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Role Of Animal Models in The Study Of Yellow Fever Disease And Vaccine

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ABSTRACT

Yellow fever (YF) is a mosquito-borne disease caused by the neurovirulent and viscerotropic YF virus (YFV). Fevers, nausea, body aches, and fatigue commonly characterize the disease, with around 15% of infected people entering fatal phases. The mosquitos that typically carry YFV roam over Africa and South America, where approximately 1 billion people among 46 countries are at risk. A live-attenuated vaccine, strain 17D, was developed in the 1930s and used ever since. However, cases of adverse events presented after the administration of YF vaccines. Additionally, certain groups cannot take the YF vaccine due to its contraindications. Previous advancements in understanding the YFV and developing the vaccine are attributable to the use of animal models. Model organisms are also critical in implementing current modifications to the YF vaccine. This review will discuss these topics in greater detail with a heavy emphasis on the contribution of animal models to the study of YF, along with alternative methods to maximizing global immunity against YF.

Introduction

Yellow fever virus (YFV) is an arbovirus carried by *Haemagogus* spp. and *Sabethes* spp. mosquitoes in South America as well as *Aede*s spp. mosquitoes found in Africa and South America. Common symptoms of the illness include fevers, nausea, body aches, and fatigue. However, around 15% of people infected with YF enter a critical phase that can lead to organ failure and death [1]. While the production and administration of the current YF vaccine have proven to be effective in protecting travelers and populations in endemic regions, there have been serious adverse events (SAEs), including death following the administration of the vaccine. Vaccine alternatives, modified vaccine administration methods, and advancements to the YF vaccine are necessary to expand immunity to as many people as possible. Using model organisms like mice, hamsters, and nonhuman primates (NHPs) in the study of YF and protection against the virus is critical. These animal models can replicate various aspects of the clinical disease and have provided insight into the pathogenic mechanisms of the virus. During the 19th century, YF was a widely feared disease for which no cause or treatment was known. Now, with the help of animal models, modifications to the YF vaccine are being tested, and alternative administration methods are being developed to protect more people from YF outbreaks than ever before.

Yellow Fever Vaccine Background Information

YF-Vax®, the only YF vaccine licensed in the United States, is manufactured by Sanofi Pasteur, the vaccine division of the French pharmaceutical company Sanofi [2]. Another YF vaccine produced by Sanofi Pasteur is Stamaril®, which has been available in the United Kingdom, Uganda, Scotland, and other countries for decades [3]. The YF vaccine is a live-attenuated virus, meaning it contains a weakened form of the yellow fever virus. The vaccine is prepared by culturing the 17D-204 strain of YFV in living avian leukosis virus-free chicken embryos [4]. 17D-204 is

one of three substrains of the 17D strain of YF. The original 17D vaccine was derived from a wild-type YFV Asibi strain that was isolated from the blood of a patient with YF in 1927 [5, 6, 7]. YFV is grown in mouse and chick embryo cells to make the YF vaccine. The final preparation of the vaccine occurs in eggs [4, 5, 8]. YFV is grown in embryo cells repeatedly to weaken the strength of the virus. Therefore, when the live virus is administered, the body can establish a protective immune response without causing illness [8]. For most people, a single dose of the YF vaccine provides lifelong immunity [9].

Need for A Next-Generation Vaccine

Contraindications

Many people are prevented from receiving the YF vaccine and thus unable to develop immunity to YF due to the vaccine's contraindications. Individuals allergic to eggs, egg products, or other vaccine components like gelatin are at risk of anaphylaxis, a severe allergic reaction [2, 4, 10]. In addition, infants younger than nine months should avoid the vaccine due to an increased risk of encephalitis–inflammation of the brain [10,11]. The vaccine should not be administered to women who are breastfeeding infants younger than nine months old, as reports have raised concerns that the YF vaccine virus can be transmitted through breast milk [11,12]. Individuals with severe immunodeficiency due to HIV/AIDS, a thymus disorder, or other causes should also avoid the YF vaccine [2, 8, 11].

Vaccine Production Process

Organizations, including the World Health Organization (WHO), have designed a global strategy known as Eliminate Yellow fever Epidemics (EYE) in response to a 2016 YF outbreak in Angola [13]. The EYE Strategy aims to protect at-risk populations and prevent the international spread of YF by promoting vaccination activities. It is estimated that between 393.7 and 492.9 million people reside in YFV transmission risk areas that must be vaccinated to achieve the recommended 80% population coverage threshold [14, 15]. Despite several YF vaccine producers, supply has been unable to keep up with demand. Only 80 million vaccines are being produced a year [16]. The YF vaccine production capacity is limited due to the labor-intensive method involving embryonated eggs used to produce the vaccine, which has been relatively unchanged since 1945 [17]. The incidence of YF appears to be increasing in under-vaccinated and historically non-endemic areas. There is an insufficient vaccine supply to meet the needs of mass vaccination campaigns.

Serious Adverse Events (SAEs)

Viscerotropic disease, a highly lethal acute illness resembling severe wild-type disease, was identified and connected to the current YF vaccine, resulting in experts calling for the development of a safer vaccine [18,19]. Data from the U.S. Vaccine Adverse Event Reporting System (VAERS) between 2007-2013 reveal that 3.8 SAEs occur per 100,000 doses [20]. Passive surveillance data from several non-endemic countries reveal rates ranging from 1.3 to 5.1 SAEs per 100,000 doses [21]. Vaccine recipients between 60-69 years old had a rate of 6.5 SAEs per 100,000 doses, and those over 70 years old had a rate of 10.3 SAEs [20]. The primary SAEs include anaphylaxis (0.2–1.8 per 100,000 doses), neurologic disease (0.1–3.9 per 100,000 doses) and viscerotropic disease (0.07–0.4 per 100,000 doses) due to 17D virus infection of the liver and visceral organs [21,22]. While cases of Yellow fever vaccine-associated viscerotropic disease (YEL-AVD) are rare events, the fatality rate is greater than 60% [23]. Older individuals may be at an increased risk for YEL-AVDs due to immune senescence or underlying chronic diseases [23]. Findings from animal models of YF disease support the hypothesis that an impaired innate immune system may allow the spreading of the 17D strain [24, 25].

Animal Models in YF Vaccine Development

Mice

Mice are one of the most commonly used animal models for analyzing human disease, and for a good reason [27]. Mice are not only biologically similar to humans but can also contract several of the same diseases for the same genetic reasons as humans. Mice are also small and have a fast generation time, making them easy to work with. Although mice are naturally immune to infections with flavivirus [26], mice can be genetically manipulated to mimic human diseases and conditions. A129 mice, for example, were modified to have a deficiency in type 1 interferon (IFN-α/β) receptors. This modification allows A129 mice to be susceptible to flavivirus infection when exposed to viscerotropic YFV [24]. After infection, the altered mice developed signs of viscerotropic disease, a symptom of YFV and a vaccine complication seen in humans.

A separate study revealed that an individual's genetic background was associated with early events elicited after YF17DD vaccination [28]. Mouse models with various genetic backgrounds can be used to more accurately assess the safety and protective efficacy of vaccines that may respond differently due to genetic differences [26].

Furthermore, mice are used to test batches of the 17D vaccine to verify safety prior to evaluation in nonhuman primates (NHPs) and people [29].

Hamsters

Another useful small animal model for preclinical testing and analysis of YFV is the Syrian golden hamster (*Mesocricetus auratus*). Similar to mice, the small size and fast generation time of golden hamsters make them easier to work with. The hamster is susceptible to viscerotropic infection with YFV derived from Asibi clinical strains after passages in hamsters [26]. The YF disease progression in hamsters is similar in many ways to humans. The Syrian golden hamster produces immune responses to infectious pathogens comparable to humans, making it an advantageous model for evaluating the pathogenesis of infection and assessing the efficacy of medications and vaccines for those pathogens [30].

A study regarding YFV infection in the Syrian hamster reveals that the results of viremia and immune response tests in hamsters after YF infection are similar to those in rhesus monkeys, a preferred animal model used to investigate many human diseases. The study also observed indications of coagulation abnormalities during the acute phase of YF infection in hamsters, which corresponds with hemorrhagic diathesis and dark-colored hematemesis, signs of YF often observed in humans [31].

Other studies have displayed marked increases in cytokines (IFN-γ, IL-2, TNF-α) for hamsters in the spleen, kidney, and heart but reduced levels in the liver, which reflect levels seen in infected humans [30].

Syrian hamsters can also be utilized to assess various treatments and vaccines, which is crucial to providing proof to support advancement toward clinical trials.

Nonhuman Primates

Nonhuman primate (NHP) models are natural hosts of YFV. Experimental infection of NHPs allows the observation of YFV in a natural host, making them helpful in investigating critical stages of disease progression and developing successful intervention strategies. In fact, the initial passage and characterization of YFV isolates were performed in rhesus macaques (*Macaca mulatta)*, a Southeast Asian monkey species [26].

YF observed in rhesus macaques models much of the disease progression in people. For instance, macaques infected with YFV presented significant pathologic changes in the liver, kidneys, and gastrointestinal tract, which occur in humans **[**32**].** Additionally, lymphopenia, a low lymphocyte count disorder, was observed in infected models– a characteristic similar to severe disease in humans after adverse vaccination events [26].

Another NHP system, the howler monkey (*Alouatta* sp.), also develops human aspects of YF disease like fatal liver failure and massive cell death as a consequence of natural YF infection [33].

NHPs not only aid in uncovering a deeper understanding of the pathogenic mechanisms of YF but also facilitate the evaluation of the 17D vaccine [26]. NHP models are essential in testing vaccine safety and are used for further evaluation after the YF vaccines prove safe in rodent studies.

Figure 1. Benefits and Contributions of Model Organisms to Yellow Fever Study.

Model Organism Type:	- A129 Mice	- Syrian Golden Hamsters	- Rhesus Macaques and Howler Monkeys
Benefits of Model Organism:	- Can be genetically manipulated to mimic human diseases and conditions. - Small. - Relatively short generation time.	- Models disease well. - Small. - Relatively short generation time.	- Similar disease progression to humans. - Ideal model for vaccine study.
Contributions of Model Organism to YF Study:	- Developed signs of a com plication of the YF vaccin seen in humans after mod fication. - Can be used to study role genetics in vaccine response. - Facilitate the evaluation of the 17D vaccine.	- Susceptible to viscerotropic infection with hamster-adapted virus. - Produces immune responses YF comparable to humans. - Facilitate the evaluation of the 17D vaccine.	- Initial passage and characte zation of YFV isolates were performed in rhesus macaques. - Facilitate the evaluation of the 17D vaccine.

YF Vaccine Alternatives

To address the limited production capacity of current YF vaccines and increase YF vaccination, vaccine manufacturers should introduce modern technology into the development process of next-generation YF vaccines.
New vaccine candidates should ideally present lower cases of SAEs and safer profiles for infants, breastfeeding women, individuals with compromised immune systems, and people of old age [34].

Recent research funded and sponsored by Sanofi Pasteur revealed the generation process and preclinical profile of vYF-247, a modified live-attenuated YF vaccine candidate. vYF-247 was selected from twenty-four other vaccine candidate substrains from the Stamaril® and YF-VAX® lineage that were created through transfection of viral genomic RNA into Vero cells cultured in serum-free media. Out of the substrains, vYF-247 possessed the preclinical profile with the lowest neurovirulence, viscerotropic effects, and immunogenicity in animal models [35]. vYF-247 presented with lower neurovirulence compared to the current YF vaccines (Stamaril® and YF-VAX®) but is still in the early stages of testing.

Another approach to counter YF infection through vaccines is using an inactivated whole virus vaccine that would likely not create the adverse side effects associated with viral replication in the live-attenuated YF vaccine. The inactivated vaccine would be produced by using the 17D virus grown in cell culture [36,37].

Besides producing new vaccines, dose-sparing methods--where the vaccine is administered at a reduced dose--are also being considered [38].

Conclusion

The landscape of the current YF vaccine prevents certain people from being able to receive its protection, and in some instances, SAEs have negatively impacted those who took the vaccine. Action is needed to modify the current vaccine to achieve a higher level of global protection, which cannot be accomplished without the help of model organisms. In addition, researchers must continue to explore alternative administration procedures like dose-sparing methods and inactivated vaccines to amplify YF immunity.

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References

1. Centers for Disease Control and Prevention. (n.d.). *Yellow fever*. Centers for Disease Control and Prevention. Retrieved August 22, 2022, from https://wwwnc.cdc.gov/travel/diseases/yellow-fever#:~:text=Yellow%20fever%20is%20a%20disease,organ%20failure%2C%20and%20sometimes%20death

2. Centers for Disease Control and Prevention. (2020, December 18). *Vaccine information*. Centers for Disease Control and Prevention. Retrieved August 22, 2022, from https://www.cdc.gov/yellowfever/healthcareproviders/vaccine-info.html#:~:text=Yellow%20Fever%20Vaccine%20Information%20for%20Healthcare%20Providers&text=Sanofi%20Pasteur%2C%20the%20manufacturer%20of,YF%2DVax%20in%20June%202021.

3. *Stamaril Yellow Fever vaccine*. Welcome to Precision Vaccinations. (n.d.). Retrieved August 22, 2022, from https://www.precisionvaccinations.com/vaccines/stamaril-yellow-fever-vaccine

4. *Package insert - YF-VAX - food and drug administration*. (n.d.). Retrieved August 22, 2022, from https://www.fda.gov/media/76015/download

5. Frierson, J. G. (2010, June). *The yellow fever vaccine: A history*. The Yale journal of biology and medicine. PMID: 20589188

6. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892770/
Davis, E. H., Thompson, J. K., Widen, S. G., & Barrett, A. D. T. (2021, July 16). *Genome characterization of yellow fever virus wild-type strain Asibi, parent to live-attenuated 17D vaccine, from three different sources*. Viruses. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8310116/. doi:10.3390/v13071383

7. *Defining stage-specific activity of potent new inhibitors of ...* (n.d.). Retrieved August 22, 2022, from https://journals.asm.org/doi/10.1128/mBio.00052-20. doi: https://doi.org/10.1128/mBio.01956-15

8. The Children's Hospital of Philadelphia. (2014, August 23). *Yellow fever vaccine*. Children's Hospital of Philadelphia. Retrieved August 22, 2022, from https://www.chop.edu/centers-programs/vaccine-education-

center/vaccine-details/yellow-fever-vaccine#:~:text=The%20yellow%20fever%20vaccine%20is%20made%20by%20growing%20yellow%20fever,and%20over%20again%20weakened%20it

9. Centers for Disease Control and Prevention. (2021, April 21). *Yellow fever vaccine*. Centers for Disease Control and Prevention. Retrieved August 22, 2022, from https://www.cdc.gov/yellowfever/vaccine/index.html

10. *Yellow fever vaccine information*. Sanofi U.S. (n.d.). Retrieved August 22, 2022, from https://www.sanofi.us/en/products-and-resources/vaccines/yellow-fever-vaccine-information

11. World Health Organization. (n.d.). *Yellow fever*. World Health Organization. Retrieved August 22, 2022, from https://www.who.int/news-room/fact-sheets/detail/yellow-fever#:~:text=Vaccination%20is%20the%20most%20important,fever%20vaccine%20is%20not%20needed

12. Neale, T. (2010, February 13). *Nursing mothers should avoid yellow fever vaccine*. Medical News. Retrieved August 22, 2022, from https://www.medpagetoday.com/infectiousdisease/generalinfectiousdisease/18466#:~:text=A%20single%20case%20report%20has,baby%20through%20her%20breast%20milk

13. *Eliminate yellow fever epidemics (eye) - world health organization*. (n.d.). Retrieved August 22, 2022, from https://apps.who.int/iris/bitstream/handle/10665/272408/9789241513661-eng.pdf

14. *193–204 no 16 weekly epidemiological record relevé ... - who*. (n.d.). Retrieved August 22, 2022, from https://apps.who.int/iris/bitstream/handle/10665/255040/WER9216.pdf?sequence=1

15. Shearer, F. M., Moyes, C. L., Pigott, D. M., Brady, O. J., Marinho, F., Deshpande, A., Longbottom, J., Browne, A. J., Kraemer, M. U. G., O'Reilly, K. M., Hombach, J., Yactayo, S., de Araújo, V. E. M., da Nóbrega, A. A., Mosser, J. F., Stanaway, J. D., Lim, S. S., Hay, S. I., Golding, N., & Reiner, R. C. (2017, November). *Global yellow fever vaccination coverage from 1970 to 2016: An adjusted retrospective analysis*. The Lancet. Infectious diseases. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5666204/. doi: 10.1016/S1473-3099(17)30419-X

16. Barrett, A. D. T., Dr. Alan D.T. Barrett on the current yellow fever epidemic and lessons about vaccine supply and global health. (08:12), Author AffiliationsFrom the Department of Pathology, Dr. Alan D.T. Barrett on the current yellow fever epidemic and lessons about vaccine supply and global health. (08:12), Derse, A. R., Davis, H. E. and C. S., Daval, C. J. R., C. T. Bramante and Others, J. P. Thornhill and Others, & S. S. Abdool Karim and N. Devnarain. (1970, August 20). *Yellow fever in Angola and beyond - the problem of vaccine supply and demand: Nejm*. New England Journal of Medicine. Retrieved August 22, 2022, from https://www.nejm.org/doi/10.1056/NEJMp1606997?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0www.ncbi.nlm.nih.gov. doi: 10.1056/NEJMp1606997

17. Ferguson, M., Shin, J., Knezevic, I., Minor, P., Barrett, A., & WHO Working Group. (2010, December 6). *Who Working Group on technical specifications for manufacture and evaluation of yellow fever vaccines, Geneva,*

Switzerland, 13-14 May 2009. Vaccine. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7131007/. doi: 10.1016/j.vaccine.2010.10.070

18. EB;, H. (n.d.). *Is it time for a new yellow fever vaccine?* Vaccine. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/20971115/. doi: 10.1016/j.vaccine.2010.10.015

19. TP;, M. (n.d.). *Review of the risks and benefits of yellow fever vaccination including some new analyses*. Expert review of vaccines. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/22551029/. doi: 10.1586/erv.12.6

20. Lindsey NP;Rabe IB;Miller ER;Fischer M;Staples JE; (n.d.). *Adverse event reports following Yellow Fever Vaccination, 2007-13*. Journal of travel medicine. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/27378369/. doi: 10.1093/jtm/taw045

21. MK;, A. I. J. S. (n.d.). *Questions regarding the safety and duration of immunity following live yellow fever vaccination*. Expert review of vaccines. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/27267203/. doi: 10.1080/14760584.2016.1198259

22. Gershman MD;Staples JE;Bentsi-Enchill AD;Breugelmans JG;Brito GS;Camacho LA;Cottin P;Domingo C;Durbin A;Gascon J;Guenaneche F;Hayes EB;Jelenik Z;Khromava A;Martins Rde M;Wilson MM;Massy N;Nasidi A;Niedrig M;Sherwat A;Tsai T;Vilella A;Wilson ME;Kohl KS; ; (n.d.). *Viscerotropic disease: Case definition and guidelines for collection, analysis, and presentation of Immunization Safety Data*. Vaccine. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/22561144/. doi: 10.1016/j.vaccine.2012.04.067

23. Tomashek, K. M., Challberg, M., Nayak, S. U., & Schiltz, H. F. (2019, November 8). *Disease resurgence, production capability issues and safety concerns in the context of an aging population: Is there a need for a new yellow fever vaccine?* Vaccines. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6963298/. doi: 10.3390/vaccines7040179

24. Meier KC;Gardner CL;Khoretonenko MV;Klimstra WB;Ryman KD; (n.d.). *A mouse model for studying viscerotropic disease caused by yellow fever virus infection*. PLoS pathogens. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/19816561/. doi: 10.1371/journal.ppat.1000614

25. JK;, E. A. K. P. (n.d.). *Dynamic viral dissemination in mice infected with yellow fever virus strain 17D*. Journal of virology. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/24027319/. doi: 10.1128/JVI.02149-13

26. Julander, J. G. (2016, April 16). *Animal models of yellow fever and their application in Clinical Research*. Current Opinion in Virology. Retrieved August 22, 2022, from https://www.sciencedirect.com/science/article/pii/S1879625716300426#sec0020. doi: https://doi.org/10.1016/j.coviro.2016.03.010

27. *What is a mouse model?* The Jackson Laboratory. (n.d.). Retrieved August 22, 2022, from https://www.jax.org/why-the-

mouse/model#:~:text=Mice%20are%20the%20most%20commonly,any%20human%20disease%20or%20condition

28. Azamor, T., da Silva, A. M. V., Melgaço, J. G., Dos Santos, A. P., Xavier-Carvalho, C., Alvarado-Arnez, L. E., Batista-Silva, L. R., de Souza Matos, D. C., Bayma, C., Missailidis, S., Ano Bom, A. P. D., Moraes, M. O., & da Costa Neves, P. C. (2021, January 12). *Activation of an effective immune response after yellow fever vaccination is associated with the genetic background and early response of IFN-γ and CLEC5A*. Viruses. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7828179/. doi: 10.3390/v13010096

29. Moulin, J.-C., Silvano, J., Barban, V., Riou, P., & Allain, C. (2013, June 10). *Yellow fever vaccine: Comparison of the neurovirulence of new 17D-204 Stamaril™ seed lots and RK 168-73 strain*. Biologicals. Retrieved August 22, 2022, from https://www.sciencedirect.com/science/article/pii/S1045105613000535. doi: https://doi.org/10.1016/j.biologicals.2013.04.005

30. Miao, J., Chard, L. S., Wang, Z., & Wang, Y. (1AD, January 1). *Syrian hamster as an animal model for the study on infectious diseases*. Frontiers. Retrieved August 22, 2022, from https://www.frontiersin.org/articles/10.3389/fimmu.2019.02329/full. doi :https://doi.org/10.3389/fimmu.2019.02329

31. Tesh, R. B., Guzman, H., da Rosa, A. P. A. T., Vasconcelos, P. F. C., Dias, L. B., Bunnell, J. E., Zhang, H., & Xiao, S.-Y. (2001, May 15). *Experimental yellow fever virus infection in the Golden Hamster (mesocricetus auratus). I. Virologic, biochemical, and immunologic studies*. OUP Academic. Retrieved August 22, 2022, from https://academic.oup.com/jid/article/183/10/1431/891721. doi: https://doi.org/10.1086/320199

32. Engelmann, F., Josset, L., Girke, T., Park, B., Barron, A., Dewane, J., Hammarlund, E., Lewis, A., Axthelm, M. K., Slifka, M. K., & Messaoudi, I. (n.d.). *Pathophysiologic and transcriptomic analyses of viscerotropic yellow fever in a rhesus macaque model*. PLOS Neglected Tropical Diseases. Retrieved August 22, 2022, from https://journals.plos.org/plosntds/article?id=10.1371%2Fjournal.pntd.0003295 doi: https://doi.org/10.1371/journal.pntd.0003295

33. Leal SG;Romano AP;Monteiro RV;Melo CB;Vasconcelos PF;Castro MB; (n.d.). *Frequency of histopathological changes in howler monkeys (alouatta sp.) naturally infected with yellow fever virus in Brazil*. Revista da Sociedade Brasileira de Medicina Tropical. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/27163561/. doi: 10.1590/0037-8682-0363-2015

34. Tomashek, K. M., Challberg, M., Nayak, S. U., & Schiltz, H. F. (2019, November 8). *Disease resurgence, production capability issues and safety concerns in the context of an aging population: Is there a need for a new yellow fever vaccine?* Vaccines. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6963298/. doi: 10.3390/vaccines7040179

35. Piras-Douce, F., Raynal, F., Raquin, A., Girerd-Chambaz, Y., Gautheron, S., Sanchez, M. E. N., Vangelisti, M., & Mantel, N. (2021, March 26). *Next generation live-attenuated yellow fever vaccine candidate: Safety and immuno-efficacy in small animal models*. Vaccine. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8047865/. doi: 10.1016/j.vaccine.2021.02.033

36. Pereira RC;Silva AN;Souza MC;Silva MV;Neves PP;Silva AA;Matos DD;Herrera MA;Yamamura AM;Freire MS;Gaspar LP;Caride E; (n.d.). *An inactivated yellow fever 17DD vaccine cultivated in Vero cell cultures*.

Vaccine. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/25862300/. doi: 10.1016/j.vaccine.2015.03.077

37. Monath TP;Lee CK;Julander JG;Brown A;Beasley DW;Watts DM;Hayman E;Guertin P;Makowiecki J;Crowell J;Levesque P;Bowick GC;Morin M;Fowler E;Trent DW; (n.d.). *Inactivated yellow fever 17D vaccine: Development and nonclinical safety, Immunogenicity and protective activity*. Vaccine. Retrieved August 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/20347059/. doi: 10.1016/j.vaccine.2010.03.023

38. Roukens, A. H., Vossen, A. C., Bredenbeek, P. J., van Dissel, J. T., & Visser, L. G. (2008, April 23). *Intradermally administered yellow fever vaccine at reduced dose induces a protective immune response: A randomized controlled non-inferiority trial*. PloS one. Retrieved August 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2297511/.