Effects of fractional dose yellow fever vaccination: a systematic review and meta-analysis

---

by

Chukwudi Arnest Nnaji

NNJCHU001

A thesis submitted to the Faculty of Health Sciences, University of Cape Town, in partial fulfilment of the requirements for the degree of Master of Public Health (MPH)

Supervisor:

Professor Charles S. Wiysonge

Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, University of Cape Town, South Africa;

Cochrane South Africa, South African Medical Research Council, Cape Town, South Africa

Co-Supervisors:

Dr Muki S. Shey

Department of Medicine, Faculty of Health Sciences, University of Cape Town, South Africa

Dr Olatunji O. Adetokunboh

Division of Epidemiology and Biostatistics, Department of Global Health, Stellenbosch University, South Africa;

Cochrane South Africa, South African Medical Research Council, Cape Town, South Africa

DECLARATION

I, Chukwudi Arnest Nnaji, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: Signed by candidate

Date: 23 October 2018

THESIS ABSTRACT

Persistent yellow fever endemicity and continued outbreaks have continued to increase vaccine demand, while straining global vaccine supply. Fractional dose vaccination is being considered as a dose-sparing strategy to mitigate current global vaccine shortages. This study therefore assessed the effects of fractional dose yellow fever vaccination, in comparison to those of the standard dose. We registered the review in the prospective register of systematic reviews; conducted a comprehensive search of published and grey literature; used standard Cochrane methods to collect and synthesise the evidence and followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidance. We stratified analyses by the strength of the fractional dose. We retrieved 2495 records from the literature search, nine of them potentially eligible. We included six eligible studies (three randomised and three quasi-randomised trials), with 2371 participants. There was no statistically significant difference in immunogenicity between participants who received fractional doses containing one-third (two trials, 547 participants: RR 1.02, 95% CI 1.00 to 1.04; I^2 = 0%), 1/5th (one trial, 155 participants: RR 1.00, 95% CI 0.98 to 1.03), 1/10th (four trials, 890 participants: RR 0.99, 95% CI 0.96 to 1.01, I^2 = 0%), and 1/50th (three trials, 661 participants: RR 0.97, 95% CI 0.92 to 1.02, I^2 = 72%) of the standard dose and those who received the full standard dose. However, immunogenicity was significantly lower among participants who received fractional doses containing 1/100th (four trials, 868 participants: RR 0.92, 95% CI 0.87 to 0.97, I^2 = 60%) and <1/100th (five trials, 1053 participants; RR 0.53, 95% CI 0.44 to 0.64, I^2= 98%) of the standard dose compared to participants who received the full standard dose. Minor adverse events following vaccination did not differ across doses, but no serious adverse events were reported in any study arm. The combined data provide moderate certainty evidence that there is little or no difference in immunogenicity between ≥1/50th fractional doses and the standard dose of yellow fever vaccines. However, these studies were of short duration ranging from four weeks to a year. These findings support the use of fractional dose vaccination as a dose-sparing strategy for yellow fever vaccination.

ACKNOWLEDGEMENTS

I would like to immensely thank my supervisor, Professor Charles S. Wiysonge for his tireless support, guidance and mentoring through every step of this thesis, from conception to completion. I am also very grateful to my co-supervisors, Dr Muki S. Shey and Dr Olatunji O. Adetokunboh, for their enormous methodological input and valuable guidance.

My gratitude also goes to Ms. Mary Shelton, the faculty's reference librarian for her valuable time and contribution to the development of the search strategies and to Dr Wiyeh Beriliy for the insightful insights she provided for the GRADE of evidence.

Finally, my profound gratitude goes to my sweetheart, Chisom; to my dear parents, Chief Michael and Mrs. Cordelia Nnaji; and to my lovely siblings, Emeka, Amobi, Ify and Uju; for their steadfast material, moral and psychological support through this journey of my postgraduate study.

To God be the glory.

DISSERTATION CONTENTS

In line with the current recommendations on the structure of MPH dissertations at UCT, the contents of this dissertation are as follows:

PART A – Protocol for a systematic review and meta-analysis of the effects of fractional dose yellow fever vaccination

PART B – Literature review: Overview of yellow fever epidemiology, vaccination and fractional dose vaccination

PART C – Journal article: A systematic review and meta-analysis of the effects of fractional dose yellow fever vaccination

PART D - Appendices

PART A: PROTOCOL

Protocol Table of Contents

Protocol abstract

Background: Persistent yellow fever endemicity and continued outbreaks have continued to increase vaccine demand, while straining limited global vaccine supply. To vaccinate susceptible populations in preventive mass-immunisation campaigns during outbreaks, fractional dose yellow fever vaccine is being considered as a dose-sparing strategy to maximise limited vaccine supplies. The purpose of this review is to assess the effects of this strategy in comparison with those of standard dose of the vaccine.

Aims: This study aims to assess the effects (immunogenicity, efficacy, effectiveness or safety) of fractional-dose yellow fever vaccination in comparison to standard dose vaccination.

Methods: We will conduct a comprehensive search of electronic databases and reference lists of relevant publications; and will follow the guidance contained in the statement on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA). Studies that compared the effects and safety of fractional dose yellow fever vaccine with those of the standard dose vaccine will be included, regardless of study design. We will pool data using random-effects meta-analyses. We will assess statistical heterogeneity using the Chi-squared test of homogeneity and quantify it using the Higgins' I^2 statistic. In the presence of statistically significant heterogeneity, we will conduct subgroup analyses, with subgroups defined by variations in study design, HIV status and region.

Discussion: This review will provide a robust evidence-base for informing global health and vaccine policy and advocacy processes on the use of factional doses of vaccines as a dose-sparing strategy to mitigate vaccine shortages, while providing clinicians and immunisation programme stakeholders with a user-friendly evidence summary. It will also help to highlight remaining evidence and research gaps on fractional dose vaccination, to inform future research efforts.

Keywords: Yellow fever; vaccine; fractional dose; dose-sparing; immunogenicity; efficacy; effectiveness; safety.

1. Background

1.1. Description of the condition

Yellow fever is a viral haemorrhagic disease of humans caused by the yellow fever virus.[1] The disease mostly occurs in tropical areas of Africa and South America, where it is endemic and intermittently epidemic.[1 2] The yellow fever virus is a prototypic member of the genus *Flavivirus* (*flavus* meaning yellow in Latin) having a relatively narrow host range, mostly humans and monkeys; and typically transmitted by *Aedes* mosquitoes.[2 3] Globally, approximately 200 000 cases of yellow fever and 30 000 deaths occur annually.[4 5] Sub-Saharan Africa, where the disease remains a major global health concern, bears 90% of the disease burden.[4] Typically, humans are infected when bitten by blood-feeding mosquitoes.[2] Susceptibility to yellow fever depends on several factors, such as previous infection with the virus and other flaviviruses; immune status, environmental, racial and genetic factors.[6 7] Transmission is largely dependent on availability of vector, vertebrate hosts and vegetation.[8]

In humans, yellow fever disease can be asymptomatic or cause a wide spectrum of diseases, from mild symptoms to severe illness with fever, nausea, vomiting, hepatitis, jaundice and, in extreme cases, haemorrhagic shock and death.[2 5] Case fatality ranges between 20 and 60%.[9] There is no known cure or specific treatment for yellow fever, hence supportive treatment remains the mainstay of clinical management.[2] Wild-type yellow fever infection can induce lifelong protection against subsequent infection.[9]

Yellow fever poses a significant threat to unvaccinated persons travelling to endemic areas.[10] With globalisation and ease of international travel, there has been documented cases of human importation of the disease from endemic areas to places where it is non-endemic.[10] Recently, imported cases have been reported in China, Kenya and Morocco.[11 12 13] Due to transmission between non-human primates and mosquitoes, and by transovarial transmission in mosquitoes, eradication of the disease is extremely difficult.[2 9]

The development of a life-attenuated yellow fever vaccine which came into use in 1938, and its wide roll-out in the 1940s, results in significant decline in the burden of disease.[9 14] The World Health Organisation (WHO) recommends vaccination in high-risk countries, as part of the routine

childhood immunisation programmes as well as in mass immunisation campaigns during outbreaks.[4] [10] Additionally, vaccination is recommended for preventive immunisation of travellers to endemic regions, making yellow fever the only disease stipulated by international health regulations (IHR) for which proof of vaccination from travellers may be required by countries as a pre-condition for entry.[4]

Yellow fever vaccine has been considered effective, immunogenic, safe and well tolerated.[4] [8] [14-17] There are numerous serological methods used to assess immune response. These include plaque reduction neutralisation, haemagglutination inhibition and complement fixation tests, as well as enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody test.[7] [9] Currently, detection and analysis of the immune response post-vaccination are mostly done using the plaque reduction neutralisation test [8] [13], and is considered to be the most specific and gold-standard method.[4]

Adverse events following immunisation are classified based on severity as either non-serious or serious adverse events. The WHO classifies an adverse event as serious if it results in persistent or significant disability; a congenital anomaly or birth defect; intervention to prevent permanent impairment, hospitalisation or prolongation of hospitalisation; or death.[18] Reported serious adverse events include anaphylactic or hypersensitivity reactions; Yellow fever vaccine-associated viscerotropic disease which mimics naturally-acquired yellow fever disease and yellow fever vaccine-associated neurologic disease, which can manifest as meningoencephalitis, Guillain-Barré syndrome, or acute disseminated encephalomyelitis.[18-21] The Brighton Collaboration case definitions provide a standardised classification of these serious adverse events using three distinct levels of diagnostic certainty, with levels 1, 2 and 3 representing the highest, intermediate and lowest levels of diagnostic certainty respectively.[21-25]

Reporting rates for serious adverse events vary, due to differences in routes of vaccine administration, case definitions, study designs, surveillance methods, frequency of reporting, diagnostic capability and availability of other health-systems resources.[26] [27] Surveillance and adverse events monitoring during preventive vaccination campaigns in eight African countries showed low adverse events incidence attributable to underreporting and other structural

challenges. There were reports of non-adherence to standard criteria for assigning causality due to the unavailability of certain laboratory investigations, with the majority of reported events not meeting the Brighton case definitions. There were also reports of improper sample labeling, faulty containers, improper storage, and delays between collection and transport.[27]

1.2. Description of the intervention

Persistent endemicity and continued outbreaks have continued to increase vaccine demand, while straining limited global vaccine supply.[4 13] Therefore, to vaccinate susceptible populations in preventive mass immunisation campaigns during outbreaks, fractional dosing of the vaccine is being considered as a dose-sparing strategy to maximise limited vaccine supplies.[4 13] Fractional dose yellow fever vaccination refers to administration of a reduced volume of vaccine dose, which has been reconstituted as per manufacturer recommendations.[4] The minimum potency recommended for standard dose should not be less than 1000 international units (IU)/dose.[13 28] However, the potency of standard doses is usually many-fold higher than the recommended potency.[4] This forms the fundamental basis of fractional dose considerations. As a dose-sparing strategy, a fractional dose yellow fever vaccine meeting the minimum potency requirement is expected to be equivalent to a standard yellow fever vaccine dose with respect to safety, immunogenicity, efficacy and effectiveness.[4 29 30]

The first practical use of fractional dose yellow fever vaccination was in response to a large yellow fever outbreak in the Democratic Republic of the Congo in mid-2016.[4 13] This was to ensure that the entire target population could be vaccinated despite limited vaccine supply. The WHO International Coordinating Group on vaccine coordinates the access to and provision of vaccines during outbreaks.[4] It currently maintains a global emergency stockpile of six million doses of yellow fever vaccine, which is currently being threatened by increasing demands.[13 31]

1.3. How the intervention might work

Fractional dose vaccination against yellow fever works by stimulating humoral immunity through neutralising antibodies against the yellow fever virus.[30] Various studies have investigated the protective effects and safety of fractional doses of the yellow fever vaccine in humans.[29 30 32-38] This review has systematically summarised the findings from these studies.

1.4. Why it is important to do this review

We have found two non-systematic reviews of fractional dose yellow fever vaccination in the grey literature.[13 28] However, we are unaware of any currently available comprehensive and systematic synthesis of the body of evidence on the effects and safety of this strategy, thus informing the need for this review. The use of meta-analysis in vaccinology has grown remarkably in recent years.[39] It helps to produce quantitatively robust and accurate effect-size measurements that are generalisable.[40 41] This review will add to the body of knowledge and fill knowledge gap, in addition to providing a robust evidence base for informing global health policy and advocacy processes for addressing vaccine shortages. Furthermore, the analytical rigour with which the review will be conducted will provide methodological guidance for subsequent reviews, while providing vaccinologists, clinicians, policy makers and other stakeholders a user-friendly evidence summary. Current international best practices in the conduct of systematic reviews and meta-analyses will be followed; including registration on the International Prospective Register of Systematic Reviews (PROSPERO) [42 43] and design of this protocol in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) guidelines.[44]

2. Objectives

This review aims to assess the effects of fractional dose yellow fever vaccination, compared to vaccination using the standard dose of the vaccine.

3. Methods

3.1. Types of studies

There will not be restriction on inclusion based on study-design; randomised trials, non-randomised trails, and observational studies will be eligible for inclusion.

3.2. Types of participants

All individuals, irrespective of age will be included.

3.3. Types of intervention

The eligible intervention will be the administration of fractional doses of any live-attenuated yellow fever vaccine, while the eligible comparison will be the administration of the standard dose of the vaccine; irrespective of vaccination schedule, route of administration, or formulation.

3.4. Types of outcome measures

3.4.1. Primary outcomes

- Immunogenicity i.e. levels of vaccine-specific virus-neutralising antibodies and rates of seroconversion, assessed at least four weeks following vaccination.[45]
- Safety i.e. adverse events following vaccination as reported by authors and standardised using the WHO and the Brighton Collaboration case definitions.[18-21]

3.4.2. Secondary 0utcomes

- Incidence of laboratory-confirmed yellow fever cases[9 46]
- Mortality

3.5. Search methods

A comprehensive electronic literature search will be conducted, from inception of each database to the date of the search. The following databases will be searched: Cochrane Library (including the Cochrane Central Register of Controlled Trials (CENTRAL) and the Database of Abstracts of Reviews of Effects (DARE)), PubMed/Medline, EBSCOhost, Scopus, CINAHL, ClinicalTrials.gov, WHO International Clinical Trials Registry Platform (ICTRP), African Index Medicus, and Latin American and Caribbean Health Sciences Literature (LILACS). No language restrictions will be applied. The reference lists of relevant publications, including WHO position papers, will also be

searched for potentially eligible studies. The websites of WHO approved yellow fever vaccine manufacturers will also be searched. Additionally, relevant grey literature will be searched for relevant vaccination field reports, policy statements, and conference abstracts and proceedings.

A provisional PubMed format search strategy is provided below (Table 1). It was developed with guidance from a reference librarian. The strategy will be adapted for other databases using appropriate controlled vocabulary and syntax.

Table 1: Provisional PubMed search strategy

Search #	**Search Texts and Syntaxes**
#1	Yellow Fever [MeSH Terms]
#2	Yellow Fever Vaccine [MeSH Terms]
#3	yellow fever vaccine OR yellow fever vaccination OR yellow fever immunisation
#4	#1 OR #2 OR #3
#5	Dose-Response Relationship, Immunologic [MeSH Terms]
#6	fractional dosing OR Fractionated dosing OR drug dose comparison OR sub-dose OR sub-dosage OR reduced dose OR reduced dosing OR reduced dosage OR dose-sparing OR immunologic dose response relationship
#7	#5 OR #6
#8	safety OR adverse reaction OR adverse event OR adverse effects OR toxicity OR reactogenicity OR immunogenicity OR immunogenic OR immune response OR seroconversion OR efficacy OR effects OR effectiveness
#9	#7 OR #8
#10	#4 AND #9

The project team will be made up of four contributors:

- Chukwudi A. Nnaji (CAN) will be the primary investigator. He will take lead in all aspects of the project and be the guarantor of the publication.
- Muki S. Shey (MSS) will contribute to duplicate screening of search outputs, study selection, data extraction from included studies, and verification of data generated. He will provide immunological insights to the review and serve as a co-supervisor for this project.
- Olatunji O. Adetokunboh (OOA) will contribute to duplicate screening of search outputs, study selection, data extraction from included studies, and verification of data generated. He will provide systematic review guidance to the primary investigator and serve as a co-supervisor for this project.
- Charles S. Wiysonge (CSW) will be a supervisor for this project and will be responsible for general guidance in conducting the review. He will arbitrate in cases where there are discrepancies in the independent assessment of eligibility and risk of bias or extracted data, that are not resolved through consensus.

3.6. Data extraction and management

Two researchers (CAN and either OOA or MSS) will independently screen the search output, to retrieve full texts of potentially eligible studies and assess their eligibility using predefined inclusion criteria. Disagreements in the eligibility assessment will be resolved through consensus between the two researchers and, if a consensus is not arrived at, CSW will arbitrate. If a study published in a language other than English is deemed eligible for inclusion, a translation will be sought.

Following the selection of eligible studies, two researchers (CAN and either OOA or MSS) will independently extract relevant data using a standardised data extraction form. Study eligibility will be re-verified at the start of data extraction. Extracted data will include:

- General details: authors' details, affiliations, and year of publication.
- Study details: design, setting, geographical location, risk of bias items
- Participant characteristics: mean age and standard deviation, age range, sex, and sample size.

- Intervention details: vaccine sub-strain, strength of fractional dose, and route of administration
- Outcome details: types of outcomes, outcome assessment methods, outcome data.

Any disagreements between the two investigators will be resolved by discussion and, if a consensus is not arrived at, CSW will arbitrate. If required data are not available from study publications, CAN will contact the authors in an attempt to obtain the missing information. Extracted data will be entered by CAN into the analysis software, Review Manager 5.3.[47] CSW will double-check the entered data for accuracy.

3.7. Risk of bias assessment

Two authors (CAN and either OOA or MSS) will independently assess each included study for risk of bias. For randomised trials, we will use the seven specific domains of the Cochrane risk of bias tool.[48 49] The seven domains include random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other issues. For each included study, the two researchers will independently describe and make judgement of "low risk" of bias, "high risk" of bias, or "unclear risk" of bias accordingly.

We will use the risk of bias in nonrandomised studies of interventions (ROBINS-I) tool for assessing risk of bias in non-randomised studies.[50] The tool covers seven distinct domains for assessing risk of bias, including confounding; selection; intervention classification; deviations from intended interventions; missing data; measurement of outcomes; and selection of reported results. It categorises risk of bias judgements as "low risk", "moderate risk", "serious risk" and "critical risk" of bias; with "low risk" corresponding to the risk of bias in a high quality randomised trial. The two researchers will compare their independent risk of bias assessments and resolve any discrepancies by discussion and consensus.; with arbitration by a third researcher (MSS or CSW).

3.8. Data analyses

3.8.1. Measures of intervention effect

We will analyse and report risk ratios (RR) with their corresponding 95% confidence intervals (CIs) for dichotomous data. For continuous outcomes, we will present mean differences (MD) with their corresponding 95% CIs.

3.8.2. Dealing with missing data

We will contact the corresponding authors to provide any unreported data relevant for analyses. If this does not yield a response, such unreported data will be classified as missing data and will be treated as such in the risk of bias assessment and analyses for the affected study. If the amount of incomplete outcome data is such that the trial is thought to be at a high risk of attrition bias, we will use imputation and perform sensitivity analyses to investigate the impact of the missing data.

3.8.3. Data synthesis

We will use the random-effects method to combine data from included studies, stratified by the strength of the fractional dose. We will assess statistical heterogeneity across included studies using the Chi-Squared test of homogeneity (with significance defined at the 10% α-level) and quantify it using the Higgins' I^2 statistic.[48 51] Where meta-analysis is not appropriate, we will present narrative reports of findings.

3.8.4. Subgroup analyses

We will explore possible causes of significant statistical heterogeneity of effects by using subgroup analyses, with subgroups defined by study design (randomised versus non-randomised), continent where study was conducted, and HIV status (positive versus negative).

3.8.5. Sensitivity analyses

We plan to perform sensitivity analyses for aspects of the review that could potentially affect the results, including high risk of bias and funnel plot asymmetry. We will consider a randomised trial to have a high risk if there was no allocation concealment, no blinding of outcome assessors, or differential loss to follow-up in intervention arms of more than 25%. For non-randomised studies, the definition of high risk of bias will focus on selection of participants, missing data, and

measurement of outcomes. If we have evidence to suggest that small study effects are influencing the results of a meta-analysis, we will conduct sensitivity analyses to assess the robustness of the meta-analysis conclusions to different assumptions about the causes of funnel plot asymmetry.

3.8.6. Assessment of reporting bias

Funnel plots of estimated differences in outcome effects against their standard errors will be done if at least 10 studies are included in the meta-analysis. As a rule of thumb, funnel plot asymmetry should be used only when there are at least 10 studies included in the meta-analysis, because fewer studies will not have adequate power to distinguish chance from real asymmetry.[48]

3.9. Grading the certainty of evidence

We will use the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to assess the certainty of the evidence.[52] We will generate GRADE evidence profiles and summary of findings tables for each primary outcome measure.

4. Ethics and dissemination

The planned systematic review will involve literature available through publicly accessible electronic databases, therefore research ethics review and approval are not required. We thus applied and obtained a waiver from the Human Research Ethics Committee of the University of Cape Town (#303/2018).

5. Dissemination plans

We will disseminate the findings of this review through Master's Degree thesis for CAN, presentation at relevant scientific meetings, and publication in a peer-reviewed journal.

6. Discussion

The use of meta-analyses in vaccinology, in the context of systematic reviews, has grown remarkably in recent years.[39] Meta-analyses help to produce quantitatively robust and accurate effect-size measurements that are generalisable.[40 41] Although randomised studies, due to their

methodological rigour, help to contribute high quality to meta-analyses, the inclusion of other study designs allows meta-analyses to include relevant studies that have the potential to address the research question as comprehensively as possible. This poses the risk of introducing studies of low quality and compromising the certainty of the evidence generated from pooled estimates. The impacts can be addressed using the risk of bias appraisal, subgroup analyses defined by study designs as well as GRADE of evidence in the interpretation of pooled estimates.

This review will add to the body of knowledge on the merits of fractional dose yellow fever vaccination. The analytical rigour with which the review will be conducted will provide methodological guidance for subsequent reviews, while providing vaccinologists, clinicians, policy makers and other stakeholders with a user-friendly evidence summary.

7. Declaration of interests

None.

8. References

1. Wiysonge CS, Nomo E, Mawo J, et al. Yellow fever control in Cameroon: where are we now and where are we going? *BMC medicine* 2008;6:3. doi: 10.1186/1741-7015-6-3 [published Online First: 2008/02/12]
2. Monath TP, Vasconcelos PF. Yellow fever. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* 2015;64:160-73. doi: 10.1016/j.jcv.2014.08.030 [published Online First: 2014/12/03]
3. Barrett AD, Higgs S. Yellow fever: a disease that has yet to be conquered. *Annual review of entomology* 2007;52:209-29. doi: 10.1146/annurev.ento.52.110405.091454 [published Online First: 2006/08/18]
4. World Health Organization (WHO). WHO position on the use of fractional doses - June 2017, addendum to vaccines and vaccination against yellow fever WHO: Position paper - June 2013. *Vaccine* 2017;35(43):5751-52. doi: 10.1016/j.vaccine.2017.06.087 [published Online First: 2017/07/12]
5. World Health Organization (WHO). Vaccines and vaccination against yellow fever: WHO Position Paper, June 2013--recommendations. *Vaccine* 2015;33(1):76-7. doi: 10.1016/j.vaccine.2014.05.040 [published Online First: 2014/05/24]
6. Gubler DJ. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? *Comparative immunology, microbiology and infectious diseases* 2004;27(5):319-30. doi: 10.1016/j.cimid.2004.03.013 [published Online First: 2004/07/01]
7. Vainio J, Cutts F. Yellow fever. WHO/EPI/GEN/9811. Geneva: World Health Organization; 1998. p. 1–87.
8. Mutebi JP, Barrett AD. The epidemiology of yellow fever in Africa. *Microbes and infection* 2002;4(14):1459-68. [published Online First: 2002/12/12]
9. Barnett ED. Yellow fever: epidemiology and prevention. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007;44(6):850-6. doi: 10.1086/511869 [published Online First: 2007/02/17]

10. Gardner CL, Ryman KD. Yellow Fever: A Reemerging Threat. *Clinics in laboratory medicine* 2010;30(1):237-60. doi: 10.1016/j.cll.2010.01.001
11. Dou XF, Zheng Y, Lyu YN, et al. [The first confirmed imported case of yellow fever in China]. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi* 2016;37(6):788-90. doi: 10.3760/cma.j.issn.0254-6450.2016.06.009 [published Online First: 2016/06/28]
12. Wilder-Smith A, Leong WY. Importation of yellow fever into China: assessing travel patterns. *Journal of travel medicine* 2017;24(4) doi: 10.1093/jtm/tax008 [published Online First: 2017/04/21]
13. World Health Organization (WHO). Fractional Dose Yellow Fever Vaccine as a Dose-sparing Option for Outbreak Response. WHO Secretariat Information Paper. Department of Immunization, Vaccines and Biologicals. WHO reference number: WHO/YF/SAGE/16.1. Published 20 July 2016. Available via: http://apps.who.int/iris/bitstream/handle/10665/246236/WHO-YF-SAGE-16.1-eng.pdf;jsessionid=F4BD11CEBB8426D52C781267F9795A8A?sequence=1 21 April 2018.
14. Norrby E. Yellow fever and Max Theiler: the only Nobel Prize for a virus vaccine. *The Journal of Experimental Medicine* 2007;204(12):2779.
15. Shearer FM, Moyes CL, Pigott DM, et al. Global yellow fever vaccination coverage from 1970 to 2016: an adjusted retrospective analysis. *The Lancet Infectious Diseases* 2017;17(11):1209-17. doi: 10.1016/S1473-3099(17)30419-X
16. Makhunga-Ramfolo N, Rheeder P. Safety and tolerability of yellow fever vaccines. *Southern African Journal of Epidemiology and Infection* 2012;27(1):16-18. doi: 10.1080/10158782.2012.11441474
17. World Health Organization (WHO). Yellow Fever: Travel and Health. Annex 1 - Update. Released 16 February 2017. Available from: http://www.who.int/ith/2017-ith-annex1.pdf Accessed on 11 November 2017.
18. Thomas RE. Yellow fever vaccine-associated viscerotropic disease: current perspectives. *Drug Design, Development and Therapy* 2016;10:3345-53. doi: 10.2147/DDDT.S99600
19. Thomas RE, Spragins W, Lorenzetti DL. How many published cases of serious adverse events after yellow fever vaccination meet Brighton Collaboration diagnostic criteria? *Vaccine* 2013;31(52):6201-9. [published Online First: 2014/01/18]
20. Thomas RE, Lorenzetti DL, Spragins W, et al. Active and passive surveillance of yellow fever vaccine 17D or 17DD-associated serious adverse events: systematic review. *Vaccine* 2011;29(28):4544-55. doi: 10.1016/j.vaccine.2011.04.055 [published Online First: 2011/05/10]
21. Rüggeberg JU, Gold MS, Bayas J-M, et al. Anaphylaxis: Case definition and guidelines for data collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;25(31):5675-84. doi: https://doi.org/10.1016/j.vaccine.2007.02.064
22. Lindsey NP, Rabe IB, Miller ER, et al. Adverse event reports following yellow fever vaccination, 2007–13. *Journal of travel medicine* 2016;23(5):taw045-taw45. doi: 10.1093/jtm/taw045
23. Gershman MD, Staples JE, Bentsi-Enchill AD, et al. Viscerotropic disease: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2012;30(33):5038-58. doi: 10.1016/j.vaccine.2012.04.067 [published Online First: 2012/05/09]
24. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barre syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29(3):599-612. doi: 10.1016/j.vaccine.2010.06.003 [published Online First: 2010/07/06]
25. Sejvar JJ, Kohl KS, Bilynsky R, et al. Encephalitis, myelitis, and acute disseminated encephalomyelitis (ADEM): case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;25(31):5771-92. doi: 10.1016/j.vaccine.2007.04.060 [published Online First: 2007/06/16]
26. Thomas RE, Lorenzetti DL, Spragins W, et al. Reporting rates of yellow fever vaccine 17D or 17DD-associated serious adverse events in pharmacovigilance data bases: systematic review. *Current drug safety* 2011;6(3):145-54. [published Online First: 2011/11/30]

27. Breugelmans JG, Lewis RF, Agbenu E, et al. Adverse events following yellow fever preventive vaccination campaigns in eight African countries from 2007 to 2010. *Vaccine* 2013;31(14):1819-29. doi: 10.1016/j.vaccine.2013.01.054 [published Online First: 2013/02/12]
28. Hickling J, Jones R. Yellow fever vaccination: The potential of dose sparing to increase vaccine supply and availability. A Technical Programme Report for Programme for Appropriate Technology in Health (PATH) Report. 2013. Available via https://www.path.org/publications/files/TS_vtg_yf_rpt.pdf Accessed on 29 December 2017.
29. Campi-Azevedo AC, de Almeida Estevam P, Coelho-dos-Reis JG, et al. Subdoses of 17DD yellow fever vaccine elicit equivalent virological/immunological kinetics timeline. *BMC Infectious Diseases* 2014;14:391-91. doi: 10.1186/1471-2334-14-391
30. Roukens AH, Vossen AC, Bredenbeek PJ, et al. Intradermally Administered Yellow Fever Vaccine at Reduced Dose Induces a Protective Immune Response: A Randomized Controlled Non-Inferiority Trial. *PLoS ONE* 2008;3(4):e1993. doi: 10.1371/journal.pone.0001993
31. Vannice K, Wilder-Smith A, Hombach J. Fractional-Dose Yellow Fever Vaccination — Advancing the Evidence Base. *New England Journal of Medicine* 2018;379(7):603-05. doi: 10.1056/NEJMp1803433
32. Martins RM, Maia MdLS, Farias RHG, et al. 17DD yellow fever vaccine: A double blind, randomized clinical trial of immunogenicity and safety on a dose-response study. *Human vaccines & immunotherapeutics* 2013;9(4):879-88. doi: 10.4161/hv.22982
33. Lopes Ode S, Guimaraes SS, de Carvalho R. Studies on yellow fever vaccine. III--Dose response in volunteers. *Journal of biological standardization* 1988;16(2):77-82. [published Online First: 1988/04/01]
34. Freestone DS, Ferris RD, Weinberg AL, et al. Stabilized 17D strain yellow fever vaccine: Dose response studies, clinical reactions and effects on hepatic function. *Journal of biological standardization* 1977;5(3):181-86. doi: https://doi.org/10.1016/S0092-1157(77)80003-6
35. Fox JP, Kossobudzki SL, Cunha JF. Field studies of the immune response to 17D yellow fever virus. Am J Hyg. 1943;38:113–38.
36. Nzolo D, Engo Biongo A, Lusakibanza M, et al. Safety profile of fractional dosing of yellow fever vaccine, experience from community based pharmacovigilance in Kinshasa, DRC2017.
37. Roukens AH, Vossen AC, van Dissel JT, et al. Reduced intradermal test dose of yellow fever vaccine induces protective immunity in individuals with egg allergy. *Vaccine* 2009;27(18):2408-9. doi: 10.1016/j.vaccine.2009.02.049 [published Online First: 2009/04/17]
38. Ahuka-Mundeke S, Casey RM, Harris JB, et al. Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak - Preliminary Report. *The New England journal of medicine* 2018 doi: 10.1056/NEJMoa1710430 [published Online First: 2018/02/15]
39. Jacobson RM, Targonski PV, Poland GA. Meta-analyses in vaccinology. *Vaccine* 2007;25(16):3153-59. doi: https://doi.org/10.1016/j.vaccine.2007.01.047
40. Lyman GH, Kuderer NM. The strengths and limitations of meta-analyses based on aggregate data. *BMC Med Res Methodol* 2005;5:14. doi: 10.1186/1471-2288-5-14 [published Online First: 2005/04/27]
41. Finney DJ A statistician looks at met-analysis .J Clin Epidemiol 1995 48(1): 87-103.
42. Booth A, Clarke M, Dooley G, et al. The nuts and bolts of PROSPERO: an international prospective register of systematic reviews. *Systematic Reviews* 2012;1:2-2. doi: 10.1186/2046-4053-1-2
43. Booth A, Clarke M, Ghersi D, et al. An international registry of systematic-review protocols. *Lancet* 2011;377(9760):108-9. doi: 10.1016/s0140-6736(10)60903-8 [published Online First: 2010/07/16]
44. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ : British Medical Journal* 2015;349 doi: 10.1136/bmj.g7647

45. Jean K, Donnelly CA, Ferguson NM, et al. A Meta-Analysis of Serological Response Associated with Yellow Fever Vaccination. *The American Journal of Tropical Medicine and Hygiene* 2016;95(6):1435-39. doi: 10.4269/ajtmh.16-0401
46. Banaszkiewicz A, Radzikowski A. Efficacy, effectiveness, immunogenicity - are not the same in vaccinology. *World Journal of Gastroenterology : WJG* 2013;19(41):7217-18. doi: 10.3748/wjg.v19.i41.7217
47. The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). RevMan [Computer program]. Version 5.3. Copenhagen:, The Nordic Cochrane Centre, The Cochrane Collaboration. 2014.
48. Higgins JPT, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
49. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343 doi: 10.1136/bmj.d5928
50. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016;355 doi: 10.1136/bmj.i4919
51. Melsen WG, Bootsma MCJ, Rovers MM, et al. The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses. *Clinical Microbiology and Infection* 2014;20(2):123-29. doi: https://doi.org/10.1111/1469-0691.12494
52. Guyatt GH, Oxman AD, Kunz R, et al. What is "quality of evidence" and why is it important to clinicians? *Bmj* 2008;336(7651):995-8. doi: 10.1136/bmj.39490.551019.BE [published Online First: 2008/05/06]

PART B: LITERATURE REVIEW

Table of Contents

1. Description of the condition

1.1. Background

Yellow fever is a viral haemorrhagic disease of humans caused by the yellow fever virus.[1] The disease mostly occurs in tropical areas of Africa and South America, where it is endemic and intermittently epidemic.[1 2] The yellow fever virus is a prototypic member of the genus *Flavivirus* (*flavus* meaning yellow in Latin) having a relatively narrow host range, mostly humans and monkeys; and typically transmitted by *Aedes* mosquitoes.[2 3] Globally, approximately 200 000 cases of yellow fever and 30 000 deaths occur annually. [4 5] Sub-Saharan Africa bears 90% of the disease burden.[4 6] Typically, humans are infected when bitten by blood-feeding mosquitoes.[2] Susceptibility to yellow fever depends on several factors, such as previous infection with the virus and other flaviviruses; immune status; and environmental, racial and genetic factors.[7 8] Transmission is largely dependent on availability of vector, vertebrate hosts and vegetation.[9 10]

1.2. Epidemiology of yellow fever

1.2.1. Causative agent

Yellow fever is caused by an arthropod vector-borne arbovirus from the flavivirus genus of the *flaviviridae* family. The virus is a prototype member of this genus. It is a positive-sense, single-strand ribonucleic acid (RNA) virus [2], with a diameter of approximately 40 nm and 5 – 10 nm surface projections.[11] Mature virions are icosahedral in shape and consist of a nucleocapsid, composed of capsid (C) protein subunits, surrounded by a lipid bilayer. The viral envelope is studded with membrane (M) protein and envelope (E) glycoproteins.[11 12] The E glycoprotein is the major component of the virion surface and is involved in most of the biologic activity, including cell-surface receptor binding, fusion and immunogenicity.[12]

1.2.2. Yellow fever vectors

In Africa, the main vectors of yellow fever are mosquitoes of the genus *Aedes*, subgenera *Stegomyia* and *Diceromyia* with seven species: *Aedes (Stegomyia) aegypti, A. (Stegomyia) africanus, A.(Stegomyia) opok, A. (Stegomyia) luteoceph alus, A. (Stegomyia) simpsoni group, A. (Diceromyia) furcifer,* and *A. (Diceromyia) taylori.*[13] *Aedes* mosquitoes are classified based on their mode of contact with humans. The domestic category consists mainly of *A. aegypti; while*

the semi-domestic category consists of wild vectors which can acquire domestic habits such as *A. furcifer, A. africanus* and *A. luteocephalus.*[8] Monkeys and galagoes (bush babies) are the main vertebrate hosts. In savannah areas, these primates are exposed to mosquito bites and develop viraemia over a maximum period of nine days.[12 13] The main vectors in South America are *A. aegypti* (urban) and *A. Haemagogus* (jungle).[13 14]

1.2.3. Transmission of yellow fever

The transmission of yellow fever is via two mechanisms; vertical and horizontal transmissions.[8] The less clear vertical mechanism of transmission occurs from orally infected females to their progeny.[15] The horizontal mechanism consists of the maintenance and amplification cycles. Which of the two cycles prevails depends on the degree of contact with the susceptible host and the associated ecological factors.[8 13] The maintenance cycle is the more stable cycle; it occurs when the vector-vertebrate contact is loose. This results in an endemic or enzootic form of yellow fever. In contrast, the amplification cycle results from closer vector–vertebrae contact and manifests in epizootic or epidemic forms of yellow fever. For transmission to occur, following a blood meal on an infected vertebrate host (e.g. monkeys or humans), the vector must remain alive long enough to allow full development and replication of the virus inside its tissues, and the virus must be inoculated with saliva into another vertebrate host during subsequent blood meals.[8]

1.2.3.1. Transmission pattern in Africa

In Africa, transmission patterns are largely dependent on the availability of invertebrate and vertebrate hosts, which are in turn determined by vegetation patterns.[9] Currently, endemic regions in Africa can be found between latitudes 15 degrees north to 15 degrees south of the equator. Endemic forms of yellow fever occur year-round and transmission is primarily between monkeys and *A. africanus.*[8 9] Due to the abundant presence of both vector and host populations, these areas are prone to high rates of sporadic outbreaks, particularly during the rainy seasons, when enzootic *Aedes* mosquitoes reach high densities.[9] Urban-type transmission may also occur with resultant outbreaks of *A. aegypti*-borne yellow fever if the virus is introduced into urban regions by infected persons or mosquitoes.[2 9]

1.2.3.2. Transmission pattern in South America

In South America, the transmission pattern consists of two cycles; jungle and urban.[2 9] The jungle cycle is mainly transmitted by *A. haemagogus,* while the urban type is transmitted by *A. aegypti*. In this region, the disease mainly affects unvaccinated persons who enter the forest for hunting or wood cutting, making it an occupational disease.[2]

1.2.3.3. Transmission pattern in Asia

Despite the substantial presence of A. *aegypti,* the Asian region is not yellow fever endemic.[2 14] Some reasons have been postulated, including the likelihood of cross-protection between flaviviruses; absence of the maintenance cycle; and variation in vector competence and behaviour in the region.[14]

1.2.3.4. Transmission among travelers

Yellow fever poses a significant threat to unvaccinated persons travelling to endemic areas.[11] With globalisation and ease of international travel, there have been documented cases of human importation of the disease from endemic areas to places where it is non-endemic.[11] In 2016, imported cases were reported in China [16 17] and Morocco.[18]

1.2.4. Risk factors for acquiring and surviving yellow fever

The susceptibility of individuals and populations to yellow fever depends on several factors. These include previous exposure to yellow fever and other flaviviruses; immune status; environmental; behavioural as well as racial and genetic factors.[7 8] Population growth, increasing forest encroachment, migration, political unrest, wars and urbanisation all contribute to increased transmission.[8 13] Previous infection and vaccination can confer life-long immunity.[14] Though an association between Human Leucocyte Antigen (HLA) haplotype and disease severity has been described [3], the role of genetic or racial factors in determining human host susceptibility to yellow fever infection remains uncertain.[2] Lower rates of case fatalities have been demonstrated in blacks than in Caucasians.[9]

1.2.5. Pathophysiology and clinical course

Yellow fever in humans varies from a mild disease in which symptoms abate rapidly after the first phase, to an invariably fatal fulminating disease.[11] Following inoculation, the virus replicates in the adjacent tissues and localised lymph nodes. An incubation period lasting 3-6 days is followed

by an abrupt onset of symptoms. In mild, abortive cases, symptoms are typically nonspecific; manifesting as fever, headache, and constitutional problems.[2 12] In such cases, patients recover in a few days with no lasting sequelae. In severe cases, patients may experience nausea, fever, chills, malaise, headache, lower back pain, and generalised myalgia. Faget's sign (increasing temperature with decreasing pulse rate) is also a common feature of severe cases.[9 19] Severe disease can also manifest as pan-systemic viral sepsis; with pyrexia (which may be higher than 39^{o}C to 40^{o}C); prostration; hepatic, renal, and myocardial injury; hemorrhage; shock; and fatality in 20 – 60% of cases.[14 19]

1.2.6. Diagnosis and treatment

Clinical diagnosis of yellow fever is based on the presence of sudden fever, relative bradycardia, and signs of jaundice in people in endemic areas or with history of recent travel to an endemic areas.[11 19] The disease, however, shares clinical features with other viral haemorrhagic fevers such as the dengue hemorrhagic fever, Lassa fever, Ebola virus disease, and Crimean-Congo hemorrhagic fever. Hence, the risk of mis-diagnosis is high in sporadic or early epidemic cases.[11 14] Clinical diagnosis is further complicated by variations in symptomatic presentation. Mild cases are difficult to recognise. Laboratory diagnosis is therefore the definitive means of ascertaining cases. The laboratory criterion for diagnosis is at least one of the following: (1) the presence of yellow fever-specific Immunoglobulin M (IgM) or four-fold or more increase in Immunoglobulin G (IgG) levels between acute and convalescent sera in the absence of recent vaccination; (2) isolation of the virus; (3) positive postmortem liver histopathology; or (4) detection of viral antigen in tissues by immunohistochemistry or polymerase chain reaction.[11 14 19]

There is no specific antiviral treatment for yellow fever.[19] Passive antibodies such as interferons have been found to have very limited antiviral effect, and only useful before or within hours of infection and for prophylaxis following exposure in laboratory or health workers [11 14 19]. Treatment is therefore primarily supportive.[11 19] A standard treatment protocol comprises of maintenance of nutrition, rehydration, ventilation support, correction of metabolic imbalance, treatment of haemorrhage, dialysis if indicated by renal failure, and treatment of secondary infections. [19]

1.2.7. Prevention of yellow fever through vector control

Vector control is one of the two main methods of preventing yellow fever, the other method being vaccination.[9] [14] Vector control methods include community-based environmental interventions like spraying of breeding sites; biological methods involving the use of autocidal ovitraps, predatory toxorhynchite mosquitoes and predatory fishes; and individual-level measures consisting of the use of insect repellent, protective clothing and mosquito nets.[14]

1.3. Global health importance of yellow fever and international health regulation (IHR)

Quantifying the burden of yellow fever disease is made challenging by the wide spectrum of clinical severity and non-specificity of symptoms making diagnosis difficult. Additionally, there are limitations in the surveillance, diagnostic capacity and reporting across much of the affected regions, meaning that the disease is substantially underreported.[20] Annual reporting of cases relies on passive surveillance and thus significantly underestimates the true incidence.[2] It is, however, estimated that 200 000 cases of the disease and 30 000 deaths occur annually.[4] [5] Sub-Saharan Africa bears approximately 90% of this burden.[4] The disease poses an enormous health burden on residents in endemic regions, non-immunised travelers entering endemic areas and persons moving within their own country from low-risk to high-risk areas.[21]

Dramatic upsurges in yellow fever outbreaks have occurred recently. These include Angola in 2015, Democratic Republic of Congo and Uganda in 2016, and Nigeria and Brazil in 2017.[4] [18] [22] Imported cases were also reported in 2016 in China and Morocco.[16] [18] The underlying reasons for the increasing epidemics are multifactorial, involving vector density; viral virulence and emergence of new virus lineage; climatic factors such as increased rainfall and high temperatures; behavioural factors; and waning immunisation coverage.[2] [14]

Yellow fever is the only disease specified in the International Health Regulations (IHR) for which countries may require a proof of vaccination from travellers as a pre-condition for entry.[4] The World Health Organisation (WHO) publishes and continually updates a list of countries with risk of outbreaks and those requiring yellow fever vaccination as part of routine immunisation programme (see Figures 1 and 2 below).[23] In practice, however, compliance with the IHR vaccination requirement is suboptimal.[4] [18] Compliance is made more challenging by porous national borders and ease of cross-border migration.[18]

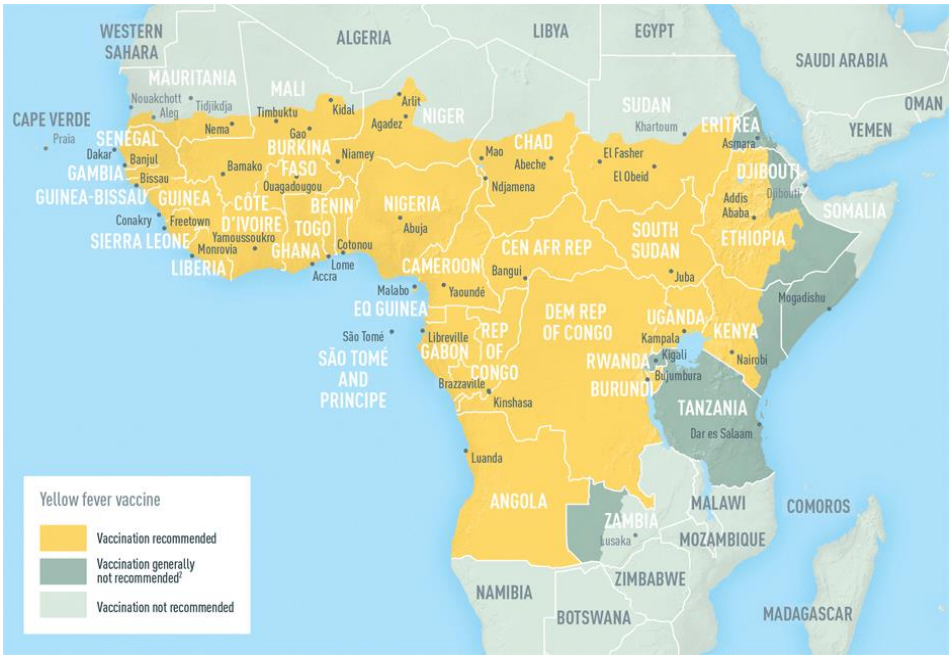

Fig. 1: Yellow fever endemicity and vaccine requirement in Africa [24]

Fig. 2: Yellow fever endemicity and vaccine requirement in South America [24]

1.4. Yellow fever vaccine

The development of a life-attenuated yellow fever vaccine which came into use in 1938, and its wide roll-out, have led to significant decline in the burden of disease.[14 25] Prevention through vaccination can limit the morbidity, mortality, and spread of outbreaks.[14 26] The WHO strongly recommends vaccination in at-risk countries, as part of the routine childhood immunisation programmes. In addition to routine immunisation, preventive mass immunisation campaigns to prevent outbreaks, and reactive mass immunisation campaigns in response to outbreaks, yellow fever vaccination is used for preventive immunisation of travellers to endemic regions.[4 11]

1.4.1. Yellow fever vaccine immunogenicity, efficacy and effectiveness

The protective effects of vaccines can be measured through different types of studies.[27-29] The measurement of a vaccine's effect in randomised controlled studies is referred to as efficacy. Efficacy studies are however not commonly conducted due to the high cost of randomised studies as well as ethical concerns of withholding vaccines from participants in the placebo arms of studies. The measurement of a vaccine's effect from observational studies is referred to as effectiveness. Immunogenicity refers to the ability of a vaccine to induce an immune response (antibody or cell-mediated) in vaccinated individuals.[27]

Yellow fever vaccine is highly immunogenic, with results of clinical trials showing that 99% of vaccine recipients developed protective levels of neutralising antibodies within four weeks.[4 9 13 23 25 30 31] Until 2013, a booster-dose of the vaccine was recommended after 10 years.[14] The concern for the tenth year booster was based on evidence of a time-dependent decrease in immunity, with one study showing that neutralising antibody titers decreased from 94% in the first year after vaccination to 75% ten years after.[21] However, this recommendation for a booster has been challenged by many studies which suggest that the duration of immunity after vaccination may last for life in as many as 80% of those vaccinated.[21 31 32] Moreover, it has been argued that even if there is some evidence that shows a decrease in neutralising antibodies over time, the proportion of the population with protective antibody titers at the end of the follow-up period was consistently at the herd-immunity threshold of >60%.[21] Consequently, in 2013, the WHO declared that booster vaccination was no longer necessary.[33 34]

There are numerous serological methods used to assess immune response following yellow fever vaccination. These include plaque reduction neutralisation, haemagglutination inhibition, complement fixation, enzyme linked immunosorbent assay, and indirect fluorescent antibody tests.[8 14] Currently, detection and analysis of the immune response post-vaccination are mostly done using the plaque reduction neutralisation [9 18], which is considered to be the most specific and gold-standard method.[4] Immunity in humans corresponds to 50–80% viral plaque reduction.[35]

1.4.2. Safety of yellow fever vaccine

Yellow fever vaccine has also been considered safe and well tolerated. Serious adverse events are rare.[21 36] Reported serious adverse events include anaphylactic or hypersensitivity reactions; yellow fever vaccine-associated viscerotropic disease and yellow fever vaccine-associated neurologic disease.[36-39] The mechanisms of these serious adverse events are poorly understood, though old age has been identified as a risk factor.[40 41] The Brighton Collaboration case definitions provide a standardised classification of serious adverse events using three distinct levels of diagnostic certainty.[39 42-45] Incidence of yellow fever vaccine-associated viscerotropic disease ranges from 0 to 0.21 cases per 100 000 vaccine doses in endemic regions, and from 0.09 to 0.40 cases per 100 000 doses in non-endemic settings. Yellow fever vaccine-associated neurologic disease is estimated to occur at a frequency of 0.8 cases per 100 000 vaccine doses administered.[4] Reporting rates for serious adverse events can vary. Systematic reviews have found that such variation may be due to differences in routes of vaccine administration, case definitions, study designs, surveillance methods, frequency of reporting, diagnostic capability, and availability of other health-systems resources.[46 47] The studies documented non-adherence to standard criteria for assigning causality due to the unavailability of certain laboratory investigations, with the majority of reported events not meeting the Brighton case definitions.[46 47] There are also challenges with mis-classification of cases due to the similarities of serious adverse event symptoms with those of common conditions like malaria or bacterial meningitis. There were also reports of improper sample labeling, faulty containers, improper storage, and delays between collection and transport.[47]

2. Fractional dose yellow fever vaccination

Fractional dose yellow fever vaccination refers to administration of a reduced volume of vaccine dose, which has been reconstituted as per manufacturer recommendations.[4] Recent and ongoing yellow fever outbreaks are sharply increasing the demand for yellow fever vaccine, mounting enormous strain on the global stockpile and putting at risk the immunisation of endemic populations.[4 48] Further compounding the global shortages is a global insufficient production capacity for the vaccine. Yellow fever vaccine is manufactured using a process that has not significantly and innovatively evolved for decades.[26 49] Production capacity is limited by a combination of commercial and technical factors, including the uncertainty and commercial unattractiveness of the yellow fever vaccine market which limits the number of manufacturers and the amount of vaccine produced by each manufacturer. [49] Between 2001 and 2009, the total demand for yellow fever vaccine increased three-fold from 34 million to 105 million doses per year.[18 49] Similarly, the list of countries with demand for the vaccine for routine immunisation increased from 33 to 44 within the same time frame.[49]

The World Health Organisation's International Coordinating Group (ICG) coordinates the supply of vaccines during outbreaks.[4] It currently maintains a global emergency stockpile of six million doses of yellow fever vaccine, which is continually replenished.[18] The stockpile was depleted three times during the 2016 outbreak and complicating this is the fact that current global yellow fever immunisation coverage is well below the 80% target expected to maintain herd immunity: with a recent study estimating that 43% of people living in high-risk settings remain unvaccinated.[50] Therefore, to vaccinate susceptible populations in preventive mass immunisation campaigns during outbreaks, fractional dosing of the vaccine is being considered as a dose-sparing strategy to maximise limited supplies.[4 18]

The first practical use of fractional doses during a mass vaccination campaign was in response to a large yellow fever outbreak in the Democratic Republic of Congo in 2016.[4 18] The minimum potency as recommended by WHO for the standard dose of yellow fever vaccines should not be less than 1000 international units (IU) per dose.[18 49] This forms the fundamental basis of fractional dose considerations, as the potency at release of the vaccine at standard doses is usually many-fold higher than the recommended potency.[4] As a dose-sparing strategy, a fractional dose

meeting the WHO minimum requirement for potency is expected to be equivalent to a standard dose of the vaccine with respect to safety, immunogenicity, efficacy and effectiveness.[4 51 52]

3. How the fractional dose of yellow fever vaccine might work

3.1. Protective effects of fractional dose yellow fever vaccine

The protective immune response evoked by the administration of fractional doses of the yellow fever vaccine has been substantially investigated.[51-58] The earliest study, a 1943 quasi-randomised controlled trial conducted by Fox and colleagues involving 550 military personnel in Belo Horizonte, Brazil, found that as low as 1/100th fractional doses were able to produce seroconversion rates of >96% which were non-inferior to the standard (i.e. full) doses.[56] This study is however limited by its demographically-restricted participants (exclusively male with no age-stratification), hence might not have been representative of the reference population from which their sample was drawn. The short follow-up duration of five weeks might also not have been adequate to account for possible differences in time-dependent immune responses. In their 1977 trial involving 68 research laboratory staff in Beckenham and Dartford, England; Freestone and colleagues compared the protective immune responses between standard and <1/100th fractional doses. They found statistically significant lower immunogenicity of <1/100th fractional doses at four weeks post-vaccination.[55] Though gender-inclusive, the study's limitation however stems from its small sample size. A 1988 trial by Lopes and co-workers assessed seroconversion among 259 military personnel with eight different sub-doses in comparison to the standard dose of yellow fever vaccine 28 days after vaccination.[54] They found that the inoculation of 200-500 IU (much lower than the minimum required standard by WHO of 1,000 IU) of the vaccine induced seroconversion in 100% of participants. Only men were included in this trial, however, which might not have been representative of the reference population from which the study sample was drawn. Furthermore, the short follow-up duration of four weeks might not have been long enough to account for temporal differences in immune responses.

In a 2008 randomised trial by Roukens and colleagues in Leiden, the Netherlands, 155 participants who received 1/5th of the full dose of the vaccine were followed up for 1 year and

assessed for immunological responses.[52] They demonstrated that from two weeks to one year after vaccination, neutralisation of viral plaques by 80% did not differ between participants who received fractional and standard doses. Sero-protection was reached in both intervention and comparison groups. The one-year follow-up duration of this study provides some evidence on the long-term protective immunity of fractional dose vaccination. The study is however limited by its sample size, lack of age-stratification of participants and the use of a modified plaque reduction neutralization test for assessing immune responses. The following year, Roukens and colleagues conducted another study to assess immune responses to reduced intradermal test-dose of yellow fever vaccine in a small cohort of seven individuals with egg allergy. They found that intradermally administered 1/5th dose of the yellow fever vaccine sufficiently induced protective immune responses in all seven subjects. The study's limitation however stems from its small sample size, lack of a comparison group, and involvement of only individuals with egg allergy.[58]

More recently, a randomised trial conducted by Martins and colleagues in 2009, involving 749 army recruits, in Rio de Janeiro, Brazil, found that 97% of participants who received as low as 1/50th of the full dose achieved seroconversion at four weeks, similar to those who received the full dose.[53] Participants were followed up for eight years, with 85.2% of them remaining seropositive; providing some evidence of prolonged protective immunity of fractional dose vaccination.[59] The limitation of this study is its demographically-restricted sample, consisting exclusively of mostly young, male military personnel. Similarly, Campi-Azevedo and colleagues, in their 2014 trial conducted on 550 participants from the same cohort of participants as in the Martins et al study, similarly showed that as low as 1/50th of the standard dose was able to trigger comparable immunogenicity as the standard dose vaccine.[51] The study was also demographically limited by age and gender of included participants.

Most recently, Ahuka-Mundeke and colleagues conducted an observational study during the preventive immunisation campaign in response to the 2016 outbreak in the Democratic Republic of the Congo; to assess immune responses following fractional dose vaccination of 716 individuals.[57] They observed that 98% of participants who received the fractional dose had

seroconverted at 28 days of follow-up. Though the study had a relatively big sample size, it was limited by not having a control group of participants who received standard dose vaccination.

Overall, these studies assessed the immunogenicity (antibody production and seroconversion rates) of fractional dose yellow fever vaccination at four to five weeks post-vaccination. Only one of the studies reported for a longer follow-up duration (one year).[52] Immune response was assessed by the plaque reduction neutralisation test with seroconversion end-points defined at 50 – 80% viral neutralisation[51-54 56] or log10 neutralisation index of ≥0.7.[55] Notably, most of the studies above were conducted in Latin America (Brazil)[51 53 54 56] and Europe (England[55] and the Netherlands[52]), with one conducted in Africa (Democratic Republic of Congo),[57] indicative of a dearth of evidence on the African continent where the disease burden is enormous.

3.2. Safety of fractional dose yellow fever vaccine

The safety of fractional dose vaccination has been investigated.[52 53 55 57 60] Local and systematic adverse events reported 0–10 days following fractional dose yellow fever vaccination included pain, hyperaemia, oedema, nausea, arthralgia, exanthema, and pruritus. In three of the studies that compared fractional and standard doses, there were no significant differences in the frequency of common non-serious adverse events.[52 53 55 57] Observational studies by Nzolo and colleagues[60] and Ahuka-Mundeke and colleagues[57] have assessed the safety of the fractional dose vaccines administered in large cohorts of at-risk populations during mass preventive vaccination campaigns in response to the 2016 outbreak in Kinshasa, the densely populated capital city of the Democratic Republic of Congo. They did not observe any serious adverse effect following fractional dose yellow fever vaccination.[4 18] Overall, none of the studies that assessed safety of fractional dose vaccination reported serious adverse events attributable to fractional dose vaccination; however, the sample sizes of the studies were not large enough and follow-up durations were not long enough to detect less common and serious adverse events. It is, therefore, hard to draw firm conclusions on the safety profile in terms of serious adverse event risks of fractional dose yellow fever vaccination.

Though it has been argued that lower viral doses in vaccines might be associated with risk of vaccine-induced viraemia,[61 49] however it has been shown that viraemia risk does not increase at lower vaccine doses.[51]

4. Why it is important to do this project

We have found two non-systematic reviews of fractional dose yellow fever vaccination in the grey literature.[18 49] However, we are not aware of any currently available comprehensive and systematic synthesis of the body of evidence on the effects of this strategy, thus informing the need for this review. None of the two grey literature documents provided a meta-analysis of the effects of fractional doses of yellow fever. The small sample sizes of identified relevant primary studies and the dearth of larger single studies further justify the need for this review. The use of meta-analysis , which has grown remarkably in vaccinology in recent years, helps to produce quantitatively robust and accurate effect-size measurements that are generalizable.[62-64] The planned systematic review will not only add to the body of existing knowledge, it will also fill a knowledge gap and provide a robust evidence base for informing relevant policy and advocacy processes. Current international best practices in the conduct of systematic reviews and meta-analyses will be followed, including prospective registration in the International Prospective Register of Systematic Reviews (PROSPERO) [65 66] and compliance with the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P)[67] and the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.[68]

5. References

1. Wiysonge CS, Nomo E, Mawo J, et al. Yellow fever control in Cameroon: where are we now and where are we going? *BMC medicine* 2008;6:3. doi: 10.1186/1741-7015-6-3 [published Online First: 2008/02/12]
2. Monath TP, Vasconcelos PF. Yellow fever. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* 2015;64:160-73. doi: 10.1016/j.jcv.2014.08.030 [published Online First: 2014/12/03]
3. Barrett AD, Higgs S. Yellow fever: a disease that has yet to be conquered. *Annual review of entomology* 2007;52:209-29. doi: 10.1146/annurev.ento.52.110405.091454 [published Online First: 2006/08/18]
4. World Health Organization (WHO). WHO position on the use of fractional doses - June 2017, addendum to vaccines and vaccination against yellow fever WHO: Position paper - June 2013. *Vaccine* 2017;35(43):5751-52. doi: 10.1016/j.vaccine.2017.06.087 [published Online First: 2017/07/12]
5. World Health Organization (WHO). Vaccines and vaccination against yellow fever: WHO Position Paper, June 2013--recommendations. *Vaccine* 2015;33(1):76-7. doi: 10.1016/j.vaccine.2014.05.040 [published Online First: 2014/05/24]
6. Markoff L. Yellow fever outbreak in Sudan. *New England Journal of Medicine* 2013;368(8):689-91. doi: 10.1056/NEJMp1300772

7. Gubler DJ. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? *Comparative immunology, microbiology and infectious diseases* 2004;27(5):319-30. doi: 10.1016/j.cimid.2004.03.013 [published Online First: 2004/07/01]
8. Casey G. Vaccines--how and why they work. *Nursing New Zealand (Wellington, NZ : 1995)* 2016;22(1):20-4. [published Online First: 2016/04/05]
9. Mutebi JP, Barrett AD. The epidemiology of yellow fever in Africa. *Microbes and infection* 2002;4(14):1459-68. [published Online First: 2002/12/12]
10. Chen LH, Hamer DH. Vaccination Challenges in Confronting the Resurgent Threat From Yellow Fever. *JAMA: Journal of the American Medical Association* 2017;318(17):1651-52. doi: 10.1001/jama.2017.14258
11. Gardner CL, Ryman KD. Yellow Fever: A Reemerging Threat. *Clinics in laboratory medicine* 2010;30(1):237-60. doi: 10.1016/j.cll.2010.01.001
12. Vasconcelos PF. [Yellow Fever]. *Revista da Sociedade Brasileira de Medicina Tropical* 2003;36(2):275-93. [published Online First: 2003/06/14]
13. Makhunga-Ramfolo N, Rheeder P. Safety and tolerability of yellow fever vaccines. *Southern African Journal of Epidemiology and Infection* 2012;27(1):16-18. doi: 10.1080/10158782.2012.11441474
14. Barnett ED. Yellow fever: epidemiology and prevention. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007;44(6):850-6. doi: 10.1086/511869 [published Online First: 2007/02/17]
15. Diallo M, Thonnon J, Fontenille D. Vertical transmission of the yellow fever virus by Aedes aegypti (Diptera, Culicidae): dynamics of infection in F1 adult progeny of orally infected females. *Am J Trop Med Hyg* 2000;62(1):151-6. [published Online First: 2000/04/13]
16. Dou XF, Zheng Y, Lyu YN, et al. [The first confirmed imported case of yellow fever in China]. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi* 2016;37(6):788-90. doi: 10.3760/cma.j.issn.0254-6450.2016.06.009 [published Online First: 2016/06/28]
17. Wilder-Smith A, Leong WY. Importation of yellow fever into China: assessing travel patterns. *Journal of travel medicine* 2017;24(4) doi: 10.1093/jtm/tax008 [published Online First: 2017/04/21]
18. World Health Organization (WHO). Fractional Dose Yellow Fever Vaccine as a Dose-sparing Option for Outbreak Response. WHO Secretariat Information Paper. Department of Immunization, Vaccines and Biologicals. WHO reference number: WHO/YF/SAGE/16.1. Published 20 July 2016. Available via: http://apps.who.int/iris/bitstream/handle/10665/246236/WHO-YF-SAGE-16.1-eng.pdf;jsessionid=F4BD11CEBB8426D52C781267F9795A8A?sequence=1 21 April 2018.
19. Monath TP. Treatment of yellow fever. *Antiviral research* 2008;78(1):116-24. doi: 10.1016/j.antiviral.2007.10.009 [published Online First: 2007/12/07]
20. Garske T, Van Kerkhove MD, Yactayo S, et al. Yellow Fever in Africa: Estimating the Burden of Disease and Impact of Mass Vaccination from Outbreak and Serological Data. *PLoS Medicine* 2014;11(5):e1001638. doi: 10.1371/journal.pmed.1001638
21. 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. *The American Journal of Tropical Medicine and Hygiene* 2013;89(3):434-44. doi: 10.4269/ajtmh.13-0264
22. Van den Broeck J, Eeckels R, Massa G. Maternal determinants of child survival in a rural African community. *Int J Epidemiol* 1996;25 doi: 10.1093/ije/25.5.998
23. Becher H, Muller O, Jahn A, et al. Risk factors of infant and child mortality in rural Burkina Faso. *Bull World Health Organ* 2004;82
24. Hobcraft J. Women's education, child welfare and child survival: a review of the evidence. *Health Transit Rev* 1993;3
25. Norrby E. Yellow fever and Max Theiler: the only Nobel Prize for a virus vaccine. *The Journal of Experimental Medicine* 2007;204(12):2779.

26. Monath TP, Woodall JP, Gubler DJ, et al. Yellow fever vaccine supply: a possible solution. *The Lancet*;387(10028):1599-600. doi: 10.1016/S0140-6736(16)30195-7
27. Banaszkiewicz A, Radzikowski A. Efficacy, effectiveness, immunogenicity - are not the same in vaccinology. *World Journal of Gastroenterology : WJG* 2013;19(41):7217-18. doi: 10.3748/wjg.v19.i41.7217
28. Weinberg GA, Szilagyi PG. Vaccine Epidemiology: Efficacy, Effectiveness, and the Translational Research Roadmap. *The Journal of Infectious Diseases* 2010;201(11):1607-10. doi: 10.1086/652404
29. Oosterhuis-Kafeja F, Beutels P, Van Damme P. Immunogenicity, efficacy, safety and effectiveness of pneumococcal conjugate vaccines (1998-2006). *Vaccine* 2007;25(12):2194-212. doi: 10.1016/j.vaccine.2006.11.032 [published Online First: 2007/02/03]
30. Shearer FM, Moyes CL, Pigott DM, et al. Global yellow fever vaccination coverage from 1970 to 2016: an adjusted retrospective analysis. *The Lancet Infectious Diseases* 2017;17(11):1209-17. doi: 10.1016/S1473-3099(17)30419-X
31. Groot H, Ribeiro RB. Neutralizing and haemagglutination-inhibiting antibodies to yellow fever 17 years after vaccination with 17D vaccine. *Bulletin of the World Health Organization* 1962;27(6):699-707.
32. Poland JD, Calisher CH, Monath TP, et al. Persistence of neutralizing antibody 30-35 years after immunization with 17D yellow fever vaccine. *Bull World Health Organ* 1981;59(6):895-900. [published Online First: 1981/01/01]
33. World Health Organization (WHO). Yellow fever vaccination booster not needed. News Release. 17 May 2013 Available via http://www.who.int/mediacentre/news/releases/2013/yellow_fever_20130517/en/ Accessed on 3 January 2018.
34. WHO. Vaccines and vaccination against yellow fever: WHO Position Paper, June 2013--recommendations. *Vaccine* 2015;33(1):76-7. doi: 10.1016/j.vaccine.2014.05.040 [published Online First: 2014/05/24]
35. Mercier-Delarue S, Durier C, Colin de Verdière N, et al. Screening test for neutralizing antibodies against yellow fever virus, based on a flavivirus pseudotype. *PLoS ONE* 2017;12(5):e0177882. doi: 10.1371/journal.pone.0177882
36. Thomas RE. Yellow fever vaccine-associated viscerotropic disease: current perspectives. *Drug Design, Development and Therapy* 2016;10:3345-53. doi: 10.2147/DDDT.S99600
37. Thomas RE, Spragins W, Lorenzetti DL. How many published cases of serious adverse events after yellow fever vaccination meet Brighton Collaboration diagnostic criteria? *Vaccine* 2013;31(52):6201-9. [published Online First: 2014/01/18]
38. Thomas RE, Lorenzetti DL, Spragins W, et al. Active and passive surveillance of yellow fever vaccine 17D or 17DD-associated serious adverse events: systematic review. *Vaccine* 2011;29(28):4544-55. doi: 10.1016/j.vaccine.2011.04.055 [published Online First: 2011/05/10]
39. Rüggeberg JU, Gold MS, Bayas J-M, et al. Anaphylaxis: Case definition and guidelines for data collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;25(31):5675-84. doi: https://doi.org/10.1016/j.vaccine.2007.02.064
40. Roukens AH, Soonawala D, Joosten SA, et al. Elderly Subjects Have a Delayed Antibody Response and Prolonged Viraemia following Yellow Fever Vaccination: A Prospective Controlled Cohort Study. *PLoS ONE* 2011;6(12):e27753. doi: 10.1371/journal.pone.0027753
41. Monath TP, Cetron MS, McCarthy K, et al. Yellow fever 17D vaccine safety and immunogenicity in the elderly. *Human vaccines* 2005;1(5):207-14. [published Online First: 2006/10/03]
42. Lindsey NP, Rabe IB, Miller ER, et al. Adverse event reports following yellow fever vaccination, 2007–13. *Journal of travel medicine* 2016;23(5):taw045-taw45. doi: 10.1093/jtm/taw045

43. Gershman MD, Staples JE, Bentsi-Enchill AD, et al. Viscerotropic disease: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2012;30(33):5038-58. doi: 10.1016/j.vaccine.2012.04.067 [published Online First: 2012/05/09]
44. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barre syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29(3):599-612. doi: 10.1016/j.vaccine.2010.06.003 [published Online First: 2010/07/06]
45. Sejvar JJ, Kohl KS, Bilynsky R, et al. Encephalitis, myelitis, and acute disseminated encephalomyelitis (ADEM): case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;25(31):5771-92. doi: 10.1016/j.vaccine.2007.04.060 [published Online First: 2007/06/16]
46. Thomas RE, Lorenzetti DL, Spragins W, et al. Reporting rates of yellow fever vaccine 17D or 17DD-associated serious adverse events in pharmacovigilance data bases: systematic review. *Current drug safety* 2011;6(3):145-54. [published Online First: 2011/11/30]
47. Breugelmans JG, Lewis RF, Agbenu E, et al. Adverse events following yellow fever preventive vaccination campaigns in eight African countries from 2007 to 2010. *Vaccine* 2013;31(14):1819-29. doi: 10.1016/j.vaccine.2013.01.054 [published Online First: 2013/02/12]
48. World Health Organization (WHO). Fractional dose yellow fever vaccine as a dose-sparing option for outbreak response. WHO Secretariat information paper. *World Health Organization Department of Immunization, Vaccines and Biologicals Geneva* 2016;WHO/YF/SAGE/16.1(20 July 2016):76-7. doi: 10.1016/j.vaccine.2014.05.040 [published Online First: 2014/05/24]
49. Hickling J, Jones R. Yellow fever vaccination: The potential of dose sparing to increase vaccine supply and availability. A Technical Programme Report for Programme for Appropriate Technology in Health (PATH) Report. 2013. Available via https://www.path.org/publications/files/TS_vtg_yf_rpt.pdf Accessed on 29 December 2017.
50. Vannice K, Wilder-Smith A, Hombach J. Fractional-Dose Yellow Fever Vaccination — Advancing the Evidence Base. *New England Journal of Medicine* 2018;379(7):603-05. doi: 10.1056/NEJMp1803433
51. Campi-Azevedo AC, de Almeida Estevam P, Coelho-dos-Reis JG, et al. Subdoses of 17DD yellow fever vaccine elicit equivalent virological/immunological kinetics timeline. *BMC Infectious Diseases* 2014;14:391-91. doi: 10.1186/1471-2334-14-391
52. Roukens AH, Vossen AC, Bredenbeek PJ, et al. Intradermally Administered Yellow Fever Vaccine at Reduced Dose Induces a Protective Immune Response: A Randomized Controlled Non-Inferiority Trial. *PLoS ONE* 2008;3(4):e1993. doi: 10.1371/journal.pone.0001993
53. Martins RM, Maia MdLS, Farias RHG, et al. 17DD yellow fever vaccine: A double blind, randomized clinical trial of immunogenicity and safety on a dose-response study. *Human vaccines & immunotherapeutics* 2013;9(4):879-88. doi: 10.4161/hv.22982
54. Lopes Ode S, Guimaraes SS, de Carvalho R. Studies on yellow fever vaccine. III--Dose response in volunteers. *Journal of biological standardization* 1988;16(2):77-82. [published Online First: 1988/04/01]
55. Freestone DS, Ferris RD, Weinberg AL, et al. Stabilized 17D strain yellow fever vaccine: Dose response studies, clinical reactions and effects on hepatic function. *Journal of biological standardization* 1977;5(3):181-86. doi: https://doi.org/10.1016/S0092-1157(77)80003-6
56. Fox JP, Kossobudzki SL, Cunha JF. Field studies of the immune response to 17D yellow fever virus. Am J Hyg. 1943;38:113–38.
57. Ahuka-Mundeke S, Casey RM, Harris JB, et al. Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak - Preliminary Report. *The New England journal of medicine* 2018 doi: 10.1056/NEJMoa1710430 [published Online First: 2018/02/15]

58. Roukens AH, Vossen AC, van Dissel JT, et al. Reduced intradermal test dose of yellow fever vaccine induces protective immunity in individuals with egg allergy. *Vaccine* 2009;27(18):2408-9. doi: 10.1016/j.vaccine.2009.02.049 [published Online First: 2009/04/17]
59. de Menezes Martins R, Maia MLS, de Lima SMB, et al. Duration of post-vaccination immunity to yellow fever in volunteers eight years after a dose-response study. *Vaccine* 2018;36(28):4112-17. doi: 10.1016/j.vaccine.2018.05.041 [published Online First: 2018/05/23]
60. Nzolo D, Engo Biongo A, Lusakibanza M, et al. Safety profile of fractional dosing of yellow fever vaccine, experience from community based pharmacovigilance in Kinshasa, DRC2017.
61. Sanchez V, Gimenez S, Tomlinson B, et al. Innate and adaptive cellular immunity in flavivirus-naive human recipients of a live-attenuated dengue serotype 3 vaccine produced in Vero cells (VDV3). *Vaccine* 2006;24(23):4914-26. doi: 10.1016/j.vaccine.2006.03.066 [published Online First: 2006/04/25]
62. Jacobson RM, Targonski PV, Poland GA. Meta-analyses in vaccinology. *Vaccine* 2007;25(16):3153-59. doi: https://doi.org/10.1016/j.vaccine.2007.01.047
63. Lyman GH, Kuderer NM. The strengths and limitations of meta-analyses based on aggregate data. *BMC Med Res Methodol* 2005;5:14. doi: 10.1186/1471-2288-5-14 [published Online First: 2005/04/27]
64. Nigeria Demographic and Health Survey, 2008. 2012, Ref Type: Online Source, [http://www.measuredhs.com/pubs/pdf/FR222/FR222.pdf].
65. Booth A, Clarke M, Dooley G, et al. The nuts and bolts of PROSPERO: an international prospective register of systematic reviews. *Systematic Reviews* 2012;1:2-2. doi: 10.1186/2046-4053-1-2
66. Booth A, Clarke M, Ghersi D, et al. An international registry of systematic-review protocols. *Lancet* 2011;377(9760):108-9. doi: 10.1016/s0140-6736(10)60903-8 [published Online First: 2010/07/16]
67. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ : British Medical Journal* 2015;349 doi: 10.1136/bmj.g7647
68. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS medicine* 2009;6(7):e1000100. doi: 10.1371/journal.pmed.1000100 [published Online First: 2009/07/22]

PART C: JOURNAL ARTICLE (COCHRANE REVIEW FORMAT)

Table of contents

Abstract

Background: Persistent yellow fever endemicity and continued outbreaks have continued to increase vaccine demand, while straining limited global vaccine supply. To vaccinate susceptible populations in preventive mass-immunisation campaigns during outbreaks, fractional dose yellow fever vaccine is being considered as a dose-sparing strategy to maximise limited vaccine supplies.

Aim: This systematic review sought to assess the effects of fractional dose yellow fever vaccination in comparison with those of standard dose vaccination.

Methods: We registered the review in the prospective register of systematic reviews (PROSPERO, number: CRD42018084214); conducted a comprehensive search of electronic databases and reference lists of relevant publications; and followed the guidance contained in the statement on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA). We included randomised trials and quasi-randomised trials, expressed each study's result as a risk ratio (RR) with its 95% confidence interval (CI), and pooled the data using the random-effects method. We stratified analyses by the strength of the fractional dose. We assessed statistical heterogeneity using the Chi-squared test of homogeneity and quantified it using the Higgins' I^2 statistic.

Results: We retrieved 2495 records from the literature search, nine of them potentially eligible. We included six eligible studies (three randomised and three quasi-randomised trials), with 2371 participants. There was no statistically significant difference in immunogenicity between participants who received fractional doses containing one-third (two trials, 547 participants: RR 1.02, 95% CI 1.00 to 1.04; I^2 = 0%), one-fifth (one trial, 155 participants: RR 1.00, 95% CI 0.98 to 1.03), one-tenth (four trials, 890 participants: RR 0.99, 95% CI 0.96 to 1.01, I^2 = 0%), and one-fiftieth (three trials, 661 participants: RR 0.97, 95% CI 0.92 to 1.02, I^2 = 72%) of the standard dose and those who received the full standard dose. However, immunogenicity was significantly lower among participants who received fractional doses containing 1/100th (four trials, 868 participants: RR 0.92, 95% CI 0.87 to 0.97, I^2 = 60%) and <1/100th (five trials, 1053 participants; RR 0.53, 95% CI 0.44 to 0.64, I^2= 98%) of the standard dose than among those who received the full standard dose. Minor adverse events following vaccination did not differ across doses, but

no serious adverse events were reported in any study arm. The combined data provide moderate certainty evidence that there is little or no difference in immunogenicity between ≥1/50th fractional doses and the standard dose of yellow fever vaccines. However, due to limited data, we are uncertain whether there are differences between standard and fractional doses of yellow fever vaccines in the incidence of severe adverse events following vaccination.

Conclusion: These findings of this review support the use of fractional dose yellow fever vaccination as a dose-sparing strategy for yellow fever vaccination.

Keywords: Yellow fever, vaccination, fractional dose, dose-sparing, immunogenicity, safety.

1. Background

Yellow fever is a viral haemorrhagic disease of humans caused by the yellow fever virus.[1] The disease mostly occurs in tropical areas of Africa and South America, where it is endemic and intermittently epidemic.[1 2] The yellow fever virus is a prototypic member of the genus *Flavivirus* (*flavus* meaning yellow in Latin) having a relatively narrow host range, mostly humans and monkeys; and typically transmitted by *Aedes* mosquitoes.[2 3] Globally, approximately 200 000 cases of yellow fever and 30 000 deaths occur annually. [4 5] Sub-Saharan Africa, where the disease remains a major global health concern, bears 90% of the disease burden.[4 6] Typically, humans are infected when bitten by blood-feeding mosquitoes.[2] Susceptibility to yellow fever depends on several factors, such as previous infection with the virus and other flaviviruses; immune status; and environmental, racial and genetic factors.[7 8] Transmission is largely dependent on availability of vector, vertebrate hosts and vegetation.[9 10]

1.1. Description of the condition

In humans, yellow fever disease can be asymptomatic or cause a wide spectrum of diseases, from mild symptoms to severe illness with fever, nausea, vomiting, hepatitis, jaundice and, in extreme cases, haemorrhagic shock and death.[2 5 11] Case fatality ranges from 20 to 60%.[12] There is no known cure or specific treatment for yellow fever, hence supportive treatment remains the mainstay of clinical management.[2] Wild-type yellow fever infection can induce lifelong protection against subsequent infection.[12]

Yellow fever poses significant threat to unvaccinated persons travelling to endemic areas,[13] and with globalisation and ease of international travel, there has been documented cases of human importation of the disease from endemic areas to places where it is non-endemic.[13] Recently, imported cases have been reported in China, Kenya and Morocco.[14 15 16] Due to transmission between non-human primates and mosquitoes, and by transovarial transmission in mosquitoes, eradication of the disease is extremely difficult.[2 9]

The development of a life-attenuated vaccine which came into use in 1938, and its wide roll-out in the 1940s, have resulted in significant decline in the burden of yellow fever disease.[12 17] The

World Health Organisation (WHO) recommends vaccination in at-risk countries, as part of the routine childhood immunisation programmes as well as in mass immunisation campaigns during outbreaks.[4 13] Additionally, vaccination is recommended for preventive immunisation of travellers to endemic regions.[4] Yellow fever vaccine has been considered highly immunogenic, with results of clinical trials showing that 99% of vaccine recipients developed protective levels of neutralising antibodies within four weeks.[4 9 17-21] Currently, the plaque reduction neutralisation test is considered to be the gold-standard serological method for assessing immune response to yellow fever vaccination.[9 16]

Yellow fever vaccine has also been considered safe and well tolerated. Serious adverse events are rare.[22 23] Reported serious adverse events include anaphylactic or hypersensitivity reactions; yellow fever vaccine-associated viscerotropic disease and yellow fever vaccine-associated neurologic disease.[22 24-26] The mechanisms of these serious adverse events are poorly understood, though older age has been identified as a risk factor.[27 28] The Brighton Collaboration case definitions provide a standardised classification of serious adverse events using three distinct levels of diagnostic certainty.[26 29-32] Incidence of yellow fever vaccine-associated viscerotropic ranges from 0 to 0.21 cases per 100 000 vaccine doses in endemic regions, and from 0.09 to 0.4 cases per 100 000 doses in non-endemic settings. Yellow fever vaccine-associated neurologic disease is estimated to occur at a frequency of 0.8 cases per 100 000 vaccine doses administered.[4]

1.2. Description of the intervention

Persistent endemicity and continued outbreaks have continued to increase vaccine demand, while straining limited global supplies.[4 16] Therefore, to vaccinate susceptible populations in preventive mass immunisation campaigns during outbreaks, fractional dosing of the vaccine is being considered as a dose-sparing strategy to maximise limited supplies.[4 16] Fractional dose yellow fever vaccination refers to administration of a reduced volume of vaccine dose, which has been reconstituted as per manufacturer recommendations.[4] While, the minimum potency recommended for standard dose should not be less than 1000 international units (IU)/dose,[16 33]

the potency of standard doses is usually many-fold higher than recommended.[4] This forms the fundamental basis of fractional dose considerations. As a dose-sparing strategy, a fractional dose of the yellow fever vaccine meeting the minimum potency requirement is expected to be equivalent to a standard yellow fever vaccine dose with respect to safety, immunogenicity, efficacy and effectiveness. [4 34 35] The first practical use of fractional doses was in response to a large yellow fever outbreak in the Democratic Republic of the Congo in mid-2016.[4 16]

1.3. How the intervention might work

Fractional dose vaccination against yellow fever works by stimulating humoral immunity through neutralising antibodies against the yellow fever virus.[35] Various studies have investigated the protective effects and safety of fractional doses of the yellow fever vaccine in humans.[34-42] This review has systematically summarised the findings from these studies.

1.4. Why it is important to do this review

We have found two non-systematic reviews of fractional dose yellow fever vaccination in the grey literature.[16 33] However, we are unaware of any currently available comprehensive and systematic synthesis of the body of evidence on the effects and safety of this strategy, thus informing the need for this review. Also, the use of meta-analysis in vaccinology has grown remarkably in recent years;[43] helping to produce quantitatively robust and accurate effect-size measurements that are generalisable.[44 45] Therefore, this review will not only add to the body of knowledge and fill knowledge gap, it will provide a robust evidence-base for informing global health and vaccine policy and advocacy processes. Furthermore, the analytical rigour with which the review was conducted will provide methodological guidance for subsequent reviews, while providing vaccinologists, clinicians and policy makers a user-friendly evidence summary.

Current international best practices in the conduct of systematic reviews and meta-analyses were followed; including registration on the International Prospective Register of Systematic Reviews (PROSPERO) [46 47] and synthesised the evidence in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA).[48]

2. Objectives

To assess the effects of fractional dose yellow fever vaccination, compared to vaccination using the standard dose of the vaccine.

3. Methods

3.1. Types of studies

There was no restriction on inclusion based on study-design.

3.2. Types of participants

All individuals, irrespective of age were eligible for inclusion.

3.3. Types of intervention

The eligible intervention was the administration of fractional doses of the live-attenuated yellow fever vaccine, while the eligible comparison was the administration of the standard dose of the vaccine; irrespective of vaccination schedule, route of administration or formulation.

3.4. Types of outcome measures

3.4.1. Primary outcomes

- Immunogenicity: i.e. levels of vaccine-specific virus-neutralising antibodies and rates of seroconversion, assessed at least four weeks following vaccination.[49]
- Safety i.e. adverse events following vaccination as reported by authors and standardised using the WHO and the Brighton Collaboration case definitions.[22 24-26]

3.4.2. Secondary 0utcomes

- Incidence of laboratory-confirmed yellow fever cases[12 50]
- Mortality

3.5. Search methods

We conducted a comprehensive search of peer-reviewed literature in multiple electronic databases from inception of each database to the date of the search. Search strategies (see Appendix 1) were developed with guidance from a reference librarian and adapted for the

various databases using appropriate controlled vocabulary and syntax. The following databases were searched: Cochrane Library (including the Cochrane Central Register of Controlled Trials (CENTRAL) and the Database of Abstracts of Reviews of Effects (DARE)), PubMed/Medline, EBSCOhost, Scopus, Cumulative Index of Nursing and Allied Health (CINAHL), ClinicalTrials.gov, WHO International Clinical Trials Registry Platform (ICTRP), African Index Medicus, and Latin American and Caribbean Health Sciences Literature (LILACS). Additionally, we conducted a hand search of reference lists of relevant studies and grey literature, including vaccine-related journals and WHO position papers, relevant vaccination field reports, policy statements and conference abstracts and proceedings.

Two researchers (Chukwudi A. Nnaji (CAN) and either Muki S. Shey (MSS) or Olatunji O. Adetokunboh (OOA)) independently screened the search output, to retrieve full texts of potentially eligible studies and assess their eligibility using predefined inclusion criteria. Disagreements in the eligibility assessment were resolved through consensus between the two researchers. If there was no consensus following discussion, Charles S. Wiysonge (CSW) would have arbitrated.

3.6. Data extraction and management

Following the selection of eligible studies, two researchers (CAN and OOA or MSS) independently extracted relevant data using a standardised data extraction form (see Appendix 2). We re-verified study eligibility at the start of data extraction.

Extracted data included:

- General details: authors' details, affiliations and year of publication.
- Study details: design, setting, geographical location and risk of bias items
- Participant characteristics: mean age and standard deviation, age range, sex, ethnicity and sample size.
- Intervention details: vaccine sub-strain, strength pf fractional dose and route of administration
- Outcome details: types of outcomes, outcome assessment methods, outcome data.

Disagreements between the two investigators were resolved by discussion. If a consensus was not arrived at, CSW would have arbitrated. If required data were not available from study publications, CAN would have contacted the authors to obtain the missing information. Extracted data were entered by CAN into the Cochrane meta-analytical software.[51] CSW double-checked the entered data for accuracy.

3.7. Risk of bias assessment

Two researchers (CAN and either OOA or MSS) independently assessed each included study for risk of bias using the seven specific domains of the risk of bias tool, as described in the Cochrane Handbook for Systematic Reviews of Interventions.[52 53] The seven domains included random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and other bias. We assessed the adequacy of random sequence generation and allocation concealment for risk of selection bias; and blinding of participants and personnel for the risk of performance bias. For the risk of detection bias, we assessed the blinding of outcome assessors, and completeness of outcome data and outcome reporting for the risks of attrition and reporting biases, respectively. For each domain, we classified the risk of bias as low if the criterion was adequately addressed, high if not adequately addressed, and unclear if the information provided was insufficient or unavailable to make an informed judgement. We summarised the assessment and categorised each included study either as having a low or a high risk of bias. Any study that had a high risk of selection, detection or attrition bias was categorised as having a high risk of bias. All other studies were considered to have a low risk of bias.

3.8. Data analyses

3.8.1. Measures of effects

We analysed and reported risk ratios (RR) with their corresponding 95% confidence intervals (CI) for dichotomous data.

3.8.2. Dealing with missing data

Study authors would have been contacted to obtain missing data if necessary.

3.8.3. Data synthesis

We used the random-effects method to combine data from included studies and stratified by the strength of the fractional dose. We assessed statistical heterogeneity across included studies using the Chi-Squared test of homogeneity (with significance defined at the 10% α-level) and quantify it using the Higgins' I^2 statistic.[52 54]

3.8.4. Subgroup analyses

We conducted subgroup analyses to explore possible causes of significant statistical heterogeneity of effects, with subgroups defined by vaccine sub-strain and continent where study was conducted.

3.8.5. Sensitivity analyses

We performed sensitivity analyses to assess the robustness of pooled estimates to risk of bias.

3.8.6. Assessment of reporting bias

We could have performed funnel plot of estimated differences in outcome effects against their standard errors if there were at least 10 studies included in the review, as required for the assessment of publication bias.[52] We however minimised the potential for reporting bias by conducting a comprehensive search of both published and grey literature.

3.9. Grading the certainty of evidence

We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to assess the certainty of the evidence on the effect of fractional dosing of yellow fever vaccines.[55] We generated GRADE evidence profiles and summary of findings tables for each outcome measure.

4. Results

4.1. Literature search results and study selection

The literature searches generated a total of 2494 records. After screening titles and abstracts of the records, we discarded 2486 clearly irrelevant records. An additional study was found through hand search of the reference lists of included studies. Of the nine potentially eligible studies, six met the study's inclusion criteria.[34-39] The remaining three were excluded for not having control groups.[40-42] Figure 1 below shows the PRISMA flow diagram of the study selection process.

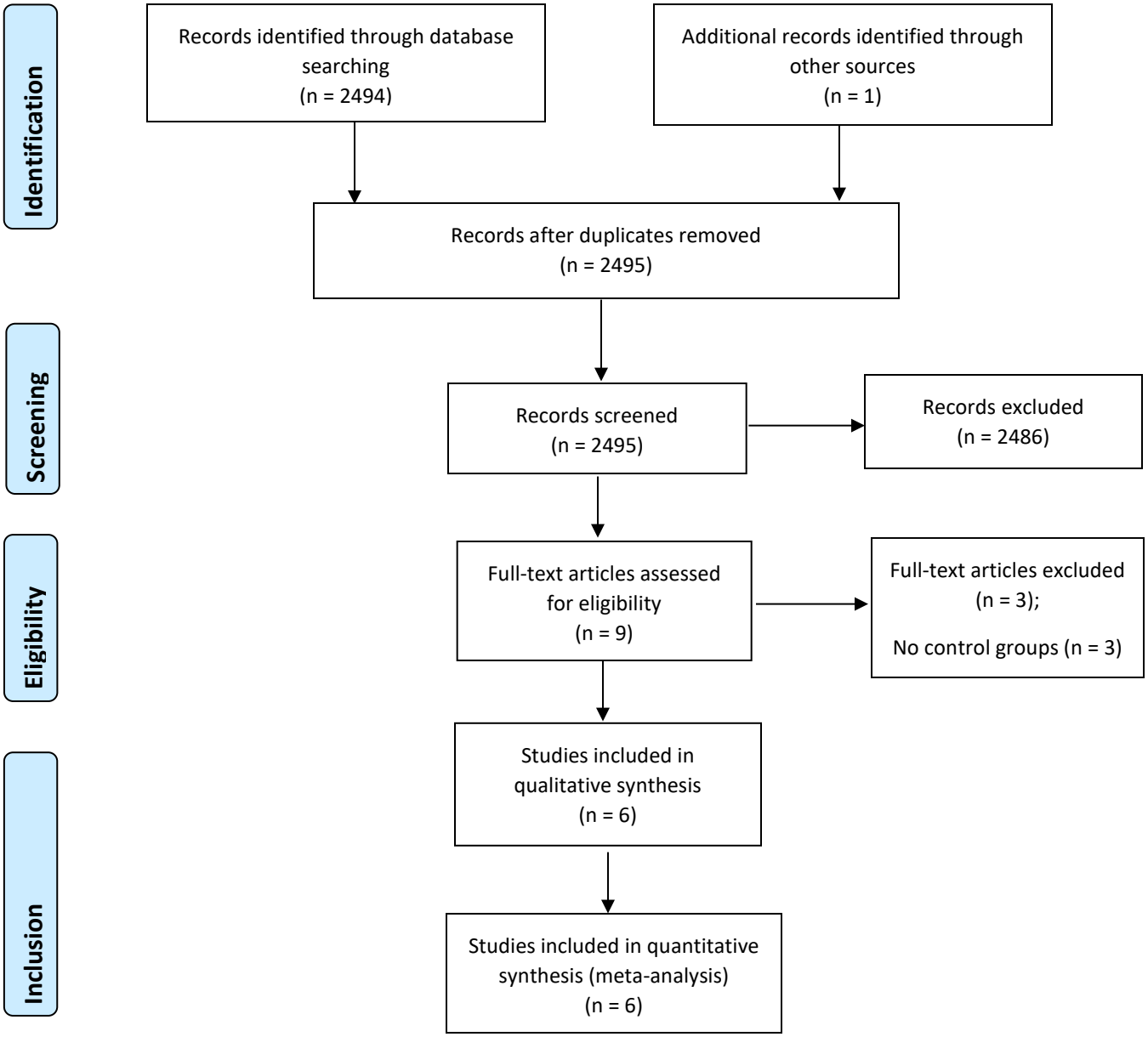

Figure 1: PRISMA flow diagram showing study selection process

4.2. Description of included studies

Of the six included studies, three are randomised controlled trials[34-36], while the others are quasi-randomised trials.[37-39] Table 1 below summarises the characteristics of the six included studies. A total of 2371 participants participated in the studies, with individual study sample sizes ranging from 68 to 749. Four of the studies were conducted in Latin America (Brazil)[34 36 37 39], while two were undertaken in Europe (England[38] and the Netherlands[35]). In terms of settings, four of the studies were conducted in military settings[34 36 37 39] and the other two involved civilian participants.[35 38] In all studies, participant were healthy adults, with reported age ranging from 18 to 59 years.

The 17D sub-strain yellow fever vaccine was administered in four studies[35 37-39], whereas the 17DD sub-strain was used in the other two.[34 36] Fractional doses with which comparison was made with their corresponding reference standard (or undiluted) doses varied by strength of dilution as well as units of vaccine doses across studies and their respective intervention arms. Fox 1943 compared standard doses with fractional doses corresponding to 1/10th; 1/100th; and <1/100th of the reference standard dose[39]; Freestone 1977 did comparison with dilution strengths of <1/100th[38]; Lopes 1988 used dilutions corresponding to 1/10th, 1/50th, 1/100th and <1/100th[37]; Roukens 2008 used 1/5th (0.1mL) of the reference standard dose[35]; while Martins 2013 and Campi-Azevedo 2014 used fractional doses corresponding to 1/3rd, 1/10th, 1/50th, 1/100th and <1/100th of the reference standard dose.[34 36] All studies assessed immunogenicity with seroconversion rates across vaccination arms. Actual antibody geometric mean titre values were reported in three studies.[34 36 37] Immune response was assessed by the plaque reduction neutralisation test with seroconversion end-points defined at 50 – 80% viral neutralisation[34-37 39] or log10 neutralisation index of ≥0.7.[38] Three studies assessed and reported vaccine safety.[35 36 38]

Table 1: Characteristics of included studies

Study	Design	Participants	Interventions and comparisons	Outcome assessment methods
Fox 1943	qRCT	550 military personnel **Sex**: all male **Age:** not described **Location:** Belo Horizonte, Brazil	**Intervention:** vaccination with five dilutions of the 17D YF vaccine; 1/10; 1/100; 1/1000; 1/10 000 and 1/100 000 **Comparison:** vaccination with standard (undiluted) dose of the 17D yellow fever vaccine.	**Seroconversion:** determined by a 50% viral plaque reduction by anti-YF neutralising antibody titres five weeks following vaccination using the plaque reduction and neutralisation test (PRNT). **Antibody Geometric Mean Titre (GMT)**: not reported **Safety:** not assessed
Freestone 1977	qRCT	68 research laboratory staff **Sex:** male and female **Age range (mean):** 18 – 59 (34.3) years **Location:** Beckenham and Dartford, England	**Intervention:** vaccination with five sub-doses of stabilised 17D YF vaccine; $10^{5.26}$ (181 970) PFU; $10^{5.27}$ (186 209) PFU; $10^{2.25}$ (178) PFU; $10^{1.49}$ (31) PFU and $10^{0.98}$ (10) PFU **Comparison:** vaccination with unstabilised standard dose of the 17D yellow fever vaccine; $10^{5.26}$ (181 970) PFU vaccine.	**Seroconversion:** determined by viral plaque reduction by anti-yellow fever neutralising antibody titres four weeks following vaccination using the PRNT. **Antibody GMT:** not reported. Instead, mean antibody response was reported and expressed as neutralising index. **Safety:** self-reported adverse events by volunteers, who were instructed to fill out a diary for adverse events during the first 8 days after vaccination.
Lopes 1988	qRCT	259 military personnel **Sex:** all male **Age range (mean):** 18 – 47 (21.6) years **Location:** Rio de Janeiro, Brazil	**Intervention:** vaccination with four diluted doses of the 17D yellow fever vaccine (1/10th, 1/60th, 1/100th and 1/1000th dilutions) **Comparison:** vaccination with full dose (>1656 PFU) of the 17D yellow fever vaccine	**Seroconversion:** determined by a 50% viral plaque reduction by anti-yellow fever neutralising antibody titres four weeks following vaccination using PRNT. **Antibody GMT:** expressed in PFU/dose **Safety:** not assessed.
Roukens 2008	RCT	155 volunteers from a university **Sex:** male and female **Age range (mean):** 20 – 50 (32) years **Location:** Leiden, The Netherlands	**Intervention:** vaccination with fractional dose (0.1mL) dose of the 17D yellow fever vaccine administered intradermally. **Comparison:** vaccination with standard dose (0.5mL) of the 17D yellow fever vaccine administered subcutaneously.	**Seroconversion:** for comparison of standard and fractional vaccinations, serum dilution at which 80% viral neutralisation occurred was taken as endpoint at two, four and eight weeks and 1 year after vaccination using modified PRNT. **Antibody GMT:** not reported. **Safety:** participants reported duration and severity of adverse events after vaccination in a three-week diary. Safety of vaccination expressed in various parameters, number of

				days events lasted and severity of event (absent, mild, moderate and severe).
Martins 2013	RCT	749 army recruits **Sex:** all male **Mean age:** 18 – 30 (19.4) years **Location:** Rio de Janeiro, Brazil	**Intervention:** vaccination with 5 sub-doses of the 17DD yellow fever vaccine 10,447 IU, 3,013 IU, 587 IU, 158 IU and 31 IU **Comparison:** vaccination with standard dose (27,476 IU) of the 17DD yellow fever vaccine	**Seroconversion:** determined by a 50% viral plaque reduction by anti-yellow fever neutralising antibody titres at 30 days (approximately four weeks) and 10 months following vaccination using PRNT. **Antibody GMT:** expressed in log10 mIU/mL and 2.7 log10 mIU/mL as the cut-off point to segregate seropositive from seronegative samples. **Safety:** volunteers filled out an adverse events diary during the first 10 days after vaccination. Intensity of adverse events were evaluated on a scale from 0 to 4, according to the Food and Drug Administration guidelines.
Campi-Azevedo 2014	RCT	590 army recruits **Sex:** all male **Mean age:** 19 years (age range not described) **Location:** Rio de Janeiro, Brazil	**Intervention:** vaccination with 5 sub-doses of the 17DD yellow fever vaccine 10,447 IU, 3,013 IU, 587 IU, 158 IU and 31 IU. **Comparison:** vaccination with standard dose (27,476 IU) of the 17DD yellow fever vaccine.	**Seroconversion:** determined by a 50% viral plaque reduction by anti-yellow fever neutralising antibody titres at 26 - 34 days (approximately four weeks) following vaccination using the plaque reduction and neutralisation test (PRNT). Serology was conducted on 885 paired samples from 590 participants. **Antibody GMT:** assessed in log10 mIU/mL but not expressed in actual numeric figures. **Safety:** not assessed.

RCT: randomised controlled trial; qRCT: quasi-randomised controlled trial.

4.3. Excluded studies

We excluded three studies for not having comparison groups; Roukens 2009 [41], Nzolo 2017[40], and Ahuka-Mundeke 2018[42]. Roukens 2009 was a cohort study that assessed immune response to reduced intradermal doses of the yellow fever vaccine in individuals with egg allergy and the other two studies assessed the immunogenicity and safety of fractional dose vaccination during a mass preventive vaccination campaign. In the appendix, a table of characteristics of excluded studies is provided.

4.4. Risk of bias in included studies

One randomised trial had adequate random sequence generation and allocation concealment.[35] Of the other five studies, one had a high risk of selection bias due to inadequate random sequence generation and allocation concealment[37], while the risk was unclear in the rest.[34 36 38 39] Two studies had a low risk of bias for blinding of participants and personnel.[35 36] Outcome assessors were not aware of intervention allocations in one study[36], but this was not the case in the remaining five.[34 35 37-39] One study had a high risk of attrition bias (loss to follow-up and post-randomisation exclusion of >25% with attrition imbalance across arms)[34], while the rest had a low risk of attrition bias. All studies had a low risk of selective outcome reporting[37], while one had an unclear risk of other biases.[37] Table 2 shows a summary of the risk of bias in included studies.

Table 2: Summary of risk of bias in included studies.

	Fox 1943	Freestone 1977	Lopes 1988	Roukens 2008	Martins 2013	Campi-Azevedo 2014
Random sequence generation (selection bias)	?	?	−	+	?	?
Allocation concealment (selection bias)	?	?	−	+	?	?
Blinding of participants and personnel (performance bias)	?	?	?	+	+	?
Blinding of outcome assessment (detection bias)	?	?	?	?	+	?
Incomplete outcome data (attrition bias)	+	+	?	+	+	−
Selective reporting (reporting bias)	+	+	+	+	+	+
Other bias	+	+	?	+	+	+

+ Low risk; − High risk; ? Unclear risk.

4.5. Measures of effect

4.5.1. Immunogenicity

4.5.1.1. Seroconversion rates in individual studies

Table 3 below describes the immunogenicity in each study according to their respective vaccine arms. In the earliest study by Fox and colleagues in 1943, Belo Horizonte, Brazil; 109 army recruits were vaccinated with standard doses of the 17D yellow fever vaccine, while 116, 103 and 222 others received 1/10th, 1/100th and <1/100th fractional doses of the reference vaccine respectively. Seroconversion rates (proportions of vaccinated participants who seroconverted at five weeks following vaccination) were found to be 100% (109/109), 98.28% (114/116), 96.12% (99/103) and 46.93% (103/222) among participants who received standard, 1/10th, 1/100th and <1/100th fractional doses respectively.[39]

In their 1977 study in Beckenham and Dartford, England; Freestone and colleagues allocated 30 and 38 participants to standard and <1/100th fractional doses of the 17D yellow fever vaccine respectively. They found seroconversion rates (assessed at four weeks following vaccination) to be 100% (30/30) and 47.37% (18/38) among those who received standard and <1/100th fractional doses respectively.[38]

In 1988, Rio de Janeiro, Brazil; Lopes and colleagues assigned 46, 49, 56, 55 and 53 military personnel to be vaccinated with standard, 1/10th, 1/50th, 1/100th and <1/100th doses of the 17D yellow fever vaccine respectively. Their seroconversion rates at four weeks following vaccination were 100% (46/46), 100% (49/49), 85.71% (48/56), 83.64% (46/55) and 41.50% (22/53) respectively.[35]

Roukens and colleagues in their 2008 trial in Leiden, the Netherlands, randomised 78 and 77 participants to receive standard and 1/5th doses of the 17D yellow fever vaccine respectively. All (100.00%) of the participants in both standard and 1/5th vaccination arms seroconverted four weeks following vaccination.[35]

In 2013, Rio de Janeiro, Brazil; Martins and colleagues randomised 131, 115, 132, 131, 122 and 118 army personnel to receive standard, 1/3rd, 1/10th, 1/50th, 1/100th and <1/100th doses of the 17DD yellow fever vaccine respectively. Their seroconversion rates at four weeks following vaccination were 97.70% (128/131), 99.13% (114/115), 97.72% (129/132), 96.95% (127/131), 88.52% (108/122) and 57.69% (79/118) respectively.[36]

In their 2014 trial, also in Rio de Janeiro, Brazil; Campi-Azevedo and colleagues randomised 157, 144, 150, 140, 145 and 149 army personnel to receive standard, 1/10th, 1/3rd, 1/50th, 1/100th and <1/100th doses of the 17DD yellow fever vaccine respectively. Those who seroconverted after four weeks were 98.09% (154/157), 100.00% (144/144), 98.00% (147/150), 97.14% (136/140), 88.97% (129/145) and 57.05% (85/149) respectively.[34]

Table 3: Seroconversion rates in individual studies

Study		**Seroconverted**	**Total vaccinated**	**Seroconversion rate (95% CI)**
Fox 1943	Standard dose	109	109	100.00 (96.67 – 100.00) %
	1/10th dose	114	116	98.28 (93.91 – 99.79) %
	1/100th dose	99	103	96.12 (90.35 – 98.93) %
	<1/100th dose	103	222	46.39 (39.67 – 53.19) %
Freestone 1977	Standard dose	30	30	100.00 (88.43 – 100.00) %
	<1/100th dose	18	38	47.37 (30.98 – 64.18) %
Lopes 1988	Standard dose	46	46	100.00 (92.29 – 100.00) %
	1/10th dose	49	49	100.00 (92.75 – 100.00) %
	1/50th dose	48	56	85.71 (73.78 – 93.62) %
	1/100th dose	46	55	83.64 (71.12 – 92.23) %
	<1/100th dose	22	53	41.50 (28.14 – 55.87) %
Roukens 2008	Standard dose	78	78	100.00 (95.38 – 100.00) %
	1/5th dose	77	77	100.00 (95.32 – 100.00) %
Martins 2013	Standard dose	128	131	97.70 (93.45 – 99.52) %
	1/3rd dose	114	115	99.13 (95.25 – 99.97) %
	1/10th dose	129	132	97.72 (93.50 – 99.53) %
	1/50th dose	127	131	96.95 (82.37 – 99.16) %
	1/100th dose	108	122	88.52 (81.49 – 93.58) %
	<1/100th dose	79	118	66.95 (57.69 – 75.33) %
Campi-Azevedo 2014	Standard dose	154	157	98.09 (94.51 – 99.60) %
	1/3rd dose	144	144	100.00 (97.47 – 100.00) %
	1/10th dose	147	150	98.00 (94.27 – 99.59) %
	1/50th dose	136	140	97.14 (92.84 – 99.22) %
	1/100th dose	129	145	88.97 (82.70 – 93.56) %
	<1/100th dose	85	149	57.05 (48.69 – 65.12) %

4.5.1.2. Meta-analyses of seroconversion rates

Since included studies involved multiple intervention arms with varying dilutions of the standard doses, we stratified meta-analyses by fractional dose strength. This helped to homogenise comparison across study arms between similar strengths of fractional doses and their corresponding standard doses. Apart from dealing with heterogeneity across vaccine arms, stratification also enabled stratum-level reporting of immunogenicity estimates for each stratum, thereby helping to deal with the unit of analyses error that could have resulted from double-counting of control groups across strata, if they were all pooled in a single meta-analysis.[52]

Two studies compared immunogenicity between 1/3rd fractional doses and the standard doses of the reference vaccine lots, with a combined sample size of 557 (259 in the intervention arms and 288 in the control groups).[34 36] Seroconversion rates of 1/3rd fractional doses compared with the reference standard doses were respectively 99.13% (114/115) and 97.71% (128/131) in Martins 2013[36]; and 100.00%(144/144) and 98.09% (154/157) in Campi-Azevedo 2014.[34] Combining data from the studies shows that there was no statistically significant difference in immunogenicity between 1/3rd and reference standard doses (two trials, 547 participants: RR 1.02, 95% CI 1.00 to 1.04, I^2= 0 %) (Figure 2 and Table 4).

One study, Roukens 2008, compared between 1/5th fractional dose and the standard dose among 77 participants in the intervention arm and 78 in the control group.[35] They reported seroconversion rates of 100.00% in both fractional and standard dose vaccine arms (77/77 and 78/78 respectively), showing that there was no statistically significant difference in immunogenicity between 1/5th and standard doses (one trial, 155 participants: RR 1.00, 95% CI 0.98 to 1.03) (Figure 2 and Table 4).

Four studies compared immunogenicity between 1/10th and standard doses of the reference vaccine lots, with a combined sample size of 890 (447 and 443 in the intervention and control arms respectively).[34 36 37 39] Seroconversion rates of 1/10th fractional doses compared with the reference standard doses were respectively 98.28% (114/116) and 100.00% (109/109) in Fox 1943[39]; 100.00% (49/49) and 100.00% (46/46) in Lopes 1988 [37]; 97.73% (129/132) and 97.71%

(128/131) in Martins 2013[36]; and 98.00% (147/150) and 98.09% (154/157) in Campi-Azevedo 2014.[34] Combining data from the studies shows that there was no statistically significant difference in immunogenicity between 1/10th fractional doses and reference standard doses (four trials, 890 participants: RR 0.99, 95% CI 0.98 to 1.01, I^2= 0 %) (Figure 2 and Table 4).

Three studies compared immunogenicity between 1/50th and standard doses among 661 participants (327 in the intervention arms and 334 in the control arms).[34 36 37] The first study, Lopes 1988, reported seroconversion rates of 85.71% (48/56) and 100.00% (46/46) seroconversion rates in the 1/50th fractional dose and standard dose arms respectively.[37] The second study, Martins 2013, reported 96.95% (127/131) and 97.71% (128/131) while the third study, Campi-Azevedo 2014 reported 97.14% (136/140) and 98.09% (154/157) in the 1/50th fractional dose and standard dose vaccine arms respectively.[34] Combining data from the studies shows that there was no statistically significant difference in immunogenicity between 1/50th fractional dose and reference standard doses (three trials, 661 participants: RR 0.97, 95% CI 0.92 to 1.02, I^2= 72 %) (Figure 2 and Table 4). The substantial between-study heterogeneity observed was driven by the Lopes 1988 study, as explored and described later in sensitivity analyses.

Immunogenicity of lower fractional doses was also investigated. Four studies compared immunogenicity between 1/100th fractional doses and the standard doses of the reference vaccine lots, with a combined sample size of 868 (425 and 443 in the intervention and control arms respectively).[34 36 37 39] Seroconversion rates of 1/100th fractional doses compared with the reference standard doses were respectively 96.12% (99/103) and 100.00% (109/109) in Fox 1943[39]; 83.64% (46/55) and 100.00% (46/46) in Lopes 1988[37]; 88.52% (108/122) and 97.71% (128/131) in Martins 2013[36]; and 88.96%(129/145) and 98.09% (154/157) in Campi-Azevedo 2014.[34] Combining data from the studies shows a statistically significant lower immunogenicity of the 1/100th fractional dose, compared with the reference standard doses (four trials, 868 participants: RR 0.92, 95% CI 0.87 to 0.97, I^2= 60 %) (Figure 2 and Table 4). The moderate between-study heterogeneity observed was driven by the Fox 1943 study, as explored and described later in sensitivity analyses.

Much lower fractional doses were also investigated. Five studies investigated the comparative immunogenicity between <1/100th fractional doses and standard doses of the reference vaccines among 1053 participants (580 and 473 in the intervention and control arms respectively).[34 36-39] Seroconversion rates of <1/100th fractional doses compared with the reference standard doses were respectively 46.40% (103/222) and 100.00% (109/109) in Fox 1943[39]; 47.37% (18/38) and 100.00% (30/30) in Freestone 1977[38]; 41.51% (22/53) and 100.00% (46/46) in Lopes 1988[37]; 66.95% (79/118) and 97.71% (128/131) in Martins 2013[36]; and 57.05% (85/149) and 98.09% (154/157) in Campi-Azevedo 2014.[34] Combining data from the studies shows a statistically significant lower immunogenicity of the <1/100th fractional doses, compared with the reference standard doses (five trials, 1053 participants: RR 0.53, 95% CI 0.44 to 0.64, I^2= 80 %) (Figure 2 and Tables 4). The considerable heterogeneity observed was mostly driven by the Fox 1943 study, as explored and described later in sensitivity analyses.

Overall, the results of the meta-analysis show that immunogenicity did not differ between participants who received 1/3rd, 1/5th, 1/10th and 1/50th fractional doses, compared with those who received the standard doses of the reference vaccines, with no substantial heterogeneity between studies. However, there was statistically significant lower immunogenicity in fractional doses lower than 1/50th (1/100th and <1/100th), compared with the reference standard dose vaccine. We assessed the certainty of the evidence across fractional dose arms using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach. Though evidence from randomised trials is considered of high certainty in the GRADE framework; we downgraded the evidence across fractional doses for various reasons (see Table 5).

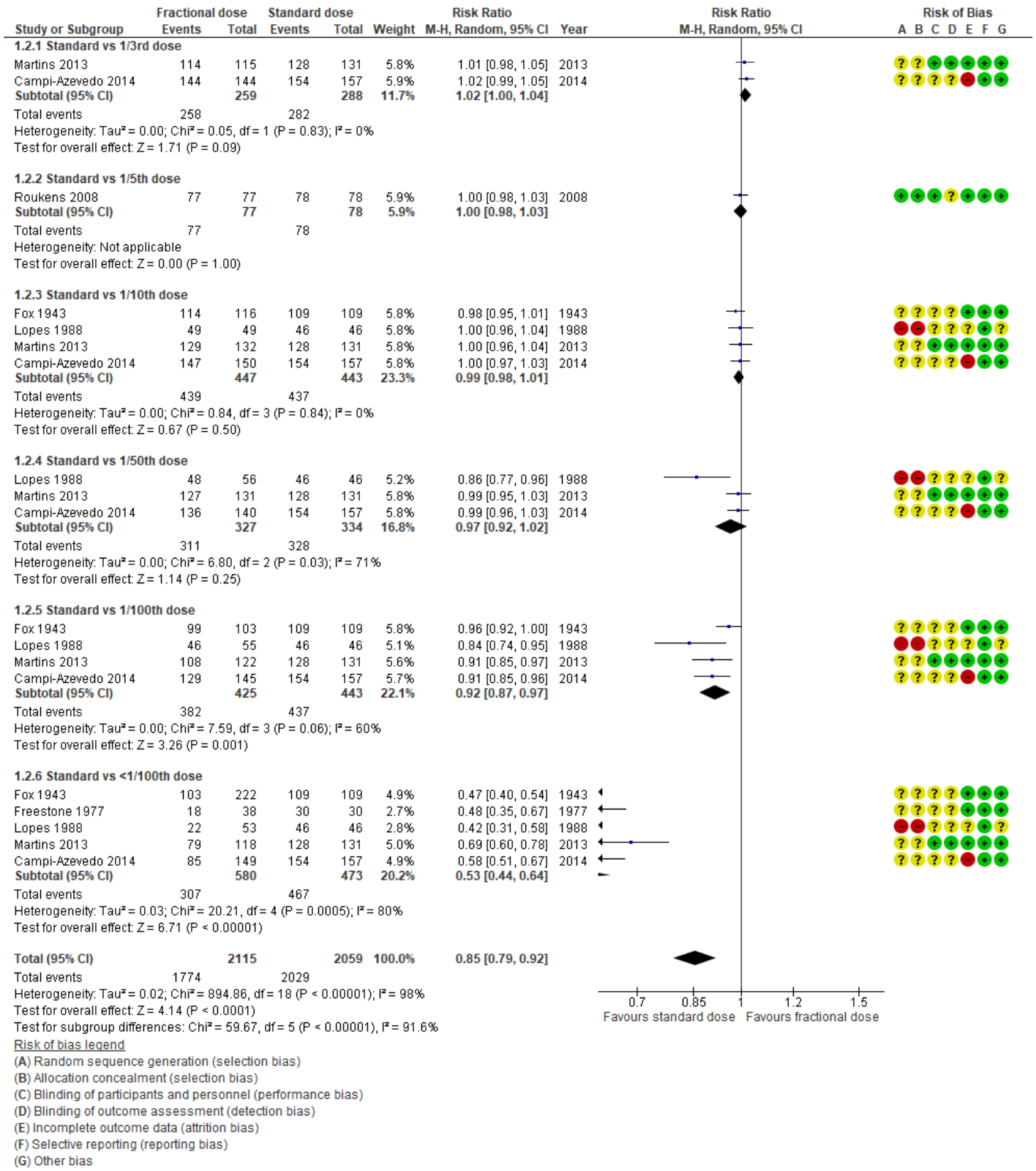

Figure 2: Forest plot showing seroconversion rates of fractional doses of the yellow fever vaccine compared to standard doses of the vaccine

Table 4: Comparative immunogenicity stratified by strength of fractional doses

	Illustrative comparative seroconversion rates (95% CI)		Relative effect (95% CI)	No of Participants (studies)
	Standard dose	Fractional dose		
Standard vs 1/3rd doses	282 / 288; 97.92 (95.52 – 99.23) %	258 / 259; 99.61 (97.87 – 99.99) %	RR 1.02 (1.00 to 1.04)	547 (2 studies)
Standard vs 1/5th doses	78 / 78; 100.00 (95.38 – 100.00) %	77 / 77; 100.00 (95.32 – 100.00) %	RR 1.00 (0.98 to 1.03)	155 (1 study)
Standard vs 1/10th doses	437 / 443; 98.65 (97.08 – 99.50) %	439 / 447; 98.21 (96.50 – 99.22) %	RR 0.99 (0.98 to 1.01)	890 (4 studies)
Standard vs 1/50th doses	328 / 334; 98.20 (96.13 – 99.34) %	311 / 327; 95.11 (92.18 – 97.18) %	RR 0.97 (0.92 to 1.02)	661 (3 studies)
Standard vs 1/100th doses	437 / 443; 98.65 (97.08 – 99.50) %	382 / 425; 89.88 (86.61 – 92.58) %	RR 0.92 (0.87 to 0.97)	868 (4 studies)
Standard vs <1/100th doses	467 / 473; 98.73 (97.26 – 99.53) %	307 / 580; 52.93 (48.78 – 57.06) %	RR 0.53 (0.44 to 0.64)	1053 (5 studies)

4.5.1.3. Subgroup analyses of seroconversion rates

4.5.1.3.1. Subgroup analyses of seroconversion rates by vaccine sub-strain

When grouped according to the constituent viral sub-strain of the vaccines, immunogenicity of ≥1/50th doses did not differ across vaccine sub-strains (six trials, 1631 participants: RR 0.99, 95% CI 0.98 to 1.01, I^2= 21%) (Figure 3).

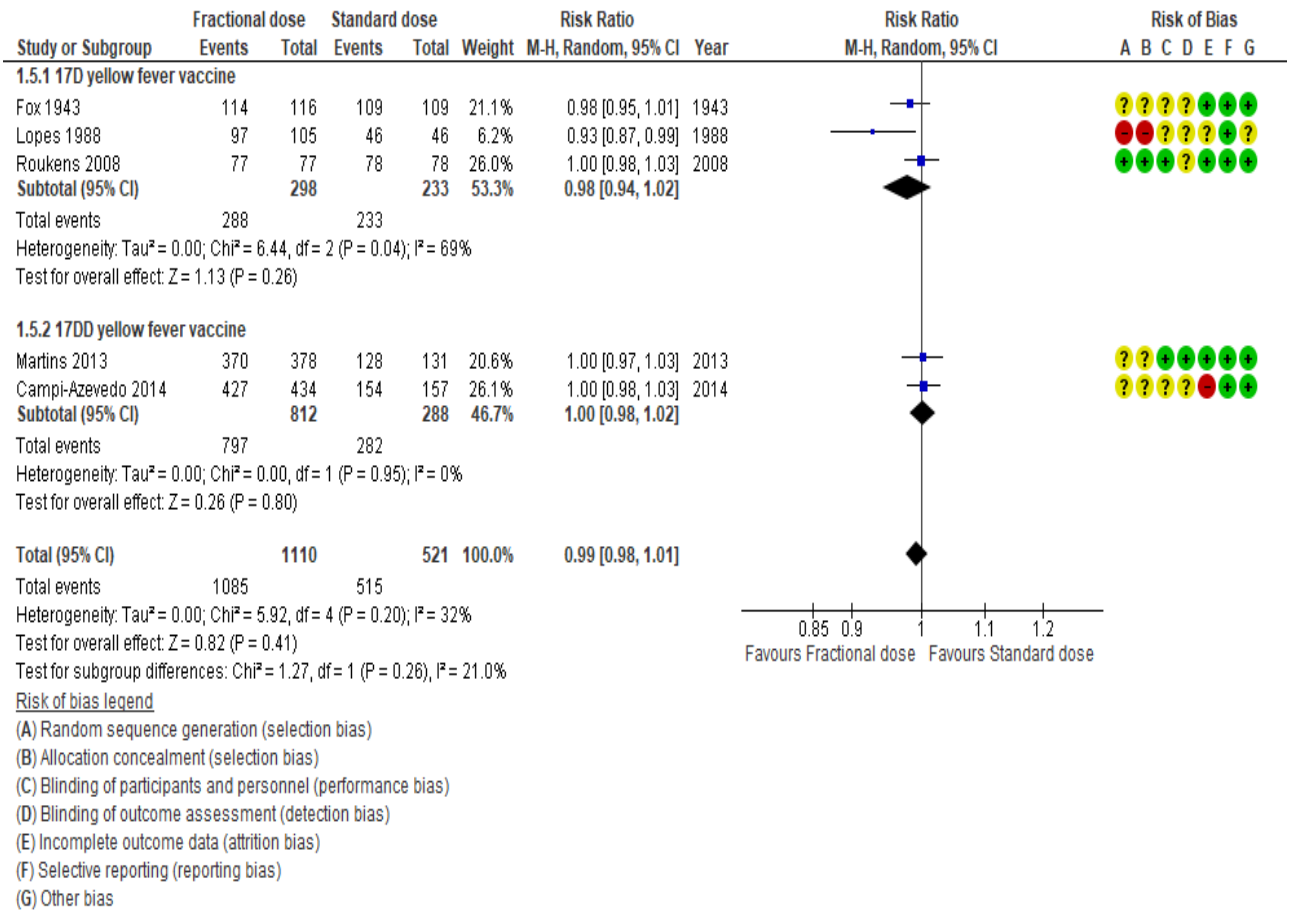

Figure 3: Subgroup immunogenicity of fractional doses of the yellow fever vaccine by vaccine sub-strain

4.5.1.3.2. Subgroup analyses of seroconversion rates by geographical region

When grouped by continents, immunogenicity of ≥1/50th doses did not differ across vaccine sub-strains (six trials, 1631 participants: RR 0.99, 95% CI 0.98 to 1.01, I^2= 0%) (Figures 4).

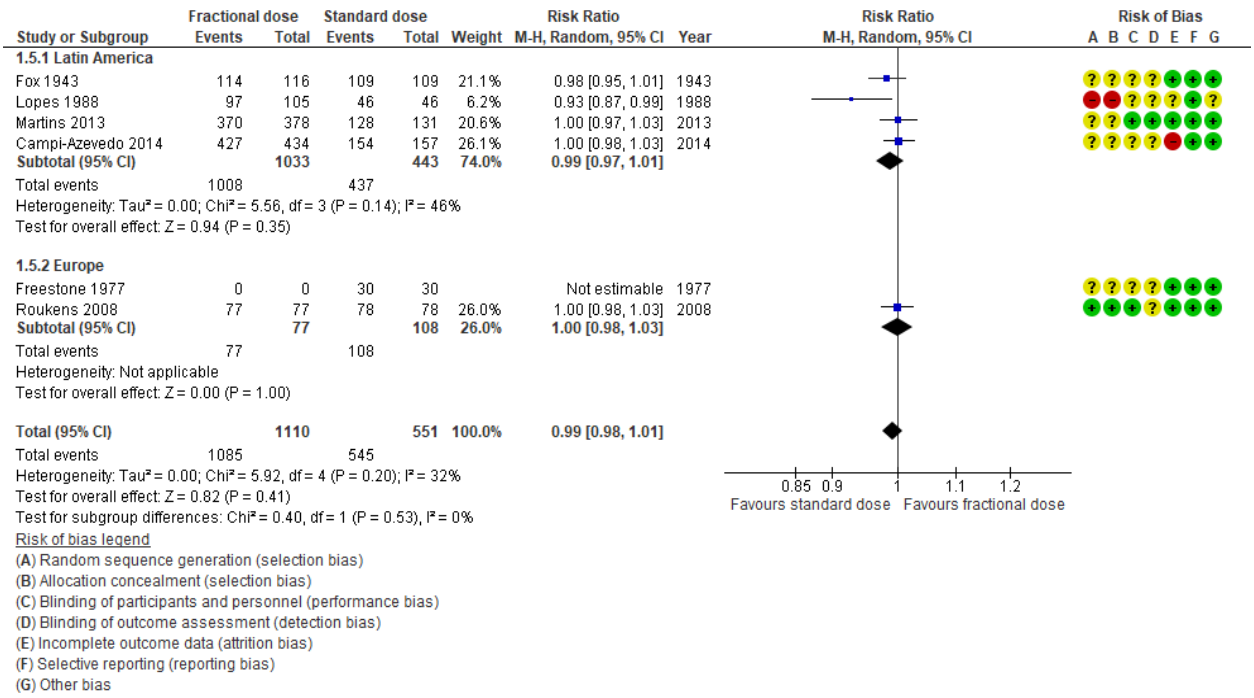

Figure 4: Subgroup immunogenicity of fractional doses by region/endemicity

4.5.1.4. Sensitivity analyses of seroconversion rates

The sensitivity analysis confirmed the robustness of pooled estimates. When excluding individual studies, the 95% confidence intervals of risk ratios maintained the absence of significant differences between standard, 1/5th and 1/10th fractional doses (95% CI lower limit ranged from 0.95 to 0.98; upper limit ranged from 1.01 to 1.05); while also maintaining observed differences between standard and 1/100th and <1/100th fractional doses (95% CI lower limit ranged from 0.35 to 0.76; upper limit ranged from 0.67 to 0.95). When restricting the comparison of the 1/50th dose to the Lopes 1988 study alone, 1/50th dose became significantly less immunogenic than the standard dose, contrary to the earlier pooled effect of non-inferiority. When restricting the analysis to studies with low risk of bias; selection bias and post-vaccination attrition of >25% did not substantially influence the pooled estimates between standard and 1/3rd, 1/10th and 1/50th fractional doses (95% CI lower limit ranged from 0.95 to 0.98; upper limit ranged from 1.01 to 1.05) and between standard and 1/100th and <1/100th fractional doses (95% CI lower limit ranged from 0.40 to 0.88; upper limit ranged from 0.73 to 0.99).

4.5.1.5. Antibody geometric mean titres

Of the six studies included, actual antibody Geometric Mean Titres (GMT) values were reported in three studies.[36-38] Due to variation in the units in which GMTs were expressed across studies, they could not be pooled and meta-analysed. Freestone 1977 reported no difference in antibody geometric mean titres across vaccine arms, with titres of 2.74 – 29.6 and 2.97 – 3.10 neutralising index (N.I) in standard and <1/100th fractional dose vaccine arms respectively.[38] Similarly, Lopes 1988 found no significant difference in antibody titres among participants who received different vaccine doses; 1656, 1955, 1778, 1636 and 1940 PFU/dose in standard, 1/10th, 1/50th, 1/100th and <1/100th doses respectively.[37] However, Martins 2013 demonstrated an incremental relation between vaccine doses and antibody titres among participants who received different vaccine doses; 13,479 mIU/mL, 12,191 mIU/mL, 11,608 mIU/mL, 12,145 mIU/mL, 6,837 mIU/mL, 1970 mIU/mL in standard, 1/3rd, 1/10th, 1/50th, 1/100th and <1/100th doses respectively.[36]

4.5.2. Adverse events following vaccination

Three of the six included studies assessed vaccine safety.[35 36 38] Freestone 1977 reported headache, redness and pain at the site of vaccinations as the most common adverse events, with no significant difference in occurrence between vaccine arms.[38] Roukens 2008 assessed participants for self-reported adverse events following vaccination, their duration and severity using in a 32-week diary. They found that fractional dose vaccination evoked itching, redness and swelling at the site of inoculation more frequently and for a significantly longer period than after standard dose vaccination. Longer pain and myalgia at the site of injection were more frequently reported in the standard dose arm. The severity of adverse events did not reveal a difference in experienced discomfort (both local and systemic) between the fractional and standard dose vaccine arms.[35] Martins 2013 assessed safety using self-reported adverse events diaries during the first 10 days after vaccination. Headache and fatigue were the most frequent symptoms (reported by more than 20% of participants). The only statistically significant difference was more frequent injection-site pain in the standard dose group (21.3%) compared with 1/3rd (11.3%), 1/10th (12.0%), 1/50th (13.3%), 1/100th (10.1%) and <1/100th (9.3%) in the fractional dose vaccine arms.[36] Of note is that none of the three studies reported any serious adverse events

following vaccination across any of the vaccine arms. Considering the limited details of safety findings, we were unable to appraise and standardise reported adverse events using the Brighton Collaboration case definitions.[24 26 29-32]. For the same reason, we are uncertain whether there are differences between standard and fractional doses of yellow fever vaccines in the incidence of severe adverse events following vaccination (very low certainty evidence, table 5).

4.5.3. Secondary outcomes

None of the included studies reported data on incidence of yellow fever and mortality.

Table 5: GRADE summary of findings for the effects of fractional dose yellow fever vaccine

Population: Healthy, immunocompetent adults
Settings: Latin America (endemic) and Europe (non-endemic)
Intervention: Vaccination with fractional doses of yellow fever vaccine
Comparison: Vaccination with the **s**tandard yellow fever vaccine

Outcomes	Illustrative comparative effects (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Certainty of evidence (GRADE)
	Standard dose	Fractional dose			
seroconversion rates; Standard vs 1/3rd doses	**98 per 100 (96 to 99)**	**100 per 100 (98 to 100)**	RR 1.02 (1.00 to 1.04)	547 (2 studies)	⊕⊕⊕⊖ moderate[1]
seroconversion rates; Standard vs 1/5th doses	**100 per 100 (95 to 100)**	**100 per 100 (95 to 100)**	RR 1.00 (0.98 to 1.03)	155 (1 study)	⊕⊕⊕⊖ moderate[2]
Seroconversion rates; Standard vs 1/10th doses	**99 per 100 (97 to 100)**	**98 per 100 (97 to 99)**	RR 0.99 (0.98 to 1.01)	890 (4 studies)	⊕⊕⊕⊖ moderate[3]
Seroconversion rates; Standard vs 1/50th doses	**98 per 100 (96 to 99)**	**95 per 100 (92 to 97)**	RR 0.97 (0.92 to 1.02)	661 (3 studies)	⊕⊕⊖⊖ low[4]
Seroconversion rates; Standard vs 1/100th doses	**99 per 100 (97 to 100)**	**90 per 100 (87 to 93)**	RR 0.92 (0.87 to 0.97)	868 (4 studies)	⊕⊕⊖⊖ low[5]
Seroconversion rates; Standard vs <1/100th doses	**99 per 100 (97 to 100)**	**53 per 100 (49 to 57)**	RR 0.53 (0.44 to 0.64)	1053 (5 studies)	⊕⊖⊖⊖ very low[6]
Serious adverse events following vaccination	**0 per 100**	**0 per 100**	n/a	972 (3 studies)	⊕⊖⊖⊖ very low[7]

CI: Confidence interval; **RR:** Risk ratio; **GRADE**: Grading of Recommendations Assessment, Development and Evaluation.
[1] Downgraded by 1 level (from high), because one of the studies has a high risk of attrition bias.
[2] Downgraded by 1 level, because it involves only one study with small sample size.
[3] Downgraded by 1 level, because one of the studies has a high risk of selection bias and another study has a high risk of attrition bias.
[4] Downgraded by 2 levels due to high risks of selection and attrition bias and inconsistency of outcomes (substantial heterogeneity and non-overlapping of confidence intervals)
[5] Downgraded by 2 levels due to high risks of selection and attrition bias and inconsistency (substantial heterogeneity)
[6] Downgraded by 2 levels due to high risks of selection and attrition bias, imprecision (wide confidence intervals) and inconsistency (substantial heterogeneity)
[7] No data reported

5. Discussion

5.1. Summary of main results

Based on six included studies representing a total of 2,371 participants, we found no statistically significant difference in immunogenicity between participants who received fractional doses containing one-third (two trials), one-fifth (one trial, 155 participants), one-tenth (four trials, 890 participants), and one-fiftieth (three trials, 661 participants) of the standard dose and those who received the full standard dose. However, immunogenicity was significantly lower among participants who received fractional doses containing 1/100th (four trials, 868 participants) and <1/100th (five trials, 1053 participants) of the standard dose and those who received the full standard dose. Thus, these pooled estimates provide moderate certainty evidence that there is little or no difference in immunogenicity between ≥1/50th fractional doses and the standard dose of yellow fever vaccines. Adverse events following vaccination did not differ across doses and no serious adverse events were reported in any study arm. Due to the limited data reported, we are uncertain whether there are differences between standard and fractional doses of yellow fever vaccines in the incidence of severe adverse events following vaccination.

5.2. Overall completeness and applicability of evidence

This systematic review includes relevant primary studies meeting inclusion criteria, identified through comprehensive and up-to-date literature search. The few eligible studies found are an indication of the dearth of evidence on the topic. To the best of our knowledge, this review is the first to systematically and meta-analytically evaluate the effect of this intervention. Notably, the three earlier (1943 – 1988) studies[37-39] included in the review employed immunological methods and vaccine dose units (plaque forming units, PFUs) different from those currently in use. In 2008, the World Health Organization (WHO) recommended the use of international units (IUs) for defining vaccine dose.[33] Consequently, the three more recent (2008 – 2014) studies[34-36] expressed antibody titres and vaccine doses in international units, thereby making comparisons difficult across the two eras. Though the World Health Organisation recommends that the minimum potency for standard dosing should not be less than 1000 international units per dose or its equivalent in plaque forming units [16 33], the relationship between PFUs and international units

of vaccine doses remains uncertain.[56 57] Therefore, to address these differences and enable comparability across studies, we categorised reported fractional doses as corresponding to 1/3rd, 1/10th, 1/50th, 1/100th or <1/100th of their reference standard doses and stratified meta-analyses accordingly.

Though this review's findings on immunogenicity are based on assessment at the fourth or fifth week after vaccination, evidence suggests that duration of immunity of fractional dose vaccination can persist for long.[35] Further research will however be needed to ascertain the long term immunity of fractional dose vaccination as well as clarify whether or not booster doses will be required in individuals who were vaccinated with fractional doses, as currently recommended for standard dose vaccination. As the participants in the included studies were mostly young adults, the findings may not be generalisable to elderly people. This is given the attendant risk of attenuated virus causing higher viraemia, that may result in severe disease in elderly subjects (age ≥60 years) who have been found to have a lower antibody response to yellow fever vaccines.[27 28] Individuals with suppressed immunity were also not represented in the study populations. Therefore, with the high burden of immunosuppression (due to HIV infection or cancer chemotherapy), especially in yellow fever endemic settings, further investigations of the effectiveness of fractional dose vaccination in these specific populations are of utmost necessity.[49]

Reported adverse events were few in the three studies that assessed vaccine safety. The non-occurrence of serious adverse events in all the studies is consistent with documented rare incidence of vaccine-associated viscerotropic and neurotropic diseases (0 to 0.8 cases per 100 000 persons vaccinated.[4] These studies however have limited statistical power and follow-up durations to detect the occurrence of such uncommon serious adverse events. Hence, the findings do not permit conclusions on the safety profile of fractional dose yellow fever vaccination. They therefore need to be verified in larger and demographically representative studies, with longer follow-up durations.

5.3. Quality of the evidence

Overall, the quality of evidence in this review is of low to moderate certainty. Though evidence from randomised trials is considered of high certainty in the GRADE framework; we downgraded the evidence for various reasons. Evidence from the 1/5th fractional doses was graded as moderate certainty evidence due to its involvement of only one study with small sample size; those of 1/3rd and 1/10th doses were also downgraded by one level for high risks of selection and attrition bias; while those of 1/50th, 1/100th and <1/100th were further downgraded for inconsistency of outcomes and imprecision in the studies involved. Evidence on safety was of very low certainty. A methodological limitation of this study is the inclusion of both randomised and quasi-randomised trials in the pooled estimates. A possible approach of addressing this could have been to down-weight quasi-randomised or adopting a Bayesian approach to pooling the estimates. These statistical methods are however beyond the scope of a mini-dissertation but could be considered for future publication of the review's findings.

5.4. Potential biases in the review process

We followed the guidance contained in the statement on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA). Potential biases were minimised by performing a comprehensive search of databases of published and grey literature, including hand searching of reference lists and conference proceedings. Screening of search outputs for potentially eligible studies, assessment of eligibility, risk of bias appraisal and data extraction were carried out in duplicates by two independent researchers.

5.5. Agreements and disagreements with other studies or reviews

These findings present evidence of moderate certainty, which are consistent with those of two previous narrative literature reviews[16 33] and observational studies[40-42] which demonstrated that fractional dose vaccination was highly immunogenic and safe. The equivalent immunogenicity of yellow fever vaccines from the 17D and 17DD vaccine sub-strains demonstrated by the subgroup analyses of this review was also consistent with earlier described findings.[58]

6. Authors' conclusions

6.1. Implications for practice

With future yellow fever outbreaks likely to require additional immunisation campaigns at large scale and further straining limited global stockpile of the yellow fever vaccine, the findings of this review support the use of fractional dosing as a dose-sparing strategy for yellow fever vaccination. There is some evidence of the long-term duration of immunity following fractional dose vaccination, with a recent study showing that 85.2% of participants remained seropositive eight years after initial vaccination with fractional doses as low as 1/50th of the standard dose.[59] Until there is substantial evidence of longer-term immunity, however, this strategy should not ideally serve as long-term vaccination strategy, nor replace established routine immunisation practices. It does not currently meet the vaccination requirements under the International Health Regulations (IHR), hence proof of vaccination for international travel still requires re-vaccination with a standard full dose.[4] The strategy is, nonetheless, particularly vital in preventive vaccination of large number of at-risk populations during outbreaks in densely-populated settings. In such situations, capacity building and training of frontline health workers and vaccinators on the effective handling and off-label use of the vaccine in fractional doses are necessary.

Yellow fever vaccines contain no preservative, hence there is a potential risk of increased contamination if vials are repeatedly punctured during immunisation sessions.[16 60] To address this concern, the use of lower-dose vials could help minimise the number of punctures, while reducing the risk of post-reconstitution contamination and mitigating vaccine waste. The variation in actual doses of yellow fever virus particles in the standard doses across all prequalified vaccine manufacturers presents a quality assurance challenge.[56 57] This needs to be addressed to ensure that fractional doses of corresponding dilution strengths are similar in viral contents across all vaccine lots. Lastly, active post-vaccination surveillance is necessary to allow for assessment of duration of protection, effectiveness, tracking of break-through cases and safety monitoring of fractional dose vaccination.

6.2. Implications for future research

This review's findings are based on assessment of protective immunity and safety of fractional doses of the yellow fever vaccine in healthy, immunocompetent and mostly young adults in a limited number of settings. There are concerns that fractional dosing might be less effective or less safe in children, elderly persons and immunocompromised individuals.[4] These deserve further research efforts. Until data relevant to such specific subgroups become available, children aged <2 years, pregnant women, and individuals with compromised immunity should preferentially be vaccinated using standard doses during preventive vaccination campaigns.

Further research is also needed to investigate the longer-term protective immunity of fractional dose vaccinations as well as well as put to rest the question as to whether or not booster doses are required in individuals who were vaccinated with fractional doses. While available clinical trial data do not suggest a need for revaccination after fractional dose vaccination, there is need for further monitoring of immunogenicity, duration of immunity, and safety to strengthen this evidence.[16 36] Lastly, available data on the effects of fractional dose yellow fever vaccination are mostly on immunogenicity and/or safety, with lack of evidence on the actual efficacy or effectiveness of the strategy in preventing incident yellow cases in those vaccinated. With efficacy studies being highly resource-intensive and in the context of resource-constraints typical of yellow fever endemic areas, integrating vaccine efficacy assessment into existing immunisation programmes and impact evaluation frameworks might be a cost-cutting approach to prospectively assessing the effectiveness of the strategy in large scales.

Acknowledgements

This work is sponsored partly by the South African Medical Research Council and the National Research Foundation of South Africa (Grant Numbers: 106035, 108571, and 111678).

Contributors

CSW conceived the study and provided supervision. CAN wrote the first draft. MSS and OOA provided co-supervision and contributed to the development of the methods and coherence of the draft. All other specific roles are as described in the methodology section.

Declaration of interests

None.

Differences between protocol and review

None.

7. References

1. Wiysonge CS, Nomo E, Mawo J, et al. Yellow fever control in Cameroon: where are we now and where are we going? *BMC medicine* 2008;6:3. doi: 10.1186/1741-7015-6-3 [published Online First: 2008/02/12]
2. Monath TP, Vasconcelos PF. Yellow fever. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* 2015;64:160-73. doi: 10.1016/j.jcv.2014.08.030 [published Online First: 2014/12/03]
3. Barrett AD, Higgs S. Yellow fever: a disease that has yet to be conquered. *Annual review of entomology* 2007;52:209-29. doi: 10.1146/annurev.ento.52.110405.091454 [published Online First: 2006/08/18]
4. World Health Organization (WHO). WHO position on the use of fractional doses - June 2017, addendum to vaccines and vaccination against yellow fever WHO: Position paper - June 2013. *Vaccine* 2017;35(43):5751-52. doi: 10.1016/j.vaccine.2017.06.087 [published Online First: 2017/07/12]
5. World Health Organization (WHO). Vaccines and vaccination against yellow fever: WHO Position Paper, June 2013--recommendations. *Vaccine* 2015;33(1):76-7. doi: 10.1016/j.vaccine.2014.05.040 [published Online First: 2014/05/24]
6. Markoff L. Yellow fever outbreak in Sudan. *New England Journal of Medicine* 2013;368(8):689-91. doi: 10.1056/NEJMp1300772
7. Gubler DJ. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? *Comparative immunology, microbiology and infectious diseases* 2004;27(5):319-30. doi: 10.1016/j.cimid.2004.03.013 [published Online First: 2004/07/01]
8. Vainio J, Cutts F. Yellow fever. WHO/EPI/GEN/9811. Geneva: World Health Organization; 1998. p. 1–87.
9. Mutebi JP, Barrett AD. The epidemiology of yellow fever in Africa. *Microbes and infection* 2002;4(14):1459-68. [published Online First: 2002/12/12]
10. Chen LH, Hamer DH. Vaccination Challenges in Confronting the Resurgent Threat From Yellow Fever. *JAMA: Journal of the American Medical Association* 2017;318(17):1651-52. doi: 10.1001/jama.2017.14258
11. Thomas RE, Lorenzetti DL, Spragins W. Mortality and Morbidity Among Military Personnel and Civilians During the 1930s and World War II From Transmission of Hepatitis During Yellow Fever Vaccination: Systematic Review. *American Journal of Public Health* 2013;103(3):e16-29. doi: 10.2105/AJPH.2012.301158
12. Barnett ED. Yellow fever: epidemiology and prevention. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007;44(6):850-6. doi: 10.1086/511869 [published Online First: 2007/02/17]
13. Gardner CL, Ryman KD. Yellow Fever: A Reemerging Threat. *Clinics in laboratory medicine* 2010;30(1):237-60. doi: 10.1016/j.cll.2010.01.001

14. Dou XF, Zheng Y, Lyu YN, et al. [The first confirmed imported case of yellow fever in China]. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi* 2016;37(6):788-90. doi: 10.3760/cma.j.issn.0254-6450.2016.06.009 [published Online First: 2016/06/28]
15. Wilder-Smith A, Leong WY. Importation of yellow fever into China: assessing travel patterns. *Journal of travel medicine* 2017;24(4) doi: 10.1093/jtm/tax008 [published Online First: 2017/04/21]
16. World Health Organization (WHO). Fractional Dose Yellow Fever Vaccine as a Dose-sparing Option for Outbreak Response. WHO Secretariat Information Paper. Department of Immunization, Vaccines and Biologicals. WHO reference number: WHO/YF/SAGE/16.1. Published 20 July 2016. Available via: http://apps.who.int/iris/bitstream/handle/10665/246236/WHO-YF-SAGE-16.1-eng.pdf;jsessionid=F4BD11CEBB8426D52C781267F9795A8A?sequence=1 21 April 2018.
17. Norrby E. Yellow fever and Max Theiler: the only Nobel Prize for a virus vaccine. *The Journal of Experimental Medicine* 2007;204(12):2779.
18. Shearer FM, Moyes CL, Pigott DM, et al. Global yellow fever vaccination coverage from 1970 to 2016: an adjusted retrospective analysis. *The Lancet Infectious Diseases* 2017;17(11):1209-17. doi: 10.1016/S1473-3099(17)30419-X
19. Makhunga-Ramfolo N, Rheeder P. Safety and tolerability of yellow fever vaccines. *Southern African Journal of Epidemiology and Infection* 2012;27(1):16-18. doi: 10.1080/10158782.2012.11441474
20. World Health Organization (WHO). Yellow Fever: Travel and Health. Annex 1 - Update. Released 16 February 2017. Available from: http://www.who.int/ith/2017-ith-annex1.pdf Accessed on 11 November 2017.
21. Groot H, Ribeiro RB. Neutralizing and haemagglutination-inhibiting antibodies to yellow fever 17 years after vaccination with 17D vaccine. *Bulletin of the World Health Organization* 1962;27(6):699-707.
22. Thomas RE. Yellow fever vaccine-associated viscerotropic disease: current perspectives. *Drug Design, Development and Therapy* 2016;10:3345-53. doi: 10.2147/DDDT.S99600
23. 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. *The American Journal of Tropical Medicine and Hygiene* 2013;89(3):434-44. doi: 10.4269/ajtmh.13-0264
24. Thomas RE, Spragins W, Lorenzetti DL. How many published cases of serious adverse events after yellow fever vaccination meet Brighton Collaboration diagnostic criteria? *Vaccine* 2013;31(52):6201-9. [published Online First: 2014/01/18]
25. Thomas RE, Lorenzetti DL, Spragins W, et al. Active and passive surveillance of yellow fever vaccine 17D or 17DD-associated serious adverse events: systematic review. *Vaccine* 2011;29(28):4544-55. doi: 10.1016/j.vaccine.2011.04.055 [published Online First: 2011/05/10]
26. Rüggeberg JU, Gold MS, Bayas J-M, et al. Anaphylaxis: Case definition and guidelines for data collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;25(31):5675-84. doi: https://doi.org/10.1016/j.vaccine.2007.02.064
27. Roukens AH, Soonawala D, Joosten SA, et al. Elderly Subjects Have a Delayed Antibody Response and Prolonged Viraemia following Yellow Fever Vaccination: A Prospective Controlled Cohort Study. *PLoS ONE* 2011;6(12):e27753. doi: 10.1371/journal.pone.0027753
28. Monath TP, Cetron MS, McCarthy K, et al. Yellow fever 17D vaccine safety and immunogenicity in the elderly. *Human vaccines* 2005;1(5):207-14. [published Online First: 2006/10/03]
29. Lindsey NP, Rabe IB, Miller ER, et al. Adverse event reports following yellow fever vaccination, 2007–13. *Journal of travel medicine* 2016;23(5):taw045-taw45. doi: 10.1093/jtm/taw045
30. Gershman MD, Staples JE, Bentsi-Enchill AD, et al. Viscerotropic disease: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2012;30(33):5038-58. doi: 10.1016/j.vaccine.2012.04.067 [published Online First: 2012/05/09]

31. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barre syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29(3):599-612. doi: 10.1016/j.vaccine.2010.06.003 [published Online First: 2010/07/06]
32. Sejvar JJ, Kohl KS, Bilynsky R, et al. Encephalitis, myelitis, and acute disseminated encephalomyelitis (ADEM): case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;25(31):5771-92. doi: 10.1016/j.vaccine.2007.04.060 [published Online First: 2007/06/16]
33. Hickling J, Jones R. Yellow fever vaccination: The potential of dose sparing to increase vaccine supply and availability. A Technical Programme Report for Programme for Appropriate Technology in Health (PATH) Report. 2013. Available via https://www.path.org/publications/files/TS_vtg_yf_rpt.pdf Accessed on 29 December 2017.
34. Campi-Azevedo AC, de Almeida Estevam P, Coelho-dos-Reis JG, et al. Subdoses of 17DD yellow fever vaccine elicit equivalent virological/immunological kinetics timeline. *BMC Infectious Diseases* 2014;14:391-91. doi: 10.1186/1471-2334-14-391
35. Roukens AH, Vossen AC, Bredenbeek PJ, et al. Intradermally Administered Yellow Fever Vaccine at Reduced Dose Induces a Protective Immune Response: A Randomized Controlled Non-Inferiority Trial. *PLoS ONE* 2008;3(4):e1993. doi: 10.1371/journal.pone.0001993
36. Martins RM, Maia MdLS, Farias RHG, et al. 17DD yellow fever vaccine: A double blind, randomized clinical trial of immunogenicity and safety on a dose-response study. *Human vaccines & immunotherapeutics* 2013;9(4):879-88. doi: 10.4161/hv.22982
37. Lopes Ode S, Guimaraes SS, de Carvalho R. Studies on yellow fever vaccine. III--Dose response in volunteers. *Journal of biological standardization* 1988;16(2):77-82. [published Online First: 1988/04/01]
38. Freestone DS, Ferris RD, Weinberg AL, et al. Stabilized 17D strain yellow fever vaccine: Dose response studies, clinical reactions and effects on hepatic function. *Journal of biological standardization* 1977;5(3):181-86. doi: https://doi.org/10.1016/S0092-1157(77)80003-6
39. Fox JP, Kossobudzki SL, Cunha JF. Field studies of the immune response to 17D yellow fever virus. Am J Hyg. 1943;38:113–38.
40. Nzolo D, Engo Biongo A, Lusakibanza M, et al. Safety profile of fractional dosing of yellow fever vaccine, experience from community based pharmacovigilance in Kinshasa, DRC2017.
41. Roukens AH, Vossen AC, van Dissel JT, et al. Reduced intradermal test dose of yellow fever vaccine induces protective immunity in individuals with egg allergy. *Vaccine* 2009;27(18):2408-9. doi: 10.1016/j.vaccine.2009.02.049 [published Online First: 2009/04/17]
42. Ahuka-Mundeke S, Casey RM, Harris JB, et al. Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak - Preliminary Report. *The New England journal of medicine* 2018 doi: 10.1056/NEJMoa1710430 [published Online First: 2018/02/15]
43. Jacobson RM, Targonski PV, Poland GA. Meta-analyses in vaccinology. *Vaccine* 2007;25(16):3153-59. doi: https://doi.org/10.1016/j.vaccine.2007.01.047
44. Lyman GH, Kuderer NM. The strengths and limitations of meta-analyses based on aggregate data. *BMC Med Res Methodol* 2005;5:14. doi: 10.1186/1471-2288-5-14 [published Online First: 2005/04/27]
45. Finney DJ A statistician looks at met-analysis .J Clin Epidemiol 1995 48(1): 87-103.
46. Booth A, Clarke M, Dooley G, et al. The nuts and bolts of PROSPERO: an international prospective register of systematic reviews. *Systematic Reviews* 2012;1:2-2. doi: 10.1186/2046-4053-1-2
47. Booth A, Clarke M, Ghersi D, et al. An international registry of systematic-review protocols. *Lancet* 2011;377(9760):108-9. doi: 10.1016/s0140-6736(10)60903-8 [published Online First: 2010/07/16]
48. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration.

PLoS medicine 2009;6(7):e1000100. doi: 10.1371/journal.pmed.1000100 [published Online First: 2009/07/22]
49. Jean K, Donnelly CA, Ferguson NM, et al. A Meta-Analysis of Serological Response Associated with Yellow Fever Vaccination. *The American Journal of Tropical Medicine and Hygiene* 2016;95(6):1435-39. doi: 10.4269/ajtmh.16-0401
50. Banaszkiewicz A, Radzikowski A. Efficacy, effectiveness, immunogenicity - are not the same in vaccinology. *World Journal of Gastroenterology : WJG* 2013;19(41):7217-18. doi: 10.3748/wjg.v19.i41.7217
51. The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). RevMan [Computer program]. Version 5.3. Copenhagen:, The Nordic Cochrane Centre, The Cochrane Collaboration. 2014.
52. Higgins JPT, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
53. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343 doi: 10.1136/bmj.d5928
54. Melsen WG, Bootsma MCJ, Rovers MM, et al. The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses. *Clinical Microbiology and Infection* 2014;20(2):123-29. doi: https://doi.org/10.1111/1469-0691.12494
55. Guyatt GH, Oxman AD, Kunz R, et al. What is "quality of evidence" and why is it important to clinicians? *Bmj* 2008;336(7651):995-8. doi: 10.1136/bmj.39490.551019.BE [published Online First: 2008/05/06]
56. World Health Organization. Global norms and standards for biological quality, safety and efficacy. Proceeding. 23: 12-15 Available via http://www.who.int/biologicals/publications/trs/areas/vaccines/yellow_fever/ECBS%202008%20WHO_TRS%20872%20%20Amndmt_Yellow%20Fever.pdf Accessed on 29 June 2018. 2009/01//. World Health Organization.
57. World Health Organization (WHO). Expert Committee on Biological Stadardization. Requirements for Yellow Fever Vaccine (Requirements for Biological Substances No. 3) Proposed Amendment Geneva, October 2008.
58. Camacho LAB, Freire MdS, Leal MdLF, et al. Immunogenicity of WHO-17D and Brazilian 17DD yellow fever vaccines: a randomized trial. *Revista de Saúde Pública* 2004;38:671-78.
59. de Menezes Martins R, Maia MLS, de Lima SMB, et al. Duration of post-vaccination immunity to yellow fever in volunteers eight years after a dose-response study. *Vaccine* 2018;36(28):4112-17. doi: 10.1016/j.vaccine.2018.05.041 [published Online First: 2018/05/23]
60. Gavi. Yellow Fever Supply and Procurement Roadmap. Update 20th March 2017. Available from: https://www.gavi.org/.../supply-procurement/yellow-fever-roadmap-public-summary/ Accessed on 11 July 2018.

PART D: APPENDICES

Appendix 1: Search strategies

1.1. PubMed

Search #	Search Texts and Syntaxes
#1	Yellow Fever [MeSH Terms]
#2	Yellow Fever Vaccine [MeSH Terms]
#3	yellow fever vaccine OR yellow fever vaccination OR yellow fever immunization
#4	#1 OR #2 OR #3
#5	Dose-Response Relationship, Immunologic [MeSH Terms]
#6	fractional dosing OR Fractionated dosing OR drug dose comparison OR sub-dose OR sub-dosage OR reduced dose OR reduced dosing OR reduced dosage OR dose-sparing OR immunologic dose response relationship
#7	#5 OR #6
#8	safety OR adverse reaction OR adverse event OR adverse effects OR toxicity OR reactogenicity OR immunogenicity OR immunogenic OR immune response OR seroconversion OR efficacy OR effects OR effectiveness
#9	#7 OR #8
#10	#4 AND #9

1.2. EBSCOhost (including CINAHL)

Search #	Search Texts and Syntaxes
S1	SU yellow fever OR SU yellow fever vaccine OR AB (yellow fever vaccine OR yellow fever vaccination OR yellow fever immuni#ation)
S2	SU dose response relationship, immunologic OR AB (fractional dosing OR Fractionated dosing OR drug dose comparison OR sub-dose OR sub-dosage OR reduced dose OR reduced dosing OR reduced dosage OR dose-sparing OR immunologic dose response relationship)
S3	AB safety OR adverse reaction OR adverse event OR adverse effects OR toxicity OR reactogenicity OR immunogenicity OR immunogenic OR immune response OR seroconversion OR efficacy OR effects OR effectiveness
S4	S2 OR S3
S5	S1 AND S4

1.3. Scopus

Search #	Search Texts and Syntaxes
#1	TITLE-ABS-KEY yellow Fever OR yellow fever vaccine OR (yellow fever vaccine OR yellow fever vaccination OR yellow fever immune?ation)
#2	TITLE-ABS-KEY dose?response relationship, immunologic OR (fractional dosing OR fractionated dosing OR drug dose comparison OR sub-dose OR sub-dosage OR reduced dose OR reduced dosing OR reduced dosage OR dose-sparing OR immunologic dose response relationship)
#3	TITLE-ABS-KEY safety OR adverse reaction OR adverse event OR adverse effects OR toxicity OR reactogenicity OR immunogenicity OR immunogenic OR immune response OR seroconversion OR efficacy OR effects OR effectiveness
#4	#2 OR #3
#5	#1 AND #4

Appendix 2: Data extraction tool

Section 1: General review information

Review Title: Effects of Fractional Dose Yellow Fever Vaccine: Systematic Review and Meta-analysis

Study ID (Surname and Year: as it will appear in RevMan):

Name of review author completing this form:

Date form completed:

Name of review author checking the data extracted to this form:
Other information and notes:

Author contact details for study	
Further information required	
Correspondence with authors successful or not; what information was received and when	
Will any additional unpublished data supplied by the authors be included in the review?	

Section 2: Methods of the study

Details of Study (to be reported in the Characteristics of Included Studies tables)

Minimum standards:

Aim of study (As stated in the trial report/s. What was the trial designed to assess?):

Study design:

Number of arms or groups (including control groups); briefly describe each:

Consumer involvement (eg. In design of study and/or intervention; in delivery of intervention; in evaluation of intervention; in interpretation of study findings)

Funding source (also include any details about possible or explicit conflicts of interest):

Informed consent obtained? (Yes/No/Unclear):

Ethical approval (Yes/No/Unclear):

Section 3: Study characteristics - Participants

The minimum standards below outline those fields on which data must be extracted, the optional items can be chosen or adapted; and decisions need to be made about what to report in the Characteristics of included studies table and what could be reported in Additional tables.

Minimum standards:

Description (eg. Patients/consumers; carers; parents of patients/consumers; health professionals; well people in the community):

Geographic location (eg. City/State/Country):

Setting (eg. Community, home, health centre, miliary facility):

Methods of recruitment of participants (How were potential participants approached and invited to participate?)

Inclusion/exclusion criteria for participation in study:

Age: range, mean (standard deviation):

Gender:

Ethnicity:

Numbers involved:

Study numbers	**Number**
Eligible for inclusion	
Excluded	
Refused to take part	
Randomised to intervention group(s)	
Randomised to control group	
Excluded post randomisation (for each group; with reasons if relevant)	
Withdrawn (for each group; with reasons if relevant)	
Lost to follow up (for each group; with reasons)	Intervention group (with reasons)
	Control group (with reasons)
Included in the analysis (for each group, for each outcome)	Outcome 1 Intervention Control
	Outcome 2 Intervention Control

Section 4: Study characteristics - Interventions

Data on interventions (and control) procedures should be collected in enough detail to allow replication of the procedures. Depending on how much detail is available, some of this information might be best reported in Additional tables within the review, as the Characteristics of Included Studies tables will otherwise become very long and unwieldy.

Data should be extracted for each relevant (included) intervention arm, as well as the control arm. Information on any co-interventions (if applicable) should also be recorded.

Minimum standards:

Item	Explanation, notes	Intervention	Control
1 Intervention name	Include a brief name or phrase that describes the intervention (including definition of any acronyms or abbreviations)		
2 Aims and rationale ('why?')	Aim(s) of intervention (as stated in the trial report/s. What was the problem that this intervention was designed to address?) Describe any theory (with key references) or rationale relevant to the intervention. (Note that for a complex intervention with different components, each component may have a different aim or rationale) Describe any information on the quality of the intervention, assessed by study authors, others, or by you - such as the evidence base supporting the intervention.		
3 What was done?	Materials: Describe the content, format(s) or media, source of materials (if possible, where they can be accessed), and any other information relevant to the physical or information materials provided to participants or in training providers of the intervention. Procedures: Describe each of the processes used in delivering the intervention (eg education, telephone follow-up, case management)		

	Note that some complex interventions require additional support activities to be implemented, and if so details of these should also be reported. Note also that some complex interventions require sequencing of activities, whereas for others the order of delivery is less critical. <u>Mode of delivery:</u> Describe the mode of delivery of the intervention, such as whether it was delivered face-to-face (eg in patient consultation, educational session, training) or at a distance (eg via phone, internet, mail); and whether the delivery was to individuals or groups of participants. <u>Cointerventions:</u> Describe the delivery of any co-interventions (Co-interventions may be separate to the intervention of interest, or they may be other similar elements in a suite of interventions which have a common purpose).		
4 Who delivered the intervention?	Describe who was involved in delivery of each component of the intervention and/or each different intervention provider. 'Intervention provider' could for example be taken to mean a health professional or it could mean a consumer peer advocate. Include description of any specific training given to providers to deliver the intervention, numbers of providers, professional background, specific pre-existing skills or experience required, quality of any specific training received to deliver the intervention, and any measures of competence or consistency in delivering the intervention recorded before or during the study.		
6 Where was the intervention provided?	Describe the features of the setting (location) that might be relevant to intervention delivery (eg country, type of clinic, primary or hospital care).		

	If the location varied this should be described, with relevant features that might affect the intervention delivery; as should any requisite features of the location that might impact on intervention delivery or feasibility (eg location close to participants' usual doctor, availability of equipment)		
7 When and how often or how much of the intervention was provided?	Describe how the intervention was delivered, such as stages, timing, frequency, number of sessions, intensity and duration of intervention delivery.		
8 Was the intervention modified or adapted?	If the intervention was changed during the study, this should be described (eg unforseen modifications required, changes in study circumstances requiring modifications to the intervention). If such modifications happen, why, what, how and when the intervention was changed should be described.		
9 How well was the intervention delivered?	Assessment of fidelity: if intervention fidelity was assessed, describe the extent to which the intervention was delivered as intended. (ie the amount or type of intervention planned for delivery might differ from what was actually delivered) If strategies to maintain intervention fidelity were planned before intervention delivery, or were used during the study, describe these, along with any materials or tools used.		

**Table is adapted from Hoffman et al (2014). Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide. BMJ; 348: P 1687.

Section 5: Study characteristics - Outcomes and comparison groups

All data reported by the included study for all eligible primary and secondary outcomes sought by the review must be reported.

Data on all relevant adverse events must also be collected and reported. These should be included as primary outcomes, unless there is a good rationale not to do so.

If adverse effects are not reported by the included study, it should be clearly reported whether adverse effects were investigated or not by the study.

Details may be best presented in Additional tables if a large volume of information is collected for each study.

Primary outcomes			
Outcome	Method of assessing outcome measures eg, phone survey, questionnaire	Method of follow-up for non-respondents	Timing of outcome assessment (including frequency, length of follow up)

Primary outcomes - adverse events (eg complaints, levels of dissatisfaction, adverse incidents, side effects, increased inequities)		
Adverse event	Method of assessment	Timing of assessment (including frequency, length of follow up)

Secondary outcomes		
Outcome	Method of assessment	Timing of outcome assessment (including frequency, length of follow up)

Section 6: Data and results

These data will be used in the "Comparisons and Data" section in RevMan (not the table "Characteristics of Included Studies") and as the basis for the "Results" section of your review text. All data are numbers (of patients/units), not percentages.

Minimum standards:

You must extract all data relevant to the outcomes specified in your selection criteria. This may be as dichotomous, continuous, and/ or other data or results.

Dichotomous outcomes

Outcome	Timing of outcome assessment (days/months)	Intervention group*		Control group		Notes
		Observed (n)	Total (N)	Observed (n)	Total (N)	

Note: add additional columns if there is more than one intervention group.

Continuous outcomes

Outcome	Timing of outcome assessment (days/months)	Intervention group			Control group			Notes
		*Mean / Mean change	Standard deviation	N	*Mean / Mean change	Standard deviation	N	