volume of 0.5 ml. Thus, a strategy was investigated to give vaccinees 0.1 ml via a tuberculin syringe such that there was more than 1,000 IU per 0.1 ml. Review of the literature showed that of the four prequalified vaccines, only the 17DD vaccine manufactured in Brazil had publications investigating dose sparing; according to two studies with the 17DD vaccine, doses as low as 587 IU achieved equivalent seroconversion, immune activation, and neutralizing antibodies as a full dose [42••, 43••]; but only subdoses as low as 3,013 IU mimicked viremia kinetics of the full dose [42••]. The WHO recommended that a subdose of >3,000 IU per 0.1 ml of the 17DD vaccine be used for immunizations in Angola and DRC on an emergency basis only in August/September 2016 [44].

Since a full dose of vaccine was not being administered, vaccinees would not receive an international YF vaccination certificate for the factional-dose immunization. Adults were the only participants in both subdose studies, therefore, it was decided that fractional doses could only be given to those over 2 years of age. Thirty-two health zones in Kinshasa, DRC with a population of over 7 million plus 15 health zones on the border between DRC and Angola were at risk for YF [39]. Therefore, an emergency campaign was initiated in August 2016 using a fractional-dose approach. One fifth of a full dose was administered subcutaneously to everyone over 2 years, while children aged 9–23 months and pregnant women received a full dose [44]; studies are ongoing to determine the immunogenicity and immune longevity to fractional dosing, providing the additional data on the immune response in children and women, which was not evaluated in the previous subdosing studies.

Conclusions

Despite a very successful vaccine being available for over 75 years, YF is still a serious public health concern for endemic areas and areas with sporadic YFV transmission. Most recently, YF captured worldwide attention due to the outbreaks in Angola and DRC that lead to imported cases in non-endemic countries. Most notable were the 11 imported cases in China, as there has never been an outbreak in Asia despite having a competent mosquito vector [34]. The concurrent outbreaks exhausted stockpiles of the 17D vaccine, requiring an emergency "fractional dose" vaccination campaign in DRC, putting into practice the findings of two studies with the 17DD vaccine substrain that showed a subdose as low as 3,013 IU was as immunogenic and antigenic as a full dose [42••, 43••], at least for the 17DD vaccine.

Vaccination with 17D vaccine is considered to be lifelong, evidenced by the detection of neutralizing antibodies and memory T cells up to 60 years following vaccination. Therefore, the YF vaccine guidance was revised and a 10-year booster vaccine was no longer recommended [26, 27]. This is a recommendation and is not followed by all endemic counties due to the possibilities of waning immunity in certain sub-populations. This requires additional studies. Significantly, the Angola and DRC emergency "fractional dose" campaign presents a unique opportunity to investigate the immune response induced in vaccinees using state-of-the-art techniques, including women and children, as well as the effect fractional dosing may have on immune longevity.

Compliance with Ethical Standards

Conflict of Interest Drs Collins declares no conflict of interest.

Dr Barrett declares an R21 grant from NIH/NIAD and serves as a member of WHO yellow fever working groups.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Monath TP, Vasconcelos PF. Yellow fever. J Clin Virol. 2013;64: 160–73.
2.•• Garske T, Van Kerkhove MD, Yactayo S, Olivier R, Lewis RF, Staples JE, et al. Yellow fever in Africa: estimating the burden of disease and impact of mass vaccination from outbreak and serological data. PLoS Med. 2014;11:e1001638. **The most current morbidity and mortality rates of yellow fever virus in Africa**.
3. Barrett AD, Higgs S. Yellow fever: a disease that has yet to be conquered. Annu Rev Entomol. 2007;52:209–29.
4. Lai S, Huang Z, Zhou H, Anders KL, Perkins TA, Yin W, et al. The changing epidemiology of dengue in China, 1990–2014: a descriptive analysis of 25 years of nationwide surveillance data. BMC Med. 2015;13:100.
5. Bryant JE, Holmes EC, Barrett AD. Out of Africa: a molecular perspective on the introduction of yellow fever virus into the Americas. PLoS Pathog. 2007;3, e75.
6. Beck A, Guzman H, Li L, et al. Phylogeographic reconstruction of African yellow fever virus isolates indicates recent simultaneous dispersal into east and west Africa. PLoS Negl Trop Dis. 2013;7, e1910.
7. World Health Organization. Yellow fever, 05.2016 http://www.who.int/mediacentre/factsheets/fs100/en/. 2016.
8. World Health Organization. Global Strategy to Elimate Yellow Fever Epidemics (EYE), 9.26.2016. http://www.who.int/immunization/sage/meetings/2016/october/2_EYE_Strategy.pdf?ua=1. 2016.
9. Pugachev KV, Guirakhoo F, Ocran SW, Mitchell F, Parsons M, Penal C, et al. High fidelity of yellow fever virus RNA polymerase. J Virol. 2004;78:1032–8.
10. Beck A, Tesh RB, Wood TG, Widen SG, Ryman KD, Barrett AD. Comparison of the live attenuated yellow fever vaccine 17D-204 strain to its virulent parental strain Asibi by deep sequencing. J Infect Dis. 2014;209:334–44.
11. Tangy F, Desprès P. Yellow fever vaccine attenuation revealed: loss of diversity. J Infect Dis. 2014;209:318–20.
12. Salmona M, Gazaigne S, Mercier-Delarue S, Garnier F, Korimbocus J, de Verdière NC, et al. Molecular characterization of the 17D-204 yellow fever vaccine. Vaccine. 2015;33:5432–6.
13. Bonaldo MC, Sequeira PCC, Galler R. The yellow fever 17D virus as a platform for new live attenuated vaccines. Hum Vaccin Immunother. 2014;10:1256–65. doi:10.4161/hv.28117.
14. Barrett AD, Teuwen DE. Yellow fever vaccine—how does it work and why do rare cases of serious adverse events take place? Curr Opin Immunol. 2009;21:308–13.
15. Gaucher D, Therrien R, Kettaf N, Angermann BR, Boucher G, Filali-Mouhim A, et al. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J Exp Med. 2008;205:3119–31.
16. Luiza-Silva M, Campi-Azevedo AC, Batista MAA, Martins MA, Avelar RS, da Silveira D, et al. Cytokine signatures of innate and adaptive immunity in 17DD yellow fever vaccinated children and its association with the level of neutralizing antibody. J Infect Dis. 2011;204:873–83.
17. Silva ML, Martins MA, Espírito-Santo LRR, Campi-Azevedo AC, Silveira-Lemos D, Ribeiro JGG, et al. Characterization of main cytokine sources from the innate and adaptive immune responses following primary 17DD yellow fever vaccination in adults. Vaccine. 2011;29:583–92.
18. Quaresma JA, Pagliari C, Medeiros DB, Medeiros DB, Duarte MI, Vasconcelos PF. Immunity and immune response, pathology and pathologic changes: progress and challenges in the immunopathology of yellow fever. Rev Med Virol. 2013;23:305–18.
19. Gotuzzo E, Yactayo S, Córdova E. Efficacy and duration of immunity after yellow fever vaccination: systematic review on the need for a booster every 10 years. Am J Trop Med Hyg. 2013;89:434–44.
20. Blom K, Braun M, Ivarsson MA, Gonzalez VD, Falconer K, Moll M, et al. Temporal dynamics of the primary human T cell response to yellow fever virus 17D as it matures from an effector- to a memory-type response. J Immunol. 2013;190:2150–8.
21.• Watson AM, Lam LK, Klimstra WB, Ryman KD. The 17D-204 vaccine strain-induced protection against virulent yellow fever virus is mediated by humoral immunity and CD4+ but not CD8+ T cells. PLoS Pathog. 2016;12:e1005786. **An in vivo mouse study that details the immune response following vaccination with 17D-204 which suggest cellular immunity, specifically, CD4 cells contribute to protection from wild-type infection**.
22. James EA, LaFond RE, Gates TJ, Mai DT, Malhotra U, Kwok WW. Yellow fever vaccination elicits broad functional CD4+ T cell responses that recognize structural and nonstructural proteins. J Virol. 2013;87:12794–804.
23. Wieten RW, Goorhuis A, Jonker EFF, de Bree GJ, de Visser AW, van Genderen JJ, et al. 17D yellow fever vaccine elicits comparable long-term immune responses in healthy individuals and immune-compromised patients. J Infect. 2016;72:713–22.
24. Fuertes Marraco SA, Soneson C, Cagnon L, Gannon PO, Allard M, Maillard SA, et al. Long-lasting stem cell-like memory CD8+ T cells with a naïve-like profile upon yellow fever vaccination. Sci Transl Med. 2015;7:282ra48.
25. Akondy RS, Johnson PL, Nakaya HI, Edupuganti S, Mulligan MJ, Lawson B, et al. Initial viral load determines the magnitude of the human CD8 T cell response to yellow fever vaccination. Proc Natl Acad Sci U S A. 2015;112:3050–5.
26. Staples JE, Gershman M, Fischer M. Yellow fever vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2010;59:1–27.
27. Collaborative group for studies on yellow fever vaccines. Duration of post-vaccination immunity against yellow fever in adults. Vaccine. 2014;32:4977–84.
28. Campi-Azevedo AC, Costa-Pereira C, Antonelli LR, Fonseca CT, Teixeira-Carvalho A, Villela-Rezende G, et al. Booster dose after 10 years is recommended following 17DD-YF primary vaccination. Hum Vaccin Immunother. 2016;12:491–502.
29. De Melo AB, da Paz MC, Magalhães M. Description of a prospective 17DD yellow fever vaccine cohort in Recife, Brazil. AmJTrop Med Hyg. 2011;85(4):739–47.
30. Hepburn MJ, Kortepeter MG, Pittman PR, Boudreau EF, Mangiafico JA, Buck PA, et al. Neutralizing antibody response to booster vaccination with the 17D yellow fever vaccine. Vaccine. 2006;24:2843–9.
31. Muyanja E, Ssemaganda A, Ngauv P, Cubas R, Perrin H, Srinivasan D, et al. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. J Clin Invest. 2014;124: 3147–58.
32. Jean K, Donnelly C, Ferguson N, Garske T. A Meta-analysis of serological response associated with yellow fever vaccination. Am J Trop Med Hyg. 2016;95(6):1435–9.
33. Amanna IJ, Slifka MK. Questions regarding the safety and duration of immunity following live yellow fever vaccination. Expert Rev Vaccines. 2016;15:1519–33.

34. Wold Health Organization. Yellow Fever Situation Report, 10.28.2016. http://apps.who.int/iris/bitstream/10665/250661/1/yellowfeversitrep28Oct16-eng.pdf?ua=1. 2016.
35. Grobbelaar AA, Weyer J, Moolla N, van Vuren PJ, Moises F, Paweska JT. Resurgence of yellow fever in Angola, 2015–2016. Emerg Infect Dis. 2016;22:1854–5.
36. •Barrett AD. Yellow fever in Angola and beyond—the problem of vaccine supply and demand. N Engl J Med. 2016;375:301–3. **Discusses 17D vaccine production and formulation, as well as, alternative approaches to vaccination storages due to the Angola and concurrent outbreaks**.
37. Chan M. Yellow fever: the resurgence of a forgotten disease. Lancet. 2016;387:2165–6.
38. Green A. Yellow fever continues to spread in Angola. Lancet. 2016;387:2493.
39. Wolrd Health Organization.World Yellow Fever Situation Report, 07.08.2016. http://apps.who.int/iris/bitstream/10665/246189/1/yellowsitrep-8Jul2016-eng.pdf. 2016.
40. World Health Organization. World Yellow Fever Situation Report, 05.08.2016 http://apps.who.int/iris/bitstream/10665/247198/1/yellowfeversitrep-5Aug2016-eng.pdf. 2016.
41. World Health Organization. WHO prequalified vaccines, 05.05.2016. https://extranet.who.int/gavi/PQ_Web/Browse.aspx?nav=3. 2016.
42.•• Campi-Azevedo AC, de Almeida EP, Coelho-Dos-Reis JG, Peruhype-Magalhães V, Willela-Rezende G, Quaresma PFF, et al. Subdoses of 17DD yellow fever vaccine elicit equivalent virological/immunological kinetics timeline. BMC Infect Dis. 2014;14:391. **Follow-up to Martins et al. [43] study that undertakes a detailed study of the innate and adaptive immune response following sub-dose vaccination with 17DD.**
43.•• Martins RM, de Maia ML, Farias RH, Camacho LA, Freire MS, Galler R, et al. 17DD yellow fever vaccine: a double blind, randomized clinical trial of immunogenicity and safety on a dose-response study. Hum Vaccin Immunother. 2013;9:879–88. **Study of fractional dosing with 17DD sub-strain, concluding that subdoses as low as 587 IU induced comparable nuetralizing antibodies as a single dose**.
44. World Health Organization Secretariat information paper. Fractional dose yellow fever vaccine as a dose-sparing option for outbreak response, 07.20.2016. http://www.who.int/iris/handle/10665/246236 2016