The use of reduced doses of the Yellow Fever vaccine in a sub-Saharan African population: Safety, Immunogenicity and Policy

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A Thesis Submitted for the degree of Doctor of Philosophy, Nuffield Department of Medicine, University of Oxford, United Kingdom.

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Dedication of thesis

"Whatever is your duty, do it as fully and as perfectly as you possibly can. And when you have finished your duty, go on to spare some time and talent in service for less fortunate people, not for any reward at all, but because it is the right thing to do."

Dr. Geoffrey William Griffin, OBE.

I dedicate this DPhil thesis to all the health care workers, including researchers, who are working towards winning the fight against all cancers and to my late Taata, Nkiro.

Abstract

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by

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Yellow Fever (YF) is a potentially lethal haemorrhagic fever of significant public and global health importance. The 17-D YF vaccines have been used successfully for several decades with excellent immunogenicity and safety profile. A recent rise in outbreaks in endemic regions has been characterised by a repeated vaccine stock shortage that has led the World Health Organisation (WHO) to recommend the use of fractional doses as a dose sparing strategy. The recommendation was made with limited data from the African region.

A review of literature of the virus, epidemiology, vaccines and the current policies of use the vaccines explored the challenges with an upsurge in disease outbreak in endemic regions and the causes of the recurrent vaccine stock shortage. The literature identified the gaps in evidence on the use of fractional doses as a dose sparing strategy as recommended by the WHO.

Randomised, controlled, non-inferiority clinical trials comparing standard dose and fractional doses were conducted in Kenya and Uganda. In one study, a pragmatic fifth of the standard dose of the 17D-213 vaccine manufactured by the Institute of Poliomyelitis and Viral Encephalitidis, Russian Federation was tested against the standard in HIV-infected individuals. The second study was a dose-response study testing de-escalated doses of the 17D 204 vaccine manufactured by the Institut Pasteur de Dakar against a standard dose in a general population

in Kenya and Uganda. The studies assessed safety and immunogenicity, as measured by neutralising antibody assays (PRNT). The studies found the fractional doses to be safe and sufficiently immunogenic with doses as low as 500 IU/dose immunogenically non-inferior to standard doses.

A systematised review of vaccine policy identified actors, mapped out policy processes and elements that influence the direction of vaccines when introducing new vaccine policies and changing existing vaccine policies. A stakeholder analysis with actors involved with YF vaccines explored their perceptions, positions, vaccine policy processes and factors that are vital to consider for wider use of fractional doses beyond the current recommendations by WHO.

In these policy analysis studies, I found that there are systematised processes for introducing and changing vaccine policies at the global and regional levels and that each vaccine policy is unique. There was general support for use of fractional doses of the YF vaccines. However, more evidence is needed for firmer recommendations. Additionally, vaccine policy processes are heavily political with advocacy, lobbying, actor coordination and communication playing important roles.

Statement of originality and roles in the project

I confirm the work recorded in this thesis as my own, with exceptions listed below.

Chapter 1: A general introduction and synopsis of the thesis

I wrote the general introduction and the synopsis of the thesis that was reviewed by George Warimwe, Sassy Molyneux and Philip Bejon.

Chapter 2: A literature review on yellow fever virus, epidemiology, vaccines and the policies for use of the vaccines

I reviewed the literature and wrote and revised drafts of the chapter that were reviewed by George Warimwe, Sassy Molyneux and Philip Bejon who provided useful comments and suggestions.

Chapter 3: Immunogenicity and safety of fractional doses of 17D-213 yellow fever vaccine among HIV-infected persons in Coastal Kenya

I led a clinical trials team based in Kilifi, Kenya in the conduct of the trial. I was responsible for training staff, coordinating and clinical trial oversight ensured ICH-GCP compliance, sample shipment to the WHO accredited reference lab, the Institut Pasteur de Dakar who ran the PRNT assays. I conducted quality checks on the PRNT data with the help of Gamou Fall and Ndeye Sakha Bob and their team from the Institut Pasteur de Dakar and Aitana Juan from Epicentre at MSF. I modified a Statistical Analysis Plan that was developed by Kyra Grant and conducted the statistical analysis with support from Benedict Orindi. I wrote the chapter that

was reviewed by George Warimwe and Philip Bejon. I plan to write a manuscript of this chapter and submit it to an appropriate peer-reviewed journal.

Chapter 4: Non-Inferiority Fractional-doses Trial for Yellow Fever vaccine using reduced doses in Kenya and Uganda (NIFTY) – immunogenicity and safety

I led two clinical trials teams – the Yellow Fever Trials team at the KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya and the MSF – Epicentre Mbarara Research Centre, Mbarara, Uganda in the conduct of the trial. I was responsible for training staff, coordinating and clinical trial oversight ensured ICH-GCP compliance across both sites. Maria N Namulwana and Aitana Juan were very instrumental in the coordination of day-to-day activities at the Mbarara clinical site. I coordinated the sample shipment to the WHO accredited reference lab, the Institut Pasteur de Dakar who ran the PRNT assays. As with the study in chapter 3, I conducted quality checks on the PRNT data with the help of Gamou Fall and Ndeye Sakha Bob from the Institut Pasteur de Dakar and Aitana Juan from MSF – Epicentre. I developed the Statistical Analysis Plan and conducted the statistical analysis with support from Benedict Orindi. I wrote the chapter that was reviewed by George Warimwe and Philip Bejon who provided helpful comments. I plan to write a manuscript of this chapter and submit it to an appropriate peer-reviewed journal.

Chapter 5: Introduction and change of vaccine policies in low- and middle-income countries; a systematised literature review

I wrote the systematised review protocol and a version of the protocol was registered with the University of York international prospective register of systematic reviews (PROSPERO: CRD42019134665). I conducted the review and wrote the initial draft. Nadia Tagoe and Peter Mugo reviewed the initial drafts of the review of the manuscript. Sassy Molyneux guided throughout the review process. I wrote the chapter based on the manuscript. The Chapter was reviewed by Sassy Molyneux, George Warimwe and Philip Bejon. I plan to update the manuscript and submit it to a peer-reviewed journal.

Chapter 6: Priorities, perspectives and processes for a prospective vaccine policy change for wider use of fractional doses of the 17D YF vaccines: a stakeholder analysis

I designed and conducted interviews with vaccine YF vaccine policy stakeholders. Transcribers at the KEMRI-Wellcome Trust Research Programme's Health Systems and Research Ethics Department transcribed the interviews. I reviewed the transcripts and conducted the qualitative data analysis with inputs from Nadia Tagoe, Nancy Kagwanja and Juliet Otieno. Sassy Molyneux guided throughout the study. I wrote the chapter that was reviewed by Sassy Molyneux, George Warimwe and Philip Bejon.

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6.1.List of Tables

1. General introduction and synopsis of the thesis

1.1. Introduction

Yellow fever (YF), a mosquito-borne virus, causes an acute infectious disease and is endemic to sub-Saharan Africa (sSA) and South America with 90% of the cases reported in sSA. The virus has an incubation period of about 3-6 days. Infection with the YF virus is characterised by a wide range of manifestations, ranging from subclinical infection with mild and non-specific symptoms, to severe, life-threatening illness with jaundice, renal failure and haemorrhage[1]. There is no definitive treatment for YF, and supportive symptomatic management is the mainstay of treatment. The yellow fever vaccine, developed in 1937, is highly effective in disease prevention. The vaccine is used routinely; to vaccinate children ≥9 months in endemic regions and adults travelling to endemic regions and in pre-emptive and reactive vaccination campaigns. A single dose of the YF vaccine sufficiently confers life-long protective immunity against YF [2].

Despite the success of this vaccine, there are only four vaccine manufacturers that are pre-qualified by the World Health Organisation (WHO). There have been recurring vaccine shortages in the last two decades. This is in part because the YF vaccine manufacture process is laborious and the current capacity to produce increased stock in response to the outbreak is limited[3]. In July 2016, the demand for YF vaccine in response to the large urban outbreaks occurring concurrently in different parts of Africa and the risk of further spread throughout the continent and to Asia led WHO to develop recommendations for the use of fractional-dose of YF vaccine as a dose-sparing strategy[4]. This recommendation was made based on limited clinical and immunological evidence on the use of fractional doses of the YF vaccines in an African population with available evidence limited to Brazilian military men and populations in Europe where YF is not endemic[5]. Conspicuously, there were no data on the use of fractional

doses in children and HIV-infected persons in sSA. The limitation in evidence was also compounded by the uncertainty surrounding minimum dose requirements. Currently, the WHO recommends YF vaccine potency not less than 1000 IU/dose. This recommendation was based on the different methods used to determine the vaccine potencies and comparison between the different methods is difficult. Therefore, despite the apparent high vaccine effectiveness observed since thresholds were determined in the 1940s, there is uncertainty regarding the precision with which the minimum dose requirements are known[6]. These gaps have been highlighted by WHO[7].

In response to a call for more data by WHO, in a study leading up to the studies in this DPhil thesis, we tested fractional doses of all the prequalified vaccine manufacturers. In our study, there were no safety concerns. Following vaccination, the participants had a comparable proportion that seroconverted when vaccinated with a fifth of the standard dose and the standard dose; the fractional dose was non-inferior to the standard full dose for all the WHO-prequalified vaccines 28 days after vaccination. All the vaccination arms grouped by vaccine manufacturer and vaccine dose (full or fractional) had 98% to 100% rates of seroconversion at 28 days post-vaccination. The difference in seroconversion of 1·71 (95% CI −2·60 to 5·28) for the vaccine manufactured by Bio-Manguinhos, −0·90 (–4·24 to 3·13) for the vaccine manufactured by Chumakov Institute, 1·82 (–2·75 to 5·39) for the vaccine manufactured by Institut Pasteur de Dakar, and 0·0 (–3·32 to 3·29) for the vaccine manufactured by Sanofi Pasteur in a trial design that had set a non-inferiority margin <10% for seroconversion[8].

The YF vaccine policies have evolved since the 1940s for optimal public health benefits. For instance; in 1969 following the adoption of the International Health Regulations, the World Health Assembly recommended vaccinations to all persons travelling to endemic regions[9]; in 2013 WHO indicated that a single dose of the YF vaccine provides lifelong protective immunity against YF disease and that a booster dose was no longer necessary[10]; and in 2016

there was a recommendation to use fractional doses when there are vaccine dose shortages[11]. However, there have been differences in the processes to change these policies. The change in 2013 to remove vaccine doses followed the review of a lot of evidence by the WHO culminating in the decision to have a formal policy change. The current recommendation to use fractional doses is conditional and not a formal change of policy as it was made based on limited data[5].

1.2. Scope of the thesis

This Doctor of Philosophy (DPhil) thesis sets out to assess the use of fractional doses in a sub-Saharan population; immunogenicity, safety and policies. This will be done in four studies which set out to : (1) assess the immunogenicity and safety of a fifth of the standard dose of the vaccine manufactured in Chumakov by the Institute of Poliomyelitis and Viral Encephalitidis, Russian Federation compared to the standard dose in HIV-infected individuals in a randomised, double-blind non-inferiority trial; (2) determine the lowest dose of YF vaccine manufactured by Institut Pasteur that is non-inferior in immunogenicity and safety in a randomised, double-blind non-inferiority dose-finding trial (1000, 500 or 250 IU/dose) compared to the full standard; (3) to map out the process for agenda setting and policy formulation for a new vaccine policy or a change in an existing policy, and factors that influence these policy processes at regional and global levels for LMICs in a systematised review; and (4) to explore the stakeholders' priorities, perceptions, interests' opportunities and challenges for a prospective policy change at the global and regional levels, to support wider use of reduced doses of YF vaccine in LMICs using a stakeholder policy analysis for prospective policy.

1.3. Objectives

1.3.1. General Objectives

- To evaluate the immunogenicity and safety of fractional doses of the 17D YF vaccine in a sub-Saharan population.
- To map out and describe the policy processes and stakeholders and their perceptions on a prospective policy for wider use of fractional doses of the 17D YF vaccine.

1.3.2. Specific objectives:

- To test the immunogenicity and safety of fractional-dose (1/5th) vaccination in HIV-infected individuals with CD4 counts ≥ 200 cells/ml as measured by seroconversion using the $PRNT_{50}$ assay at 28 days post-vaccination in Kenya.
- To determine the lowest dose (1000, 500 and 250 IU/dose) of the YF vaccine that is non-inferior to a standard dose (~13000IU/dose) in an adult population in Africa using $PRNT_{50}$ assay at 28 days post-vaccination in Kenya and Uganda.
- To conduct a systematized review of published literature to map out the potential factors influencing the introduction of new vaccines and/or a shift in vaccine interventions into policy at global and regional policy levels for LMICs.
- To explore the stakeholders' priorities, perceptions, interests' opportunities and challenges for a prospective policy change at the global and regional levels, to support wider use of reduced doses of YF vaccine in LMICs.

1.4. Justification of the studies

The studies for this DPhil thesis include multidisciplinary studies with mixed methods that include clinical trials and policy analysis work. These studies are necessitated by the paucity of data on the immunogenicity and safety of fractional doses in a sSA population, including HIV-infected persons. Although we have demonstrated non-inferiority in all the four WHO pre-qualified YF vaccines in Kenya and Uganda, our population was limited to individuals who were not infected with HIV. It is key to test the fractional ($1/5^{th}$) doses of the standard doses as defined in the current recommendation in HIV-infected persons in sSA. The use of fractional doses as recommended is still above the minimum requirement by the WHO since most of the vaccine manufacturers often release the vaccine in excess of the requirement with at least 5000IU/dose. There are still uncertainties on the lowest dose that provides comparable protective immunity with the standard dose. Some evidence has shown that doses as low as 518IU/dose provide comparable immunogenicity to doses over fifty-times higher (27,476IU/dose). It is important to determine the lowest dose that is non-inferior to a standard dose among populations in sSA.

The current policies for use of fractional doses are conditional and still marred with uncertainties related to evidence gaps. The processes and factors that lead to the change in policy are not clearly outlined for policies for the YF vaccines. In my thesis, I attempt to map out the YF vaccine policy stakeholders at a macro-level (global and regional) and examine the processes, priorities, perceptions and interests for a prospective formal vaccine policy change for wider use of fractional doses of the YF vaccine.

1.5. Organisation and structure of the DPhil thesis

Chapter 2 of the thesis is a comprehensive review of available literature on YF; the YF virus, epidemiology and clinical diseases, the YF vaccines – here I have focused on the 17D YF vaccines; immunogenicity, safety and policies for use of these vaccines including the

circumstances around the current recommendation to use fractional doses. I also identify the gaps that my thesis sets out to address.

Chapter 3 details a study where the immunogenicity and safety of fractional doses of the 17D-213 vaccine were assessed among HIV-infected persons in comparison with the standard dose. The chapter describes pieces of work leading up to the trial organised into a background, methodology of the study (the study site, population, study design, study outcomes and the statistical analysis) the immunogenicity and safety results and a discussion of the findings including the limitations of the study.

Chapter 4 details a dose-finding study using one of the 17D YF vaccines manufactured by the Institut Pasteur de Dakar. This study tests 3 lower doses in comparison with a standard dose in a double-blind randomised non-inferiority study. This chapter as with the previous one is organised into a background, methodology, results including some exploratory analysis and a discussion of the findings including limitations of the study.

Chapter 5 is a systematised review of policy processes to introduce and change vaccine policies in low- and middle-income countries. The chapter described the vaccine policy development processes in place at the global and regional levels for LMICs. This chapter also maps out the stakeholders and factors that influence these vaccine policy processes. The chapter is broadly organised into a brief background, the systematised method of the literature review, the results and a discussion of these results.

Chapter 6 focuses on policy analysis for a prospective policy to recommend the wider use of fractional doses. Using a stakeholder analysis approach, the chapter describes actor perceptions, priorities and interests for a vaccine policy in favour of wider use of fractional doses. The chapter is organised into background, methods, results and a discussion of the findings.

Chapter 7 synthesises the findings from all the studies in this thesis and links up the evidence generated from the clinical trials, the current vaccine policies and a prospective policy change to allow wider use of fractional doses. I will also highlight the public health importance and the addition of my DPhil studies to the literature. I will also provide the direction of future research acknowledging the strengths and limitations of the studies.

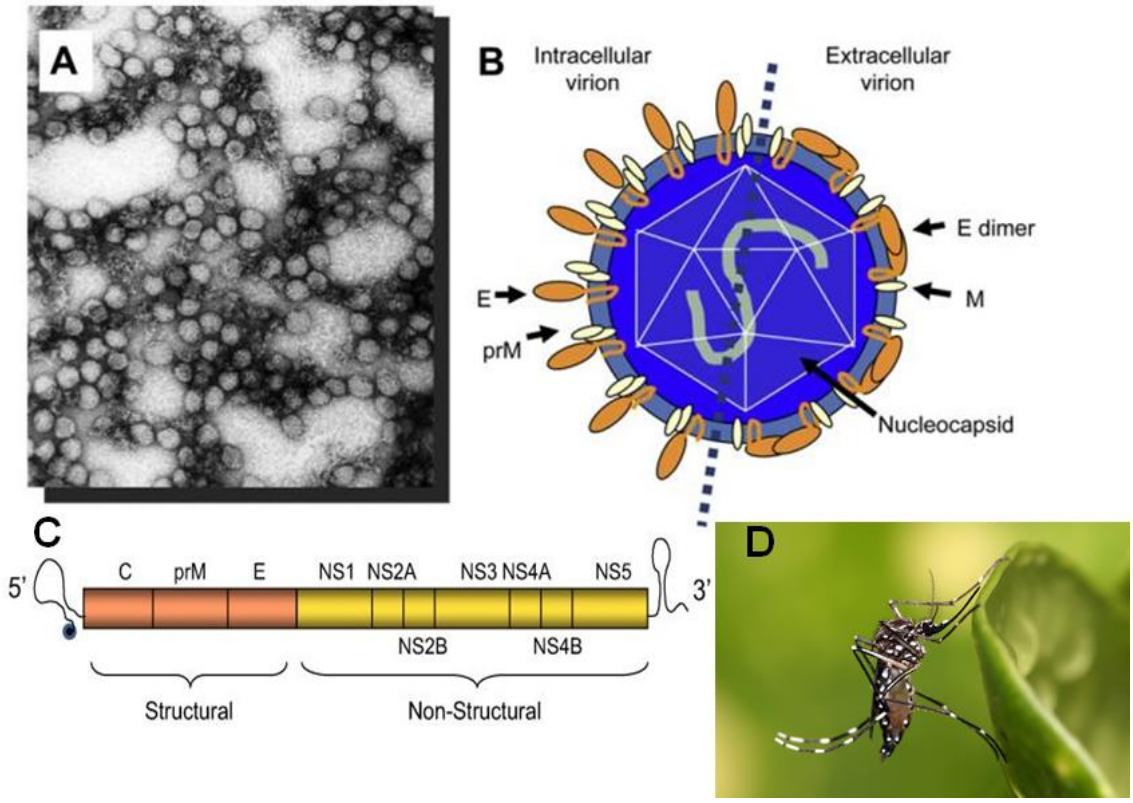
2. A literature review on yellow fever virus, epidemiology, vaccines and the policies for use of the vaccines

In this chapter, I review the available literature on Yellow Fever (YF), YF vaccines and current data on the use of fractional doses of YF vaccines; immunogenicity, safety and vaccine policy for the use of the dose-sparing strategy.

2.1. The Yellow fever virus

YF is a haemorrhagic fever caused by the Yellow Fever virus (YFV), a mosquito-borne, prototype flavivirus that is endemic in the tropical regions of Africa and tropical central and south America. The 40-60 nm enveloped single-stranded positive-sense RNA virus, approximately 10-11kb, is antigenically conserved (single serotype) with 7 distinguishable major genotypes; 3 in East, Central and Southern (Angola) Africa, 2 in West Africa and 2 in South America. The west African strains are closer in relation to the Americas' strains than with East Africa strains [12 13]. The virion is comprised of 3 structural proteins (C, PrM and E) and seven non-structural proteins (NS1, NS2A, NS2B, SN3, NS4A, NS4B and NS5) which are vital in assembly, host cell binding and entry, viral protein processing and viral replication (Figure 2.1) [14].

Figure 2. 1 YFV and the *aedes aegypti* mosquito



A) YFV particles as seen under an electron microscope; B) A schematic of the virion showing the spherical capsid with the RNA material and on the surface – the PrM, M and E heterodimers; C) The schema of the structural and non-structural proteins and D) Aedes aegypti that is responsible for large urban outbreaks.

Figure adapted from Heinz and Stiasny [14] and Aedes aegypti image credit Muhammad Mahdi Karim, https://commons.wikimedia.org/wiki/File:Aedes_aegypti.jpg .

Mosquitoes are the main vectors of infection. There are three main transmission cycles; sylvatic (enzootic zone) intermediate (emergence zone) and urban cycle (epidemic zone; Figure 2.2). The sylvatic cycle primarily involves disease transmission in non-human primates (NHPs). The virus is maintained in the enzootic cycle between mosquitoes (*Aedes spp* in Africa, *Haemogogus spp* and *Sabethes spp* in South America) and NHPs. Mosquitoes here are the primary vector with NHPs acting as amplifying hosts. When a mosquito bites, it intradermally inoculates an individual with an estimated 1,000 to 100,000 viral particles [15]. The virus replicates in the amplifying host and the mosquitoes acquire the virus by feeding on a viremic host. In this cycle, humans are infected incidentally when they enter forests for recreational activities or occupational reasons. An intermediate cycle (Savannah cycle) has been described in Africa and is considered a 'zone of emergence' reflecting evolution from a jungle disease to human disease. This cycle may be associated with high anthropophilic *Aedes* mosquito densities encountered by humans when they intrude into an ongoing jungle cycle to become infected. The urban cycle is responsible for large outbreaks and involves virus transmission when viremic persons, infected in the sylvatic or intermediate cycle, enter an urban environment with a large non-immune population and competent vectors present, usually, *Aedes aegypti* [16]. Such outbreaks have been experienced in recent years in Angola and the Democratic Republic of Congo in 2016 [17]. The human-to-human spread of the virus in an outbreak or epidemic in the absence of mosquitoes has not been reported. However, some evidence suggests that there may be possible transmission via breastmilk following maternal vaccination [18 19].

Vector competence (the transmission rate among virus-exposed mosquitoes) and vectorial capacity which considers environmental and vector factors such as biting patterns, vector behaviour and lifespan are important factors in disease dynamics. The transmissibility of the virus is highly dependent on temperature and humidity and the availability of non-immune

hosts. There have been reports of transovarial transmission in *Aedes Aegypti* but its contribution to overall YF epidemiology is unclear [20].

Figure 2. 2 of the cycles of transmission of YFV

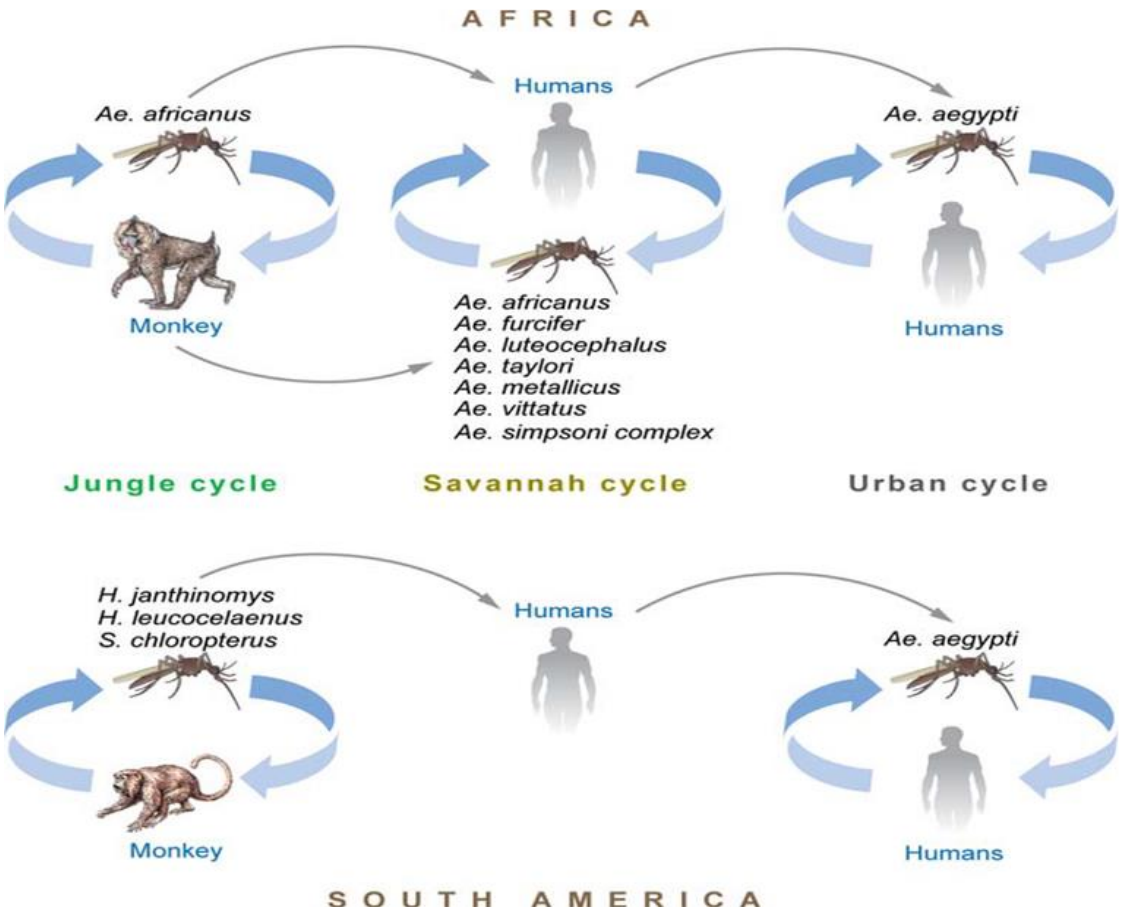


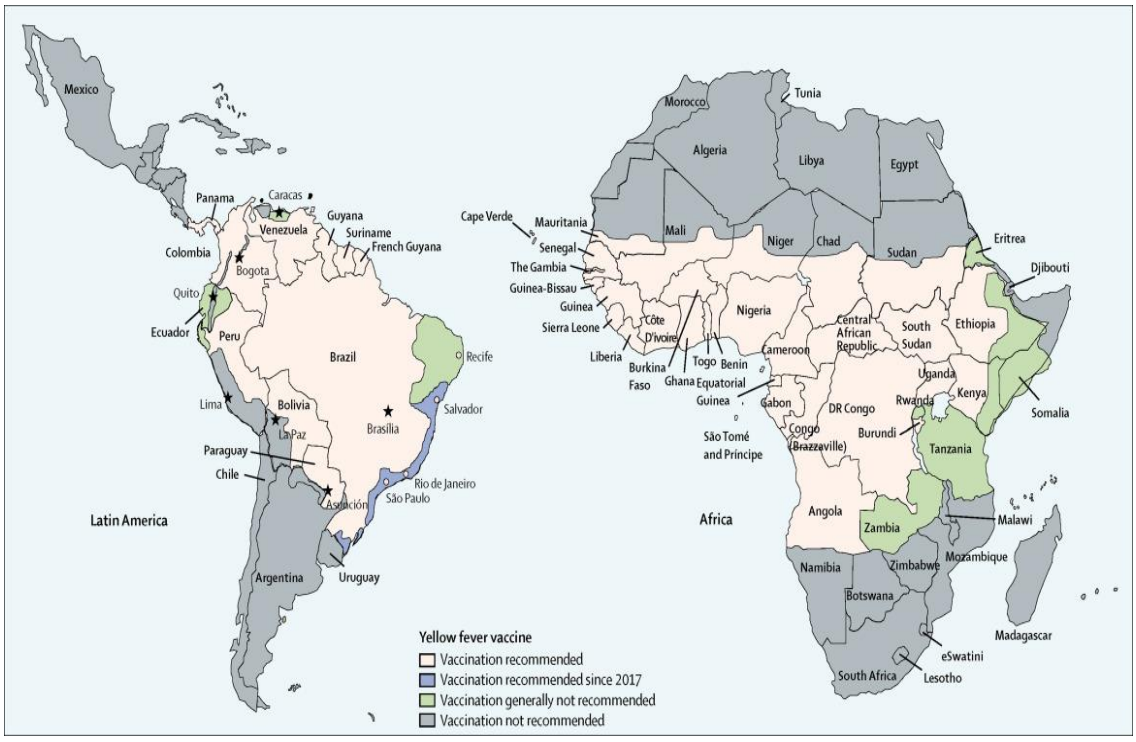
Figure adapted from Barret and Higgs[21]

2.2. Epidemiology of yellow fever

The incidence of endemic YF disease is not fully known partly because of the wide range of non-specific symptoms of disease and limited YF diagnostic facilities [3]. Transmission between humans occurs predominantly in sporadic and often massive epidemics [22 23]. There

are approximately one billion people at risk of infection in the endemic regions. This number is increasing with the virus emerging in regions where YF was not endemic [24]. Yellow fever emergence and re-emergence is attributed to poor and inconsistent vaccination coverage and incomplete surveillance of the epidemic and inter-epidemic periods. There are generally fewer cases reported in South America compared to Africa which accounts for about 90% of the global disease burden. This has been thought to be in part due to relatively low vector density and relatively high vaccination coverage in endemic areas of South America [16]. Figure 2.3 shows YF endemic regions and vaccination requirements.

Figure 2. 3 shows geographical areas with risk of yellow fever virus transmission in Latin America and Africa



Map illustrated by Reno et al. [25]

In 2013, seroepidemiological data from highly endemic regions estimated about 130,000 cases with viscerotropic disease and 78,000 deaths in Africa only [26]. Recently, there have been large outbreaks occurring in Angola, DRC and Nigeria in December 2015, July 2016 and 2018 respectively [4]. Before these, outbreaks were predominantly in West and Eastern Africa [27 28]. In Kenya specifically, there was a large outbreak that occurred in 1992 in Kerio valley necessitating mass vaccination in Baringo, Keiyo, Koibatek and Marakwet districts [29]. There have been no cases of YF reported since the introduction of YF vaccination into the EPI in these districts – there is limited evidence on whether this is due to herd immunity or reduced vector numbers leading to lower virus transmission. In Eastern, Central and Southern Africa, there are fewer outbreaks reported. In Uganda for instance, there have been more frequent but relatively small outbreaks with attack rates of <1% of the population at risk. Larger outbreaks have been reported in Ethiopia, Angola, DRC, Kenya and Sudan with attack rates of 10% and 8.8% of the population at risk, respectively. YF outbreaks are not uniform even in areas reporting similar endemicity [30 31]. In West Africa, there have been significantly more outbreaks reported in Nigeria, Senegal, Guinea, Cote d'Ivoire, Ghana, Mali, Cameroon, Burkina Faso and Gabon over the years. [13 26].

Although YF is endemic in about 13 countries in the Americas, Brazil is most affected by YF epidemics and has experienced an increased number of urban outbreaks between 2017 and 2018 due to persistently present disease in NHPs that has expanded endemic zones [32 33]. Before these recent outbreaks, the last urban outbreak in Brazil was reported in the 1940s. Endemic countries such as Peru, French Guiana, Panama, Guyana, Suriname, Bolivia, Peru and Trinidad and Tobago have not reported major outbreaks in the last 2 decades [34]. There are significantly lower mortality rates in Africa compared to South America (up to 60% versus 20% respectively) which has been attributed to the evolution of the virus and host in the region and

possibly a higher burden of other co-circulating flaviviruses which may confer cross-protection [24].

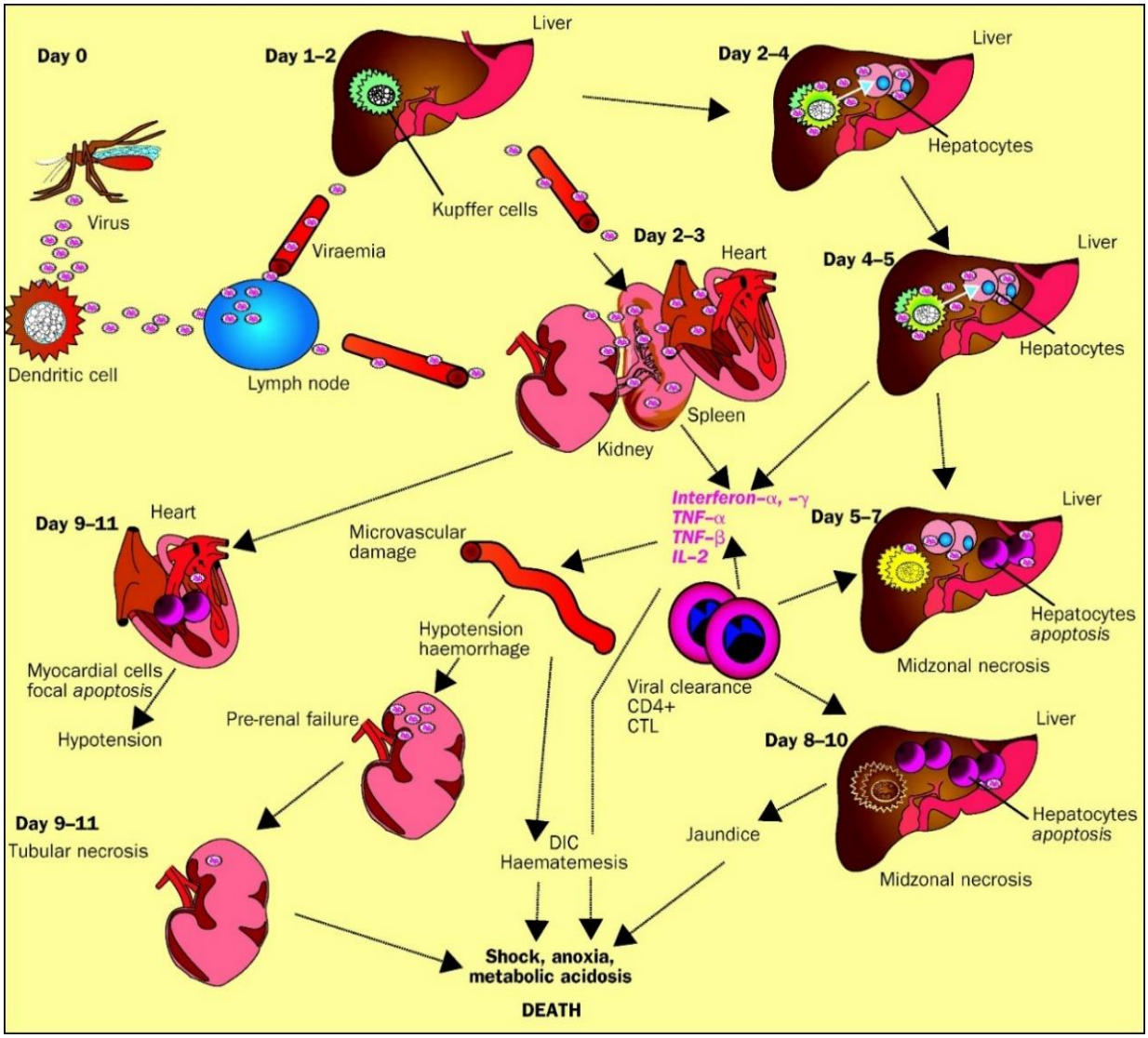
Interestingly, YF is conspicuously absent in Asia despite the presence of a competent vector and co-circulating flavivirus e.g. Dengue, Japanese Encephalitis (JEV) and Zika viruses that have similar factors that influence transmission dynamics [35]. The absence of YF in this region is poorly understood – susceptible populations have been reported to be infected with YF in Africa and some instances imported the virus to Asia without establishing endemicity even within a susceptible population [36]. In 2016, during the YF outbreak in Angola, 11 Chinese construction workers were infected and subsequently exported YF on their return to China. This was the first documented YF exportation from Africa to Asia [37]. Considering the large immunologically naïve population of over 2 billion people inhabiting large cities in Asia, the successful introduction of YF would be catastrophic [38].

The recent increase in cases may be due to a concert of factors including; global warming which has caused environmental changes that provide better conditions for the vector to thrive [39], elements of globalization such as increased international travel and urbanization with an increased risk of exporting an infection from one location to another, and relaxation of immunization requirements in endemic regions whether for routine programmes or travel to endemic regions - this has potentially reduced the population immunity levels [3 40]. The WHO Epidemic Readiness and Intervention team has identified a potential trend with these outbreaks. They seem to occur with recurring periodicity depending on the geographic location of the endemic region; with relatively frequent outbreaks in West Africa, outbreaks that are separated by about 5 years in South America, and rare outbreaks in East Africa [41].

2.3. Clinical disease and immunopathology

YF has a wide range of symptoms; subclinical infection, abortive – non-specific febrile illness without jaundice and life-threatening disease with fever, jaundice, organ failure and haemorrhage. The initial changes are often missed due to the non-specific clinical presentation. There are three clinical stages described in the classical YF cases 3-7 days after the infecting mosquito bite; infection, remission and intoxication. The period of infection (viremic period) is characterized by acute onset fever (~39°C), headache, malaise, congestion of conjunctivae and face, myalgia, nausea and vomiting. During this period the blood is infectious to biting mosquitoes and they can transmit the virus to non-immune persons. Approximately 85% of infected individuals experience these nonspecific symptoms for < 1 week [42]. Faget's sign, sphygmothermic dissociation that presents as a paradoxical slow heart rate in comparison to fever, is seen in this phase and can be diagnostic of YF [43]. Most of these cases recover without sequelae in the remission phase. This phase is often short-lived (~1-2 days) where patients feel better with a general sense of recovery. 15% of cases enter a toxic phase (viscerotropic disease) characterized by sudden and severe typical signs of multiple organ failure involving the liver, the kidneys and the heart; jaundice, oliguria, cardiovascular instability and haemorrhagic diathesis that manifests as episodes of hematemesis, melena, petechiae, ecchymosis and other haemorrhagic manifestations [44]. The hallmarks of the disease are related to hepatic injury leading to a range of clinical presentations from jaundice to hepatic failure [2]. Hepatic dysfunction here is associated with Aspartate Aminotransferase (AST) levels exceeding Alanine Aminotransferase (ALT) levels and resultant jaundice. In patients who survive the hepatitis phase, renal failure predominates. A cytokine storm due to the upsurge of pro-inflammatory cytokines is thought to be the main cause of the multiple organ dysfunction (Figure 2.4) [45 46].

Figure 2. 4 illustrates the inflammatory cascade in the pathophysiology of YF.



The virus replicates intracellularly often followed by viral clearance within about 10 days. Recurrent or persistent viremia may cause an inflammatory cascade that affects the liver and kidneys causing hepatocyte apoptosis and associated hepatic midzonal necrosis and tubular necrosis respectively. This results in bleeding dyscrasias, shock and death [45].

Specifically, TNF-α, IFN-γ, and TGF-β have been demonstrated in severe cases that become fatal. These and other cytokines including IL1-α/β, IL-4, IL-8 and IL-10 are expressed in lower levels in the liver [47]. Although the exact role of cytokines in YF is not completely understood, TNF-α and IFN-γ have been shown to bind to their respective receptors in the hepatic cells to cause cell death by intrinsic and extrinsic pathways of apoptosis [48]. TGF-β has immunosuppressor effects and is pro-apoptotic and has been thought to induce tissue damage. CD4+ lymphocytes acting via the MHC II are important in the activation of several immune cells and cytokines particularly in the liver. Renal and cardiac injuries may also be related to these immunological effects. These cytokines have been postulated to cause endothelial cell damage in the capillary bed leading to bleeding diathesis [47]. Similar immunopathology has been reported for other haemorrhagic fevers e.g. Dengue haemorrhagic fever. Death is predominantly caused by complications related to haemorrhage. Interestingly, the virus does not ordinarily infect the central nervous system (CNS) and this has been demonstrated by the absence of inflammatory changes post-mortem which would suggest viral neuro-invasion or encephalitis. Brain examination post-mortem reveals microscopic perivascular haemorrhages and oedema likely due to metabolic changes[42]. The CNS symptoms such as reduced consciousness or seizures are thought to be due to hypoglycaemia, metabolic acidosis and hypoperfusion [2 42].

Viremia is controlled by cytolysis and neutralization of circulating virus that involves both the cellular and the humoral immunity components. Viremia is correlated with disease symptomatology as it peaks between days 3 and 5 [49]. Viremia is highest during the infection stage. The remission stage is characterized by clearance of the viremia. Due to viral clearance by the synergistic effect of the immune complex through cytolysis and antibody neutralization of circulating virus, viremia disappears in the toxic phase. However, Kallas et al. reported viremia in some patients during the Brazil outbreak and associated high viral loads with

mortality [50]. It is unclear why a proportion of people enter the intoxication phase and develop severe symptoms while the rest of the patients are asymptomatic or present with mild and self-limiting symptoms. A further 15% of cases will suffer a relapse after an initial phase.

YF is associated with a high case fatality rate of up to 50% of severe disease with infants, immunocompromised persons and the elderly at the highest risk [42]. Wilder-Smith et al. in their review has identified shock, anuria, hypothermia, delirium and coma among poor prognostic signs of disease [2]. Previous data indicate that about 80%, 70% and 79% of patients who develop melena, bleeding gums and other haemorrhages respectively die and almost 100% of patients who develop anuria die [51]. Those that survive natural infection acquire long-lived and arguably lifelong immunity to YF.

Disease surveillance requires diagnostic capacity. Lack of appropriate and efficient diagnostics may delay recognizing an outbreak considering that Johansson et al. estimate that for every severe case there are about one in 70 infections that are asymptomatic [52]. Lab diagnosis can be done on serum or urine samples to assess for viremia. During early infection, YFV RNA often can be detected in the serum by virus isolation or nucleic acid amplification testing (e.g., reverse transcription-polymerase chain reaction [RT-PCR]). Diagnosis can also be confirmed by serological assays to detect virus-specific IgM and IgG antibodies. A positive IgM result in a single sample is presumptive of recent infection. Serologic cross-reactions occur with other flaviviruses; positive results should therefore be confirmed with a more specific test, usually plaque reduction neutralization test (PRNT) [53]. Laboratory confirmation requires a YF-specific seroconversion. There is limited data on the Flaviviral antibody interactions and interference in Africa where several flaviviruses (e.g. dengue, Zika, St. Louis encephalitis, and Japanese encephalitis complex viruses) are in co-circulation [54]. There is no definitive treatment for YF, and supportive care; which includes nutritional support, fluid resuscitation to manage

hypoglycaemia, treating bleeding disorders and dialysis, is critically important in the treatment of cases and has been shown to improve prognosis. Ribavirin, tiazofurin, carboxamide, pyrazoline compounds have been evaluated as candidate antivirals against YF. Ribavirin, the most widely available of these has only been shown to be effective in vitro in very high concentrations that may not be clinically achievable. Further studies are ongoing [4 55].

2.4.Yellow Fever Vaccines

Disease prevention is the most effective way to reduce disease burden. There is a very successful live-attenuated YF vaccine – probably among the most successful vaccines. Vaccination is the most effective method of disease prevention with a single dose of the vaccine providing life-long immunity [4 56]. Several other strategies have also been used with significant success. Vector control strategies include pyrethroid insecticide spraying, larvicides for larval control, insect growth regulators, sterile insect technology and bacterial toxins such as *Wolbachia* to reduce egg viability. Vaccinating NHP reservoirs has been proposed with widespread scepticism on the feasibility of such a strategy. Some of these strategies are made even more difficult by the changes in climate over time to warmer and more humid conditions and subsequent introduction of the vectors in regions where they were previously not endemic or had been eradicated [57].

2.4.1. Discovery of the 17D YF vaccine; a retrospective

The 18th, 19th and early 20th-century Western hemisphere, including the West African coast, was plagued by YF, then known as 'Yellow jack', whose aetiology was unknown and treatment undefined[58]. Between the end of the 19th century and the start of the 20th century, there were

tremendous efforts by scientists - including Adrian Stokes, Walter Reed and Max Theiler, and their colleagues - to understand the disease. Walter Reed's experiments, which involved inoculating subjects with tiny doses (equivalent to 1 to 4 mosquito bites) of a live agent, showed reduced deaths in immunized persons. However, during the experiments, previously healthy subjects died following inoculation, leading to the experiments being stopped[59]. At the same time, Noguchi Hideyo was working on a vaccine against yellow fever. Noguchi believed that YF and Weil's disease were caused by agents of *Leptospira spp.* He was reported to have been 'successful' in making a vaccine based on the *Leptospira spp* bacteria which was then produced by the Rockefeller Institute and used extensively in the Americas and Africa. However, there were concerns when his results could not be replicated by other investigators[60]. Max Theiler working with Andrew Watson disproved Noguchi's theory of a bacterial cause and demonstrated that the causative agent was a virus – which they named the yellow fever virus (YFV)[61]. Unfortunately, and ironically, Noguchi died in 1928 after being infected with YFV in Ghana where he had gone to further his research on YFV and *Leptospira*[62]. Max Theiler was also later infected with Yellow Fever but recovered and gained immunity[63].

In 1927, a 28-year-old Asibi – a Ghanaian, provided blood while suffering a mild form of YF – which he survived. Investigators, including Adrian Stokes, who were on an expedition to study YF using the isolated Asibi strain, used Indian Rhesus macaques (*Macaca mulatta)* in their experiments because African monkeys did not get sick. Their experiments, which involved inoculating monkeys with a weak form of the live virus and using immune serum from South America, protected NHPs in Africa[64]. This was the first suggestion that one vaccine could be used to offer protection globally across the strains and that the inoculate would only work in a weakened (not killed) form of the virus. At around the same time in Dakar, Francois Mayali, a young boy, contracted mild YF and French investigators used his blood to inoculate a rhesus monkey. The monkey developed severe disease[65]. The isolated strain became called

the ‘French strain’. The scientists noted that the viruses could be preserved in sub-zero temperatures. Taking advantage of preservation, the team attempted attenuation of various virus batches in different tissues including mice brains and liver tissue, noting that attenuation was associated with increased neurotropism but reduced viscerotropism in Rhesus monkeys.

During this time many more cases of YF acquired from accidental laboratory infection were reported with a significant number of people dying. YFV was one of the most hazardous viruses to work with at the time. This made a vaccine even more important to protect the lab and field workers[66]. After several trials, the mouse-derived French vaccine given with immune serum became the standard vaccine. As the vaccine was used more widely, scientists noted that the systemic and neurologic reactions were becoming more severe with the French strain vaccine[67]. Alternative attenuations were attempted with certain oils or egg yolk and the mixture freeze-dried into powder which would be reconstituted in saline for inoculation as a single dose. Combined with the Smallpox vaccine, the French vaccine was delivered as scarification and was easy to implement in mass campaigns[68]. There seemed to be significantly fewer adverse events following immunisation (AEFIs); this could have been due to incomplete field evaluation of post-vaccination reactions and the fact that the virus was attenuated further. Over the years of use, the French vaccine was associated with fears of neurologic reactions[66 69].

Theiler and Haagen continued to work with the Asibi strain, attempting to make it safer for use. Theiler et al. noted that the Asibi strain, on the 100th sub-culture in the nervous tissue-deprived chick embryo, was safer and did not result in neurotropism. This was due to a chance mutation that resulted in the 17D strain which did not require protective immune serum[70]. Unfavourable mutations in some subcultures that caused more severe AEFIs led to the introduction of the seed lot system[71]. The seed lot system is one where strains of the 17D mutant are created from the parent strains and preserved for future use. Over the years, the French

vaccine became less popular due to neurotropism and eventually, in 1982 the French vaccine was discontinued[66 72].

2.4.2. Characteristics of the 17D yellow fever vaccines

Only vaccines from the 17D strain, a derivative of the Asibi wildtype virus known to be clear from contamination and manufactured from limited seed lots, have been in use since 1982[73]. Four vaccines have been pre-qualified by the WHO, produced by four vaccine manufacturers; Institut Pasteur de Dakar in Senegal, Sanofi Pasteur in France, Chumakov by the Russian Federation and the Bio Manguinhos Fiocruz in Brazil. These manufacturers use three sub-strains all derived from the 17D strain; 17D-204, 17DD, and 17D-213 (see Table 2.1).

Table 2. 1 of release potencies of YF vaccines by WHO prequalified manufacturers.

Manufacturer (PQ)	**Sub-strain**	**Release potency (approx. IU/dose)**
Sanofi Pasteur, SA	17D-204	6,300 - 40,000
Bio-Manguinhos/Fiocruz	17DD	>5,000
Institut Pasteur de Dakar	17D-204	>5,000
Federal State Unitary Enterprise of Chumakov IPVE	17D-213	>10,000

The vaccine manufacturers produce vaccines with release potencies above approximately 5000IUs and no specific upper limit is set by WHO.

A single dose of this vaccine provides life-long immunity[74]. Immunity measured by neutralising antibodies is elicited within 30 days of vaccination. According to the WHO requirements, the minimum potency is 1000IU (International Units) per dose – there is no maximum potency limit. The 17D-204 is derived from the 204th passage of the Asibi strain in chicken tissue and the vaccine virus is produced between passages 234–238. The 17DD sub-strain is derived from the 195th passage of Asibi and chicken tissue and then through a separate passage to be used at passage 285–288. Finally, the 17D-213 strain is derived from 17D-204 at passage 235 by the Robert Koch Institute in Germany and is used at passage 238–239. These strains have subtle genomic and phenotypic differences, but no major differences in attenuation and immunogenicity [75] [76]. The vaccines are freeze-dried, single-dose products administered intramuscularly or subcutaneously in a volume of 0.5mls. Production of these vaccines relies on laborious technology developed in the early 20th century by Max Theiler and has had very little improvement [66]. This basic technology utilises specific pathogen-free (SPF) chicken embryonated eggs. These methods have limitations, outlined in table 1.2, to increase production within an outbreak [77].

Table 2. 2 shows the factors that do or have previously limited production of YF vaccine

Limiting Factor	Cause	Consequences
• **A small number of vaccine manufacturers – 4 prequalified manufacturers.**	• Unpredictable and unstable demand of YF vaccine • Poor forecasting due to the sporadic nature of the disease and short-term demand forecast • Uncertainties regarding funding for YF purchasing	• Reluctance to invest heavily in the production of YF vaccines dues to concerns of producing excess vaccines without assured purchase.
• **Competition for production capacity by more lucrative vaccines**	• Limited facilities for vaccine production leading to competition for capacity	• The more economically lucrative vaccines are attractive and favoured by manufacturers.
• **A limited supply of SPF eggs**	• Live-attenuated influenza vaccines also use SPF eggs produced by the limited SPF eggs suppliers	• Long lead time required for eggs supply
• **Availability of vaccine see stocks**	• The gradual depletion of existing seed stocks over time.	• The capacity to produce ned YF seed stocks is limited.
• **Lyophilization and fill to finish capacity**	• The process of lyophilization to fill/finish can take a few days per batched cycle.	• The lyophilization process limits the number of vials that can be produced.

Table adapted from the report by PATH [77].

The primary limitations to the production are related to the supply of SPF eggs with primary chick embryos. The seven- to nine-day-old chick embryos in these eggs are infected with the virus and incubated for 3-4 days before harvesting. The process involves centrifugation, dilution, the addition of stabilizers and preservatives. Each embryo produces 100-300 vaccine doses. The eggs used in the process must come from SPF chicken flocks whose availability is limited, making it difficult to rapidly scale up vaccine production.

The supply of SPF eggs is further limited by the fact that the eggs are also used to produce influenza vaccines which are highly successful commercially and often pay more for the SPF

eggs, therefore getting prioritised by the egg producers. Often technical difficulties have also reduced the yield of primary embryo cells from SPF egg batches. The vaccine seed stock is undergoing gradual depletion. The YF seed 17DD strain (993FB013Z), the working seed for Bio-Manguinhos, was derived from the master seed lot no. 102/84 – only four vials of the master seed remain, and 40 vials of the secondary seed. Working seed lot 993FB013Z comprising 12,861 vials was available in 2009 and was projected to last 19 years, with an annual production of 50 million doses[41]. The Institut Pasteur de Dakar reported in 2009 that there was only one vial of the master seed and the working seed was limited [41]. This annual production has since increased but adds a further limitation on the production of the vaccine. The WHO's International Coordinating Group (ICG) on Vaccine Provision keeps a stockpile of six million doses every year for epidemics [78]. Total forecasts through UNICEF for all vaccination activities in 2014-2017 averaged 62 million doses a year for routine use, which exceeds current supply requirements by approximately 42% [79].

2.4.3. Immunogenicity of the 17D yellow fever vaccines

The 17D vaccine is one of the most effective and immunogenic vaccines. Vaccination results in strong humoral immunity with neutralizing antibodies and significant antiviral activity by T cell immunity [80]. There is little evidence of vaccine failures, less than 20 reported cases of more than a 500million doses, with over 99% of vaccinees generating protective antibodies about a month after vaccination [81]. Seroconversion rates appear to be similar regardless of vaccine sub-strain and manufacturer, though vaccine immunogenicity appears to be somewhat lower in children [82 83].

There are no comprehensive data on the actual upper limit of how long these antibodies last. The correlates and surrogates of protection in humans are not fully understood [84]. In the

1970s, Mason et al. tested antibody responses in Indian macaques with different doses in a dose-finding and immunogenicity (using PRNT assay) study. The study determined 0.7 LNI as the correlate of protective immunity in the monkeys after vaccination with 17D-vaccine [85]. This has since been considered the surrogate of protection in humans [6]. This is in part due to the ethical concerns with studies to establish these measures in humans. Although there is little data on vaccine failures, there has been confidence in the assumption as regards the surrogates of protection. Post-marketing monitoring studies have revealed that during large vaccination campaigns, the YF vaccine failure rate is relatively low [81].

Although I will discuss vaccine policy further in section 2.5 below, it is important to note here that previously, the WHO recommended a booster every 10 years for persons visiting endemic regions from non-endemic regions. However, this recommendation was reviewed to remove the booster and the YF vaccine is considered to provide life-long immunity [86]. This recommendation was made after a review revealed that there is a role of cellular immunity, specifically T cells in providing antiviral activity and protection even in the absence of neutralizing antibody responses from humoral immunity [74]. Niedrig et al. and Reinhardt et al. have extensively described these T cell activities and demonstrated significant antiviral immunity [87 88]. This recommendation has been criticised with some researchers arguing that neutralising antibody is the most appropriate measure to monitor protection. This is compounded by our limited understanding of the mechanism of protection of the vaccine [6]. Some studies that have measured longevity of antibodies beyond 10 years suggest that antibodies persist for up to 35 years post-vaccination [89]. However, these data may be biased for age, sex, race, endemicity and technical differences in measures of immunity. Additionally, the effect of co-circulating flavivirus antibodies are not fully understood and information is inconsistent [90-92].

2.4.4. Safety of the 17D yellow fever vaccines

Adverse events and serious adverse events

The YF-17D vaccine has an excellent safety profile with few concerns raised over more than 80 years of use. This has been confirmed by data from multiple studies and immunization campaigns over the years where millions of vaccine doses have been given to different populations [93] [94]. The risk of complications is related to vaccinees' factors such as immunocompetence, age and allergy to eggs and other vaccine ingredients [89]. As such adverse events and serious adverse events are associated with these factors. The adverse events following immunization (AEFI) are usually a mild and self-limiting headache, myalgia, fever, malaise, pain and reaction at the injection site [94] [95]. These events may occur in combination. For these reasons, the WHO has provided contraindications for the YF vaccine. These include children under 6 months of age, known allergy to eggs or any other vaccine ingredient, immunodeficiencies due to immunosuppressive and immunomodulatory therapy, transplant patients, symptomatic HIV patients with CD4+ <200cells/mm^3 and patients with primary immunodeficiencies. Children between 6 to 8 months, adults older than 60 years, pregnant women and breastfeeding women are considered at a higher risk of serious adverse events (SAEs) but are not contraindicated to YF vaccine [78]. The severity of the AEFI is considerably higher with the first dose of the vaccine [96] [97].

Serious adverse events typically presenting as yellow fever vaccine-associated neurological disease (YEL-AND) and yellow fever vaccine-associated viscerotropic disease (YEL-AVD) often leading to death are extremely rare with the majority of the studies done reporting none [97]. In a systematic review, Roger E. Thomas et al. revealed that out of nine studies looking at the safety of YFV 17D, there were no serious adverse events or deaths reported [98]. However,

some of the studies reviewed did not report any adverse events at all in children which casts doubt about the completeness of the assessments and the credibility of these reports especially given that the vaccine was co-administered with other immunisation programmes vaccines [82] [99].

Many Persons Living with HIV (PLWHIV) often live and travel to YF endemic areas. YF vaccination is recommended for asymptomatic persons with a CD4 ≥200cells/mm^3. There is limited data on the immunogenicity and safety of the YF vaccine in HIV-infected persons. A study done by Veit et al. in 2017 among a Swiss cohort showed that the vaccine response in persons with suppressed viral load was consistently high even 10 years post-vaccination[100] [101]. There were also no safety concerns in the study; the AEFIs described were similar to those reported in the general population. Studies investigating the safety in this population are limited and there is no clear risk identified and the sensitivity of the study designs to detect serious AEFI has not been established[102-104]. There are no data from controlled trials on immunogenicity and safety in African populations living with HIV.

Viremia

Viremia is an important marker of safety following vaccination as systemic events have been shown to coincide with the period of viremia[96]. This may suggest viremia as a predictor of adverse events. However, investigation of the link is difficult due to the rarity of events and incomplete post-vaccination surveillance[96]. The bio-distribution of vaccine virus after vaccination is complex and viremia is likely to be transient and likely to be missed by a single sample collection. However, there is evidence showing that vaccine virus particles may persist for a long time after vaccination – up to 36 days after vaccination and other evidence showing frequent and prolonged shedding of vaccine RNA in the urine of vaccinees [105] [106]. The elderly

also have prolonged viremia after vaccination [107]. Positivity of viremia by RT-PCR ≥ 14 days after vaccination is one criterion for confirmation of YEL-AVD, in the Brighton criteria [108]. Roukens et al. showed that some revaccinated subjects did not develop detectable viremia while primi-vaccinees recorded significantly higher viremia. They also showed that viremia is comparable for intradermal and subcutaneous vaccine administration. Further, they described marginal differences in occurrence and intensity of some of the AEFIs depending on the route of administration of the vaccines comparing the conventional subcutaneous and the intradermal routes [109].

2.5.YF vaccine policy and regulations for use of 17D yellow fever vaccines

Vaccine usage is generally guided by vaccine policies and regulations operating at the global, regional and national levels with WHO being the key authority on vaccination policies as described in the sections above where changes in how the 17D YF vaccine is used are prescribed by the WHO. Vaccine policies are decisions, guidance and recommendations by institutions and/or organizations for vaccination plans, interventions, actions and strategies to achieve specific goals for public health benefit towards longevity and increasing life years without disability. Vaccines policies are complex and dynamic formal prescriptions. There are however exceptions to these formal policies that are in the form of recommendations especially for vaccines against diseases of epidemic and pandemic potential. These exceptions to policy are conditional and not considered formal policies, such as the case with the use of fractional doses. I will discuss fractional doses in detail in section 2.5.3 below.

2.5.1. Vaccination strategies of the 17D yellow fever vaccines

There are three well-established YF vaccination strategies worldwide; 1) routine immunization – a) childhood vaccination in endemic regions administered as part of EPI at 9-12 months often together with the Measles containing vaccine, and b) routine vaccination of non-immune visitors travelling from non-endemic to endemic regions, 2) preventive campaigns which often act as catch up campaigns in the endemic regions, and 3) emergency (reactive) outbreak response campaigns during active campaigns [22]. Vaccination is recommended for children above the age of nine months but infants as young as six months are vaccinated for outbreak control or in populations at very high risk of YF [110 111].

Despite these strategies, there are still sporadic and massive outbreaks with parts of Africa and South America characterised by unpredictable focal periodicity and uncertain risks for large outbreaks [24 26]. This is in part due to incomplete vaccination and under-reporting and related poor disease intelligence [3 21]. Such large outbreaks have been reported in the last five years in Angola, DRC and Nigeria in Africa and Brazil, Paraguay and other previously non-endemic regions in South America [33 112 113].

The main limitation to disease prevention strategies currently is the inadequate vaccine supplies. UNICEF stated that their annual procurement can cover routine immunization requirements, emergency stockpiles and some limited additional campaigns but not sufficient to cover the demands in large epidemics or outbreaks. This recurrent shortage is still seen despite efforts to address the shortages. In 2000, a global shortage of YF vaccines led to the development of the International Coordination Group (ICG) on vaccine provision for YF. The ICG aims to manage and coordinate the provision of emergency vaccine supplies during outbreaks and ensure the best allocation of limited resources [114]. In 2000, a stockpile of 2 million doses was reserved for outbreak response. This stockpile was increased from 2 to 6

million doses in 2014. However, the stockpile was depleted twice in 2016 during the Angola and DRC epidemics necessitating fractional dosing as a dose-sparing strategy of available stock [4 78].

In response to these crises, Global partners (including GAVI, UNICEF and WHO) developed the Eliminate Yellow fever Epidemics (EYE) strategy – a global and comprehensive long term (2017-2026) strategy targeting the most vulnerable countries [115 116]. The strategy addresses global risk by building resilience in urban centres and preparedness in areas with potential for outbreaks ensuring reliable vaccine supply. The strategy consists of three strategic objectives; to protect at-risk populations, to prevent international spread and to contain outbreaks rapidly. It is supported by five cross-cutting competencies to ensure its roll-out and success. These are research and development for better tools and practices, affordable vaccines and sustained vaccine market, strong political commitment at global, regional and country levels, high-level governance with long-term partnerships and synergies with other health programmes and sectors [116]. An estimated 450 million doses are needed to achieve >80% coverage in YF-affected areas, while the current annual YF vaccine production is only 80 million doses [4 117 118]. Thus, increasing global vaccine supply (including the use of fractional doses) is the backbone of the delivery of the EYE strategy.

2.5.2. Vaccine policy changes

Vaccine policies and strategies based on these policies are often subject to changes based on new evidence in an iterative process of policy that involve agenda setting, policy formulation, policy legitimization, policy implementation and policy evaluation. In response to the emerging evidence, there have been a series of changes in YF vaccination recommendations

in the last 10 years (Figure 2.5) [119]. There are coordinated efforts by the WHO and its global partners when reviewing evidence to make a decision that leads to vaccine policy change.

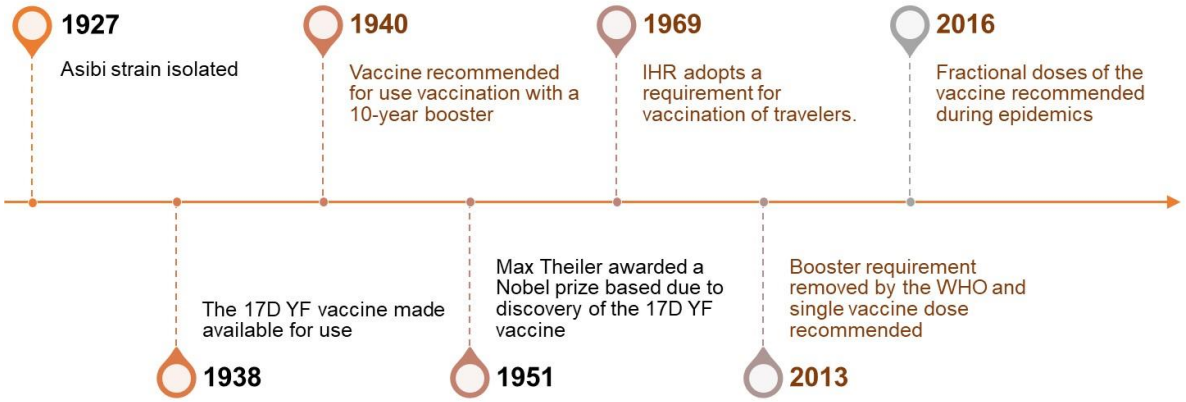
Appropriate and timely changes to vaccine policies are key to controlling diseases of global health and global security importance, including informing epidemic control strategies such as the EYE. Decision-making processes for such diseases happen at the global and regional levels but their implementation ultimately depends on national-level decisions. Although a systematized process has been described at the global level and provides a platform for vaccine policy development, each policy is unique for different regions depending on the burden of disease, priorities and other factors relating to actor beliefs and perceptions[120]. Usually, a decision or recommendation made at the WHO (global office) is published as a policy position for different vaccines and then this is adopted by different regions or countries depending on prevailing priorities[121].

Although it is crucial that communities, as part of the stakeholders, perceptions are understood and used to highlight any community-based issues that need to be considered during policy development, decisions during an epidemic often follow the top-down theory of implementation which envisages clear division between policy development and implementation – here the policies are developed at the macro level and implemented through a largely linear, rational process in which subordinate levels of a policy system put into practice the intentions of higher levels [122].

In 2012 the Advisory Committee on Immunization Practices (ACIP) examined evidence that demonstrated that YF vaccine immunity persists longer than previously thought and could provide life-long immunity and recommended the removal of the 10-year booster requirement [74 123]. In 2013, WHO's Strategic Advisory Group of Experts (SAGE) in Immunization concluded that a single primary dose of yellow fever vaccine is sufficient to

confer sustained immunity and lifelong protection against yellow fever disease [86]. The World Health Assembly went ahead to adopt this recommendation to remove the 10-year booster dose requirement from the International Health Regulations within two years of the recommendations [119].

Figure 2. 5 of the 17-D YF vaccine historical events



The events in black are general historical events while those in brown are related to policy events

Following the 2016 YF epidemic, the WHO allowed for use of fractional doses in the DRC to deal with the vaccine shortage [78 124]. This recommendation was made even with limited evidence of the immunogenicity and efficacy of fractional doses of the YF vaccine in this population. This process was different compared to the process that led to the removal of the ten-year vaccine dose booster as described above. This recommendation was an exception to the prevailing policy due to the urgency and dire need to increase vaccine dose supply at the time. This recommendation was meant to be a short-term measure. However, as discussed in

the next section, this strategy went ahead to be used in other instances due to the persistent vaccine shortages and increased number of outbreaks.

2.5.3. Fractional dosing

Fractional dosing is a dose-sparing strategy utilising a fraction of the total dose prescribed. Previously, fractional doses have been utilised as a fifth of the standard dose of the Rabies vaccine, Polio, Measles vaccines and more recently the YF vaccines[125]. The use of fractional doses in vaccination is aimed at increasing the number of people vaccinated where there is insufficient vaccine dose supply, or where the vaccine is expensive.

In 2012, the Program for Appropriate Technology in Health (PATH), a global body involved with developing innovations and implementing solutions that improve health, identified YF vaccine dose shortages as a significant threat to global health. In a report on the potential of dose sparing to increase vaccine supply and availability of the YF vaccine, PATH argued that a regulatory strategy needs to be developed to support policy and regulators to allow for the use of fractional doses [77]. Policymakers are unlikely to change vaccine policies without compelling clinical evidence on non-inferiority in immune responses and safety with formal regulatory approval of such changes. It has been suggested that a formal change of label to allow the use of fractional doses and route of administration to subcutaneous and/or intradermal method is the most likely preferred approach for policymakers [77 126]. Otherwise, the use of fractional doses can only be used off-label.

Despite the limited evidence of the immunogenicity and efficacy of fractional doses of YF vaccine, the global vaccine shortage led to the WHO recommending one-fifth of a standard dose in a reactive mass vaccination campaign during the YF outbreak in DRC, accounting for

approximately 5 million doses [5 124]. The outbreaks of YF in Angola and DRC were contained by mass vaccination of these fractional doses, and there were no reports of serious adverse reactions. In a study by CDC during the mass vaccination with fractional doses in Kinshasa, Casey R. et al. reported that the immunologic response to a fractional dose of the 17DD yellow fever vaccine was appropriate for a response to a yellow fever outbreak among children 2 years of age or older and among non-pregnant adults. However, this trial was not controlled and therefore no data on randomized controlled studies or otherwise on efficacy and safety of fractional doses in children were available [127]. Despite a large number of vaccinated individuals, the lack of a control group precludes firm conclusions regarding vaccine efficacy. Previous limited studies have found fractional doses to be sufficiently immunogenic eliciting responses above the internationally recognized threshold for protection [109 128 129]. Some of these data suggest that a fifth of the standard dose is sufficiently immunogenic. Other data support immunogenicity at even lower doses of the pragmatic fifth (518IU/dose) [130]. However, as with Casey et al.'s study, the other studies had various limitations. One of the main studies was limited because the population was homogenous including young male military men and the results from this population may not be applied to the general population and in specific populations such as children and persons living with HIV [128]. Another study was conducted in a population where YF is not endemic [109].

At the point of recommendations by whom to use fractional doses, there were no data from African populations on the efficacy of fractional doses from randomized controlled clinical trials [5 84 89]. The WHO through the initiative for vaccine research (IVR) after consultation on the research agenda for fractional dose use set key research priorities which included [7]:

- Can all four WHO-prequalified yellow fever vaccines be used as fractional doses with non-inferior immunogenicity when compared to standard vaccine doses?

- Are fractional vaccine doses associated with increased incidence of severe adverse events when compared to the standard vaccine dose?
- What is the minimum yellow fever vaccine dose that is non-inferior to the standard dose?
- Can reduced doses of yellow fever vaccine provide sufficient immunogenicity in infants and children under the age of 5 years?
- Are reduced doses of yellow fever vaccine sufficiently immunogenic in human immunodeficiency virus (HIV)-infected individuals with CD4 counts ≥ 200 cells/ml?
- What is the longevity of the immunity provided by the reduced doses of the yellow fever vaccine?
- Is the immune response to YF fractional dose comparable across different genetic backgrounds?

2.5.4. A prospective policy change

Although fractional dosing has been effectively used as a dose-sparing strategy since the recommendation by the WHO, it currently does not satisfy International Health Regulations [11]. With the current and growing clinical evidence on the efficacy of fractional doses of the YF vaccines and the recurring shortages in the supply of the YF vaccine, there are possibilities of a formal change in vaccination policy during epidemics and possibly in routine use. Some countries such as Canada and the USA have used fractional doses when faced with vaccine shortages to vaccinate travellers [131].

Policy changes are often influenced by many factors among them the nature of the proposed policy change and its implications, the socio-economic, political and epidemiological context, and the policy processes and actors involved (stakeholders) [132]. In decision-making when

planning for a vaccine policy change, it is important to identify the appropriate stakeholders and understand their perceptions on a proposed policy [122]. In the health policy analysis framework, described by Walt and Gilson [133], actors are often influenced in policy decision-making by context – situational, structural, cultural and international – within which they live and work. With the importance of future endorsement of policies for wider use of fractional doses of the YF vaccine, it is key to explore the perceptions of the stakeholders on a prospective policy. Lack of support for a strategy, such as fractional dosing as a dose sparing strategy, due to limited knowledge and negative perceptions may delay the uptake while others reject vaccine strategies frustrating the preventive efforts especially in outbreaks [134].

Specifically, for YF control in Africa, WHO regional office for Africa (AFRO) has the mandate to lead the decision-making processes on adoption and subsequent implementation in Africa. There is little data on the process of changing vaccine policies or even introducing new policies in the African region. There is, therefore, a need for a better understanding of factors influencing how policy change is implemented in this region, and regional actors' perceptions and priorities especially in the context of an epidemic.

2.6. Summary

The literature review above highlights the characteristics, immunopathogenesis, epidemiology, vaccine and vaccination strategies including fractional dosing for YF. My thesis focuses on the immunogenicity and safety of fractional doses of the YF vaccine and policy for use of this dose-sparing strategy. Prior to my DPhil project, I was involved in a clinical trial to assess the immunogenicity and safety of fractional doses of the WHO-prequalified (PQ) vaccine manufacturers [8]. I will discuss this work in detail in the 3rd chapter, but the study provides crucial evidence on the generalisability of using fractional doses for all the PQ vaccines. There

are still several gaps regarding the use of fractional doses. My thesis attempts to address some of these gaps. The data in that study was limited to adults (18-59yrs) of good health status and thus limits our conclusions for use in other populations including children, HIV-infected persons and older populations. There are no published data available on the immunogenicity of fractional doses among HIV-infected persons. The first area of my thesis builds on the first study to assess the immunogenicity and safety of fractional doses in HIV-infected persons[8 135].

The second limitation is that the fractional doses used were all above the WHO recommendation for vaccination and the lowest dose that provides sufficient immunity is still unknown. Additionally, data on interference (if any) from antibodies against co-circulating flaviviruses is scarce. In my thesis, I investigate the immunogenicity, safety and immunological kinetics of titrated lower doses and any interference of pre-existing immunity to co-circulating flavivirus before vaccination. Finally, the policies for use of fractional doses are still conditional and still marred with uncertainties related to these gaps. In my thesis, I attempt to map out the YF vaccine policy stakeholders at a macro-level (global and regional) and examine the processes, priorities, perceptions and interests for a prospective formal vaccine policy change for use of fractional doses of the YF vaccine.

3. Immunogenicity and safety of fractional doses of 17D-213 yellow fever vaccine among HIV-infected persons in Coastal Kenya

3.1. Introduction

In the introduction of this chapter, I describe a series of studies in Kenya and Uganda to test the immunogenicity and safety of fractional doses of the 17D-YF vaccine in different populations. I will then describe in detail the clinical trial conducted among Human Immuno-deficiency Virus (HIV)-infected individuals. In the first chapter, I highlighted the gaps related to the use of fractional doses. These data gaps were identified by the WHO as priorities to improve the understanding and the utilisation of use of fractional doses as a dose-sparing strategy to address vaccine shortage [7]. Responding to the WHO's accelerated global research agenda on the use of fractional YF vaccines doses, a consortium of researchers affiliated to the KEMRI-Wellcome Trust Research Programme, the University of Oxford, Epicenter – Médecins Sans Frontières and the Institut Pasteur de Dakar designed and ran a series of non-inferiority randomised trials to test the immunogenicity and safety of a fifth of the standard dose of each of the prequalified manufacturers in Kilifi, Kenya and Mbarara, Uganda[135]. One of these studies was the YEFE study, a phase IV randomised, double-blinded non-inferiority trial on the immunogenicity and safety of fractional doses of yellow fever vaccines, to compare the immunogenicity and safety of a fifth of the dose to the respective standard vaccine dose using all the four WHO-prequalified vaccines.

3.1.1. The YEFE adult study recruiting HIV-uninfected persons

In the study, 960 participants were recruited in Kenya and Uganda between Nov 2017 and Feb 2018. The participants were randomly assigned to one of the four WHO-prequalified vaccines and a standard or fractional dose; 120 participants to standard dose and 120 to the fractional dose of each of the four vaccine manufacturers (see figure 3.1). In the study, the primary endpoint was a pairwise comparison of the change in antibody as determined by 50% reduction of plaques by neutralising antibodies measured by the Plaque Reduction Neutralisation Test ($PRNT_{50}$) at 28 (±3) days after vaccination compared to the baseline (this method is discussed in more detail in section 3.2.4). The primary outcome was non-inferiority of the proportion of vaccinees that seroconverted when vaccinated with the fractional dose compared with standard-dose [8 135]. The study showed that one-fifth of all the four WHO-prequalified 17D-YF vaccines were sufficiently immunogenic meeting a set non-inferiority threshold of -10% [8]. The absolute difference in seroconversion between fractional and standard doses by the vaccine was 1·71% (95% CI –2·60 to 5·28) for Bio-Manguinhos, –0·90% (–4·24 to 3·13) for Chumakov Institute, 1·82% (–2·75 to 5·39) for Institut Pasteur de Dakar, and 0·0% (–3·32 to 3·29) for Sanofi Pasteur. At 28 (±3) days after vaccination, almost all participants showed neutralising antibodies, and these high antibody titres were sustained up to 365 (±14) days after vaccination. Non-inferiority of the fractional doses was maintained at one-year post-vaccination. Although Adverse Events Following Immunisation (AEFIs) were common (58 – 63%), there were no major safety concerns as the AEFIs were mild or moderate and self-limiting. The common AEFIs were headache (22.2%), fatigue (13.7%), myalgia (13.3%) and pyrexia (9%) [8].

This study was a sufficiently powered randomised controlled trial assessing immunogenicity and safety of fractional doses and the first to assess head-to-head all the prequalified manufacturers in an African population by the time of writing this chapter[136]. This study provides crucial evidence on the use of fractional doses for all four WHO-prequalified

vaccines. However, several questions remain regarding the use of fractional doses. Some of these questions relate to the longevity of protection by fractional doses are related to the generalisability of these data to special populations; different paediatric populations and HIV-infected individuals and the actual minimum vaccine dose that is required to provide sufficient immunity[5]. Despite the efficacy of YF vaccines, regardless of the dose, the immunological mechanisms may differ between populations due to differences in immunological maturity and immunological mechanisms of vaccination [84 137-140].

3.1.2. HIV epidemiology

Although there has been a lot of focus and progress on prevention and treatment, HIV remains the most important cause of acquired immunodeficiencies globally (see table 3.1) [141]. An estimated 38 million people are living with HIV. The large number is partly due to the life-prolonging Anti-Retroviral Therapy (ART) and associated significant decline in Acquired Immunodeficiency Syndrome (AIDS)-related deaths. There is a significant burden of HIV and associated acquired immunodeficiencies in sub-Saharan Africa and tropical South America where YF is endemic. In 2019, about 29 million (over two thirds) of HIV-infected individuals lived in these regions [142].

In Kenya, where the trial reported in this chapter was conducted, it is estimated that about 1.6 million people were living with HIV in 2018 and about 46,000 new infections per year [143]. In this report, the coastal region of Kenya accounted for an HIV prevalence of about 5.6%, with a more than three-fold higher prevalence among the 'most-at-risk-population'; including men who have sex with men (MSM), injecting drug users (IDU) and female sex workers [144 145]. Although YF is not common on the coast of Kenya, it is persistently endemic in the Rift Valley region, about 700 km North-West of the coastal region with relatively easy access, where routine immunisation is part of the Ministry of Health's Expanded Program of Immunisation

(EPI). Other flaviviruses, such as the dengue virus causes sporadic outbreaks on the coast of Kenya causing intermittent and significant outbreaks in the region[146 147].

Table 3. 1 shows the number of people living with HIV estimates by WHO region adapted from UNAIDS 2019 report.

Summary of the global HIV epidemic (2018) N (95% CI)		
WHO Region	**Estimated number of people living with HIV**	**ART coverage (%)**
African Region*	**25 700 000** [22 200 000-29 500 000]	**64** [48-76]
Eastern and Southern Africa	20 300 000 [18 000 000-22 800 000]	**67** [52-79]
Western and Central Africa	5 400 000 [4 300 000-6 700 000]	**49** [35-64]
Region of the Americas*	**3 500 000** [3 000 000-4 200 000]	**67** [49-82]
Eastern Mediterranean Region	**400 000** [290 000-570 000]	**21** [13-31]
European Region	**2 500 000** [2 300 000-2 800 000]	**55** [43-64]
South-East Asia Region	**3 800 000** [3 100 000-4 900 000]	**53** [39-71]
Western Pacific Region	**1 900 000** [1 700 000-2 100 000]	**59** [47-69]
Global	**37 900 000** [32 700 000-44 000 000]	**62** [47-74]

*Highlighted is part of the region where YF is endemic

3.1.3. Use of YF vaccine in PLHIV

The WHO recommends yellow fever vaccination for HIV-infected persons ≥9 months who are asymptomatic and with CD4+ cell counts ≥200cells/ml[10 140]. This recommendation applies to routine programs of immunisation for travel and as part of EPI and vaccination campaigns during epidemics. This recommendation is supported by data on immunogenicity and safety of yellow fever vaccine among HIV-infected persons with CD4+ ≥200 cells/ml [89 148-150]. The YF vaccine, a live attenuated vaccine, is contraindicated in persons with severe immunodeficiencies including persons with severe HIV infection and AIDS in principle due to

its potential for virulence and ability to cause severe adverse events including YEF-AVD and YEF-AND [10 151].

There are significant benefits for mass pre-emptive and reactive vaccination campaigns in YF endemic regions to tackle epidemics [116]. In YF endemic areas such as in Africa, the populations' HIV status is not always known and HIV testing or clinical diagnosis for the advanced disease may not be pragmatic or even appropriate during a vaccination campaign in the context of an outbreak [152]. During the recent outbreaks in parts of Africa and South America, fractional doses (one-fifth of the standard doses) were used as a dose sparing strategy where about 1-10% of these populations are infected with HIV [11 127 153].

Although standard doses of the YF vaccine are safe in persons living with HIV, vaccination may result in delayed and lower rates of seroconversion hence lower immunogenicity, efficacy and herd immunity compared to HIV-noninfected populations [101 151 154]. Higher CD4+ cells and lower HIV RNA levels at the time of immunisation correlate with a better immune response to the YF vaccine[150 151]. Infection with HIV has been thought to lower the duration of protection in first-time vaccinees [155]. Co-infection with other co-circulating flaviviruses may also result in antibody interference [156].

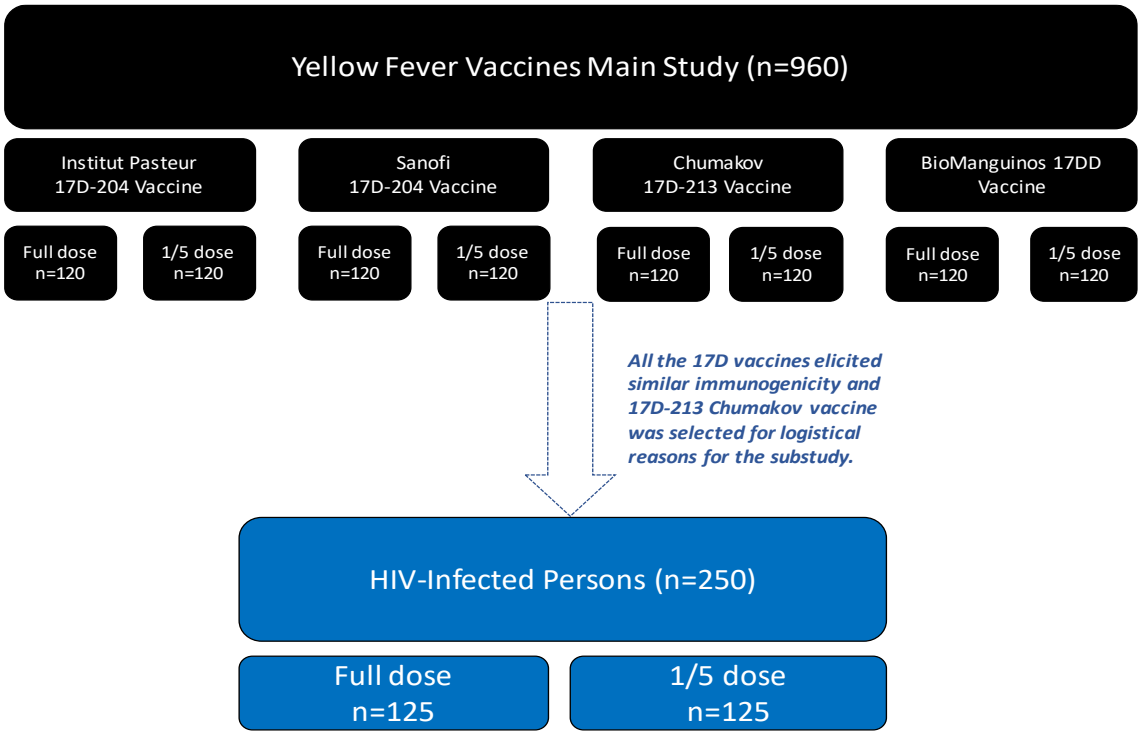
The current evidence regarding seroconversion rate, duration of humoral and cellular immunity after vaccination and revaccination in PLHIV is limited to a few, low-quality studies and even rarer data on the use of fractional doses in PLHIV[155]. In my literature search, I did not come across any dose-response studies in PLHIV. The currently available data limit rationalisation of developing comprehensive yellow fever vaccination guidelines specific for PLHIV [155].

3.2. Methods

The Chumakov vaccine (17D-213), manufactured by the Institute of Poliomyelitis and Viral Encephalitis, Russian Federation, was selected to be evaluated in PLHIV in Kilifi, Kenya. The

Chumakov vaccine was selected based on logistical considerations given that all four vaccines had similar safety, immunogenicity and efficacy including for fractional doses (see above). Considerations made were related to the fact that at the time, the outbreak response stockpile was composed of vaccines from Sanofi and Chumakov. Further, there were studies planned using the Bio-Manguinhos vaccine and the Institut Pasteur de Dakar vaccine. The 17D-213 vaccine by Chumakov was used to vaccinate YF sero-naïve HIV-infected persons with CD4+ counts ≥200 cells/ml. Participants were randomised to two arms to receive either the standard dose or fractional dose (1/5th) in a ratio of 1:1 (see figure 3.1). This trial was designed to answer questions on the use of fractional doses of vaccines with potencies as close as possible to the minimum release potency specification for each of the four WHO-prequalified YF vaccines[135].

Figure 3. 1 shows the study design for the YEFE study.



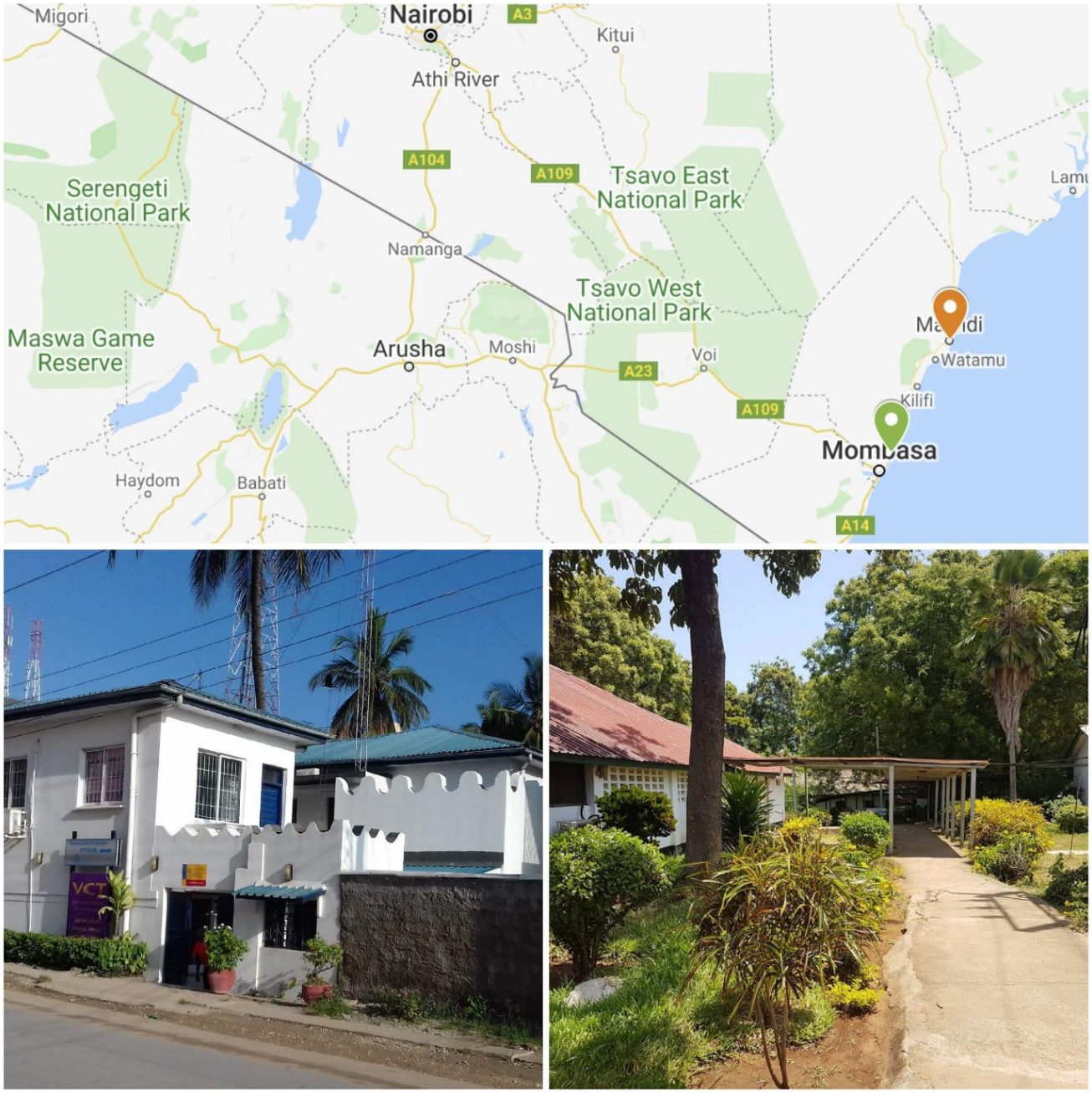
3.2.1. Ethics statement

For this sub-study, an amendment was made to the original clinical trial protocol to allow for the recruitment of HIV-infected persons only. The amendment was made to the protocol at the Kilifi site only and received ethical approval from the KEMRI's Scientific and Ethics Review Unit (SERU), the Oxford Tropical Research Ethics Committee (OxTREC) and the WHO Ethics Review Committee, and regulatory approval from the Pharmacy and Poisons Board of Kenya (PPB) [135] (details in appendix 1).

3.2.2. Study participation

Participants were recruited from comprehensive care centres in Mtwapa and Malindi on the coast of Kenya, 17km and 115km northeast of Mombasa, respectively (see figure 3. 2). The study aimed to recruit HIV-infected volunteers who were on ART treatment, and as such, we developed strategies to inform these communities about the study. A community engagement plan specific for the study was developed at each of the comprehensive care centre sites. In this plan, we involved the local sub-national health management teams, local administration, comprehensive care providers at the centres and the members attending the comprehensive care clinics. Potential participants were identified and sensitised for the trials. Willing volunteers provided signed informed consent before any study-specific procedures were undertaken to assess their suitability based on the inclusion and exclusion criteria in table 3.2. Antibodies to YF virus at baseline were used in the study outcome analysis but not as an exclusion criterion during screening.

Figure 3. 2 Map showing the location of the study clinics



Mtwapa comprehensive care clinic (top panel, in green tag and bottom left) and the Malindi comprehensive care clinic (in orange tag and bottom right).

Individuals aged ≥18<60 years, with no contraindication for vaccination or previous history of YF infection or vaccination and with CD4+ levels ≥ 200 cells/mm^3 were recruited based on the inclusion and exclusion criteria in table 3.2. The participants were vaccinated and followed up for a year after vaccination at day 0, day 10 (±1), day 28 (±3) and day 365 (±14). Procedures for these visits have been included in table 3.3 and figure 3.3.

Table 3. 2 shows the inclusion and the exclusion criteria

Inclusion criteria
• Individuals aged ≥18 - <60 years of age. • Providing informed consent to participate in the study • HIV positive on serological testing, on ART and without symptoms suggestive of current clinical immunosuppression and CD 4 count ≥200 within the last 6 months.
Exclusion criteria
• Known contraindications to YF vaccination such as allergies to egg protein, immunodeficiency due to symptomatic (i.e. clinical stage 3 or 4) HIV infection, known thymus disorder, such as thymoma and myasthenia gravis • Acute febrile disease on the day of vaccination • Previous YF vaccination or infection as determined from history • Pregnancy (as determined by a urine test on the proposed day of vaccination) and lactating women • Planning to migrate out of the study areas before the end of the study follow-up • Planning to travel to a country requiring a YF vaccination certificate within the first year after vaccination.

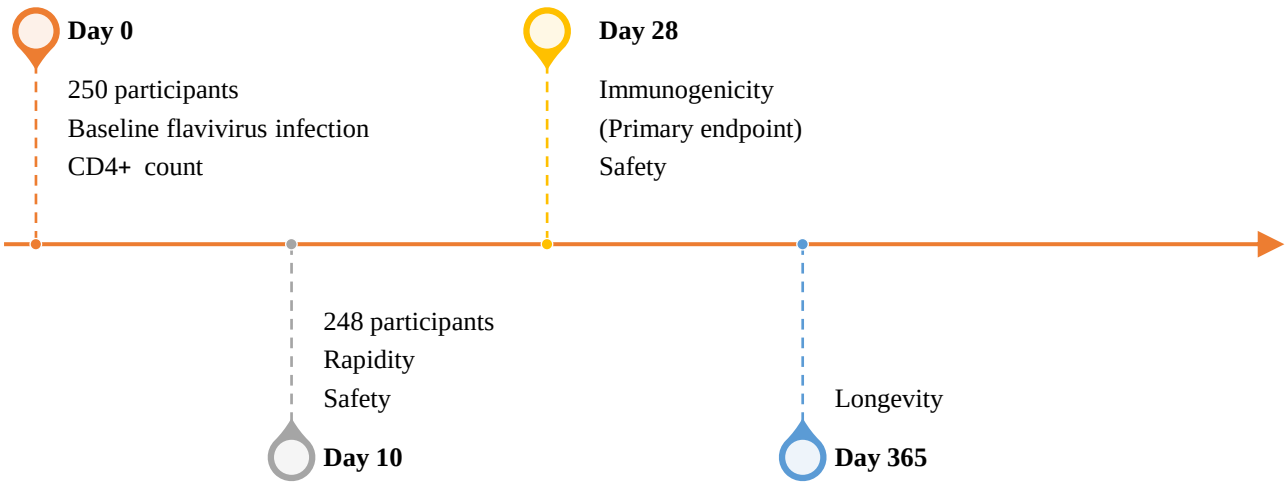
Table 3. 3 shows the YEFE_HIV study activities timeline

Procedure	Screening Day -60*	Day 0	Day 10 (+/- 1 day)	Day 28 (+/- 3 days)	Day 365 (+/-14 days)
Informed Consent	X				
CD4+ count baseline (2mls blood sample)		X			
Pregnancy test**		X		X	
Demography	X	X			
Vital signs	X	X	X	X	X
History and Physical exam	X	X	X	X	X
Randomization		X			
Vaccination		X			
Blood sample for immunology (4 mls)		X	X	X	X
Cumulative blood volume		6 ml	10ml	14ml	18ml

*Before vaccination; Screening can occur between 0 and 60 days before vaccination. Screening and vaccination can occur on the same day.

** Urine pregnancy test performed on female participants of reproductive age.

Figure 3. 3 shows the serum sample collection time points post-vaccination



3.2.3. Study procedures

Upon recruitment, baseline blood samples were collected to assess CD4+ levels and to assess the presence of existing YF antibodies from previous infection or vaccination before randomisation and vaccination. The CD4+ levels at baseline were measured using rapid diagnostic tests as per the Government of Kenya guidelines[157]. Although viral RNA may be a correlate of immunogenicity and is an established predictor of HIV/AIDS progression, we did not test for HIV viremia in this study. The trial objectives related to the effect of CD4+ level on immunogenicity, judging this to be a more direct measure of the effector immune response. However, some participants had viral load data available from a separate concurrent study. Participants were randomised to receive either a standard dose or a fractional dose of the 17D-213 YF vaccine. The potency for the vaccine was tested at the National Institute for Biological Standards and Control (NIBSC), a biological standardisation agency in the UK and are presented in table 3.4. The potency tests were done with and without pre-incubation at 37ºC. These tests are recommended by the WHO to establish the stability of vaccines and the status of the vaccine infectivity when exposed to high temperatures[158].

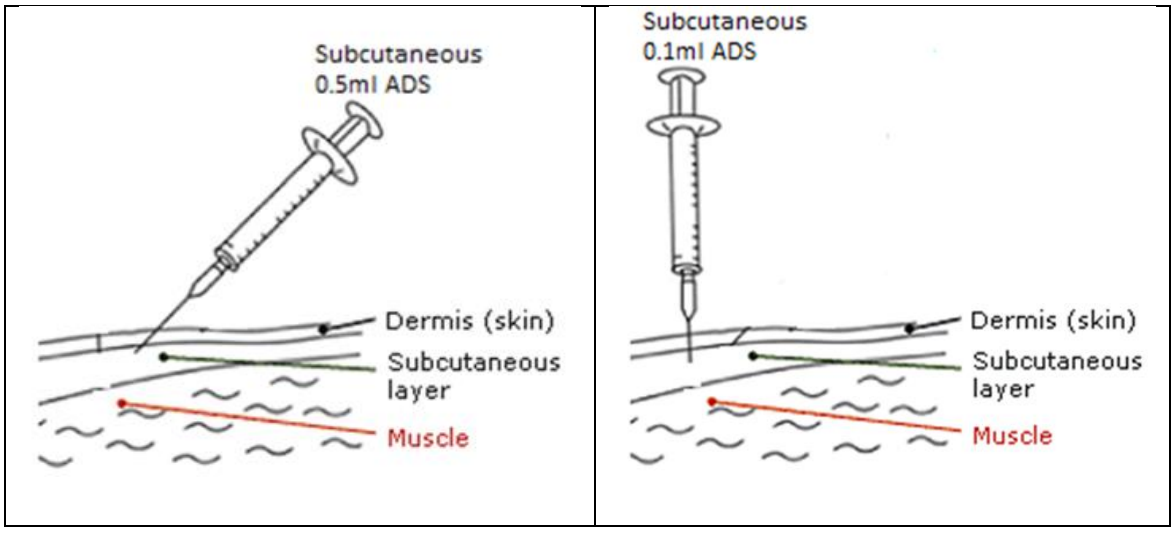
Table 3. 4 shows the potency of the YF vaccine lot 598.

Manufacturer	**Test**	**Assay 1**	**Assay 2**	**Assay 3**	**Mean (SD) (logIU/dose)**	**(IU/dose)**
Vaccine lot 598 FSUE, Russia	Potency test	4.88	4.85	4.77	**4.83 (**0.06)	**~67,608 IU/dose**
	Thermostability test at +37˚C	4.42	4.4	4.34	**4.39 (**0.04)	**~24,547 IU/dose**

The standard dose of the vaccine was shown to have a potency of 4.83 logIU/dose (~67,608IU/dose) and 4.39 logIU/dose (~24,457IU/dose) using the thermostability test at +37°C (Personal communication, NIBSC). The fractional dose (a fifth) of the standard dose corresponds to ~13,521IU/dose and ~4,909 IU/dose respectively.

The randomisation sequence was pre-generated and concealed in a scratch-off booklet by an independent firm (DiagnoSearch LifeSciences, Mumbai, India). Participants and study personnel following safety and immunological outcomes were blinded to the allocations throughout the study. The vaccines were prepared at the start of each vaccination day by the unblinded personnel (the vaccinating nurse and pharmacist). The standard dose was dispensed in a 0.5ml AD (syringe needle size 25G × 3/4") at about 45° angle which is the standard method to achieve a subcutaneous delivery while the fractional dose (1/5th) was dispensed in a 0.1ml AD syringe (needle size 26G × 3/8") at about 90° angle to achieve subcutaneous delivery (figure 3.4)[159 160]. The route of administration was informed by the WHO's research priority areas on the use of fractional doses[7]. The volume in the syringe was masked using opaque tape. Upon vaccination, the participant was observed for up to an hour to assess for any immediate reactions.

Figure 3. 4 illustrating the standard technique for subcutaneous administration of the vaccine (left) and an alternative technique using a smaller needle



Further, 4mls of venous blood samples were collected for serum isolation at day-10 post-vaccination to assess rapidity of immunological protection, at day-28 post-vaccination to assess immunogenicity (primary outcome) and at one-year post-vaccination to assess the longevity of

the antibodies. This schedule is summarised in figure 3.3. Upon collection, the whole blood samples were transported from the trial clinic to the lab within 4 hours and separated by centrifugation to collect serum. The serum samples were stored at -80°C at the KEMRI-Wellcome Trust Research Programme's repository and later shipped to Institute Pasteur de Dakar, Senegal for virus neutralisation assays.

Safety was assessed by recording reactogenicity events as adverse events (AEs) occurring in the first 28 days after vaccination. AEs were solicited at the study-specific visits at vaccination day (day 0), day 10 and day 28 post-vaccination and any unscheduled visits within the first 28 days of the trial. Serious adverse events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) were monitored throughout the study period.

3.2.4. PRNT assay

Immunogenicity was tested using a standardised PRNT assay. This method is labour-intensive and requires highly experienced technicians but remains the gold standard for evaluating immunity using functional (neutralising) antibodies (nAb) in response to YF vaccination [84]. This study used the $PRNT_{50}$ as a primary endpoint and $PRNT_{90}$ as a secondary outcome [135]. I sent the serum samples for this study to the WHO YF reference lab at Institut Pasteur de Dakar, Senegal who have an established PRNT assay[53 161]. The PRNT assay at IPD is performed with a 96-well cell culture plate to quickly test a large number of sera samples and is based on a standardised amount of YF virus (17D-204) that forms about 100 plaques when the virus infects cells in culture. Functional antibodies with the ability to inhibit infection in susceptible cells are added to the standardised virus and bind to the virus to form a virus-antibody complex. The complex is applied to a monolayer, to measure the antibodies that do not have neutralisation capability. While comparing the mixture with a virus only control (without any antibodies), the serum is added to the monolayer culture at varying dilutions and the

correspondence for this is between 50-90%[162]. There is no consensus on the optimal plaque reduction and most studies report 50%, 80% or 90% PRNT titres. Although the $PRNT_{90}$ read-out has more specificity, there is an increased likelihood to report false-negative results in participants who elicit lower virus-specific antibody titres[6 84]. A readout of $PRNT_{50}$ titres was selected for this study since it is at the midpoint of the S-shaped neutralisation curve with suitable sensitivity and specificity. As such $PRNT_{50}$ is the sera dilution capable of reducing cytopathic activity, measured by plaques formed, by 50%. This allows comparability of the data with other clinical trials data [6]. Clinical trials testing immunogenicity of flavivirus vaccines report titres of 1 in 10 as a correlate of protection as measured by $PRNT_{50}$ [163 164]. The step-by-step protocol for this is described in appendix 2. Results were sent to me for analysis (see below).

3.2.5. Statistical considerations

The study was designed with a 90% power to detect non-inferiority of the fractional dose compared to the standard of the 17D-213 YF vaccine. Assumptions were made for the HIV positive sub-study; 83% prevalence of seroconversion (compared to 95% in HIV negative adults), 90% power, one-sided test 2.5% significance (α) and a delta of -0.10 (i.e. -10%). The 10% non-inferiority margin was determined based on HIV positive population constituting a minority within a larger population, and therefore having a smaller overall impact on herd immunity in the population if there is any reduction in immunogenicity[26 165]. The study also allowed for a 20% loss to follow up and for unevaluable participants. The overall sample size was 250 for the sub-study recruiting HIV-infected individuals (see Figure 3.1). These calculations were made for the primary endpoint of non-inferiority. Analyses of all other efficacy and safety endpoints were considered as secondary outcomes and no adjustments were made for multiple comparisons [135].

There were three populations defined for analysis. The Per-Protocol (PP) population was defined as participants who gave a blood sample at baseline and at 28 (± 3) days post-vaccination, who were seronegative ($PRNT_{50}$ < 1:10) to YF virus at baseline, and for whom the eligibility criteria were appropriately applied. Immunogenicity endpoints were assessed for the subset of the PP population that self-reported no prior flavivirus (Dengue, Zika, West Nile virus) infection. The Intent-to-Treat (ITT) population was defined as the group of randomised participants who received a study vaccine and gave at least one post-vaccination blood sample. Immunogenicity endpoints were assessed for the subset of the ITT population who had pre-existing YF neutralising antibodies ($PRNT_{50}$≥1:10) at baseline. Participants who were enrolled based on a previous CD4+ count (within 6 months) and found to have CD4+ <200 cells/mm³ at enrolment would be excluded from PP analysis and included in the ITT population. The Safety population included all participants who were vaccinated with either the full or fractional dose. This population was the focus of surveillance and analysis of any AEs actively in the first month of vaccination and SAEs passively throughout the study. Safety analyses include all the participants in this population regardless of the availability of individual endpoint data. Reporting of the safety-related events was standardised by coding using Medical Dictionary for Regulatory Activities (MedDRA), v20.0.

3.2.6. Study outcomes

As described in the statistical considerations for the study, the primary outcome for this non-inferiority trial was the proportion of participants who seroconvert by day-28 post-vaccination in a pairwise comparison for the fractional dose compared to the standard dose in the PP population. Seroconversion was defined as a ≥4-fold rise in neutralizing antibody titre between pre-immunization and post-vaccination samples as measured by $PRNT_{50}$. Seronegative samples (those whose $PRNT_{50}$ was below the Limit of Quantification (LOQ), <1:10) were

assigned a nominal value of LOQ/2 such that a 4-fold increase for a participant who is <1:10 at baseline corresponds with a titre of 20[6 8 84 135].

Safety outcomes

Safety outcomes were assessed and any untoward event regardless of seriousness was actively monitored and recorded within 30 minutes post-vaccination, at the scheduled study visits (day 2-7, day 10, day 28) and any unscheduled visits within the first month of vaccination. Participants were also advised to report any reactions of concern to the research clinic during the entire duration of the trial. Any serious adverse events (SAEs), defined by the guidelines for Good Clinical Practice of the International Conference on Harmonisation (GCP-ICH) as 'any untoward medical occurrence that either resulted in death; was life-threatening; required in-patient or extended hospitalisation; resulted in persistent or significant disability or incapacity, or a congenital anomaly in participant's offspring, or was a medically important event that may jeopardise the participant or require intervention to avoid one of the above outcomes'[166], were recorded for the entire period of the study.

Each safety event was coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary version 20.0 Systems Organ Classes and Preferred Terms. The events were assessed for the degree of certainty with which it could be attributed to vaccination based on previous reports of similar events after YF vaccination and the temporal association of the event with vaccination. Events were classified as related if there was a reasonable possibility that vaccination contributed to the adverse event. Events that did not follow a reasonable temporal trajectory and there was no suspicion that the events were related to vaccination were classified as unrelated. Any event for which a determination could not be made was considered unclassified. Unexpected SAEs determined to be related to vaccination would be clarified as Suspected Unexpected Serious Adverse Reactions (SUSARs).

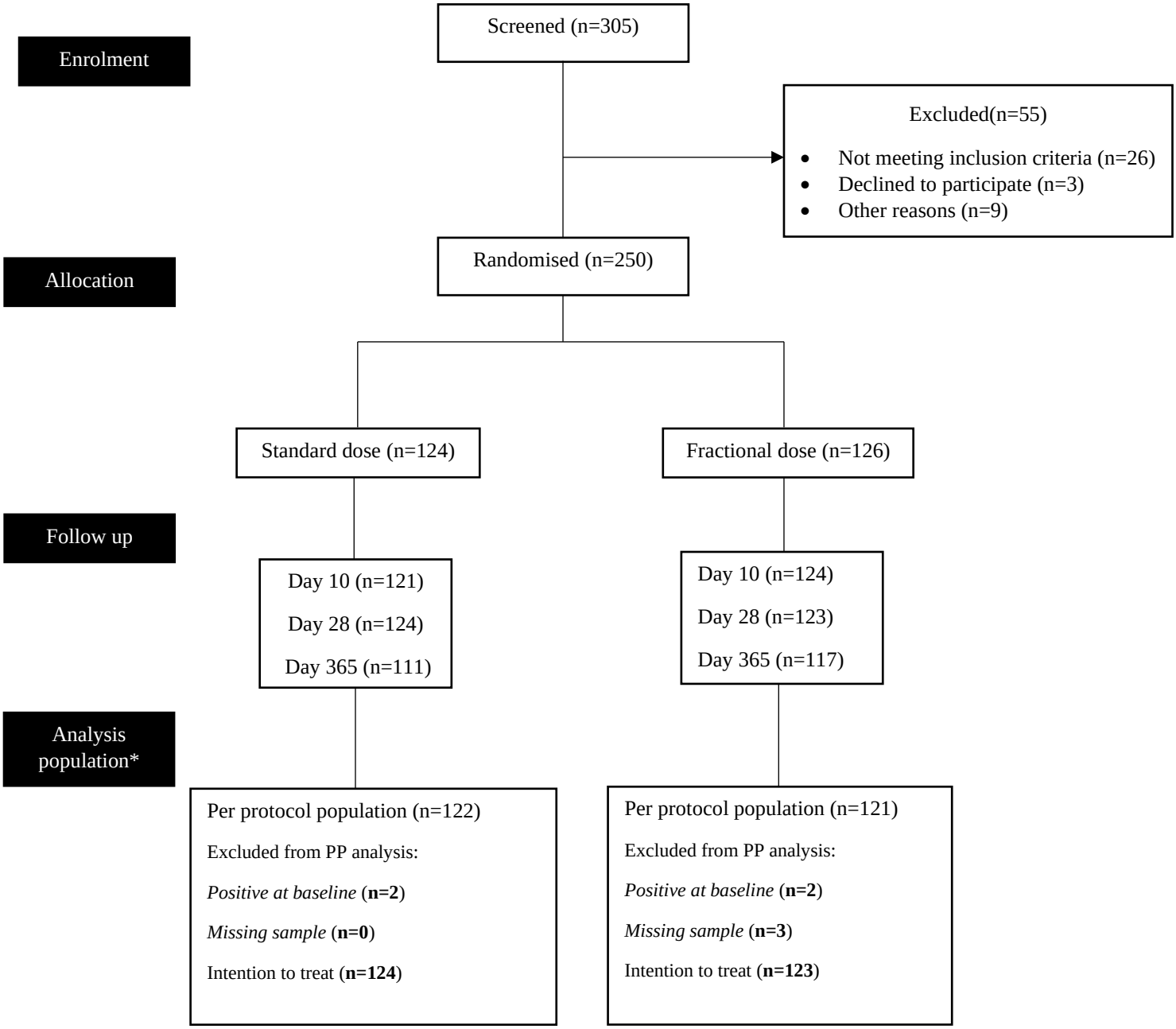
3.3. Results

3.3.1. Recruitment and participants' characteristics

Between January and May 2019, we screened 305 participants, and included, randomised and vaccinated 250 participants in the study. Although we expected an equal distribution of randomised participants per arm (i.e. as shown in figure 3.1), the randomisation algorithm resulted in n=124 participants being allocated the standard dose while n=126 were allocated the fractional dose of the 17D-213 Chumakov vaccine.

The Consort diagram (figure 3.5) illustrates the participants that were followed up for the period of the study. The study had generally low attrition rates with 5 participants missing their day-10 visit and 3 participants missing their day-28 follow up visit. Although the one-year follow up was interrupted by restrictions related to the COVID-19 pandemic, 228 (91.2%) completed their study follow-up; 83 (33.2%) were followed up within their one-year window (±14 days) and 145 (58%) were followed up 3 – 5 months after their scheduled visit due to study site closure following the COVID-19 pandemic. Twenty-two participants (8.8%) were lost to follow up. The most frequent reason for attrition was related to the safety uncertainties due to the COVID-19 pandemic.

Figure 3. 5 Consort flowchart of the recruitment and follow up of the YEFE_HIV sub-study



*Primary end point

Table 3.5 presents the characteristics of the participants by vaccine dose administered. The mean age was 37 years at enrolment and 51.6% of them were male. Four participants had pre-existing antibodies against the YF virus as measured by $PRNT_{50}$; out of these only one participant reported a history of flavivirus infection. All the participants were categorised as stage 1 HIV infection based on the WHO clinical stage classification of HIV[167]. The participants had a CD4+ count mean of 627.3 (SD 269.0). All the participants were on Anti-Retroviral Therapy (ART) and viremia was suppressed with undetectable viral loads for HIV.

Table 3. 5 Baseline demographics and characteristics by vaccine dose

Variable	**Vaccine dose group**		
	Standard dose	**Fractional dose**	**Combined**
Sex	Mean (SD)	Mean (SD)	Mean (SD)
Female	68 (54.84)	53 (42.06)	121 (48.40)
Male	56 (45.16)	73 (57.94)	129 (51.60)
Age	Mean (SD) 37.4 (9.8)	Mean (SD) 36.5 (10.3)	Mean (SD) 37.0 (10.0)
Temperature at recruitment	36.4 (0.6)	36.4 (0.6)	36.4 (0.6)
Residence			
Mombasa	23 (18.55)	26 (20.06)	49 (19.60)
South Coast	11 (8.87)	9 (7.14)	20 (8.00)
Kilifi	5 (4.03)	9 (7.14)	14 (5.60)
Malindi	77 (62.09)	76 (60.31)	153 (61.20)
Other	8 (6.45)	6 (4.76)	14 (5.60)
History of previous flavivirus infection	N (%)	N (%)	N (%)
Yes	1 (0.81)	0 (0.00)	1 (0.40)
No	123 (99.19)	126 (100)	249 (99.60)
Seropositive at baseline	2 (1.61)	2 (1.59)	4 (1.60)
CD4+ cell count	Mean (SD) 635.4 (270.04)	Mean (SD) 619.2 (268.87)	Mean (SD) 627.3 (269.03)
Underlying significant medical history	N (%)	N (%)	N (%)
Yes	24 (19.35)	17 (13.49)	41 (16.40)
No	100 (80.65)	109 (86.51)	209 (83.60)

3.3.2. Vaccine safety

Adverse events

A total of 187 adverse events were reported with 110 participants reporting at least one event, 53 participants two events, 18 participants three events, 5 participants had 4 events reported and one participant reported 5 AEs (Table 3.6). Solicited AEs comprised 56% of the total events reported. The AEs reported were either mild (97.3%) or moderate (2.7%) and were self-limiting. Sixty per cent of the events were not related to the vaccination or participating in the study. There were no major differences in the AEs reported by sex, age or CD4+ count. There were no immediate adverse events reported. Table 3.7 lists all the adverse events reported and figure 3.6 shows the number of solicited adverse events per vaccine dose.

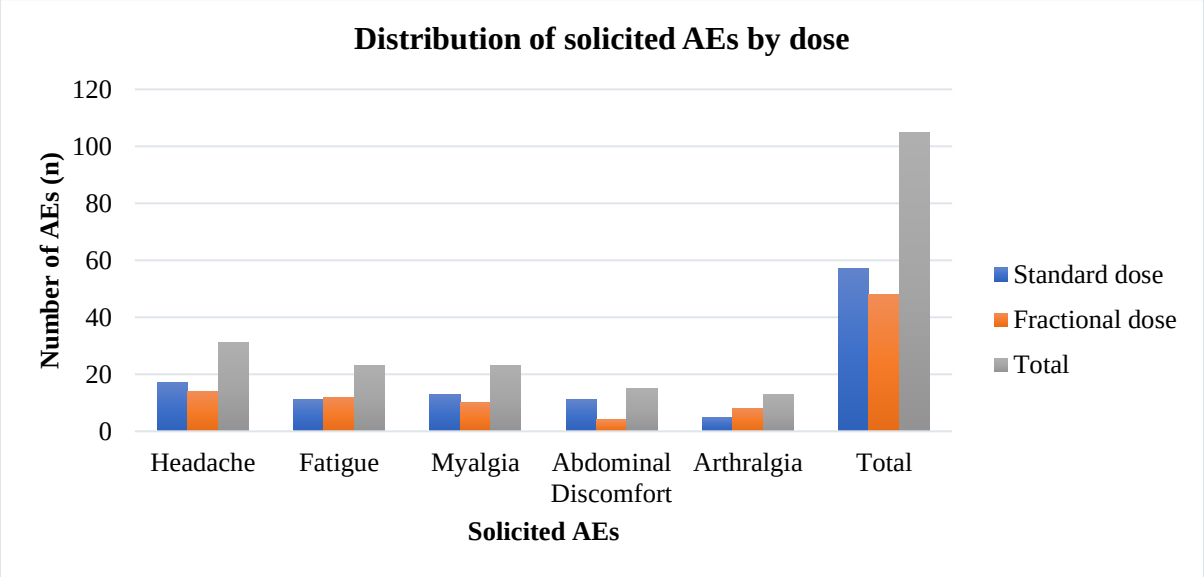
Table 3. 6 Summary of Adverse Events for all vaccinated participants

Label	**Dose group**		
	Standard dose	**Fractional dose**	**Total**
No of AEs reported			
1 AE	61 (55.5)	49 (44.5)	110 (58.8)
2 AEs	31 (58.5)	22 (41.5)	53 (28.3)
3 AEs	10 (55.6)	8 (44.4)	18 (9.6)
4 AEs	2 (40)	3 (60)	5 (2.7)
5 AEs	1 (100)	0 (0)	1 (0.5)
Total	**105 (56.15)**	**82 (43.85)**	**187**
Severity of AEs			
Mild	101 (96.19)	81 (98.78)	182 (97.33)
Moderate	4 (3.81)	1 (1.22)	5 (2.67)
AE relatedness			
Related	47 (44.76))	28 (34.15)	75 (40.11)
Not related	58 (55.24)	54 (65.85)	112 (59.89)
Sex			
Female	61 (58.10)	39 (47.56)	100 (53.48)
Male	44 (41.90)	43 (52.44)	87 (46.52)

Table 3. 7 Adverse events reported by preferred term

Adverse event preferred term	Study Group		
	Standard dose	**Fractional dose**	**Total**
Headache	17	14	31
Fatigue	11	12	23
Myalgia	13	10	23
Abdominal Discomfort	11	4	15
Cough	9	5	14
Arthralgia	5	8	13
Rhinorrhoea	6	4	10
Dizziness	7	2	9
Pyrexia	4	4	8
Gastroenteritis	3	1	4
Nausea	1	3	4
Diarrhoea	2	1	3
Skin infection	1	2	3
Tonsillitis	1	2	3
Dermatitis	1	1	2
Fungal Skin Infection	1	1	2
Injury	2	0	2
Lower Respiratory Tract infection	1	1	2
Urinary Tract Infection	1	1	2
Vaccination Site Discomfort	2	0	2
Anorectal Disorder	0	1	1
Asthma	0	1	1
Dysentery	1	0	1
Dysfunctional Uterine Bleeding	1	0	1
Ear Pain	1	0	1
External Ear Disorder	0	1	1
Eye Disorder	0	1	1
Furuncle	0	1	1
Lower Urinary Tract infection	1	0	1
Skin Irritation	1	0	1
Skin Lesion	0	1	1
Stomatitis	0	1	1
Vaginal Candidiasis	1	0	1
Wound Sepsis	1	0	1
Total	**105**	**82**	**187**

Figure 3. 6 Graph showing the number of solicited events reported



Serious adverse events

There were seven serious adverse events reported throughout the trial, none of which were related to vaccination or participation in the vaccine trial. Table 3.8 summarises the details of the SAEs. The SAEs were reported to the ethical and regulatory bodies.

Table 3. 8 Serious adverse events reported

Participant ID	Age	Dose group	SAE description	Outcome
3063	28	Standard dose	Acute kidney injury secondary to tenofovir nephropathy	Recovered without sequelae
3064	30	Standard dose	Sub-Dural haematoma	Fatal or died
3097	47	Standard dose	Tibia fracture	Recovered with sequelae
3179	45	Standard dose	Death	Fatal or died
3184	24	Fractional dose	Burn infection	Recovered with sequelae
3207	24	Fractional dose	Trauma	Recovered without sequelae
3247	30	Standard dose	Sudden death, cause unknown	Fatal or died

3.3.3. Immunogenicity results

Antibody titres

Table 3.9 presents the immunological results of the per-protocol population analysis.

There were high seroconversion rates ((84.9% and 74.6%), (95.9% and 98.4%) and (97.4% and 98.2%)) and high antibody titres with GMTs of 94.2 and 50.8, 1634.2 and 1460.3 and 1164.6 and 905.1 for the standard dose and the fractional dose at day 10, day 28 and day 365, respectively. The geometric mean fold increases (GMFI) were 18.8 and 10.2, 326.8 and 292.1 and 232.9 and 181 at day 10, day 28 and day 365 for the standard and fractional doses, respectively. The geometric mean ratio comparing the standard dose and the fractional dose was 0.53, 0.89 and 0.78 at day10, day 28 and day 365 respectively. The corresponding seroconversion rates, GMT and GMFI as measured by $PRNT_{90}$ for the per-protocol population were expectedly lower for the standard dose and the fractional doses.

The ITT population analysis data, which included all vaccinated participants that had a post-vaccination sample regardless of whether they were YF seropositive at baseline or not, are included in table 3.10. The seroconversion rates, the GMT and GMF using $PRNT_{50}$ titres and the $PRNT_{90}$ titres of the ITT population were comparable with those in the PP population.

Table 3. 9 Immunological outcomes for the per-protocol population

Dose Level	Per-protocol Analysis			
	Seroconversion rate	**GMT**	**GMFI**	**GM ratio**
Immunological parameters using $PRNT_{50}$				
Day 10 ($PRNT_{50}$)				
Standard dose (n=119)	84.9% (77.2 – 90.8%)	94.2 (68.6 – 129.0)	18.8 (13.8 – 25.8)	0.53 (0.35 – 0.83)
Fractional dose (n=122)	74.6% (65.9 – 82.0%)	50.8 (37.5 – 68.8)	10.2 (7.5 – 13. 8)	
Day 28 ($PRNT_{50}$)				
Standard dose (n=122)	98.4% (94.2 – 99.8%)	1634.2 (1184.5 – 2254.6)	326.8 (236.9 – 450.9)	0.89 (0.55 – 1.45)
Fractional dose (n=121)	95.9% (90.6 – 98.6%)	1460.3 (1014.9 – 2101.1)	292.1 (203.0 – 430.2)	
Day 365 ($PRNT_{50}$)				
Standard dose (n=110)	98.2% (93.5 – 99.8%)	1164.6 (851.9 – 1592.0)	232.9 (170.4 – 318.4)	0.78 (0.49 – 1.23)
Fractional dose (n=116)	97.4% (93.6 – 99.5%)	905.1 (643.3 – 1273.4)	181.0 (128.7 – 254.7)	
Immunological parameters using $PRNT_{90}$				
Day 10 ($PRNT_{90}$)				
Standard dose (n=119)	28.6% (20.7 – 37.6%)	9.9 (8.5 – 11.6)	2.0 (1.7 - 2.3)	0.79 (0.65 – 0.95)
Fractional dose (n=122)	17.2% (11.0 – 25.1%)	7.9 (7.0 – 8.9)	1.6 (1.4 – 1.8)	
Day 28 ($PRNT_{90}$)				
Standard dose (n=122)	82.0% (74.0 – 88.3%)	69.8 (51.7 – 94.3)	14.0 (10.3 – 18.9)	0.97 (0.64 – 1.48)
Fractional dose (n=121)	80.2% (71.9 – 86.9%)	68.1 (50.9 – 91.3)	13.6 (10.1 – 18.3)	
Day 365 ($PRNT_{90}$)				
Standard dose (n=110)	98.2% (93.5 – 99.8%)	46.2 (37.3 – 57.3)	9.2 (7.5 – 11.5)	0.95 (0.67 – 1.32)
Fractional dose (n=116)	97.4% (94.9 – 99.3%)	43.8 (33.9 – 56.4)	8.8 (6.8 – 11.3)	

The per-protocol population excluded participants who had antibodies titre ≥10 at baseline as measured by $PRNT_{50}$ (n = 4) and did not have a post-vaccination sample at study-specific timepoint. The per-protocol analysis included 241, 243 and 226 at day 10, day 28, and day 365 respectively.

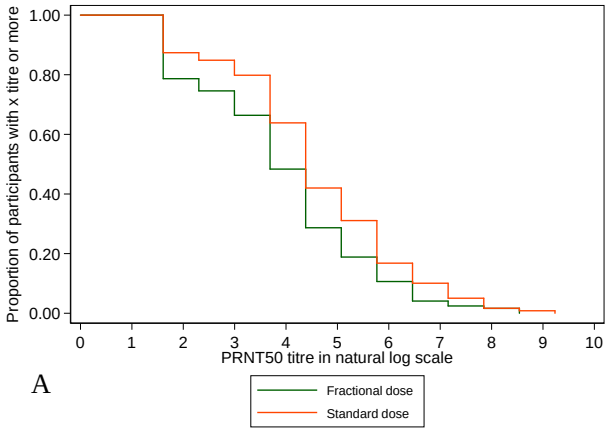
Table 3. 10 Immunological outcomes for the intention-to-treat population

Dose Level	Intention-to-Treat Analysis			
	Seroconversion rate	**GMT**	**GMFI**	**GM ratio**
Immunological parameters using $PRNT_{50}$				
Day 10 ($PRNT_{50}$)				
Standard dose (n=121)	85.1% (77.5 – 90.9%)	98.3 (71.6 – 135.0)	18.3 (13.3 – 25.0)	0.54 (0.35 – 0.83)
Fractional dose (n=124)	75.0% (66.4 – 82.3%)	51.2 (37.9 – 69.0)	9.8 (7.3 – 13.3)	
Day 28 ($PRNT_{50}$)				
Standard dose (n=124)	98.4% (94.3 – 99.8%)	1609.7 (1172.1 – 2210.7)	299.3 (212.3 – 422.2)	0.94 (0.57 – 1.54)
Fractional dose (n=123)	95.9% (90.8 – 98.7%)	1465.4 (1024.4 – 2096.1)	281.7 (196.3 – 404.4)	
Day 365 ($PRNT_{50}$)				
Standard dose (n=111)	98.2% (93.6 – 99.8%)	1165.5 (855.1 – 1588.8)	219.0 (157.0 – 305.5)	0.83 (0.52 – 1.33)
Fractional dose (n=117)	97.4% (93.7 – 99.5%)	924.1 (657.1 – 1299.5)	181.6 (129.4 – 254.7)	
Immunological parameters using $PRNT_{90}$				
Day 10 ($PRNT_{90}$)				
Standard dose (n=121)	16.9% (10.8 – 24.7%)	10.4 (8.8 - 12.3)	2.0 (1.8 – 2.4)	0.77 (0.63 – 0.93)
Fractional dose (n=124)	29.8% (21.8 – 28.7%)	7.9 (7.0 – 8.8)	1.6 (1.4 – 1.8)	
Day 28 ($PRNT_{90}$)				
Standard dose (n=124)	82.3% (74.4 – 88.5%)	70.0 (52.0 – 94.0)	13.8 (10.2 – 18.5)	0.99 (0.66 – 1.50)
Fractional dose (n=123)	80.5% (72.4 – 87.1%)	68.7 (51.5 – 91.7)	13.7 (10.3 – 18.3)	
Day 365 ($PRNT_{90}$)				
Standard dose (n=111)	82.9% (74.6 – 89.4%)	46.5 (37.6 – 57.4)	9.1 (7.4 – 11.3)	0.98 (0.70 – 1.36)
Fractional dose (n=117)	79.5% (71.0 – 86.4%)	44.5 (34.5 – 57.4)	8.9 (6.9 – 11.5)	

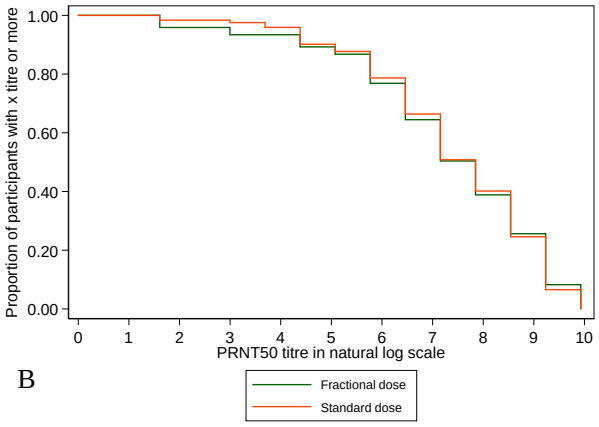
The intention-to-treat population included all vaccinated participants with a post-vaccination sample and included 245, 247 and 228 at day 10, day 28, and day 365 respectively.

Figure 3.7 illustrates the reverse cumulative distribution of the proportion of the participants with log-transformed titres for the $PRNT_{50}$ at day 10 (A), day 28 (B) and day 365 (C). The trend of fractional doses titres was consistently lower relative to the standard dose titres. The difference in the trends of the distribution by vaccine dose at day 10 was substantial using the chi-squared (χ^2) test (p-value = 0.01) while there were no statistically significant differences at day 28 and day 365 (p-values of 0.94 and 0.57 respectively).

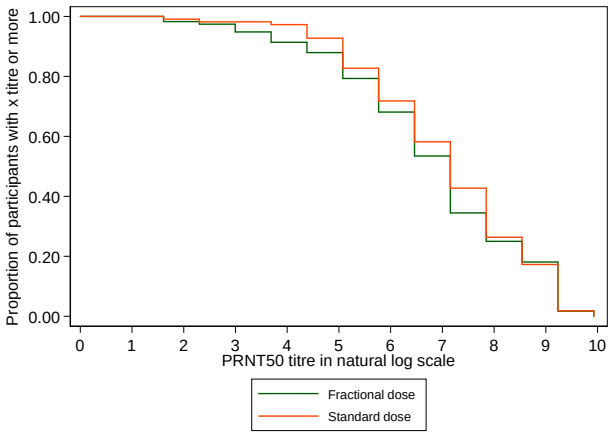
Figure 3. 7 the reverse cumulative distribution plots of titres at day 10, day 28 and day 365 post-vaccination by $PRNT_{50}$ the PP population.



A



B



C

A: Day 10 (p-value = 0.01)

B: Day 28 (p-value = 0.94)

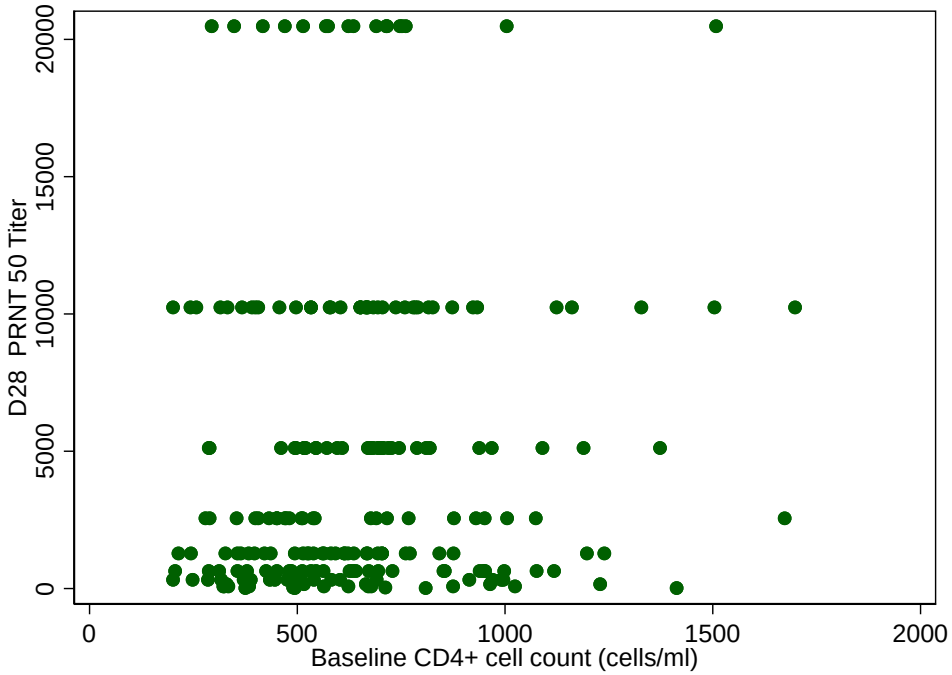
C: Day 365 (p-value = 0.57)

CD4+ count and immunogenicity

All the participants had CD4+ levels >200cells/ml with 85 participants (34%) <500 cells/ml, 107 participants (43%) >500 ≤750, 35 participants (15%) >750 ≤ 1000 and 21 participants >1000 cells/ml. The median CD4+ count was 598.5 cell/ml (201 – 1698 cells/ml).

Of all the vaccinated participants who were sampled on day 28, 240 participants tested positive and therefore had an antibody titre. I set a null hypothesis; 'there is no correlation between the levels of baseline CD4+ cell count and the $PRNT_{50}$ titre at day 28'. Figure 3.8, a scatter plot of the CD4+ count and the $PRNT_{50}$ titre at day 28, did not show any visually obvious relationship between the CD4+ count and the antibody titres.

Figure 3. 8 of a scatter plot of CD4+ count and the $PRNT_{50}$ titre at day 28



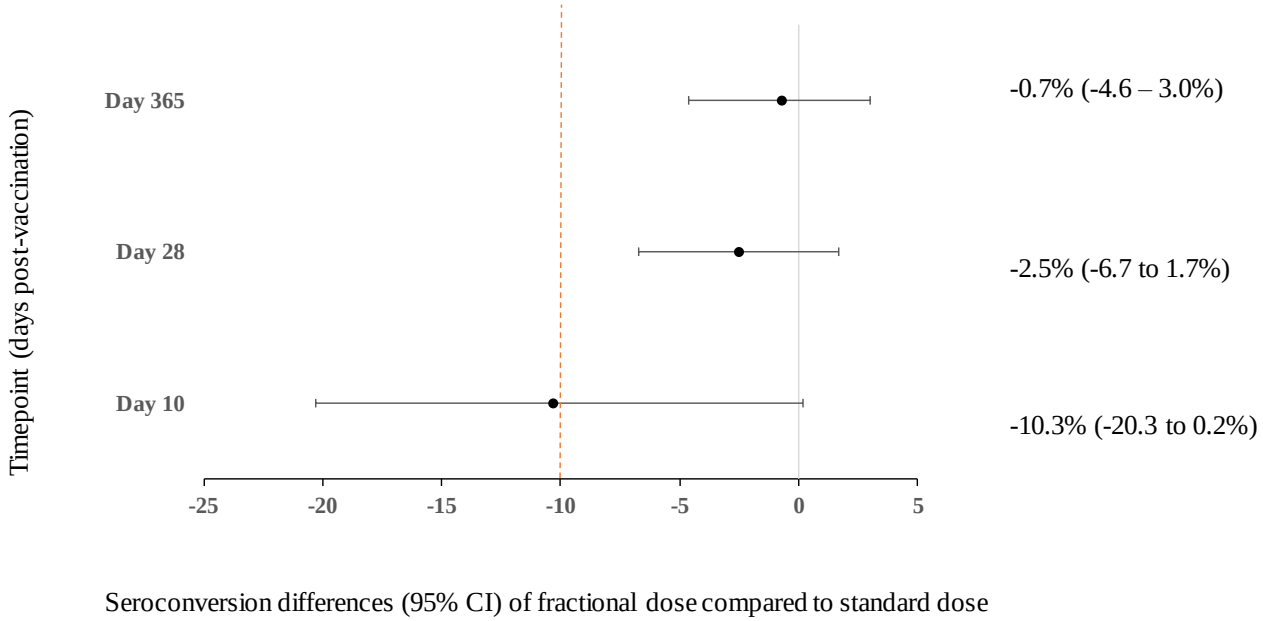
Since the antibody titres are in rank-order, I used Spearman's rank-order correlation test (Rho), to test for the null hypothesis. While statistically significant (p-value = 0.01), the correlation

between the CD4+count and the antibody titre although positive was weak (Spearman's rho = 0.16).

Non-inferiority assessment

The differences in the seroconversion rates for the fractional dose compared to the standard dose using $PRNT_{50}$ titres were -10.3% (95%CI -20.3 to 0.2%) at day 10, -2.5% (95% CI -6.7 to 1.7%) at day 28 (primary outcome) and -0.7% (95%CI -4.6 – 3.0%) at day 365 post-vaccination (see figure 3.9). Non-inferiority, defined as -10%, was met at day 28 post-vaccination and maintained beyond one-year post-vaccination. The seroconversion rate difference at day 10 was <-10% (-10.3%) and a lower confidence interval of -20.3% and as such, failed to demonstrate non-inferiority of the fractional dose to standard dose for this secondary outcome.

Figure 3. 9 Seroconversion differences, confidence intervals and the non-inferiority margin for the PP population ($PRNT_{50}$). The two vertical lines indicate non-inferiority margin (interrupted) and zero (block).



3.4. Discussion

To the best of my knowledge, this chapter presents the results of the first randomised controlled non-inferiority trial assessing fractional doses and standard doses of YF vaccines in a population of HIV-infected individuals in sub-Saharan Africa. This study affirms that fractional doses can be used in HIV-infected individuals using the current WHO's recommendations during epidemics. In this study, I was able to show that $1/5^{th}$ of the standard dose of the 17D-213 YF vaccine manufactured by the Chumakov Institute of Poliomyelitis and Viral Encephalitidis in Russia, is safe and immunogenically non-inferior to the standard dose in HIV-infected individuals at 28 (±3) days post-vaccination. These results are consistent with the evidence from the preceding study in HIV-uninfected individuals [8]. There was good compliance to the protocol among the trial participants with an attrition rate of 8.8% at one-year post-vaccination. The baseline positivity for YF as measured by $PRNT_{50}$ was 1.6%. YF virus has not been reported on the coast of Kenya and the positivity could be due to participants who had been previously vaccinated or cross-reactivity by other flaviviruses circulating in the coast of Kenya such as the Dengue virus[146 147]. There were local and systemic AEs reported in both vaccine dose groups with participants in the fractional doses group reporting fewer events. There were no safety concerns since all the AEs reported were either mild or moderate in severity and resolved spontaneously without sequelae and none of the SAEs reported was related to the vaccine administration or participation in the study. The safety profile reported was consistent with previous studies on HIV-infected and HIV-uninfected individuals [101 104 130 151]. There have been safety concerns in individuals with very low CD4+ T-cell counts (<100cells/ml) due to reports of fatal cases of YF encephalitis post-vaccination and increased risks of developing YEL-AND and YEL-AVD has been documented in this sub-group [168 169].

A single dose of the fractional dose (one-fifth of the standard dose) induced robust immunity with high neutralising antibody titres compared with the standard dose. Although the GMTs

reported here were comparable for both study arms with overlapping confidence intervals, the standard dose induced higher GMTs across all study-specific time points compared to the fractional doses. These results were consistent with the preceding study on HIV-uninfected individuals [8]. As expected, and reported in previous studies, the GMTs reported in the HIV+ population were much lower than in the HIV-negative population[140 170]. While more studies are needed to better understand this immune response, it may be an early indication of the need for a booster vaccine for long-term immunity. The WHO recently recommended that only one dose of the YF vaccine is required for life-long immunity, but this recommendation may not generalize to all groups. There were significantly lower GMTs reported at day-10 post-vaccination compared to the other time points. The GMTs peaked at day-28 and were maintained at day-365. The primary outcome, a pairwise comparison of the seroconversion rate at baseline and 28 days post-vaccination, as measured by $PRNT_{50}$ illustrated high seroconversion rates in all vaccinated participants that were maintained at one-year post-vaccination. According to WHO's International Health Regulations (IHR's) guidance, vaccinees are considered protected at 10 days post-vaccination. In this study, the rapidity of protection was measured at 10 days post-vaccination. 74.6% of the participants in the fractional dose group seroconverted at day-10 post-vaccination compared to 84.9% in the standard-dose group. Non-inferiority of seroconversion was demonstrated at 28-days post-vaccination and maintained at day 365 while fractional doses failed to demonstrate non-inferiority of rapidity in seroconversion. Although there are concerns for low seroconversion rates at day-10 in the context of reactive vaccination campaigns during an outbreak, fractional doses have previously been used successfully to control outbreaks [127].

This evidence on longevity is consistent with data from previous studies in HIV-positive and HIV-negative individuals [8 100 155 171]. It is essential to follow up with the participants from this study for longer to establish how long the immune protection persists. The current evidence

certainly allows for use of fractional doses for a limited period with the possibility of a booster dose if the protection is short-lived. However, if the long-term protection of fractional doses is found to be comparable to that of the standard doses as described by Gotuzzo et al., they can be recommended for wider use in HIV-infected and uninfected individuals such as for routine immunisation strategies, including vaccination for travel [74].

There has been conflicting evidence on the role of CD4+ T-cells and HIV viral load on immunogenicity post-vaccination with the majority of the studies reporting improved responses to YF in individuals with suppressed HIV RNA viremia[100] and high CD4+ T-cell, while others argue that the CD4+ levels do not determine immunogenicity [150]. Although there was a weak positive correlation association reported between immunogenicity and the CD4+ cell count in this study, all the participants had CD4+ cell count ≥200 cells/ml and a majority had undetectable levels of HIV viremia. Additionally, CD4+ levels >200 cells per ml have been shown to have robust immunological responses comparable to HIV-negative individuals[140 149]. Since all participants with HIV viremia data were below detectable levels, I could not assess the correlation between the viral load and immunogenicity. There is high coverage of ART in HIV-infected individuals globally [142]. However, some resource-limited settings still experience delayed and suboptimal testing for HIV and are late in getting on treatment with ART[172 173]. As such, it is important to ensure that all the HIV-infected vaccinees have been on ARTs and have been adherent and compliant with their medication on instances where their CD4+ cells and viral loads cannot be determined such as during reactive campaigns [174]. The study also was able to demonstrate the longevity of antibodies 12 months to 17 months post-vaccination.

Although the evidence generated on the use of fractional doses is largely positive, there needs to be a re-definition of optimal fractional doses. In the current guidance by WHO, fractional doses have been defined as one-fifth of the standard dose as released by the vaccine manufacturer [124]. This definition is however difficult to generalise since the vaccine

manufacturers release the YF vaccine at varying potencies. The fractional doses as defined consequently may vary significantly. In the preceding study, for instance, the vaccine manufacturers released the vaccines with potencies of 6 761 IU/dose for the Institut Pasteur de Dakar, 16 596 IU/dose for the Sanofi Pasteur, 38 905, for the Bio-Manguinhos-Fiocruz and 43 652 IU/dose for the Chumakov vaccines whose corresponding fractional doses were 1 352 IU/dose, 3 319 IU/dose, 7 781 IU/dose and 8 730 IU/dose respectively[8]. A fractional dose of the Chumakov vaccine and the Bio-Manguinhos vaccine as used in the study was higher than a standard dose of the Institut Pasteur de Dakar. This compounds the difficulties in generalising the evidence from pragmatic fractions of the vaccines. There is a need to establish the actual lower doses that are immunogenic. Chapter 4 discusses a study assessing lower doses (1 000, 500IU and 250 IU against a standard dose).

3.5. Study limitations

This study is limited by the fact that it did not assess individuals with CD4+ T-cells <200cell/mm^3. In epidemic settings where fractional doses are used, HIV-infected persons who are severely immunocompromised may not get the optimal benefit of vaccination either by fractional doses or standard doses. The results from this trial cannot, therefore, make strong conclusions on the safety and immunogenicity of fractional doses (or standard doses) in this sub-population. The study was limited to about 12 – 17 months of follow up for longevity. The results I have reported here only describe humoral immunity describing functional antibodies. Cellular immunology can be used to characterise immunological protection beyond the quantities and function of antibodies [175].

3.6. Conclusion

Fractional doses of the 17D-213 YF vaccine manufactured by Chumakov are safe and efficacious with good tolerability and immunologically non-inferior to the standard doses in HIV-infected individuals with CD4+ cells ≥200 cells/mm^3 and undetectable HIV viremia in plasma. The evidence presented here is consistent with the evidence in HIV-uninfected individuals where the fractional doses are not any worse than the standard doses. There is therefore a need for policymakers to rethink the definition and the use of fractional doses beyond epidemic settings to increase global vaccine supply. Further, there was a study conducted in Mbarara, Uganda using the same vaccine batch in children ≥9 to <60 months of age without HIV-infection[135]. Based on the results from this study, it would be important to further determine the use of fractional doses in children who are infected with HIV to be confident when making policies to recommend the wider use of fractional doses in all affected populations.

4. Non-Inferiority Fractional-doses Trial for Yellow fever vaccine using reduced doses in Kenya and Uganda (NIFTY) – immunogenicity and safety.

4.1. Introduction

In chapter three, I discuss the safety and immunogenicity of fractional doses of the four WHO-prequalified YF vaccines in two different populations, i.e., HIV negative and HIV positive populations. I show that vaccination with 1/5th of the standard dose (where the standard dose was defined as that supplied by the vaccine manufacturer) provides sufficient immunity which is comparable to that of the standard dose of the manufacturers' minimum release potencies, with no significant differences in safety and immunogenicity between the vaccines produced from different manufacturers nor between HIV-negative and HIV-positive populations[8]. This evidence is consistent with evidence from a limited number of studies[107 109 130]. However, the fractional dose potencies varied between vaccine manufacturers[8]. Evidence from these studies supports the WHO recommendation to use 1/5th of the current standard dose of vaccine for outbreak control. However, many vials still contain excess YF vaccine such that 1/5th of a vial is still likely to be substantially above the current minimum potency requirements of 1000 IU/dose [6 136]. Hence the data in chapter three answers the pragmatic question of whether healthcare providers can effectively use 1/5th of the doses as currently supplied by vaccine manufacturers, but the data do not allow us to re-appraise this minimum dose requirement.

There are uncertainties regarding the minimum dose recommended by the WHO. The vaccine dose-response studies for which the current recommendations are based used older formulations and there is uncertainty on how PFU or LD_{50} in that study relate to the current standards (IU/dose). In collaborative research across national and international control labs, a correlation between the PFU and LD_{50} using Vero cells was established with a corrective

factor of about 0.3 in the early 2000s. This corrective factor may however not be valuable in comparisons between labs as there exist differences in conversion[176 177]. The minimum potency and dose recommendation was established based on experience with lots that varied in titre and non-standardised measurements[178-180]. Currently, the potency is measured in IU, a unit of potency for the YF vaccine, based on the infectivity of the attenuated virus preparation as defined by the formation in a suitable tissue culture monolayer in parallel with an accepted working standard, calibrated in IU against the International Standard for yellow fever vaccine [179].

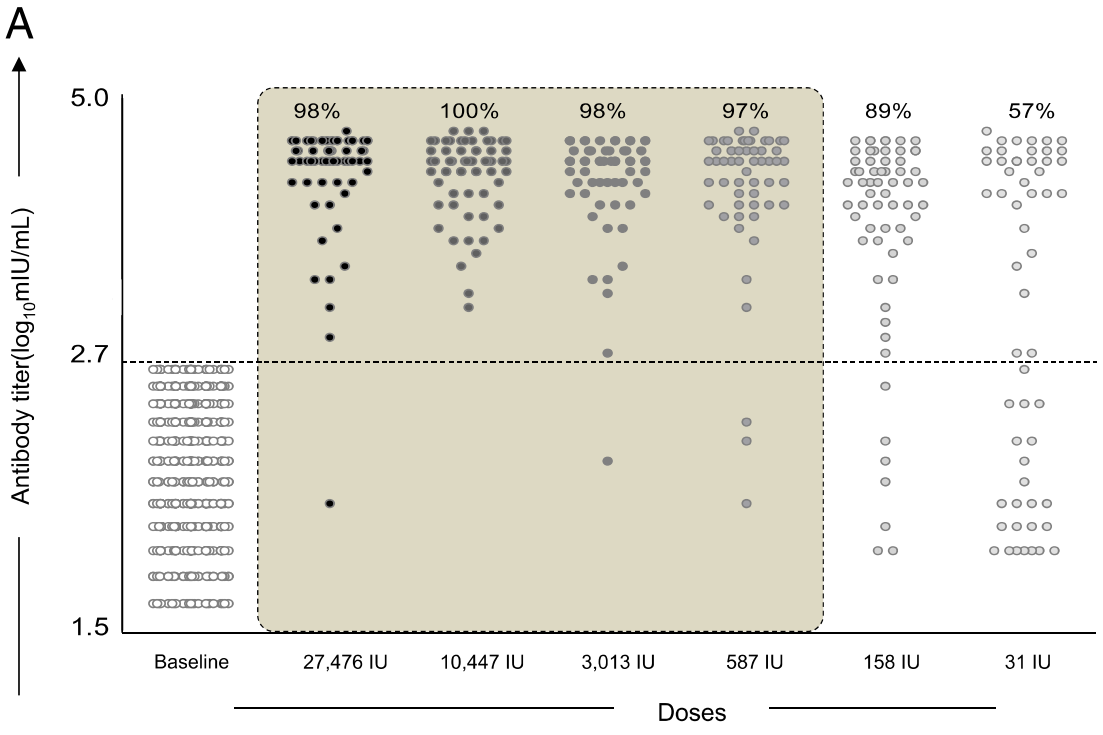
In 1988, a study by Lopes et al. tested different dilutions of different product lots in healthy military males. The study defined immunisation as the development of specific neutralising antibodies to a titre of at least 1:32 within 28 days of vaccination using plaque reduction neutralisation tests[129]. The different lots tested were produced according to WHO technical guidance [181] and Lopes' study showed that there was no difference in immunogenicity and safety between lots indicating that production was consistent. Volunteers vaccinated with titres above 200 plaque-forming units (PFU) induced seroconversion in 100% of participants and titres 100-200 PFUs induced conversion of 94% of participants and a lower percentage with lower viral doses [129]. Converting potency from PFU or LD_{50} to current standards is difficult [179]. Further, the sample size of volunteers vaccinated with each dose was not evenly distributed and relatively small for some doses and was not powered to detect a statistically significant difference.

In 2003, a potency of $10^{4.5}$ IU per ampoule (equivalent to $4.2\log_{10}$ per 0.5 ml) was established as the first international standard for yellow fever vaccine[177]. Five years later, WHO amended the requirements for yellow fever vaccine so that the vaccine potency could be expressed in IU per dose, and the dose recommended for use in humans should not be less than 3.0 $\log_{10}$ IU. However, there is no guidance on an upper limit specification and the final dose usually exceeds

the 3.0 log_{10} IU minimum specification substantially to account for potential potency losses during manufacture and the three years vaccine shelf-life[179].

A phase IV, dose-finding trial was previously carried out in Brazil in response to vaccine shortage during the 2008-2009 YF epidemic[182]. In this trial, the YF vaccine was administered to 900 young healthy adult males in descending doses, ranging from 27,476 IU/dose to 31 IU/dose. A dose of 587 IU resulted in seroconversion in 97.7% (95% CI 92.4 - 99.2%) of the participants and a dose of 158 IU/dose was able to elicit protection in a high number of vaccinees (88.5%; 95%CI 81.5-93.6), Figure 4.1, and these responses were sustained over a period of eight years [128 183].

Figure 4. 1 shows that a dose of 587IU was as immunogenic as a 27,476IU/dose [128]



While this evidence underpins WHO recommendations of using fractional doses of YF vaccine to control epidemics, no published data define the minimum dose (in IU/dose) that is non-inferior to the standard dose among populations living in YF endemic regions of Africa [184 185].

A major limitation of the trial was that it was not formally analysed for non-inferiority and may not have been powered sufficiently to detect differences in seroconversion per dose. The study was also performed in healthy young adult volunteers who were male military recruits and not a diverse population living in YF endemic regions. [5 128 183]. Data from diverse populations will provide more confidence in the current recommendations on the use of fractional doses of the YF vaccine during epidemics when there is a vaccine shortage.

I, therefore, set out to determine the lowest dose (1000, 500 or 250 IU/dose) of the 17D-204 YF vaccine manufactured by the Institut Pasteur de Dakar vaccine that would be non-inferior to a standard dose (~13803IU/dose) measured by seroconversion using the $PRNT_{50}$ assay at 28 days post-vaccination among unvaccinated adults in Kilifi, Kenya and Mbarara, Uganda. The dose of 1000IU/dose is based on the current WHO minimum vaccine potency. The 500IU/dose was informed by the Brazilian dose-response study and a 250IU/dose was selected as substantially lower than the WHO's minimum recommendation. I hypothesised that the rate of seroconversion among vaccinees receiving 1000, 500 or 250 IU/dose of vaccine will be lower than that in vaccinees receiving the standard dose by >10% as measured by plaque reduction neutralisation antibody test 28 days post-vaccination. In designing non-inferiority studies the power of the study is established based on the uncertainties that surround the retention of the null hypothesis as opposed to rejection the null in superiority studies.

The data from this study will be scientifically explanatory regarding the minimum dose required for further practice. Such data have the potential to influence the WHO recommendation for the minimum release dose specifications and possibly lead to the revision of the minimum dose and potency requirements of the 17D YF vaccine. Additionally, such data will provide further confidence in the current recommendations on the use of fractional doses of the YF vaccine during epidemics when there is a vaccine shortage.

4.2. Methods

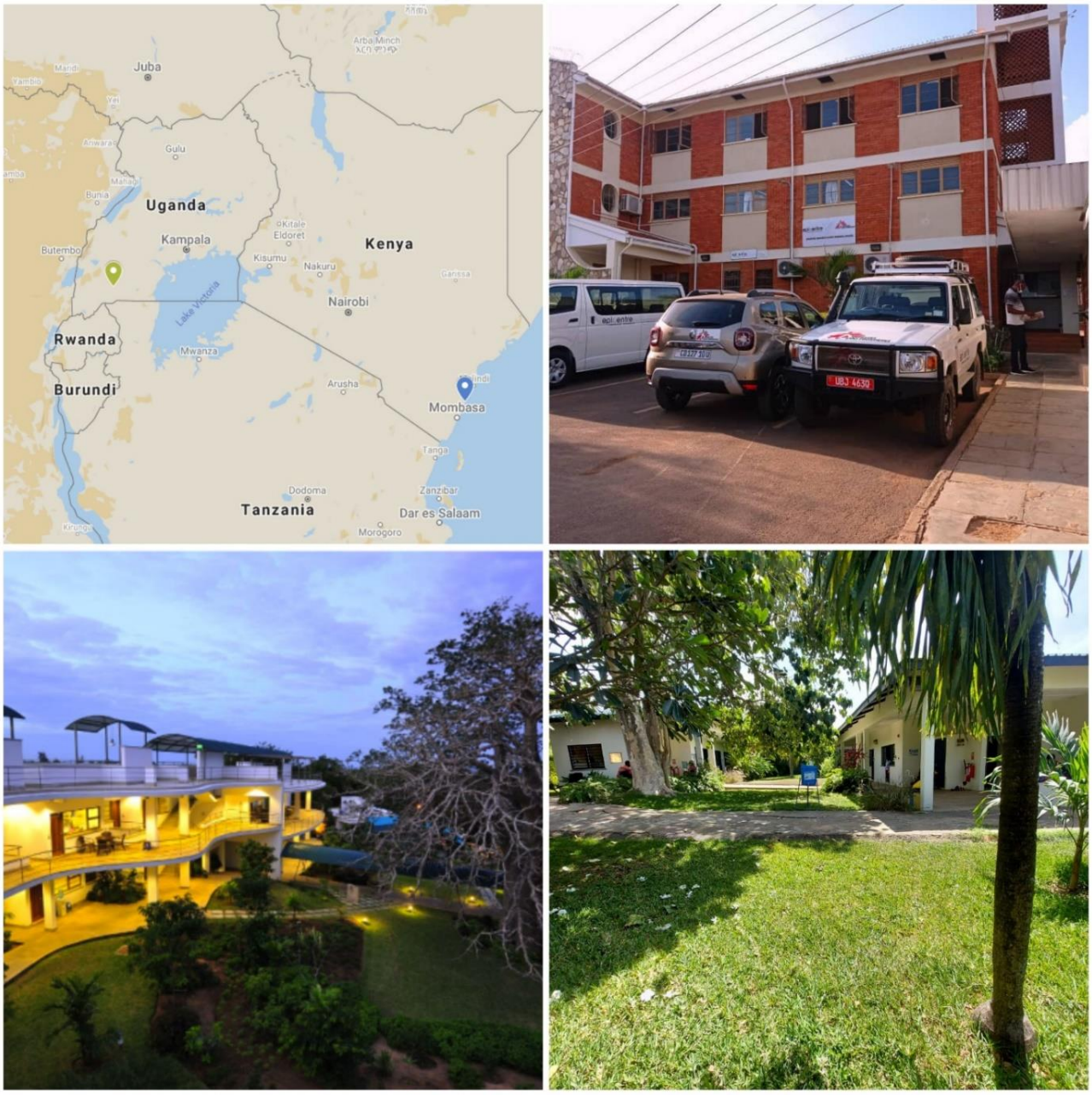
4.2.1. Study design and participants

I led the design and implementation of a phase IV randomised, double-blinded, controlled, two-centre non-inferiority trial, 'Non-Inferiority Fractional-doses Trial for Yellow fever vaccine (termed NIFTY)'. The trial was conducted at the KEMRI-Wellcome Trust Research Programme's Clinical Trials Facility in Kilifi county, Kenya and the Epicentre research centre in Mbarara district, Uganda. Kilifi is approximately 60 kilometres northeast of Mombasa. Mbarara is a district approximately 270 kilometres southwest of Kampala. The town of Mbarara is 125 kilometres west of Masaka – a town where cases of YF were reported in 2016[186] (see figure 4.2). These trial sites were part of the NIFTY consortium by the University of Oxford, the KEMRI-Wellcome Trust Research Programme, Epicentre, the Uganda Virus Research Institute and the Institut Paster de Dakar who provided vaccines for testing[135].

Prior to the recruitment of study participants, the general community members were informed of this trial using locally adapted community engagement strategies. These strategies involved community member meetings to discuss the study design, the rationale of conducting fractional doses studies, expectations for participation and the possible impact of the data generated from the study. Participants were recruited if they were between the ages of 18 – 60 years, either HIV negative on a standard serological screening test or HIV-positive and on treatment but without symptoms suggestive of clinical immunosuppression and a CD4 count greater than 200 and had no contraindication for vaccination according to the WHO guidelines[78 149]; were not pregnant or lactating, had no history of infection or vaccination with YF vaccine and were able to comply with the study procedures [135]. The recruited study participants were then randomised in parallel groups for vaccination with full standard dose (~13803 IU) or with either 1000, 500

or 250 IU (i.e. 4 arms) with a 1:1:1:1 allocation ratio (see Table 4.1). Computer-generated random numbers with fixed blocks were prepared by an independent firm (DiagnoSearch LifeSciences, Mumbai, India) allocating to a dose in equal allocations.

Figure 4. 2 Map of clinical research sites



Top left: The location of the NIFTY study sites in Mbarara, Uganda (green) and Kilifi, Kenya (blue); Top right; The Epicentre Research centre in Mbarara; Bottom left; The KEMRI-

Wellcome Trust Research Programme (KWTRP) and Bottom right: The KWTRP Research Clinic.

Table 4. 1 Planned participant recruitment and randomisation

Population	Allocation	Participants in Kilifi (n)	Participants in Mbarara (n)	Total per allocation
Adults (n=480)	Full dose	60	60	**120**
	1000 IU	60	60	**120**
	500 IU	60	60	**120**
	250 IU	60	60	**120**

Sample size calculation provided in section 4.2.5

The masked scratch booklets were only handled by the unblinded team; vaccinating nurses, an independent medical monitor and a vaccination supervisor (pharmacist and the senior nurse) at each of the sites.

I was responsible for all regulatory submissions. This trial was approved by the Scientific & Ethics Review Unit, Kenya Medical Research Institute (Kenya); Oxford Tropical Research Ethics Committee (United Kingdom); Mbarara University of Science and Technology Research Ethics Committee (Uganda); and the Uganda National Council of Sciences and Technology (Uganda). Regulatory approval was granted by the National Drug Authority in Uganda and the Pharmacy and Poisons Board of Kenya. Details of the ethical and regulatory approval documents are described in appendix 3. Study participants provided written informed consent.

4.2.2. Study Procedures

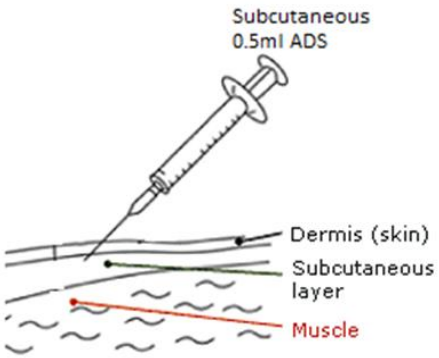
I was directly responsible for all procedures in the clinic. Participants were screened within 30 days of vaccination. Most of the participants were screened and vaccinated on the same day. Pre-vaccination, baseline samples were collected to assess the presence of existing YF antibodies from previous infection or vaccination. The lab test to check for pre-existing antibodies was not done in real-time and there was not part of the exclusion criteria. Instead, there was a 20% increase in the study sample size to account for pre-existing antibodies against YF at the base. The vaccine was diluted to prepare the vaccine to either ~13803, 1022, 502 or 251 IU/dose following the manufacturer's instructions in table 4.2. The vaccine potencies and dilutions were measured independently at the National Institute for Biological Standards and Control (NIBSC), the UK as instructed by the vaccine manufacturer to increase the confidence in the dilution process. (see Table 4.2).

Table 4. 2 Comparison of vaccine potency according to the manufacturer's instructions

Lot 2512	**Solvent Volume**	**Volume to be discarded from the solvent vial**	**Volume added to solvent vial from undiluted vaccine vial**	**Institut Pasteur Dakar**		**NIBSC**	
				IU/dose	logIU/dose	IU/dose	logIU/dose
Undiluted vaccine vial: 4.14 log IU/dose	5 ml	-	-	13803	4.14	19498	4.29
Dilution 1000 IU/dose	10 ml	3.75 ml	0.5 ml	1022	3.01	1022	3.01
Dilution 500 IU/dose	10 ml	4.7 ml	0.2 ml	502	2.70	457	2.66
Dilution 250 ml/dose	10 ml	4.6 ml	0.1 ml	251	2.40	235	2.37

On each vaccination day, the vaccines were prepared following the instructions provided by the manufacturer and the NIBSC. The prepared vaccines were labelled and kept at 2-8°C until administration and discarded after six hours from the time of dilution if not used. All the vaccine doses were administered subcutaneously as 0.5mls at a 45° injection angle using auto-disable syringes with a 25G × 3/4" needle size (see figure 4.3). The volume of the vaccine was diluted to be 0.5mls for all doses therefore there was no need to mask the content of the syringe. The vaccinees and all other study staff were not aware of the dose allocation throughout the study.

Figure 4. 3 Standard subcutaneous administration at 45° injection angle using a 0.5ml syringe with a 25G needle size.



After vaccination, the participants were observed for at least 30 minutes post-vaccination to assess for any immediate adverse events. The participants were then followed up as per the protocol. Participants were randomised and sparsely sampled for a study visit on one of the days between day 2 and day 7 post-vaccination to assess for safety. A serum sample was collected to assess viremia kinetics and control by vaccine dose. All participants were scheduled for a study visit at 10 days and 28 days post-vaccination to assess for safety and

samples were collected to evaluate rapidity and immunogenicity (the primary endpoint) respectively. Finally, at one- and two-years post-vaccination to assess the longevity of the immune response. Table 4.3 has a summary of the study-specific procedures – the data presented in this chapter were collected during the shaded period of the table.

Samples collected during this study included 4 ml of serum samples at all study clinic visits and an additional 6 ml of Peripheral Blood Mononuclear Cells (PBMCs) at day 0, day 10, day 28, one year and two years post-vaccination (Figure 4.4).

Table 4. 3 NIFTY Study procedures and schedule

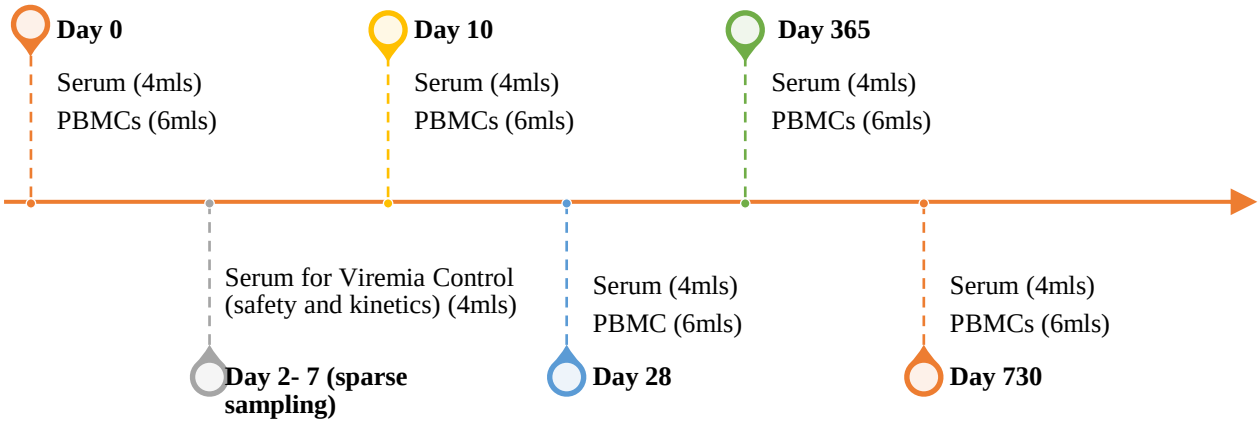
Procedure	**Screening Day -30***	**Day 0**	**Day 2, 3, 4, 5, 6, 7****	**Day 10 (+/- 1 day)**	**Day 28 (+/- 3 days)**	**Day 365 (+/-14 days)**	**Day 730 (+/- 28 days)**
Informed Consent	X						
HIV Antibody test	X						
Pregnancy test	X	X			X***		
Demography	X	X					
Vital signs	X	X		X	X	X	X
History and Physical exam	X			X	X	X	X
Randomisation		X					
Vaccination		X					
Blood sample (10mls/4mls)		X (10ml)	X (4ml)	X (10ml)	X (10ml)	X (10ml)	X (10ml)
Cumulative blood volume		10ml	14ml	24ml	34ml	44ml	54ml
Urine sample collection	X				X***		

*Before vaccination – screening can occur 0-30 days before vaccination. Screening and vaccination may occur on the same day. Participants that are not recruited within 30 days will be re-screened.

**Blood samples (4mls) will be collected for 20 randomly selected participants from each arm on days 2,3,4,5,6 and 7. Cumulative blood volumes for each of these participants are shown.

***A urine sample to conduct a pregnancy test will be done as indicated by a clinical assessment for female participants that present with symptoms and signs of pregnancy at the discretion of the study clinician.

Figure 4. 4 NIFTY study sample collection scheme



4.2.3. PRNT Assays

Similar to the studies described in Chapter 3, the PRNT assays for this study were conducted at the WHO-accredited regional YF reference lab at the Institut Pasteur de Dakar in Senegal using well-described standardised methods[53]. The step-by-step protocol using a varying concentration of serum and a constant virus concentration is described in detail in the previous chapter and the details are in appendix 2. I was not responsible for conducting these assays and received the data from Institut Pasteur de Dakar.

4.2.4. Study outcomes

My study outcomes related to immunogenicity at 28 days post-vaccination and safety throughout the trial as detailed below.

Immunogenicity outcomes

The primary outcome is a non-inferiority in the proportion of unvaccinated volunteers who seroconverted within 28 days of vaccination as measured by a 50% reduction in plaque count ($PRNT_{50}$) comparing each of the lower doses to the standard dose. Seroconversion in this study is defined as a four-fold increase of antibody titres at day-28 from the baseline (day-0) in the per-protocol (PP) study population. Secondary outcomes include assessing non-inferiority of the proportion of participants who seroconverted using a 90% plaque reduction ($PRNT_{90}$), describing Geometric Mean Titres (GMT) and Geometric Mean Fold Increase (GMFI) using $PRNT_{50}$ and $PRNT_{90}$ and seroconversion using $PRNT_{90}$ in the PP and the Intentions to Treat (ITT) study population. GMTs were defined as the average antibody titre for each of the vaccine dose groups subjects calculated by multiplying all values and taking the n^{th} root of this number where n is the number of subjects included in the analysis subgroup. For this study, the PP group is defined as participants who were seronegative for YF antibodies at baseline, had a PRNT result at day-28 and complied with the study protocol. The ITT group here refers to any vaccinated participant with PRNT results at baseline and day 28, whether they had pre-existing antibodies against YF or not.

Safety outcomes

The safety outcomes were assessed as described in chapter 3 section 3.2.6.1 at day 10, 28 and any unscheduled visits within the first month of the study for AEs and throughout the period of the study for SAEs.

4.2.5. Statistics

Sample size estimation

The study design assumed a 95% rate of seroconversion, a 0.025 alpha for a one-sided test and with 90% power to detect a non-inferiority margin of (-10%) based on the public health

consequence of waning population immunity[26 165]. Sample size calculation and simulations based on these assumptions yielded a sample size of 100 participants per arm. The details of the sample size simulations are in appendix 4. The sample size was increased by 20% to account for participants lost to follow up, any participants whose results would be unevaluable and participants with pre-existing antibodies against YF at baseline. Hence the total sample size of 480 for the four vaccine dose groups (see Table 4.1). There were no adjustments for statistical significance for the secondary and exploratory outcomes. The sample size calculation was performed using art2bin on Stata version 15 with simulations and cross-checked using Power Analysis & Sample Size software (PASS) version 14.

Statistical analysis

I wrote a statistical analysis plan before unblinding it to guide analysis (see appendix 5). The analysis of the participants consented, screened, randomised and vaccinated were performed descriptively to provide the PP and ITT groups according to the protocol. This process was per the guidelines provided by Consolidated Standards of Reporting Trials (CONSORT) whose output was the consort diagram (see figure 4.5). Demographics and baseline characteristics were described and compared between vaccine groups.

The primary outcome analysis was a pairwise comparison of the rate of seroconversion, defined as a ≥4-fold rise in $PRNT_{50}$ titre between day 0 and day 28 samples, for each of the lower doses and the standard dose in the PP population. Any PRNT value below the Limit of Quantification (LOQ) (i.e. <1:10) was reported as a negative result and for this analysis was converted to LOQ/2. Thus a 4 -fold increase for a subject who is negative (<1:10) at baseline, is a titre of 20. On the other hand, titres >20480 were designated 20480 as the upper limit and the highest serial dilution. Statistical significance of seroconversion was tested using the Agresti and Coull method, a method used to calculate confidence intervals for binomial proportions based on the

Wald formula[187]. A non-inferiority test using Dunnett's test was performed for the difference in seroconversion rates in the standard dose and each of the sub-doses using a two-sided 95% CI using $\log_{10}$ GMT. The fractional doses were considered non-inferior if the lower bound of the CI for the difference in seroconversion was greater than −10%.

Secondary outcome analysis includes non-inferiority tests used in the ITT population and using $PRNT_{90}$ GMTs in the PP and ITT populations. The GMT and Geometric Mean fold Increase (GMFI) were log-transformed and the differences were generated using t-distribution. There were additional analyses comparing time from preparation to vaccination and the rate of seroconversion for the PP and ITT populations. Duration of time from dilution to injection was calculated as the difference between the time the vaccine was prepared and the time of vaccination. The time was made in hours with 6hrs as the expected maximum of the duration.

Since the trial within which the work reported in this chapter is nested includes objectives beyond this DPhil, the trial is ongoing. To write up this chapter, the data relating to the outcomes described above for this chapter were temporarily locked and extracted in April 2021. The data was then cleaned, and analysis was performed using Stata Version 15 and R version 3.6.1.

4.3. Results

4.3.1. Recruitment, participants' baseline characteristics and follow up

Between November 2019 and March 2020, 518 participants were screened in Kilifi (n=266) and Mbarara (n=252), randomised and vaccinated 480 participants (n=240 at each site) and followed up as per protocol[135] (figure 4.5). The most common reasons for exclusion were the inability to stay in the study area for the duration of the study and chronic diseases. There were no major protocol deviations that led to the discontinuation of participants from the study. During the follow up some participants missed their visits and therefore did not provide safety

data and a blood sample (one participant at day 2-7 follow up, one other participant at day 10 and fifteen participants in Kilifi (n=2) and Mbarara (n=13) at day 28). Twelve of the fifteen missed their study visits in Mbarara on day 28 due to the movement restrictions as a measure to curb the COVID-19 pandemic in the region[188].

Figure 4. 5 Consort Diagram of the NIFTY Trial

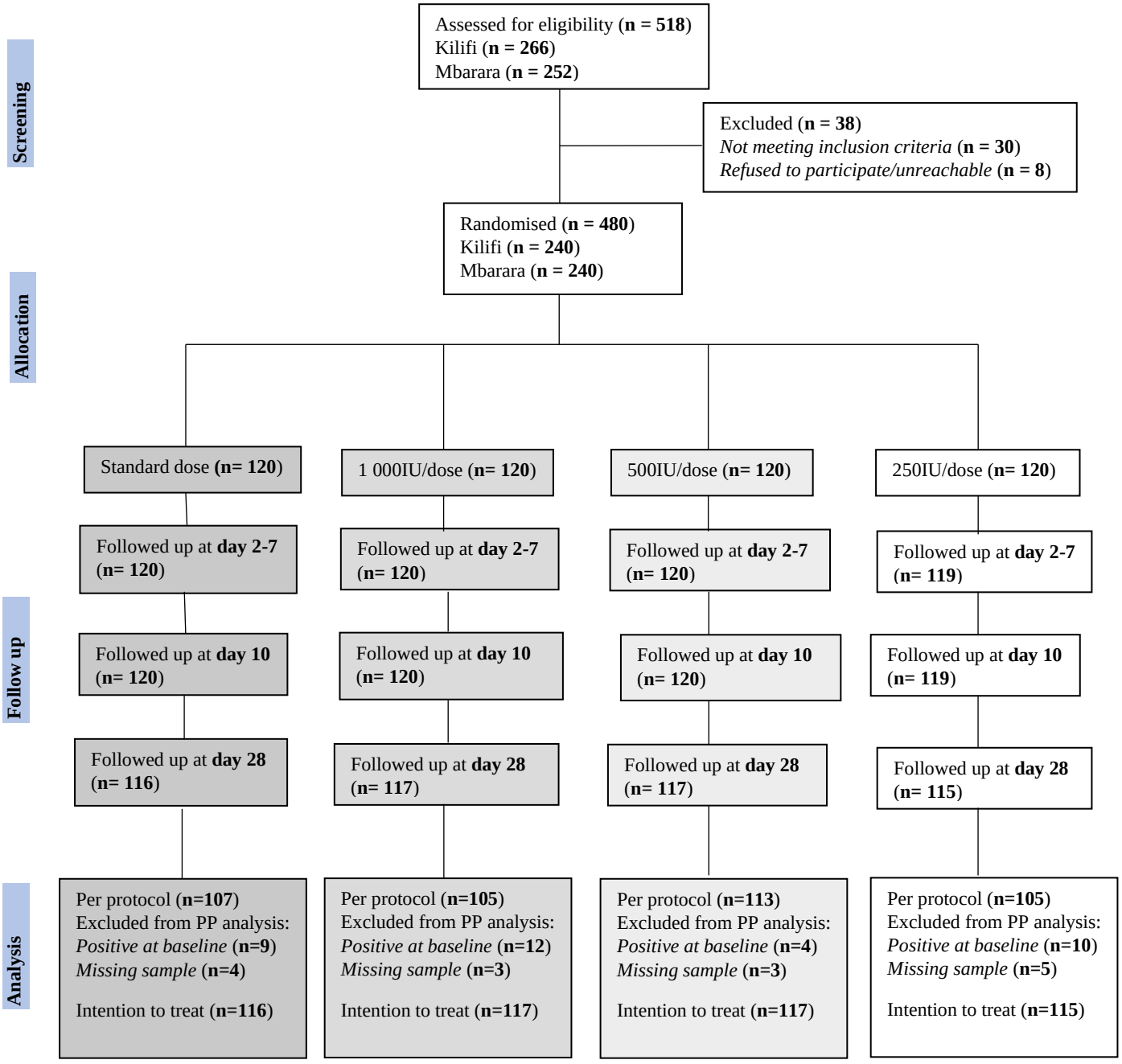


Table 4. 4 Distribution of participants by demographic and baseline characteristics across the groups.

Variable	Dose group				
	250 IU (n=120)	**500 IU (n=120)**	**1000 IU (n=120)**	**Standard Dose (n=120)**	**Combined**
Age (years)	Mean=40.2; SD =11.0	Mean=38.5; SD=11.7	Mean=40.4; SD=11.6	Mean=39.8; SD=11.9	Mean = 39.7; SD = 11.5
Gender					
Male	78 (65.0)	68 (56.7)	71 (59.2)	79 (65.8)	296 (61.7)
Female	42 (35.0)	52 (43.3)	49 (40.8)	41 (34.2)	184 (38.3)
Temperature	36.4 (0.5)	36.4 (0.5)	36.3 (0.4)	36.4 (0.5)	Mean = 36.4; SD = 0.5
Area of residence					
Kilifi (n=240)					
Ngombeni	8 (6.7)	5 (4.2)	9 (7.5)	7 (5.8)	29 (6.0)
Ziani	15 (12.5)	21 (17.5)	16 (13.3)	18 (15)	70 (14.6)
Maweni	12 (10.2)	12 (10.2)	17 (14.2)	8 (6.7)	49 (10.2)
Chumani	23 (19.2)	17 (14.2)	15 (12.5)	22 (18.3)	77 (16.0)
Other	2 (1.7)	5 (4.2)	3 (2.5)	5 (4.2)	15 (3.1)
Mbarara (n=240)					
Rwanyamahembe	49 (40.8)	48 (40)	47 (39.2)	51 (42.5)	195 (40.6)
Bukiro	10 (8.3)	12 (10)	13 (10.8)	9 (7.5)	44 (9.2)
Bwizibwera	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Yellow Fever infection					
No	120 (100)	120 (100)	120 (100)	120 (100)	480 (0.0)
Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Previous medical illness					
No	71 (59.2)	66 (55.0)	76 (63.3)	64 (53.3)	277 (57.7)
Yes	48 (40.0)	54 (45.0)	44 (36.7)	56 (46.7)	202 (42.1)
Unknown	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
HIV status at baseline					
Negative	112 (93.3)	108 (90)	115 (95.8)	107 (89.2)	442 (92.1)
Positive	8 (6.7)	12 (10)	5 (4.2)	13 (10.8)	38 (7.9)
CD4 Cell count	Mean=659.3; SD=118.3	Mean=642.3; SD=245.5	Mean=942.8; SD=323.6	Mean=627.6; SD=281.7	Mean=680.4; SD=261.7

Data are number (per cent), except for continuous variables

There were more male participants (61.7%) recruited to the study. The mean age at vaccination was 39.7 (SD=11.5). The mean temperature of participants recruited at baseline was 36.4 (SD=0.5). There was no previous history of flavivirus infection (YF, Zika, Dengue or West Nile viruses) reported. However, 38 participants tested HIV-positive at baseline (Figure 4.5). Thirty-eight of the participants (Kilifi (n=13) and Mbarara (n=25)) vaccinated (7.9%) were HIV positive at baseline, with mean CD4 levels of 680.4 (SD=261.7). (Table 4.4)

4.3.2. Vaccine safety

Adverse events

Table 4.5 shows the summary of all adverse events for the participants that were recruited to the study, randomised and vaccinated by vaccine dose arm groups. The rate of overall adverse events was similar for each of the vaccine doses given. Most of the AEs (99.4%) were mild-to-moderate and self-limiting; all the AEs reported resolved without sequelae. Although there were more AEs reported by the participants who were vaccinated with 500IU/dose, they we not statistically significant. There were more AEs reported in Mbarara compared to the Kilifi site both for the number of AEs reported and the number of subjects who reported at least one AEs per site. There were no significant differences in the number of AEs relatedness to the study treatment by vaccine dose (Table 4.6). Of the solicited AEs, headache, gastrointestinal problems and myalgia were most reported (Table 4.7).

Table 4.7 lists all the adverse events reported by the preferred term as defined by the MedDRA dictionary. Most of the adverse events were not related to the vaccine. There were three immediate adverse events as assessed within the first hour of vaccination (2 in the 250 IU/dose arm and 1 in the 500 IU/dose arm) – all reported in the Mbarara site. All three were females of an average age of 41 years.

Table 4. 5 Summary of All Adverse Events for vaccinated participants.

Topics	250 IU	500 IU	1000 IU	Standard Dose	Total N=480
Number of AEs reported	119	163	136	125	543
Number of Subjects with AEs [1]	65	75	68	65	273
Number of SAEs reported	0	0	2	1	3
Number of Subjects with SAEs [1]	0	0	2	1	3
Number of AEs by Site*					
Kilifi	31 (26.1%)	48 (29.4%)	39 (28.7%)	40 (32.0%)	158 (29.1%)
Mbarara	88 (73.9%)	115 (70.6%)	97 (71.3%)	85 (68.0%)	385 (70.9%)
Subjects with AEs by Site **					
Kilifi	23 (19.2%)	25 (20.8%)	25 (20.8%)	28 (23.3%)	101 (21%)
Mbarara	42 (35%)	50 (41.7%)	43 (35.8%)	37 (30.8%)	172 (35.8%)
Number of AEs by Severity*					
Mild	89 (74.8%)	120 (73.6%)	105 (77.2%)	99 (79.2%)	413 (76.1%)
Moderate	30 (25.2%)	42 (25.8%)	29 (21.3%)	26 (20.8%)	127 (23.4%)
Severe	0 (0%)	1 (0.6%)	2 (1.5%)	0 (0%)	3 (0.6%)
Subjects with AEs by Severity [2] **					
Mild	61 (50.8%)	63 (52.5%)	60 (50%)	59 (49.2%)	243 (50.6%)
Moderate	19 (15.8%)	31 (25.8%)	19 (15.8%)	19 (15.8%)	88 (18.3%)
Severe	0 (0%)	1 (0.8%)	2 (1.7%)	0 (0%)	3 (0.6%)
Number of AEs by Relatedness to study treatment*					
Related	28 (23.5%)	26 (16%)	29 (21.3%)	27 (21.6%)	110 (20.3%)
Probably Related	15 (12.6%)	25 (15.3%)	17 (12.5%)	13 (10.4%)	70 (12.9%)
Not related	76 (63.9%)	112 (68.7%)	90 (66.2%)	85 (68%)	363 (66.9%)
Subjects with AEs by Relatedness to study treatment [2] **					
Related	22 (18.3%)	20 (16.7%)	18 (15%)	20 (16.7%)	80 (16.7%)
Probably related	13 (10.8%)	21 (17.5%)	13 (10.8%)	13 (10.8%)	60 (12.5%)
Not related	47 (39.2%)	62 (51.7%)	56 (46.7%)	51 (42.5%)	216 (45%)

[1] Subjects who experience one or more AEs or SAEs are counted only once.

[2] Subjects are counted only once within a particular severity grade or relatedness category.

* Percentages are based on the number of AEs reported for each treatment arm.

** Percentages are based on N for each treatment arm

Table 4. 6 Summary of All Adverse Events by relatedness and site

		Study site	
Category	Total	Kilifi	Mbarara
Number of AEs by Relatedness to study treatment*			
Related	110	62 (56.4%)	48 (43.6%)
Probably related	70	23 (32.9%)	47 (67.1%)
Not related	363	73 (20.1%)	290 (79.9%)
Subjects with AEs by Relatedness to study treatment [2] **			
Related	80	46 (57.5%)	34 (42.5%)
Probably related	60	20 (33.3%)	40 (66.7%)
Not related	216	61 (28.2%)	155 (71.8%)

[1] Subjects who experience one or more AEs or SAEs are counted only once.

[2] Subjects are counted only once within a particular severity grade or relatedness category.

* Percentages are based on the number of AEs reported for each relatedness category

** Percentages are based on N with AE for each relatedness category

Table 4. 7 Adverse Events reported by preferred term

Adverse Event	Dose				
	250 IU	500 IU	1000 IU	Standard Dose	Total
Headache	16	21	27	13	77
Nasopharyngitis	19	15	17	14	65
Gastroenteritis	5	19	12	13	49
Dizziness	9	9	9	13	40
Myalgia	4	10	11	4	29
Cough	4	7	7	4	22
Fatigue	5	2	6	8	21
Neuropathy Peripheral	7	4	5	2	18
Rhinitis	2	9	1	5	17
Urinary Tract Infection	5	5	3	2	15
Arthralgia	3	4	2	5	14
Vulvovaginal Candidiasis	3	6	2	3	14
Back Pain	4	1	4	3	12
Pyrexia	0	4	3	2	9
Malaria	2	3	1	2	8
Nausea	2	1	4	1	8
Soft Tissue Injury	1	3	1	3	8
Abdominal Pain	1	3	2	1	7
Dermatitis	2	2	0	2	6

Dyspepsia	1	3	1	1	6
Rash	3	1	1	1	6
Tinea Infection	2	2	2	0	6
Conjunctivitis	3	2	0	0	5
Pregnancy	1	1	0	2	4
Tinnitus	1	2	1	0	4
Tonsillitis	0	1	1	2	4
Decreased Appetite	1	2	0	0	3
Pelvic Inflammatory Disease	1	1	0	1	3
Toothache	0	1	0	2	3
Vision Blurred	0	1	0	2	3
Abscess	0	0	1	1	2
Chills	1	1	0	0	2
Conjunctivitis	1	0	1	0	2
Diarrhoea	0	1	0	1	2
Furuncle	2	0	0	0	2
Genital Herpes	0	0	1	1	2
Gingivitis	0	0	2	0	2
Hyperphagia	1	0	1	0	2
Hypersensitivity	0	2	0	0	2
Hypertension	0	0	2	0	2
Injection Site Reaction	1	0	0	1	2
Libido Decreased	0	1	1	0	2
Oral Herpes	0	1	1	0	2
Pruritus	1	0	1	0	2
Vomiting	0	1	0	1	2
Abdominal Distension	1	0	0	0	1
Angina Pectoris	0	1	0	0	1
Bacterial Vaginosis	0	1	0	0	1
Dysuria	0	1	0	0	1
Ear Infection	0	0	0	1	1
Ear Pain	0	0	1	0	1
Eczema	0	1	0	0	1
Glossitis	0	1	0	0	1
Haematoma	0	1	0	0	1
Hallucination	1	0	0	0	1
Helminthic Infection	0	0	0	1	1
Hordeolum	0	0	0	1	1
Hypersomnia	1	0	0	0	1
Metrorrhagia	0	0	0	1	1
Muscular Weakness	1	0	0	0	1
Oedema	0	1	0	0	1
Oesophageal Candidiasis	0	1	0	0	1
Orchitis	0	0	0	1	1
Palpitations	0	1	0	0	1
Pollakiuria	0	1	0	0	1
Premenstrual Syndrome	0	0	0	1	1
Scabies	1	0	0	0	1
Skin Ulcer	0	0	0	1	1
Swelling Face	0	0	0	1	1
Syncope	0	1	0	0	1
Torticollis	0	0	1	0	1
Urticaria	0	0	0	1	1
Total	**119**	**163**	**136**	**125**	**543**

Serious adverse events

Table 4.8 summarises the SAEs reported in the study until April 2021. There were three serious adverse events (SAEs) (Standard dose=1; 1000 IU =2, with the latter two being from Mbarara) by April 2021; the first was due to a road traffic accident where the participant died upon arriving at the hospital, the second SAE was due to hospital admission for the management of Malaria - the participant recovered without sequelae, and the last was due to a surgical repair of a pre-existing umbilical hernia. None of the SAEs was related to the investigational product. These were reported to the ethical and regulatory bodies.

Table 4. 8 Table summarising the SAEs reported until April 2021

Participant ID	Age at SAE onset	Site	Study arm	MedDRA Preferred Term	Outcome of event	Related
K185	28	Kilifi	Standard Dose	**Accidental death**	Fatal/died	No
M081	45	Mbarara	1000 IU	**Malaria**	Recovered without sequelae	No
M129	47	Mbarara	1000 IU	**Umbilical hernia**	Recovered without sequelae	No

Reduced doses of YF vaccines and pregnancy

Six participants reported pregnancy during the first-month post-vaccination. Of these, three were inadvertently vaccinated in early pregnancy (Kilifi=2, Mbarara=1) while the other three became pregnant within the first 28 days of vaccination (Kilifi=1, Mbarara=2). These participants were followed up throughout the study and the outcomes were recorded. Two of the three participants that were vaccinated in early pregnancy terminated their pregnancies while the other participant carried the pregnancy to term and reported a normal delivery and

puerperium. One of the participants who reported pregnancy within the first month of vaccination had an unremarkable pregnancy with normal deliveries and puerperium, one terminated her pregnancy and the other participant was lost to follow up.

4.3.3. Vaccine-induced immunogenicity

Antibody titres

Seroconversion rates, as measured by the $PRNT_{50}$ titres, were high in all dose groups – with 93.3% to 100% of the participants seroconverting in all treatment groups in the PP population. GMTs by $PRNT_{50}$ were high in all treatment groups with titres of 781 to 1813 in the 250 IU/dose group, 1400 to 2472 in the 500 IU/dose group, 1615 to 2917 in the 1000 IU/dose group and 1261 to 2152 in the standard-dose group. The seroconversion rates, as measured by $PRNT_{90,}$ were also generally high although relatively lower than those using $PRNT_{50}$ titres. The GMTs were significantly lower in the $PRNT_{90}$ with titres between 44.2 and 64.3 in the PP population for all vaccine dose arms (see table 4.9). Using the Agresti and Coull method, the proportion that seroconverted was shown to be statistically significant for the 250 IU/dose group in the $PRNT_{50}$ and $PRNT_{90}$ titres. The $PRNT_{50}$ GMTs in the 250 IU/dose arm were relatively lower compared to the other vaccine dose groups. Pairwise comparison of the neutralising antibody titres at baseline and 28 days post-vaccination recorded an increase in geometric means across all the vaccine dose groups. Corresponding GMT and GMFI were lower using $PRNT_{90}$ titres relative to the $PRNT_{50}$ titres (Table 4.9). High antibody titres and seroconversion was also reported in the HIV-infected sub-group. The results for the ITT results are included in Table 4.9. Figure 4.6 show the reverse cumulative distribution of the proportion of seroconversion using the $PRNT_{50}$ titres and the $PRNT_{90}$ titres. The corresponding trends in the ITT population are comparable.

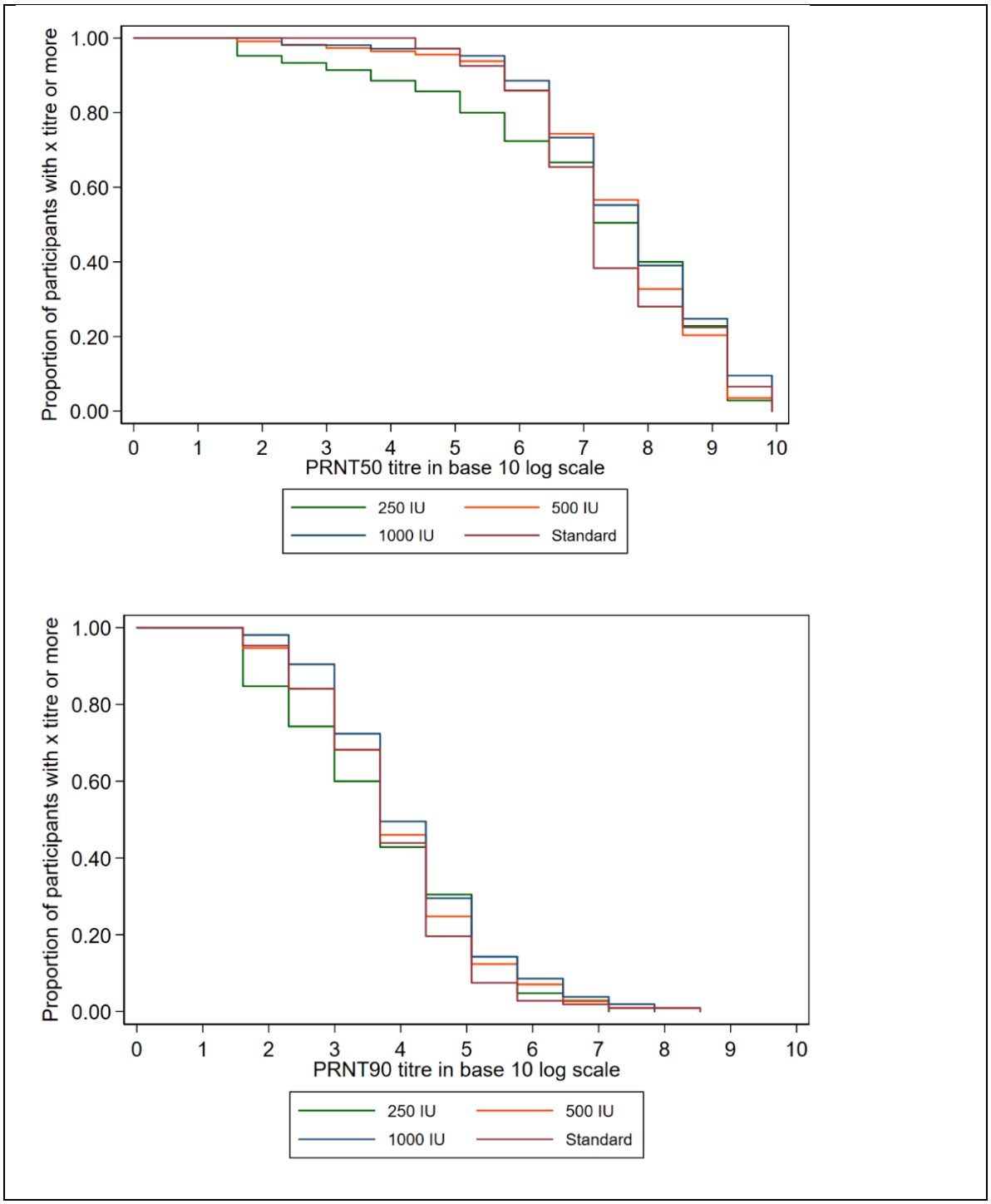
Table 4. 9 Immunological Parameters for Per-protocol analysis (upper panel) and Intent-to-treat Analysis (lower panel).

Dose Level	Per-protocol Analysis (Total N=430)		
	Seroconversion rate	GMT	GMFI
$PRNT_{50}$			
250 IU (N=105)	93.3% (86.7-97.0%) *	1190.3 (781.4-1813.3)	238.1 (156.3-362.7)
500 IU (N=113)	98.2% (93.4-99.9%)	1860.9 (1400.5-2472.6)	372.2 (280.1-494.5)
1000 IU (N=105)	98.1% (92.9-99.9%)	2170.5 (1615.1-2917.1)	434.1 (323.0-583.4)
Standard (N=107)	100% (95.8-100%)	1647.9 (1261.5-2152.7)	329.6 (252.3-430.5)
$PRNT_{90}$			
250 IU (N=105)	74.3% (65.1-81.7%)	44.2 (33.1-58.9)	8.8 (6.6-11.8)
500 IU (N=113)	84.1% (76.1-89.8%)	53.4 (41.6-68.4)	10.7 (8.3-13.7)
1000 IU (N=105)	90.5% (83.2-94.9%)	64.3 (50.1-82.6)	12.9 (10-16.5)
Standard (N=107)	84.1% (75.9-89.9%)	47.6 (37.9-59.9)	9.5 (7.6-12)
Dose Level	Intention-to-treat Analysis (Total N=465)		
	Seroconversion rate	GMT	GMFI
$PRNT_{50}$			
250 IU (N=115)	93.9% (87.8-97.2%) *	1303.4 (878.4-1934)	231.1 (155.8-342.6)
500 IU (N=117)	98.3% (93.6-99.9%)	1773 (1336.5-2352.1)	342.2 (256.1-457.2)
1000 IU (N=117)	98.3% (93.6-99.9%)	2181.6 (1661.2-2865)	383 (288.6-508.3)
Standard (N=116)	99.1% (94.8-100%)	1549.7 (1183.2-2029.7)	270.1 (200.1-364.8)
$PRNT_{90}$			
250 IU (N=115)	75.7% (67-82.6%)	47.4 (35.9-62.4)	9.4 (7.2-12.4)
500 IU (N=117)	82.9% (75-88.7%)	51.9 (40.7-66.2)	10.4 (8.1-13.2)
1000 IU (N=117)	90.6% (83.8-94.8%)	62.4 (49.4-78.8)	12.3 (9.7-15.5)
Standard (N=116)	82.8% (74.8-88.6%)	46.2 (36.9-57.8)	9.0 (7.2-11.3)

* =significantly different than the full standard dose; † 95% confidence intervals estimated using Agresti-Coull method;

GMT = Geometric mean titre; GMFI=Geometric mean fold increase

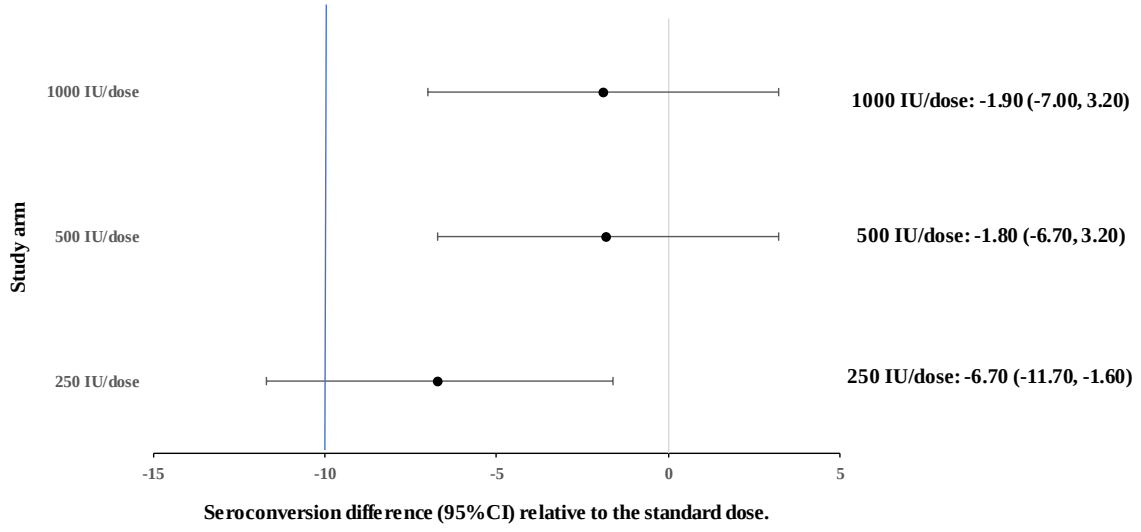
Figure 4. 6 reverse cumulative of the proportion of seroconversion using $PRNT_{50}$ and $PRNT_{90}$ in the PP population.



Non-inferiority assessment

In the PP population, the differences in seroconversion rates at day 28 between each of the lower doses (1000IU/dose, 500IU/dose and 250IU/dose) were -1.9% (95%CI -7 to 3.2%), -1.8% (95%CI -6.7 to 3.2%) and -6.7% (95%CI -11.7 to -1.6%) respectively in comparison to the standard dose (figure 4.7). In the ITT group, these differences were -0.8% (95%CI -5.7 to 4%), -0.8% (95%CI -5.7 to 4%) and -5.2% (95%CI -10.1; -0.3%) respectively see table 4.10). Non-inferiority defined by a margin of -10% was met by the 500IU/dose and 1 000IU/dose in both the PP and ITT populations. The seroconversion rate difference in the 250IU/dose group had 95% CIs that included values worse than the non-inferiority margin of -10% for the PP population (-11.7%) and the ITT populations (-10.1%) and therefore non-inferiority could not be demonstrated. The significance of the difference in seroconversion was tested using the Dunnett method[189 190].

Figure 4. 7 Seroconversion differences, confidence intervals and the non-inferiority margin for PP population ($PRNT_{50}$). The two vertical lines indicate non-inferiority margin (blue) and zero (black).



Although non-inferiority for this trial was not determined based on the GMT ratios, I undertook an analysis of the GMT ratios to compare the different doses to the standard dose. The GMT ratios had less than two-fold difference between the different lower vaccine dose groups and the standard dose as indicated by the GMT ratios (table 4.10). There were no statistically significant differences detected between the PP and the ITT population and between titres as measured by the $PRTN_{50}$ and $PRNT_{90}$ using the Dunnett test.

Table 4. 10 Non-inferiority of seroconversion rate in each lower dose vs the standard dose.

Dose Level	**Value**			
	Per-protocol Analysis		**Intention-to-treat Analysis**	
	Seroconversion difference	GMT ratios	Seroconversion difference	GMT ratios
$PRNT_{50}$				
250 IU	**-6.7% (-11.7; -1.6%) ***	0.72 (0.44-1.18)	**-5.2% (-10.1; -0.3%) ***	0.84 (0.52; 1.35)
500 IU	-1.8% (-6.7; 3.2%)	1.13 (0.77-1.66)	-0.8% (-5.7; 4%)	1.14 (0.78; 1.69)
1000 IU	-1.9% (-7; 3.2%)	1.32 (0.89-1.96)	-0.8% (-5.7; 4%)	1.41 (0.96; 2.06)
Standard	Reference	Reference	Reference	Reference
$PRNT_{90}$				
250 IU	-9.8% (-21.8; 2.1%)	0.93 (0.64-1.34)	-7.1% (-18.6; 4.4%)	1.03 (0.72; 1.46)
500 IU	0% (-11.8; 11.7%)	1.12 (0.8-1.57)	0.1% (-11.3; 11.6%)	1.12 (0.81; 1.56)
1000 IU	6.4% (-5.6; 18.3%)	1.35 (0.96-1.89)	7.8% (-3.7; 19.3%)	1.35 (0.98; 1.86)
Standard	Reference	Reference	Reference	Reference

* Significantly different than the standard dose using the Dunnett test. GMT = Geometric mean titre.

Assessment comparing time from dilution to injection and seroconversion

During a meeting to discuss interim results, there was a concern that time to vaccination may affect the rate of seroconversion. Additional analyses comparing time from preparation to vaccination and rate of seroconversion showed no statistically significant differences in terms of duration using Dunnett's test comparing the different doses with the standard dose. The median (range) time from dilution to injection was 1.08h (range: 0.02-4.97h) in the per-

protocol population and 1.12 (range: 0.02-4.97h) for the intention-to-treat population (table 4.11).

Table 4. 11 Median (interquartile range) for the duration (hrs.) from dilution to injection by dose.

	Per-protocol population		Intention-to-treat population	
Dose	**N**	**Median duration (IQR)**	**N**	**Median duration (IQR)**
250 IU	105	1.05 (0.45-1.63)	115	1.05 (0.45-1.68)
500 IU	113	1.05 (0.53-1.90)	117	1.05 (0.53-1.90)
1000 IU	105	1.02 (0.57-1.77)	117	1.02 (0.57-1.77)
Standard	107	1.20 (0.73-2.00)	116	1.28 (0.73-2.02)
Total	430	1.08 (0.58-1.88)	465	1.12 (0.58-1.87)

*Dunnett test comparing each dose level with standard dose.

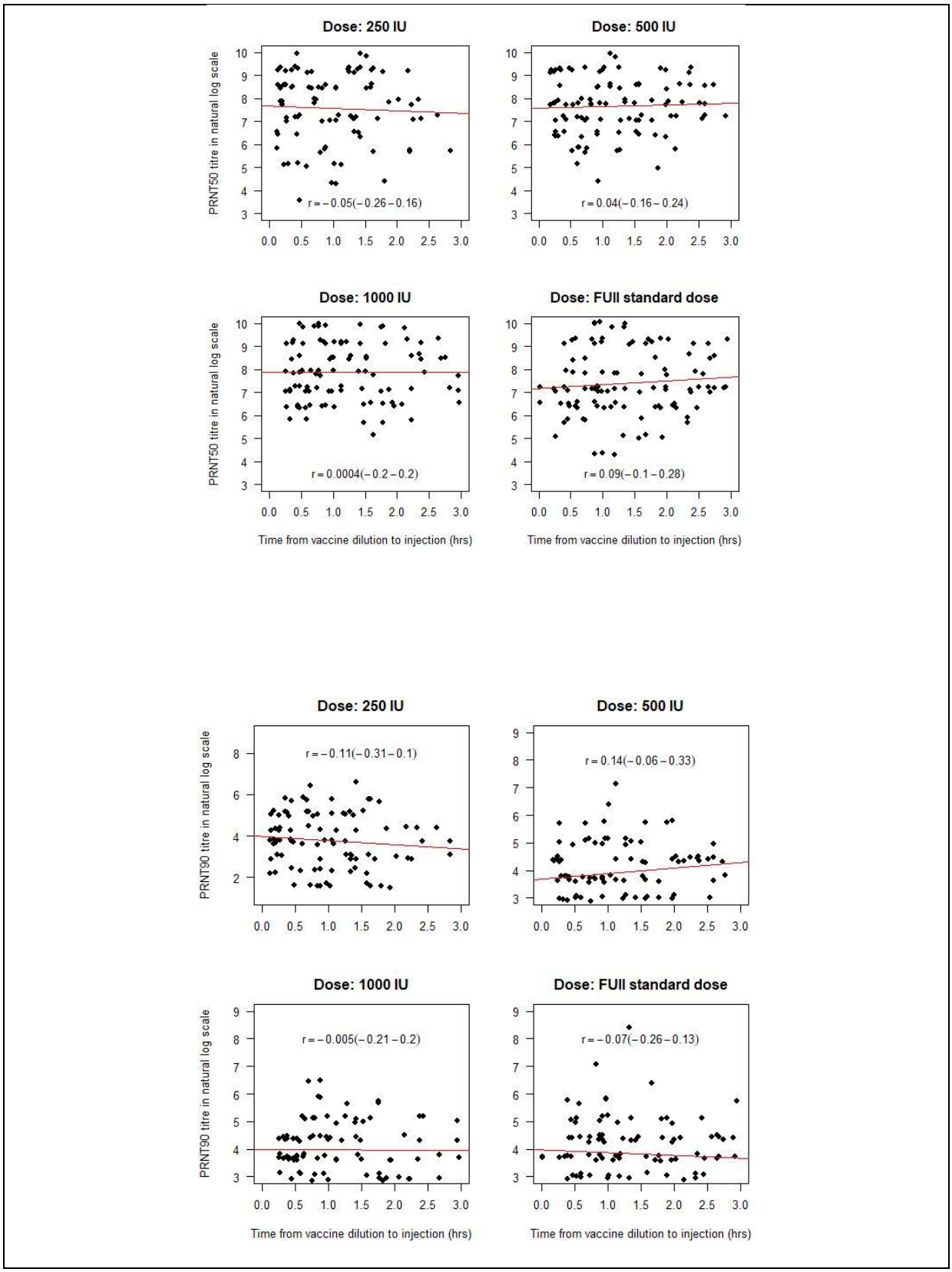
Table 4.12 presents the logistic regression model and the linear regression model for seroconversion rate with dose and duration and their interaction (calculated as the product of duration and dose) as predictors in the PP population. The logistic regression models (using Firth regression) showed unstable odds ratios and 95% CIs too wide to make any statistical conclusions. More so with $PRNT_{50}$ titres where there were high seroconversion rates compared to the $PRNT_{90}$ titres that had relatively lower seroconversion rates.

Table 4. 12 Linear regression (lower section) for seroconversion with dose and duration as predictors (PP population)

	Linear Regression			
	$PRNT_{50}$		**$PRNT_{90}$**	
	Estimate (95%CI)	**P value**	**Estimate (95%CI)**	**P value**
Intercept	7.21 (6.60, 7.81)	<0.001	3.88 (3.4, 4.36)	<0.001
Duration	0.14 (-0.22, 0.50)	0.450	-0.01 (-0.30, 0.27)	0.930
Dose				
250 IU	0.52 (-0.29, 1.32)	0.205	0.18 (-0.46, 0.82)	0.581
500 IU	0.09 (-0.72, 0.89)	0.832	-0.16 (-0.8, 0.49)	0.634
1000 IU	0.43 (-0.39, 1.25)	0.304	0.16 (-0.49, 0.81)	0.630
Standard	Reference		Reference	
Duration x Dose				
250 IU x Duration	-0.68 (-1.18, -0.17)	0.009	-0.22 (-0.62, 0.19)	0.292
500 IU x Duration	0.04 (-0.45, 0.53)	0.867	0.21 (-0.18, 0.6)	0.299
1000 IU x Duration	-0.10 (-0.60, 0.39)	0.679	0.11 (-0.29, 0.5)	0.601
Standard x Duration	Reference		Reference	

Using the linear regression model, there was a positive relationship between the time from vaccine dilution to injection (independent variable) and antibody titres for the 250 IU/dose group and a negative relationship for 500 IU/dose, 1000 IU/dose and the standard dose. The coefficient of determination (R^2) were however small ($R^2_{250IU/dose}$ = -0.05, $R^2_{500IU/dose}$ = 0.04, $R^2_{1000IU/dose}$ = 0.00 and $R^2_{std\ dose}$ = 0.09) (see figure 4.8). After accounting for outliers (time > 3hrs), there was no significant difference in the relationship or statistical significance for the $PRNT_{50}$. The relationship was positive for 1000 IU/dose and negative for all other dose groups using $PRNT_{90}$.

Figure 4. 8 Scatter plot with and linear regression model of $PRNT_{50}$ and $PRNT_{90}$ titres in natural log and time from vaccine dilution to injection in the PP population.



4.4. Discussion

The results for this chapter are based on the first dose-response study using the 17D-204 vaccines in an East African population comparing them with a standard dose. There was generally good compliance to the study protocol with a very low attrition rate of 15 participants missing their day-28 study visit, out of whom 12 were due to the COVID-19 pandemic restrictions. Data from this chapter adds to the growing body of evidence on the safety and immunogenicity of fractional doses [5 109 127 128 130 136]. Baseline positivity seroprevalence of 7.9% was shown despite the fact that neither YF infection, other flavivirus infections nor previous vaccinations were reported by history at baseline. A possible explanation is that the participants may have had asymptomatic infections of YF or other flavivirus infections. The majority of flavivirus infections are asymptomatic and self-limiting[191]. This could indicate the seroprevalence of flavivirus in the regions the participants were recruited from. Studies conducted in Kilifi and Mbarara identified viral infections, including flaviviruses, as possible causes of undifferentiated febrile illness[146 147 192]. Another plausible explanation is that the participants may have misrepresented the clinical events that rely on their memory and for which confirmation is difficult [193 194].

There were frequent AEs with 273 participants (56.8%) reporting at least one AE within the first month of vaccination. In total, 543 AEs were reported. There were generally more people reporting AEs and total AEs reported in Mbarara compared to Kilifi. However, the solicited AEs were similar in both sites. There were more vaccine-related AEs reported in Kilifi (56.4%) compared to Mbarara. A possible explanation of these differences is the clinician's reporting of the AEs by site; the clinicians in Kilifi reported AEs that were more likely associated with the vaccine. Safety reporting in clinical trials is complex and difficult to standardise when evaluating and reporting AEs across different sites[195 196]. All vaccine dose groups had similar trends in safety events. Most of the AEs reported (99.4%) were mild and moderate, and self-

limiting. There were three SAEs reported in the study – none of which was related to the vaccine administration. Concerns over vaccinating pregnant women with YF vaccines have been described in the literature[197-201]. Most of the participants that were inadvertently vaccinated while pregnant or became pregnant within the first month voluntarily terminated their pregnancies while each of the participants that carried the pregnancies to term reported a normal pregnancy, delivery and puerperium. All the vaccine doses administered are therefore considered safe.

Studies of immunogenicity often report $PRNT_{50}$, $PRNT_{80}$ and $PRNT_{90}$ titres. While the $PRNT_{90}$ study is more specific it suffers low sensitivity and can often lead to false-negative titres results. $PRNT_{50}$ on the other hand although less specific it so sensitive as to detect antibodies even for lower virus-specific antibody titres[84]. Although the WHO recommends that the PRNT titres should be based on at least a 50% or greater plaque reduction, they have set titres using $PRNT_{50}$ as correlates of protection [53 202]. The primary endpoints described in this study are based on this recommendation. All vaccine doses induced high antibody titres with high seroconversion rates (93.3% to 100%) in vaccinated participants at one-month post-vaccination across the PP and ITT analysis populations. The high seroconversion rates by $PRNT_{50}$ were consistent with other studies that have tested lower doses of YF vaccines [8 128 171]. Nearly all participants vaccinated with a singular dose of the different doses elicited functional antibodies. There are no defined correlates of protection in humans due to ethical considerations[80]. Therefore, detectable titres are considered protective based on studies in macaques and hamsters [85 203]. Detectable levels of antibodies (>10) are important in providing adequate protection, including in the context of an outbreak, to control outbreaks[127 204]. High GMTs were described in all the vaccine dose groups – with those elicited in the 500IU/dose and 1000 IU/dose groups marginally higher than those of the standard dose for both the PP and the ITT groups as measured by both the $PRNT_{50}$ and the $PRNT_{90}$ assay. High antibody titres and seroconversion

rates were also reported in the HIV-infected sub-group recruited in this study. These data are consistent with data from the previous trial testing fractional doses (1/5) against the standard dose[8] and the data reported in the previous chapter. In the referenced study we had shown that there were no differences between vaccine dose manufacturers and therefore the results from this study could be generalisable across all vaccine manufacturers. Other studies have also previously shown major similarities between the 17D-YF vaccines and subtle differences comparing the safety and immunological responses [205].

Of the lower doses tested, 500IU/dose and 1000 IU/dose were non-inferior to the standard dose (i.e. ~13,000 IU) for immunogenicity measured a month after vaccination. The study confirms that the 1000IU/dose recommended by WHO elicits sufficient immunity [10 41] but also raises the question of whether 500IU/dose should be used as the minimum requirement. The 250IU/dose group failed to meet the non-inferiority criteria marginally. This data increases the confidence to use fractional doses at even lower doses of the WHO recommendation in the settings where vaccine supply is insufficient. Based on these data, the use of 500IU/dose would increase the vaccine supply by more than 26-fold using this batch of vaccines. Although these data show that 500IU/dose is sufficiently immunogenic within the first month. In the afore-mentioned study, we showed that there were significantly lower GMTs and seroconversion rates (53.2% to 77.5%) at 10 days post-vaccination using fractional doses and standard doses. However, due to the low rates of seroconversion, the study was insufficiently powered (35%) to draw any significant conclusions on non-inferiority at day 10 [8].

The trial within which this work is nested has followed participants up for one year and planning a follow-up visit at 2 years post-vaccination. Data on antibody longevity at one and two years is not presented in this DPhil project. Previous studies assessing for the longevity of fractional doses have shown high antibody titres at one year [6] and up to 8 years post-vaccination and that there are no significant differences to the participants that received standard doses [5]

[183]. Although the results for this assessment can be anticipated, the lab analysis for these time points will be complete in early 2022.

The success demonstrated in fractional doses of the 17D-YF vaccines ability to induce sufficient immunity is attributed to the fact the vaccine manufacturers overfill the vaccine vials and ampoules to account for the loss of viral particles during the shelf life [179 206]. During the titration of the vaccines to achieve the lower doses, the vaccine manufacturer reported that they noted that the lower doses lose some potency the longer they were stored within the 6-hour window of vaccination (Dr. Antoine Diatta, during the NIFTY's Trial Steering committee meeting in June 2021). Vaccine doses that were delivered to the participants may, in fact, be less than the indicated doses in table 4.2. In the analysis comparing time from dilution to injection of the vaccine and seroconversion, there was no strong correlation of time as a predictor of antibody titres in the different vaccine dose groups (500 IU/dose and 1000 IU/dose) compared to the standard dose. The unstable odds ratios and the wide confidence interval described using the logistic regression models by $PRNT_{50}$ could be attributed to near-perfect predictions due to the high seroconversion rates by $PRNT_{50}$ as compared to the $PRNT_{90}$. The logistic analysis estimates may often encounter separation using covariates that predict near-perfectly the outcome – such as with the $PRNT_{50}$ titres which may predict 100% seroconversion in some instances. These models may be misleading and visual inspection of seroconversion rates and time to vaccination are more reliable. However, the statistical significance (P=0.009) using the linear regression model and interaction of duration between vaccine preparation and injection and dose arm may suggest that the 250IU/dose may have been non-inferior if all participants in this group were vaccinated within an hour of preparation. There is a need for lab experiments to assess the vaccine viral quantities of the lower doses in a time series to complement the data from the field. However, the results from these experiments can only add to the confidence in utilisation of the lower doses (500IU/dose and 1000IU/dose).

4.5. Study strengths and limitations

The main strength of the study reported in this chapter is the randomised controlled double-blind parallel allocation design. Randomised controlled trials are considered the gold standard when conducting non-inferiority trials to assess vaccine effectiveness [207-209]. The non-inferiority design of the study compares the test doses with standard doses which have been studied for several decades. The study is the first to test different doses in a generally heterogeneous sub-Saharan African population. The sample size for the study included an attrition rate allowance of 20% and therefore a 90% power to detect differences in outcomes[135]. Although the data presented is limited to humoral immune responses using PRNT assays, the assay is the gold standard to quantify functional antibodies. The primary endpoint presented here is based on a 50% reduction of plaques. Assays describing 75%, 80% and 90 % percentage have higher specificity and sensitivity (94.5% and 100% respectively) while antibodies measured by $PRNT_{50}$ have acceptable sensitivity of 91.1% but suffer a specificity of 72.9% [53]. There is a paucity of evidence describing protective immunity beyond the mere presence or absence of functional antibodies. Work beyond this DPhil project is planned to assess immune responses using systems serology and cellular immunology[135 175]. This work will be complementary to the data reported in this chapter.

4.6. Conclusion

The results from this study support the use of YF vaccine doses as low as 500IU/dose in adults. This adds to the needed confidence to use fractional doses where there are vaccine dose shortages. Currently, vaccine manufacturers often release vaccines with potencies >5000IU/dose[8]. There is a need for vaccine manufacturers to reconsider the minimum release

potency to allow for even lower release potencies. Such a change in packaging will drastically and readily increase the vaccines stock. This will however require amendments in the manufacturing processes, particularly the steps involving the bulk harvest to filling and lyophilisation. Additional quality assessment and revisions for the pre-qualifications may be required for this to be licensed. There is a need for policymakers to further explore the growing evidence on successes in the utilisation of reduced doses of YF vaccines to recommend alternative policies that allow for wider use of this dose sparing strategy.

The processes to introduce a change in vaccine policies are complex and have not been described in any detail in the literature. There is a specific need to explore the policy environment for the YF vaccine policies – processes and other considerations when a policy change is anticipated [210]. The next two chapters will focus on prospective policy analysis to forecast the actual processes and the role of policymakers, advocates, researchers and stakeholders to influence policy change. Chapter 5 of this thesis reviews the processes and elements that influence policy change in an attempt to understand how policy actors respond to and engage in policy processes and strategies to influence other policy actors and policy change processes. Chapter 6 reports on a stakeholder analysis that aims to further map out the YF vaccine policy stakeholders at the global and regional levels and examine their priorities, perceptions and interests and the processes, for a prospective formal vaccine policy change that allows wider use of fractional doses of the YF vaccine.

5. Introduction and change of vaccine policies in low- and middle-income countries; a systematised literature review.

5.1.Introduction

In the literature review chapter, I discussed the current vaccine policies that guide the use of YF vaccines including fractional doses. Since the invention of vaccines in the late 18th Century, vaccination policies have been developed to eradicate disease and/or create herd immunity in populations at risk. These vaccine policies are informed by the evidence generated in the field and often change to optimise public health benefits. In control strategies for YF for instance, vaccine policies have been adapted following new evidence to inform strategies such as the EYE strategy. YF vaccine policies have had a formal policy change, for example in 2013 when the WHO through SAGE removed the requirement of a ten-year booster[10], and an informal policy recommendation which was conditional; in 2016 the WHO recommended the use of fractional doses of YF vaccine during epidemics and where there are vaccine dose shortages[11]. However, the availability of evidence is not always followed by a change in policies and some changes in policies can use limited data depending on the urgency of a policy change such as with the recommendation to use fractional doses. The 3rd and the 4th chapters focus on the clinical and immunological evidence for the immunogenicity and safety of fractional doses and other reduced doses of the YF vaccine. In this chapter, I report on the systematised review which we conducted to identify the role of different elements, including the role of evidence.

Emerging and re-emerging viruses, such as the YF virus, are arguably the most important infectious agents of epidemic and pandemic potential globally. Diseases caused by these viruses pose a significant risk to global health and global security especially with advances in globalization and the associated ease of international travel [211-213]. A large burden of these viruses is in Low-and-Middle-Income-Countries (LMICs), where their emergence and re-

emergence is driven by a range of biological, socio-economic, ecological and environmental factors [214].

Vaccines are a critical tool for public health interventions and have previously been used to prevent, and even eradicate, diseases worldwide [215]. The use of vaccines is guided by policies at global, regional and national levels, including routine use in travel and Expanded Programs of Immunisation (EPI), in reactive and pre-emptive vaccination during outbreaks, epidemics and pandemics, and preventive campaigns [216]. Vaccine policies are complex and heavily contextual especially for emerging and re-emerging diseases in LMICs where there are varying and often indeterminate resources for control of outbreaks, epidemics and pandemics[216 217].

Due to the global threat, these diseases pose, strategies for disease control - including the introduction of new vaccines and changes in existing vaccine policies - are often made at the global and/or regional levels and subsequently adapted by countries[216]. The WHO has a normative role in developing guidance (policies) for vaccines and immunisations; introducing new vaccines in developing countries and adapting vaccine policies to emerging evidence to optimise the public health impact of the vaccine. These roles and mandates are limited by many factors. The heuristic stages framework for public policies outlines six stages in the policymaking process: agenda-setting, policy formulation, policy legitimization, policy implementation, post-implementation evaluation and policy maintenance [217 218]. There is a formulaic decision-making process covering the three initial heuristic stages at the global level by WHO which is predominantly guided by scientific evidence [219]. However, the way in which even these stages of the process unfold in practice is often influenced by factors such as context, resource constraints, and power and politics [132].

In recent years new vaccine policies have been introduced and changes to existing vaccines have been rapidly implemented in an effort to control epidemics and pandemics caused by

agents such as yellow fever and Ebola [220] [221] [222]. Especially given the current COVID-19 pandemic, there is a critical need to map out vaccine policy processes in order to provide clear templates for action and identify areas for positive change to support timely disease control.

In this chapter, I present a systematised review of the literature on the introduction of new vaccine policies, and changes of existing vaccine policies, in LMICs. Focusing on global and regional levels and the early stages of the policymaking process, we aimed to map out the process for agenda-setting and policy formulation for a new vaccine policy or a change in an existing policy, and factors that influence these policy processes at regional and global levels for LMICs.

The research questions for this literature review were;

1. What is the process for agenda-setting and policy formulation for a new or a change vaccine policy in LMICs?
2. Who are the actors, their roles and their influences in these policy processes?
3. What are the other factors that influence the policy processes independent of the actors?
4. How do these processes unfold in theory compared to real-life?

5.2.Methods

5.2.1. Review protocol and registration

Given the dearth of published literature on this topic, we conducted a 'systematised review', an approach that models the systematic review process while allowing for the inclusion of commentaries, policy documents and expert perspectives and opinions [223]. We registered a

version of the review protocol with the University of York international prospective register of systematic reviews (PROSPERO: CRD42019134665).

5.2.2. Data Sources, Search Strategy and Selection of Papers

We systematically searched PubMed, Scopus, Web of Science and CINAHL without limitation for the year of publication. Before settling on a search strategy, we performed a preliminary search and piloted the study selection process. In the process of refining the search strategy, we attempted some search words on these databases. Initially, included all policies but had an overwhelming number of articles on other public policies including political policies that were not related to either health or vaccine policies. The primary focus was to understand the process of changing vaccine policies or shift in vaccine policies or interventions in LMICs during vaccine shortages – the initial search for change or shift in vaccine policies yielded hardly any results and this required the expansion of the search strategy to include processes for the introduction of new interventions, including new vaccine policies. The focus was also expanded to include standard processes under normal settings, including the process for decision making in routine immunisation such as the EPI. Although the motivation to conduct this review was to inform policies for the YF vaccine, the search strategy included all LMICs and was not limited to Africa and South America where YF is endemic. In the end, the search words include terms for ‘vaccine’ AND ‘health policies’ AND ‘introduction’ OR ‘change’ AND ‘interventions’ OR ‘strategies’ AND ‘LMICs’ including regional focuses that would include LMICs. The search was optimized by using a combination of variants of the search terms *(Table 5.1).* The final search results were saved in an EndNote library.

Articles were included if they focused on LMICs, agenda-setting and policy formulation processes in the introduction or change of vaccine policies, and factors influencing those processes.

Table 5. 1 Search terms for the systematised review

Variants combined by 'OR'	**Variants combined by 'AND'** → AND					
	Vaccin*	Health	Polic*	Introduc*	Interven*	LMIC
	Immuni*			New	Strategy	Low* income
				Shift	Routin*	Low* and middle income
				Change	EPI	Developing countr*
						Global
						Africa
						Asia
						Latin America
						Caribbean
						South America
						Tropic*

OR

***Output**: (*((((((vaccin* OR immuni*)) AND health) AND polic*) AND (Introduc* OR New OR Shift OR Change)) AND (Interven* OR Strategy OR Routin* OR EPI)) AND (LMIC OR Low* income OR Low* and middle income OR Developing countr* OR Global OR Africa OR Asia OR Latin America OR Caribbean OR South America OR Tropic*)

Following a set of inclusion and exclusion criteria (Table 5.2), we included all study designs, commentaries and policy documents. We excluded articles that focused on high-income countries (HICs), on policy legitimization, implementation and evaluation, and on policy processes for other products such as antibiotics, herbal medication and new drugs. We also excluded articles that focused on national-level policy processes and those evaluating cost-

effectiveness, economic feasibility and economic impact. Further, we excluded non-English articles and those that did not have full texts available. Some additional papers were identified after a manual search from the references of articles included for full-text review.

Table 5. 2 Inclusion and exclusion criteria

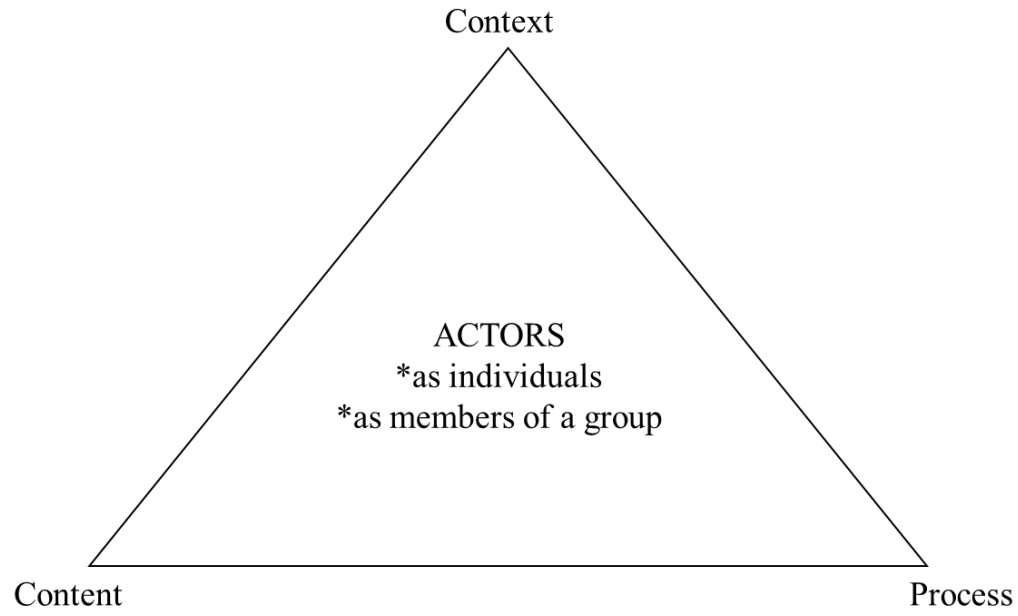
	Inclusion criteria	Exclusion criteria
Studies	Qualitative, Systematic reviews and mixed-method studies Quantitative studies Commentaries	Non-English articles Articles without full text
Participants and context	Policymakers Vaccine stakeholders Advisory groups and their members Technical working groups and their members. Actors in LMICs	Work in High-Income Countries
Focus/ phenomena of interest	Introduction of vaccine into policy, routine use or Expanded Programme for Immunization, change in strategy or shift in the use of existing vaccine regimens	Assessment of the economic impact Other products e.g. antibiotics, herbal medication, new drugs
Outcomes	Factors influencing change or introduction of vaccine policies Studies reporting on aspects that influence the development of vaccine policies.	

5.2.3. Data Extraction and Analysis

This review involved two other independent reviewers, who together my supervisors reviewed the search terms and search process. With one of the reviewers, I reviewed the articles by title and abstract and full text articles for inclusion and any disagreements were resolved by discussion by the 2nd reviewer. My supervisors provided oversight and guidance on the review process. We extracted data in a matrix format using an Excel™ workbook to facilitate sorting and filtering. Matrix columns included: author(s), year of publication, type of study and data collection methods, focus and objective of the article, geographical focus, the process(es) described, actors described, factors that influenced the policy process(es) described and key findings or conclusions from the articles. We used Walt and Gilson's policy triangle (Figure

5.1) as the main analytical framework [133 224]. This framework considers key elements of a given overall policy process; content, context and micro-processes; with policy actors at the centre of the triangle. The framework has been used extensively in both retrospective and prospective policy analysis. Data were extracted according to the elements of the policy triangle.

Figure 5. 1 Walt and Gilson's policy triangle framework



5.3.Results

5.3.1. Selection of literature

Our search, including papers up to February 2021, yielded 7031 documents. After removing 1100 duplicates, 5931 were screened using titles and abstracts (Figure 5.2). We excluded 5736 papers that did not fit our inclusion criteria (Table 5.2). Out of 195 potentially relevant articles, we removed six that either did not have the full text available or were non-English. Of the 189 full-text articles that were reviewed, 154 were excluded before data extraction. Data were extracted from 35 articles, over the course of which we excluded a further 14 articles with

reasons outlined in the matrix and included in table 5.4. We included 20 articles in this review (Table 5.3).

We included opinion articles and reviews by experts for which we did not assess for bias and did not score the articles using any of the scoring systems. This was due to the few eligible articles that were included in the review.

Figure 5. 2 Article screening and selection process.

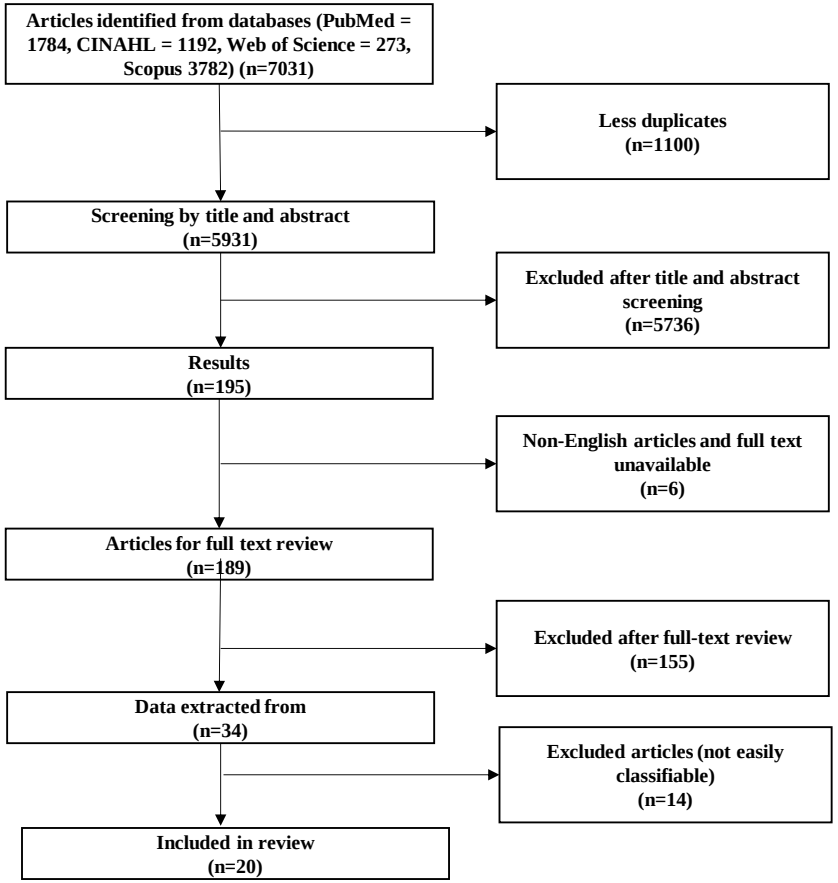


Table 5. 3 details of the studies included in this review

Author/Year	Title	The objective of the study; focus and process(es) described
Ranah Hajjeh, 2011[225]	Accelerating the introduction of new vaccines: barriers to introduction and lessons learned from the recent Haemophilus influenzae type b vaccine experience	Introducing new Hib vaccines, the Hib initiative; accelerating decision-making process to introduce vaccine decision making processes.
Jessica C. Shearer, et al., 2010[226]	Accelerating Policy Decisions to Adopt Haemophilus influenzae Type b Vaccine: A Global, Multivariable Analysis	
Leslie P Jamka, et al., 2019[227]	Accelerating Typhoid Conjugate Vaccine Introduction: What can be learnt from prior new vaccine introduction initiatives?	Typhoid conjugate vaccine by the Typhoid Accelerating Consortium (TyVAC); describes the iterative process of the strategy and experience of TyVAC.
World Health Organisation, 2017[228]	From Vaccine Development to Policy: A Brief Review of WHO Vaccine-Related Activities and Advisory Processes (2017	Vaccine development to global policy process at the WHO.
Phillipe Duclos, et al., 2013[229]	Establishing global policy recommendations: the role of Strategic Advisory Group of Experts on Immunisation	The process of development of immunisation policy recommendations at the global level and some of their impacts; the process leading up to the publications o the WHO position papers, which provide WHO recommendations on vaccine use.
Richard Mahoney, 2004[230]	Policy Analysis: An Essential Research Tool for Introduction of Vaccines in Developing Countries	Role of translational research in new vaccine development and introduction.
Giersing B.K., et al., 2017[231]	Challenges of vaccine presentation and delivery: How can we design vaccines to have optimal programmatic impact?	The process and considerations for a change in delivery method of pre-existing vaccines; a programmatic focus.
Milstien Julie, et al., 2010[232]	WHO policy development processes for a new vaccine: case study of malaria vaccines	Analysis of the WHO policy development process to predict its course for a malaria vaccine.
de Oliveira Lucia H. et al., 2013[233]	Systematic documentation of new vaccine introduction in selected countries of the Latin American Region	The process of new vaccine introduction in Latin America and the Caribbean with a focus on Rota Virus and Pneumococcal Conjugate Vaccine.
Bryson Maggie et al., 2010[234]	Global immunisation policy making process	Findings from a global survey on national immunization policy development and how it influences the policy process at the global level.

Kochhar S. et al., 2013[235]	Introducing new vaccines in developing countries	Challenges and opportunities to consider while introducing new vaccines in developing countries
Makinen Marty et al., 2012[236]	New vaccine adoption in lower-middle-income countries	Factors considered when of making new vaccine policies; constraints and enabling factors and the decision-making process in recent vaccine adoptions
Andrus JK, et al., 2011[237]	Challenges to building capacity for evidence based new vaccine policy in developing countries	The ProVac Initiative experience in accelerating the policy process in Latin America and the Caribbean.
Menning Lisa, et al., 2017[238]	Communications, Immunizations, and Polio vaccines: Lessons from the global perspective on generating will, informing decision-making and planning and engaging local support.	Describes the lessons from engaging local actors for global perspective support and the description of the 'decision package'.
de Quadros Ciro, 2013[239]	Historical Perspectives on new vaccine introduction in Latin America and the Caribbean	The historical considerations in decision-making process for new vaccine introduction in Latin America and the Caribbean
Gordon W Scott et al., 2012[240]	Introducing multiple vaccine in low-and lower-middle-income countries: issues, opportunities and challenges	Explores the interest, opportunities and challenges for the proactive planning of multiple vaccine introduction within a single planning cycle and the underlying processes for planning the introduction of new vaccines.
Alan Brooks and Antoinette Ba-Nguz, 2012[241]	Country Planning for health interventions under development: lessons from the malaria vaccine decision-making framework and implications for other new interventions.	Malaria vaccine planning in advance of availability of intervention; the evidence and processes that national experts identify as needing to take place for countries to decide.
Williams E.A. et al., 2016[242]	Anticipating policy considerations for a future HIV vaccine	The vaccination policies and strategies that policy makers involved in vaccine introductions would advise were a candidate HIV vaccine to become available
Piso B. and Wild C., 2009[243]	Decision Support in Vaccination Policies	Vaccine policy decision making summarised in a 7-step framework and the factors considered for the introduction
Jon Andrus and Damian Walker, 2013[244]	Evidence base for new vaccine introduction in Latin America and the Caribbean	The evidence and processes for decision-making for new vaccines introduction n LAC

155 articles were excluded after a full-text review. The decision to exclude these articles was guided by the inclusion and exclusion criteria. Some articles (n=14) however were not easily classifiable and the decision to exclude was made in discussion with my supervisors and two independent scientists who had prior experience in the systematic review process and policy analysis. These have been described in table 5.4. Although some of the articles had references that were helpful to understand the process of policy development, they were excluded because they focused on a country-level decision-making process and the processes and functions of the National Immunisation Technical Advisory Groups (NITAGs). Some although included global and regional actors, they focused on strengthening the national capacity of decision making. Other articles had a regional and global focus but described the processes of policy implementation and policy evaluation. A few of the articles focused on the fiscal ability of some countries and funding models for vaccine programs. Although all these are important considerations to the successful policy implementation and provide important linkage with the global policy development processes. Articles were also excluded if they did not make reference to processes for introducing or changing vaccine policies.

Table 5. 4 Articles that were not easily classifiable based on the inclusion and exclusion criteria and the reason for exclusion.

Author/Year	Title	Reason for exclusion
DeRoeck Denise, 2004 [245]	The importance of engaging policymakers at the outset to guide research on and introduction of vaccines: the use of policymakers' surveys	Articles focus on country-level processes
Carsten Mantel and Susan Wang, 2012 [246]	The privilege and responsibility of having choices: decision-making for new vaccines in developing countries	
Ba-Nguz Antoinette et al., 2017 [247]	Role of National Immunization Technical Advisory Groups (NITAGs) in the introduction of inactivated Polio vaccine experience of the Indonesia and Uganda NITAGs	
Gallagher K.E. et al., 2018 [248]	Vaccine programme stakeholder perspectives on a hypothetical single-dose human papillomavirus (HPV) vaccine schedule in low and middle-income countries	
Mala Peter et al., 2015 [249]	Ideal and reality: do countries adopt and follow recommended procedures in comprehensive multiyear planning guidelines for national immunization programmes?	
Meinda Moree and Sarah Ewart, 2004 [250]	Policy challenges in Malaria vaccine introduction	
Burns E. Julianne et al., 2009 [251]	Descriptive analysis of immunizations policy decision making in the Americas.	
Shen K Angela et al., 2016 [252]	Country ownership and GAVI transition: comprehensive approaches to supporting new vaccine introduction	The article focuses on the financial considerations and funding programs for vaccination programs
Jauregui B. et al., 2018 [253]	ProVac global initiative: a vision shaped by ten years of supporting evidence-based policy decisions	The article focuses on strengthening national capacity of the decision-making process
Jauregui B. et al., 2011 [254]	Strengthening the technical capacity at country-level to make informed policy decisions on new vaccine introduction: lessons learned by PAHO's ProVac Initiative	
Batson Amie, 1998 [255]	Sustainable introduction of affordable new vaccines: the targeting strategy	The article does not describe the process of introducing or changing an intervention. The article did not describe the process of introducing or changing an intervention (such as vaccine policies).
Clemens John et al., 1996 [256]	Evaluating new vaccines for developing countries; efficacy or effectiveness	

Watson M, Faron de Goer E, 2016[257]	Are good intentions putting the vaccination ecosystem at risk	
Guignard et al., 2019[258]	Introducing new vaccines in LMICs: challenges and approaches	

5.3.2. Characteristics of the included articles

Most articles were based on empirical research, were global in scope and focused on the introduction of new vaccines (Table 5.5).

Table 5. 5 Characteristics of included articles

Characteristic	Description	No. of articles
Type of publication	Empirical research	13
	Commentary and expert reviews	6
	Policy Document	1
Geographic focus	Global	15
	Region*	5
	Central and South America	*4*
	Africa	*1*
	Asia	*2*
	Western Pacific	*1*
	Eastern Mediterranean	*1*
Policy process	Introduction of new policy	19
	Change of Policy	1

* Some papers mentioned multiple regions

5.3.3. An overview of actors, their roles and vaccine policy processes

A list of the key actors and their roles in introducing new vaccine policies and making changes to existing ones are presented in Table 5.6. The WHO is the leading institution providing guidance on vaccine policies globally, with six regional offices; in Africa (WHO AFRO), the Americas (Pan American Health Organization- PAHO), South-East Asia, Europe, Eastern Mediterranean (EMRO) and Western Pacific. WHO-AFRO, PAHO, EMRO and the South-East Asia regional offices serve the majority of LMICs [259]. The WHO working groups and their members cut across the global and regional levels with largely similar roles at the two levels.

Table 5. 6 Vaccine policy actors

Actor	Affiliation	Role of Actor
WHO [228 229 234]	SAGE	Coordinate the review of evidence and decision-making processes at the WHO. The group's output is often adapted as the 'vaccine policy'.
	Immunizations Vaccines and Biologicals	Provide recommendations for products to be considered by SAGE
	ECBS	Set norms and standards for product manufacturing, licensing and maintaining quality for vaccines.
	GAVCS	Conduct independent risk assessment pre-licensure and post-licensure and provide scientific advice to WHO on vaccine safety issues of global and regional concern
	IPAC	Makes recommendations on programmatic suitability of vaccine products that are near licensure or post-licensure to improve efficiency and public health benefits
	IVIR-AC	Takes up research at early clinical stages for vaccines considering the burden of disease and potential economic impact of the immunization programs to minimize barriers of implementing potential policies and improve economic feasibility for the vaccine products.
	PDVAC	Provides strategic advice to WHO on vaccines in phase 2 or earlier clinical evaluation for new or second-generation vaccines of diseases with significant burden in LMICs.
	PSPQ-SC	Advises the WHO on the programmatic suitability of prequalified vaccines and the process for prequalification.
Global Health Initiatives, Institutes and other initiatives, consortia and networks	GIVS[229]	Provides a framework for the increase and sustaining high vaccine coverage and integrating immunisation programs with other health services.
	IVI[230]	The institute's mandate is to advise on neglected diseases, their vaccine development and introduction in LMICs
	STAG and MVI[232]	To develop Malaria vaccine policy development and to identify the data and the timing required for a WHO policy position on malaria vaccines.
	Hib initiative[225]	Accelerate evidence -based decisions to introduce Hib vaccines through research, communications and advocacy and coordination among all stakeholders
	TyVAC[227]	To develop a strategy for successful vaccine introduction in order to reduce the burden of typhoid by accelerating vaccine introductions.
	UNICEF[233 236 238]	Provide support such as pooled procurement mechanisms for GAVI LMICs.

	CDC[238 239 243]	Provide general global recommendations on immunization strategies
	PATH[230 241]	Conducts research and assessment of opportunities, barriers and programmatic considerations for recommendations to introduce vaccines in LMICs.
	TFGH[238]	With other partners provide communication related activities to generate awareness and political commitments in the adopting countries and regions and to further support he national program communications and training
Regional groups	RITAGS and Members of regional WHO working groups[229 239 243]	Provide expert advisory support to PAHO and its member states on the technical aspects of the Expanded Program for Immunization. The regional groups also set the tone for vaccine introduction in the regions.
Donors and Funders [225-227 235]	BMGF, GAVI, USAID, World Bank, Rotary International, UKAID	Provide funding for vaccine and immunization programs in LMICs. The support also includes procurement and logistic support for the countries. The different funders have guidelines and conditions that often must be met by recipient countries.
Others and Individuals [230 233]	Vaccine Manufacturers [236]	Provide sufficient vaccines to cater for the global demand at a reasonable cost while maintaining their financial interests
	Drug Regulatory Authority[228 233]	Provide registration of the vaccine product and provides for a regulatory process including quality control procedures.
	Researchers, Advisors, EPI coordinators Political players	Provide epistemic support to other actors either independently or as part of the other organizations. Politicians provide for political good will to support certain policies.

Expert Committee on Biological Standardization (ECBS); Global Advisory Committee on Vaccine Safety (GAVCS); Immunization Practices Advisory Committee (IPAC); Immunization and Vaccine Related Implementation Research Advisory Committee (IVIR-AC); Product Development for Vaccines Advisory Committee (PDVAC) and Programmatic Suitability of Prequalified Vaccines Standing Committee (PSPQ-SC); Program for Appropriate Technologies in Health (PATH) ; Centres for Disease Control and Prevention (CDC); Bill and Melinda Gates Foundation (BMGF); The Fund for Global Health (TFGH); Regional Immunization Technical Advisory Group (RITAG); Expanded Programme of Immunization (EPI); Strategic Advisory Group of Experts (SAGE); United Nations Children's Fund (UNICEF); Global Immunization Vision and Strategy (GIVS); International Vaccine Institute (IVI); Global Alliance for Vaccines and Immunization (GAVI); Strategic and Technical Advisory Group (STAG); Malaria Vaccine Initiative (MVI)

Other key actors' roles include financing, coordinating, research and programmatic support, regulatory and quality control.

Global Process

Fifteen papers discussed different aspects of the global process. These papers describe a formulaic, almost generic, the global process for developing and introducing new vaccine policies and making changes to existing ones. This iterative process is led by the Strategic Advisory Group of Experts (SAGE), an independent body established by WHO as its principal advisory group for vaccines and immunizations. Its members are independent experts with significant experience and achievements in various fields related to immunization [227-229].

The agenda-setting process for vaccine policy introduction and change involves a general consensus on priority vaccine candidates or vaccine policies that require change [229]. The WHO and other actors, whose roles are outlined in Table 5.6, in a series of meetings identify diseases of global importance, then identify available vaccines or sanction development of vaccines against the disease(s) of interest and facilitate the development of the vaccines. The product (vaccine) is then registered by a functional Drug Regulatory Authority (DRA) [228]. Evidence on vaccine safety, efficacy, feasibility, and programmatic suitability is presented in a 'background paper' provided by the vaccine manufacturers or specific initiatives and/or networks and is crucial for policy formulation. The evidence is reviewed by SAGE's independent immunization working groups to inform the optimal use of the vaccine [243]. The output of this process is the SAGE recommendations to WHO, and are published as the vaccine position paper[229 232].

The position paper provides a framework and recommendations that can be adapted by Regional Immunization Technical Advisory Groups (RITAGs) and National Immunization

Technical Advisory Groups (NITAGs) for LMICs in order to take into account contextual factors such as epidemiological, economic and programmatic elements [229]. The WHO and partners such as vaccine-specific consortia, initiative and networks co-ordinate global advocacy and fundraising for the new vaccine interventions that are recommended for LMICs [241]. The outputs of these engagements are communicated in weekly epidemiological and regular situation reports [229].

Although there were limited data on the processes for changing an existing policy, policy changes go through similar stages as introducing a new vaccine policy. For vaccines that are already in use, findings from regular post-licensure evaluations are considered by SAGE. A significant finding may get on the agenda for discussion and existing policies may be updated to optimize public health benefits, such as with the yellow fever vaccine (see Box 5.1) [74 124].

Box 5.1: Yellow fever vaccine policy changes at the global level

Yellow fever vaccine has been used successfully for decades and is administered as a single-dose vaccine with ten-year boosters to maintain immunity. In 2013, the WHO's working group on yellow fever vaccine assessed the need for booster doses after evidence emerged that a single dose provided protection for longer than previously thought. SAGE through a vaccine position paper made a formal policy change recommending that boosters were not needed in the general population – a single vaccine dose provided life-long immunity.
In 2016 there was a massive and unprecedented outbreak in Angola and DRC with vaccine stock shortages where the emergency vaccine stock was depleted thrice. The WHO SAGE, after a review of available but limited data, recommended the use of reduced doses as a dose-sparing strategy in a vaccine position paper in 2016. This change was introduced by WHO as an emergency measure, despite the paucity of data on the effectiveness and efficacy of fractional doses of yellow fever vaccine in sub-Saharan African populations. Although this change was successful in controlling the epidemic, the WHO highlighted gaps in data and there have been ongoing efforts to address them[184 260 135].

Regional processes

All five articles that described vaccine policy processes at the regional level focused on the Americas region which is served by PAHO. There were no descriptions of processes at the African, South-East Asian, Eastern Mediterranean or Western Pacific regions. However, one article identified that global level processes are more harmonized than at regional levels

serving LMICs [234]. Regarding the vaccine policy processes undertaken by PAHO, it was revealed that although the processes are somewhat formulaic, they vary from vaccine to vaccine [233 235]. Processes are driven largely by the region's immunization priorities, set through regular meetings where all PAHO member countries, represented by their Ministers of Health, set public health priorities [237]. PAHO coordinates a plan of action aimed at facilitating the adoption of prioritised vaccine interventions. These processes are backed by a vaccine law ensuring funding by the member states [233 240] supported by PAHO's revolving funds [239].

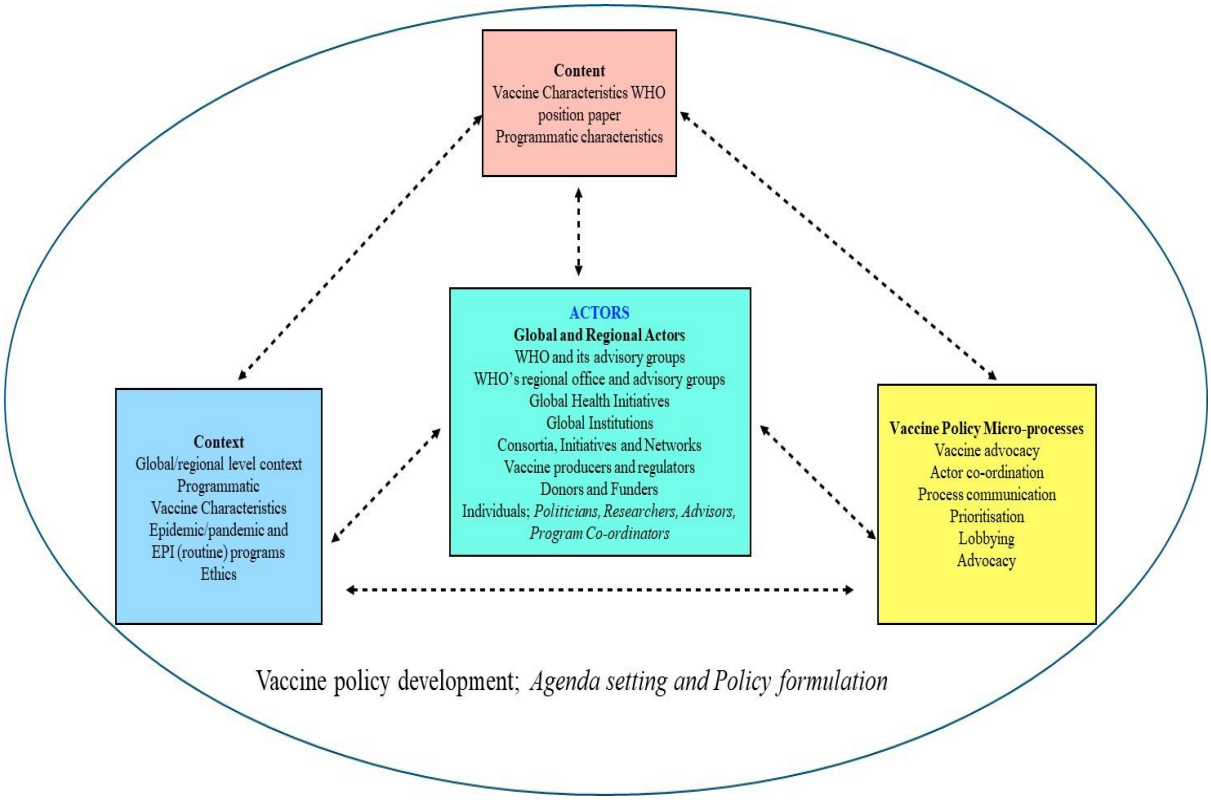
Although PAHO and the inter-agency committee coordinates these activities, member countries have the autonomy to decide whether or not to adopt the policies and whether or not to adapt to their contexts [239]. To support national-level processes, PAHO offers direct technical support to national-level decision-makers and national technical advisory groups. There are also opportunities to support LMIC national experts to learn from the United States and Canada by attending PAHO's High-Income Countries' advisory group meetings [237]. PAHO and its partners established the Promotion of evidence-based decision-making for the introduction of new Vaccines (ProVac) Network in 2004, following a request by its member countries to support them in integrating feasibility studies into the decision-making process to introduce new immunization policies [244]. The network has supported the development, review and adaptation of models and tools that support transparent evidence-based decision making in LMICs in the region [261].

5.3.4. Factors influencing new vaccine policy processes in practice

The processes described above presents an overview of the actors and processes involved in new vaccine policies, but in practice, these processes vary depending on content (such as the

nature of the actual or intended vaccine or policy change), the global and regional context (such as the epidemiological and routine vaccine situation), the micro-processes involved in different activities (such as communication and advocacy actions), and actor relations and co-ordination (Figure 5.3). In the following sections, I describe these elements in turn, as they emerge from the literature, but recognise that they are inter-related and mutually influencing, as illustrated in Box 5.2. As above, the focus is on the early stages of vaccine policy development: agenda-setting and policy formulation.

Figure 5. 3 Elements of vaccine policy development; agenda-setting and at global and regional levels for LMICs.



Content

Content refers to the form and focus of policies [262]. The content of the vaccine policies at the global level is established by high-quality evidence and strong recommendations through the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system [219] [228] [229]. The vaccine policy content can be influenced by the vaccine characteristics such as potency, method of administration and composition, and programmatic characteristics such as human and physical resources and anticipated availability of the vaccines to allow for policy adoption into programs. All these factors are considered when determining vaccination frequencies, population to vaccinate, the modes of vaccination and schedules for vaccination during policy formulation [231] [233] [235] [237] [238]. The content of vaccine policies is presented in the WHO's position paper at the global and adopted at regional and national levels [229].

Context

Context refers to circumstances and settings within which and for which the policies are made, including features from which actors derive power. Many contextual factors influence if and how agenda-setting and policy formulation for vaccine policy introduction or change unfolds in line with agenda-setting theory. Technical, operational and societal factors, referred to as the 'decision package' by Andrus et al, are important contextual considerations for policies at all levels [237]. Although these factors are primarily related to policy implementation, structural contexts such as existing programmatic infrastructure and economic sustainability (vaccine supply and funding) were reported to be carefully considered when setting the agenda and formulating policy [232] [243].

Piso and Wild, also discussed equity of access to vaccines, ethical and legal considerations as important factors related to implementation that are considered during agenda-setting and

policy formulation [240] [243]. For example, de Quadros argues that laws that mandate the governments to fund and support immunization programs in the Americas contribute significantly to the successful introduction of new vaccines in the region [239].

Power and relationships between actors

The actors and their roles have been outlined in Table 5.6 above. The key influences of actors on the way in which vaccine policy introduction or changes unfold are linked to their interests and the relationships between them [263].

The relationship between actors within the global level of WHO is quite clearly defined by the formulaic, almost generic, global process, as described above [228]. The relationships between actors within the different regional levels are less clearly described and are unique to each region. For instance, PAHO has been described as more active in shaping decision making and planning processes in the Americas, especially in South America[240], compared to the other regions.

The relationships between the actors at the global and regional levels are also significantly influenced by the coordinating agencies, bodies, initiatives and networks (formal and informal) which may differ from vaccine to vaccine [240] [241].

Political actors can offer critical support for the prioritisation of specific vaccines in countries and wider regions, feeding into regional and global processes. In some instances, political players may push a vaccine to the top of the agenda. de Oliveira et al. identified that, in fact, in Nicaragua, the introduction of the Rotavirus vaccine was initiated as a political decision during an outbreak of acute diarrhoea and later supported by technical aspects [233]. In Morocco, the first lady's foundation – which is focused on cancer awareness and management - was key in

putting the human papillomavirus (HPV) vaccine on the agenda for adoption and led to the inclusion of the vaccines in the national immunization plan [236].

In some instances, in combination with other factors, high-level political support may lead to the adoption of policies direct from the global level to the national level, skipping the regional level, as described by Mahoney (Box 5.2) [230].

Box 5.2 High-level political support to introduce Hepatitis B vaccine in Indonesia

In 1984, following the death of a close golfing friend of the President of Indonesia due to liver cancer secondary to Hepatitis B (HB) infection [context], the President asked the Minister of Health to 'do something about the disease' [actors' interest and relationships]. The Minister of Health approached PATH, funded by USAID, to identify the newly developed HB vaccine. The Minister was personally involved in reviewing and identifying the vaccine and in developing an immunization programme adopted from the existing WHO policy on HB vaccine [micro-processes]. The high-level political support [actors' interests and relationships] for this vaccine saw the prioritization of feasibility studies and capacity development [micro-processes] to produce the vaccine in the country for sustainable vaccine supply. Vaccine delivery policies were also formulated in an expedited manner [micro-processes] and received significant support from the community-level leadership [actors' interests and relationships]. Although the vaccine process to introduce the Hepatitis B vaccine in Indonesia was essential and led at the national level, it relied heavily on actors and processes outlined at the global level and benefited from high-level political support.

Micro processes

We also identified micro-processes that influence how the agenda-setting and policy formulation processes unfold in practice. Among these are prioritisation, process communication and coordination, procedural and regulatory processes, advocacy and lobbying. Policy process coordination and communication provides for the engagement of actors to prioritise, lobby and advocate for vaccine policies while fostering participation and meaningful policy dialogue among vaccine stakeholders during these iterative processes [227 234 225 238]. Regarding prioritisation, diseases of global and regional importance, for example, during an epidemic or a pandemic can be prioritised depending on the urgency of the vaccine policy (introduction or change) [229 237 244]. The yellow fever vaccine policy, for instance, has had

changes to respond to vaccine shortage and emerging data on antibody longevity (Box 5.1) [74] [220]. Duclos et al. described the importance of vaccine product regulatory and registration pathways in the agenda-setting and policy formulation process - the stepwise framework at the WHO is dependent on the registration of a vaccine product to recommend its use [229]. This is informed by the availability of data and strength of advocacy and the ability to lobby for specific vaccines, which is related to the actors' power. Policies are made at the global level for adoption by regions and countries, and there is intentional support for the successful adoption of the policies [241]. At the regional level, adoption of vaccine policies and positive experiences by some neighbouring countries motivate adoption by other countries in the region [225 226 236].

Agenda setting and policy formulation processes appear to be led and coordinated best by advisory groups or initiatives. For instance, consortia such as the Typhoid Vaccine Acceleration Consortium (TyVAC) were important in collating data and advocacy to accelerate the introduction of the Typhoid vaccine in LMICs [227]. In another example, the (Haemophilus Influenza type B) Hib Initiative – a consortium with John Hopkins Bloomberg School of Public Health, the London School of Hygiene and Tropical Medicine, WHO and CDC, established by GAVI under the Accelerated Vaccine Initiative was key in accelerating evidence-based decisions to introduce Hib vaccines, which led to a significant surge in Hib vaccine adoption [225]. However, Bryson et al. warn that a disease-specific advisory group may inadvertently act as an advocacy group and lobby for their disease of interest without considering others, and this might misrepresent the burden of disease in some cases [234].

5.4. Discussion

To our knowledge, this is the first review to map out the process for vaccine policy development at the global and regional levels for LMICs. We show that the WHO leads these processes at the global level through a broadly robust, transparent, iterative and formulaic process. The regional level processes are not as widely documented, although there is more information available for PAHO as regards processes in South America than for other regions in the rest of the Global South.

While global vaccine policy processes are transparent systematic and evidence-based, with actors' roles clearly defined, the review illustrates that the pathways for developing policy recommendations are complex, influenced by the numerous actors directly involved, the perceived urgency of the disease, and broader political interests (Figure 5.3). The introduction of new and changes to existing vaccine policies is not a linear process. The evidence-based decision making and the pathways including those for regulatory approval, policy recommendation and vaccine policy introduction and changes to the policies happen on a continuum in LMICs and are complex, requiring engagement with multiple, diverse stakeholders[244]. Actors' interests and their ability to advocate for change are shaped by their involvement in formal and informal networks, initiatives and consortia, which can play a role in pushing for relevant data, communicating priorities and needs, and coordinating and documenting views and information. For this reason, there is the potential importance of setting up formal and informal networks to facilitate policy diffusion within the policy environment[226]. Importantly, effective communication has the ability to generate and maintain policy dialogue while keeping the deferent stakeholders in the know of the progress of the policy processes[238]. Technical advisory groups play an important role at all levels in leading many processes, but informal networks and relationships potentially also play a critical role. The latter may be more

important at the regional compared to the global level, given the less formularised processes in place. Political decisions can have a significant influence on the prioritisation of policies.

Routinely, vaccines are evaluated in the post-licensure phase, safety, effectiveness, and impact data are regularly assessed, and SAGE revisits and updates existing policy as appropriate to optimize the public health benefit. In emergency settings, there may be an urgency to fast-track a policy process to allow interventions against the public health emergency. However, there exist uncertainties regarding the long-term consequences and future utilisation of these strategies. In such instances, even the most rational approach is limited by the franticness associated with the emergency. The existence of overwhelming global and regional support may overlook the national processes such as with Ebola, YF and Cholera[264].

It is important to include global, regional and local actors' representatives, vaccine developers, manufacturers, researchers, policy implementors – including politicians and other policymakers – during agenda-setting and policy formulation processes. Although this review did not focus on a formal power analysis, various helpful frameworks such as VeneKlasen and Miller's [265] expressions of power framework could be applied specifically in future analyses to examine different types (invisible power, hidden power and visible power) and forms of power ('power with', 'power within', 'power to' and the less democratic form – 'power over') held by different actors. In addition, it is important for researchers, vaccine advocates and activists to conduct a stakeholder analysis of specific vaccines in specific contexts to work out the type of evidence and data the policymakers are interested in, communication and actions that might be needed for the successful introduction of vaccine policies.

The global and regional level policies are only recommendations and their implementation ultimately depend on national-level decisions. It is not only important to consider and include national actors but also to consider national factors. The decision-making process regarding

new vaccine introduction at the national level does not always follow a systematic approach and often LMICs require technical support in the decision-making processes. Beyond the evidence available, the national-level decision-making processes often have to put into account international and local politics informed by the global organisation, including funders and regional bodies. Donor and technical agencies have a significant influence on the priorities through their financial and technical support. As such, global and/or regional driven strategies may be more decisive with relatively better support for implementations. Vaccine policies may be initiated by local or international politics and later supported by the technical aspect of the policies.

Programmatic, ethical and legal considerations – as part of context – are key to the successful introduction of a vaccine policy. Appropriate and timely vaccine policies are essential to control diseases of global health and global security importance, including disease eradication strategies. A globally coordinated and comprehensive approach introduction of new vaccine policies and changes in vaccine policies would be greatly applicable. The model described in the PAHO region where there is a closer collaboration of the regional and national stakeholders to determine the most appropriate regional priorities Country and regional stakeholders should collaborate closely in determining the most suitable and appropriate vaccines to be selected for introduction to maximize the effect of the immunization program.

Ultimately, although a systematised process provides a platform for vaccine policy development, each policy is unique. These findings have wider relevance to guide researchers and other stakeholders on important elements of the vaccine policy and factors that facilitate vaccine policy introduction or change.

5.5. Study strengths and limitations

The systematic approach employed in this review summarises and synthesises currently available evidence on the vaccine policy development process (vaccine agenda setting and policy formulation) at the global and regional levels for LMICs. The output of this study is a template for the vaccine policy development (new vaccine policies and change in existing vaccine policies) processes for LMICs at the global and regional levels. The template highlights the key actors and other elements such as context and content and how they interact in the process of policy development. The review identifies micro-processes (actor coordination, prioritisation, communication and advocacy) within the heuristic stages as key considerations in the success of each of the macro-processes. Due to its focus on the policy development stages (agenda-setting and policy formulation), the review provides a detailed account of these stages.

This study is limited to vaccine policy processes at the global and regional levels for LMICs and does not discuss the processes at national and sub-national levels or processes in high-income countries. The country-level vaccine policy processes are important considerations but in the context of public health emergencies, the availability of a global or regional level recommendation accelerates and, in some instances, might bypass the national level decision-making mechanisms. In addition, the study includes policy documents and expert opinion articles for which are often subjective and difficult to assess for bias.

In the next chapter, I will conduct a stakeholder analysis specific seeking to understand the interests, perceptions, position, attitude and role of these actors in the prospective policy for wider use of fractional doses. The key stakeholders have been identified from this review. I will explore in detail the role of evidence we have generated in the vaccine policy development process.

6. Priorities, perspectives and processes for a prospective vaccine policy change for wider use of fractional doses of the 17D YF vaccines: a stakeholder analysis

6.1. Introduction

In the last chapter, I reported on a systematised review of the introduction and change of general vaccine policies. This chapter presents the qualitative policy analysis I designed on the potential for a prospective policy change at the global and regional levels to support the wider use of reduced doses of YF vaccine in LMICs. In the background, I discuss the current and potential use of fractional doses of YF vaccines, the vaccine policy development processes at regional and global levels for LMICs, and the key stakeholders involved. I then present the stakeholder analysis (i.e. methods) I used to explore key stakeholders' priorities, perceptions, and interests, as well as opportunities and challenges for policy change. This is followed by the synthesised findings of the stakeholder analysis, and finally by a discussion of these findings.

6.1.1. Background

As already discussed in detail in chapter 2 and chapter 5, vaccination has potentially huge value in promoting personal and societal health. For instance, the full implementation of the WHO's Global Vaccine Action Plan (2011-2020) using existing vaccines and vaccination strategies would see over 2.5 million lives saved every year [266]. Vaccination strategies are particularly important in helping control epidemic-prone vaccine-preventable diseases (VPDs), which constitute public health emergencies that often trigger humanitarian crises and disruption to health systems[267 268]. Specifically for YF, the implementation of the EYE strategy would see about 1.4 billion people vaccinated by 2026 in forty countries to establish and maintain a high

level of immunity among the populations at risk[116]. However, achieving this target requires that more vaccines are produced in ten years than have been available since the vaccine was made[269].

In chapters three and four, I presented and discussed evidence from randomised clinical trials in East Africa on the use of reduced doses of the 17D YF vaccines in different populations. I argued that reduced doses of the YF vaccines can be used with confidence in populations living in YF endemic regions and that they are safe and efficacious in HIV-noninfected and HIV-infected individuals with CD4 levels >200cells/ml. The current evidence shows that reduced doses (as low as 500IU/dose) are as safe and immunogenic as the standard doses. Some countries have opted for the off-label use of fractional doses in responding to outbreaks and a few countries have considered using fractional doses outside of emergency situations[5 131]. Use of fractional doses within the context of EYE will significantly increase the vaccination rates. However, the role of fractional doses is not clearly outlined within the strategy[116].

To allow for utilisation of the dose-sparing strategy within the EYE and other contexts, it is important to consider how to include the use of fractional doses in the strategy. This process is heavily dependent on stakeholders' characteristics and their influence in the decision-making process in relation to associated policies[251 270]. In this study, I adopted a definition of stakeholders as actors (persons or organisations) with an interest and influence in YF vaccine policies. I also considered the five stages of the vaccine policy-making process; agenda setting, policy formulation, adoption, implementation and post-implementation evaluation [271]. As described in chapter five, although conceptualised sequentially, these stages are iterative and non-linear [272]. In the policy analysis work for this DPhil I focus on the initial stages of policy development at the global and regional levels; agenda-setting and policy formulation – the factors and elements that inform these processes, including considerations related to the other stages of policy processes such as implementation.

One of the findings from the previous chapter is that the processes to introduce a new vaccine policy or to change a certain policy follow similar stages. Agenda setting refers to the stage where policy issues that would otherwise be ignored are sifted and emerge for attention by stakeholders involved in policy-related decision-making[273]. During this stage, actors identify a problem by collecting evidence to describe the magnitude of the problem to present to policymakers. Kingdon's multiple streams theory describes three semi-independent streams (political, problems and policy events) that flow together across realms and come together in order for a policy to emerge or to change[274]. This public policy theory has been used extensively to understand health policies including vaccine policies. The definition of the streams has been defined in various ways in different contexts over the years[275]. For this study, the problem stream refers to policy problems in society that potentially require attention, such as the vaccine supply shortage and increased disease outbreaks; the policy stream pertains to the many potential policy solutions that are defined by communities of policymakers, experts and lobby groups, such as the use of fractional doses, and the politics stream refers to factors such as epidemics and pandemics that may have elements of political influence. Political influence may refer to the global policy environment which is shaped in large measure by actor power and by how different actors relate. A policy window is a point where the three streams converge to get a certain issue onto the agenda[274]. Policy formulation is a stage where options for policy are considered as possible solutions to a certain issue in the agenda. It involves the determination of the impact and role of existing policies and the potential for alternative or additional policies. During this stage, stakeholders' advocacy strategies and power can strongly influence the direction of the policy.

Vaccine policy processes as with other public policy processes are highly dependent on stakeholders' interests, position and power that may differ from policy to policy. Actor power has been described in the literature as a central element that drives policy processes towards

changing or introducing new health policies[210]. Elster's framework is one approach to understand how the actions and decisions of actors are influenced by actor-specific factors such as rationality, beliefs, interest and social norms to fulfil public expectations[276]. Although, the set of human motivations can be classified in an indefinite number of ways, their roles, power and leverage, concerns and perceived challenges are based on their experiences[277].

As described in chapter 5, the uniqueness of each vaccine policy relates to the urgency of a policy decision, the burden of disease, the affected regions and populations, the vaccine characteristics and stakeholders' priorities, interests and ability to lobby for policies. As such, for each policy, it is important to understand stakeholders' roles, responsibilities, interests and priorities in decision-making processes at all the policy process stages. Prospective stakeholder analysis can be instrumental in anticipating policy processes and direction, and supporting designing policy changes in ways that address stakeholders' concerns [278] [279]. Although evidenced-based policy decisions underpin good policies, stakeholder analysis can therefore be an additional layer that can help to ensure that available evidence is taken up by decision makers[280]. In this chapter, I report findings of a stakeholder analysis, described by Varvasovszky and Brugha as an approach for generating knowledge about actors to understand their perceptions, intentions, relations and interests while assessing their influence on decision-making processes[278].

6.2.Methods

In this policy analysis, I employed qualitative methods of data collection and analysis. The questions and foci were informed by the findings of the systematised review of published literature reported in the previous chapter, which did not focus on YF specifically. I conducted in-depth interviews with stakeholders involved in YF vaccine policy decisions to explore their perspectives on the use of fractional doses of the 17D YF vaccines. The scope of the analysis

was limited to vaccine policy development processes for LMICs at the global and regional levels. As with the literature review, I adapted the WHO's definition of health policy and defined vaccine policies as decisions, guidance and recommendations by institutions and/or organisations for vaccination plans, interventions, actions and strategies to achieve specific goals for public health benefit towards longevity and increasing life years without disability [210]. Data were collected through document review and individual semi-structured interviews.

6.2.1. Literature review

I reviewed published articles and policy documents for the introduction and change of vaccine policies in LMICs to identify stakeholders, their roles, power and other factors that influence vaccine policy processes (as reported in the previous chapter). The literature review was complemented by key informant interviews which is a qualitative research method that involves interviewing a selected group of experts on a certain subject, to gather information and ideas, including on problems and possible solutions. I chose to conduct individual semi-structured interviews as they have been described as ideal to understand underlying motivations and attitudes towards a certain issue while providing flexibility to explore new ideas and solutions from highly knowledgeable stakeholders [281].

6.2.2. In-depth interviews

I aimed to interview key stakeholders and their representatives as identified through the systematised review (table 5.6) and brainstorming with my supervisors. During the interviews, initial key informants identified and referred to other potential stakeholders involved in vaccine policy development processes in line with the 'snowball sampling' approach[282]. I developed an interview guide (appendix 6) that sought to understand stakeholders' priorities, perceptions,

interests, opportunities and challenges for a prospective policy change. The guide was adapted over the course of interviews as new issues were identified.

In the semi-structured interviews, I discussed with the interviewees actor related factors (actor power and leverage, role, policy position and attitudes, relationships, concerns and perceived challenges), vaccine policy contexts and content. I further sought to understand how these elements influence the decision-making process and the direction of the policy process to formally recommend the wider use of fractional doses of the YF vaccines.

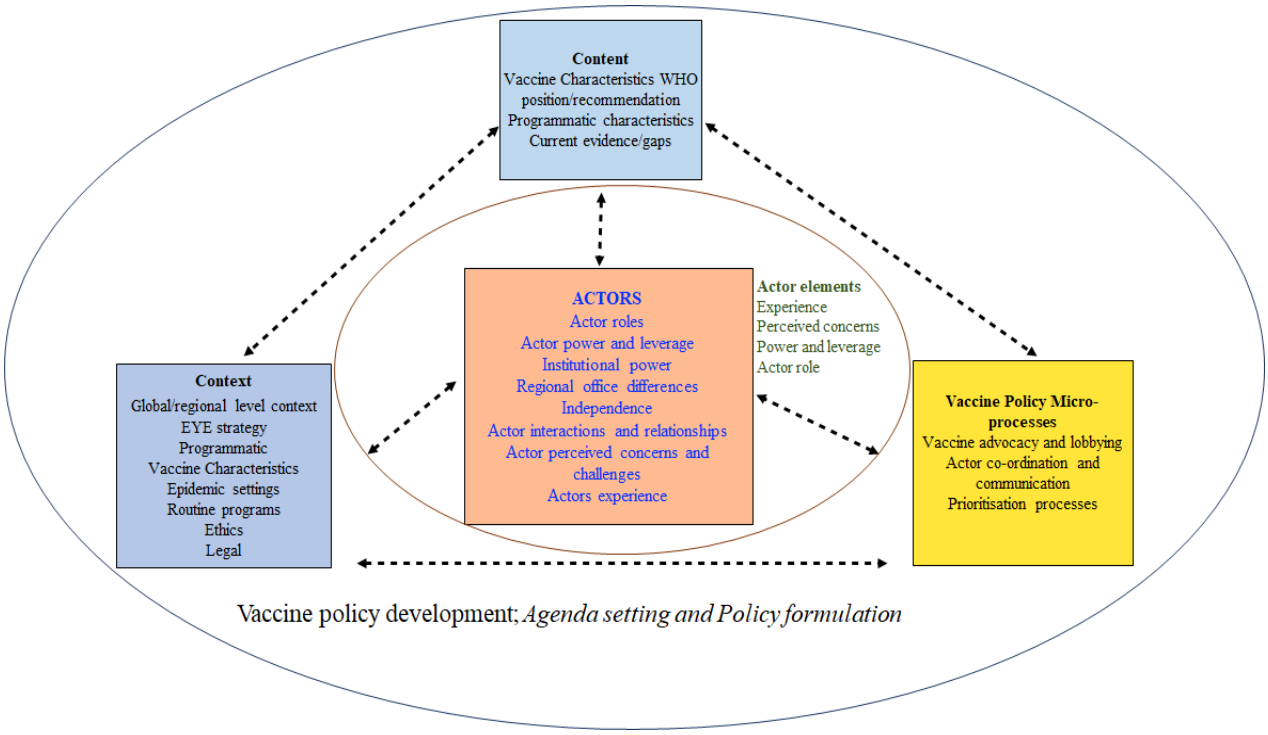
Before the interviews, each participant reviewed and provided informed consent either by written consent or by word of mouth (which was recorded during the interview). The interviews were conducted in English virtually on Microsoft Teams or Zoom and recorded. The recordings were then transcribed verbatim at the KEMRI-Wellcome Trust's Health Systems and Research Ethics Department by the transcription team. All identifiers in the audio recordings were removed during transcription and replaced with codes. The data were then stored on a secure password-protected laptop under the KEMRI-Wellcome Trust Research Programme regulations as part of the NIFTY project database[135].

6.2.3. Analysis

Data analysis was conducted in NVIVO 1.5, a qualitative data analysis computer software package used to organise, analyse and find insights in unstructured or qualitative data, and complemented by Microsoft Word and Excel workbooks. I used a framework analysis and thematic synthesis approach[283]. In the familiarisation stage, for each of the interviews, I read the interview transcripts as I checked through for the accuracy of the transcriptions. I coded the transcripts inductively line by line on NVivo based on the talking points of the interview guide which was based on several frameworks that evolved from the frameworks described from the systematised review; Walt and Gilson's policy triangle, heuristic stages of policy processes

(where the focus for this study was agenda-setting and policy formulation), Kingdon's multiple streams theory and Elster's framework. Themes and corresponding subthemes were generated based on this conceptual framework (see figure 6.1)

Figure 6. 1 Conceptual framework



Due to the importance of actor power in policy processes, I drew in my questions and analysis of responses on VeneKlasen and Miller's theory that distinguishes several different dimensions of power; 'power over', 'power to', 'power with' and 'power within'[265]. 'Power over' relates to the influence of actors with more power to affect the actions of people with less power. 'Power to' is the unique ability and potential for actors to shape the policies towards an objective. 'Power with' refers to mutual support and collaboration by actors to promote equitable relations. 'Power within' refers to the self-knowledge and awareness of self-worth. I also drew on Arts and Tatenhove's[284] three forms of power; relational power, dispositional power and structural power[285]. Relational power refers to the dynamics and interactions among

actors, the resources they have access to and outcomes with a premise that there always exists a form of dominance in every relationship[284]. Dispositional power is the shaping of power by institutional rules and regulations and resources[285]. Art and Tatenhove argue that the authority and control of resources is a source of actor legitimacy[284]. Structural power on the other hand refers to the societal structures that give individuals, institutions and organisations domination and legitimisation[284].

6.3. Results

Twenty stakeholders were invited via email and telephone to participate in the study, 15 responded and I was able to complete nine key informant interviews (see table 6.1). 6 participants who responded were not interviewed because they were unavailable due to work related to the COVID-19 pandemic response. The interviews were 25 minutes to 85 minutes long with a mean of 45 minutes per interview.

Table 6. 1 Summary of stakeholders (actors) who were invited to interview and those that were interviewed

Stakeholders invited to interview	
Stakeholder/actor	**Comment**
Vaccinologist and researcher	Interviewed as YFP 001
Research funder	Interviewed as YFP 002
Epidemiologist at Epicentre	Interviewed as YFP 003
Emergency response expert MSF, Epicentre and WHO	Interviewed as YFP 004
Epidemiologist MSF and WHO	Interviewed as YFP 005
WHO SAGE member and research and immunisation program funder	Interviewed as YFP 006
Clinical epidemiologist	Interviewed as YFP 007
Media/science and policy communication	Interviewed as YFP 008
YF expert, WHO and EYE Advisor	Interviewed as YFP 009
Member of RITAG (AFRO)	Responded but did not find time to attend the interview

Research and Vaccination Programs funder	Responded but did not find time to attend the interview
Member RITAG (AFRO)	Responded but did not find time to attend the interview
Vaccination Programs funder	Responded but did not find time to attend the interview
Vaccine production and supply	Responded but did not want to have the comments on record
Vaccine production and supply	Responded but did not want to have the comments on record
Division of Emergency Preparedness and Response (AFRO)	Did not respond
Programmatic Evaluation of Fractional doses (PATH)	Did not respond
The African Health Observatory Platform on Health Systems & Policies	Did not respond
Africa Vaccine Manufacturing Initiative	Did not respond
Africa Medicine Agency and Vaccine Regulation in Africa and Regulatory Forum	Did not respond

In this section, I present the findings from the interviews focusing on each of the main areas of the conceptual framework in turn. Given that the actors are in the centre of the conceptual framework I begin with this area. I report key stakeholder (actor) characteristics and how actors utilise them in order to achieve a result of interest. I also report on their concerns and challenges they anticipate with fractional dose policies, and their positions with regards to a policy change to allow wider use of fractional doses. I then summarise the stakeholders' understanding of the content of the vaccine policy on the use of fractional doses of YF vaccines and elements that influence YF vaccine policy content including vaccine dosing, vaccine manufacturing process, vaccine stability, and evidence. This is followed by findings on different vaccine policy contexts and the micro-processes of vaccine policy change.

6.3.1. Actors, their roles, power and leverage

A variety of vaccine policy actors were identified at the global, regional and national levels for the YF vaccines. Their roles, power and interactions were described as important in understanding the vaccine policy processes and how policies navigate the highly political environment.

The WHO and its agencies

The WHO's authority in vaccine policy recommendations and guidance was confirmed by interviewees for the YF vaccine policies including the International Health Regulations (IHR)[9]. It was added that the WHO should ideally update these regulations at least every 5 years in a vaccine position paper published on their website. The WHO and its agencies at the global level or through their regional offices (PAHO for South America and AFRO for Africa) provide the YF vaccine policy guidance for countries to adopt. Country departments of health or their equivalent are responsible to adopt and implement these vaccine policies using laid out national-level decision-making processes that vary from country to country. WHO's in-country offices support national decision-making processes and link up the support across different countries' implementation of the policies.

"…WHO provides this guidance … and it is up to the country to decide what they choose to implement…" YFP003.

"…WHO is an NGO and its role is to make recommendations, it does not make any regulations and it is up to countries to make the call on what regulations they put in place based on these recommendations…" YFP009

Most interviewees pointed out WHO's role to lead vaccination strategies aimed at achieving public health benefit strategies such as the EYE strategy for YF[116]. The interviewees indicated that PAHO and AFRO operate differently; PAHO operates more autonomously and operates at the regional level relatively independent of the global office compared to AFRO. Some interviewees, however, noted a shift in how AFRO works in regard to global health issues that affect the African region in a similar autonomous manner. They reported that there are ongoing efforts by AFRO to promote an Afrocentric vaccine ecosystem which includes building vaccine manufacturing capacity, and the development of regulatory frameworks through the African Vaccine Regulatory Forum (AVAREF) and the African Medicine Agency (AMA)[286].

Interviewees noted that although this shift was initiated before the COVID-19 pandemic, vaccine access inequities for the African continent has necessitated expediting the operationalisation of this shift [286].

In the setting of a yellow fever epidemic, WHO's International Coordination Group (ICG) for YF vaccines manages the vaccine stock and provides vaccines to countries that need them for emergency response and in some instances where there are multiple outbreaks prioritise where the vaccines are needed more.

The WHO and its agencies have structural and dispositional power on vaccine policies primarily driven from the institutionalised mandate within which the vaccine policy processes are nested. The WHO has the mandate to provide strategies to address or deal with global health issues. This asserts the institutional legitimacy of the WHO, its bodies and agencies. For example, the ICG has the 'power to' review the vaccine needs during an epidemic and make a decision on the vaccine supply to nations and regions that are affected, although in practice the ICG has not denied vaccine supplies to any country experiencing an outbreak.

A weakness of the WHO highlighted by several interviewees is the relationship and levels of engagement with vaccine manufacturers. The relational power dynamics between the WHO and vaccine manufacturers is complex and relies on mutual goodwill. For use of fractional doses of the YF vaccine, there has been reluctance from some vaccine manufacturers to change production processes. The WHO has had less direct engagement with manufacturers and the pharmaceutical industry to minimise appearing biased.

"…I guess one of the limitations of WHO which I don't know if it's seen it as a strength because of the risk of bias but there is the level of engagement with manufacturers and pharmaceutical industry I think because of the risk they are concerned of appearing biased, means they probably have a bit more of a hands-off approach, less direct engagement…" YFP002.

The WHO's legitimacy and authority on vaccine policies however dominate the relationship with vaccine manufacturers. While vaccine manufacturers have the 'power to' decide whether they want to change how the YF 17D vaccine is manufactured or packaged, the WHO has the 'power to' overwrite the manufacturers' recommendation for off-label use of the manufacturer's product – the basis for the current utilisation of fractional doses for YF vaccines. Although the WHO and its agencies may have 'power over' vaccine manufacturers in this example, there are diplomatic efforts during decision-making processes to promote collaboration between the stakeholders. This is an example of VeneKlasen and Miller's 'power with' dimension.

"…it won't be a friendly conversation if the WHO starts to support fractional dosing of vaccines or off label use of vaccines, and there is a risk that the manufacturers can restrict supply because at the end of the day they hold the supply of the product. But I think the public relations aspect of it comes in and it comes down to the kind of political lobbying and this can call for corporate social responsibility that will, might then… sway the debate and allow…off label use…" YFP007.

The WHO has a role to convene experts to advise on vaccine policies. The independence of this expertise within WHO's advisory groups is seen as a source of trust as well as dispositional and structural power in WHO.

"…they are meant to do in terms of evidence that we help support generate and are meant to have an unbiased viewpoint by bringing in independent experts and to be able to assess that. So, it's not the WHO staff – it's the independent next person they bring in – some of whom we fund – and also so that gives them that power of being able to cite their independence and of expert opinion..." YFP002.

Nevertheless, even with recommendations by subject matter experts, policy decisions were described as political.

"…these decisions are largely political, and the politicians in policy are quite conservative in how they make decisions for policy especially outside the context of emergencies…" YFP009.

Other government-mandated and independent technical bodies

Advisory groups such as Regional Immunisation Technical Advisory Groups (RITAGs) and National Immunisation Technical Advisory Groups (NITAGs) are mandated by governments to contextualise the recommendations to their specific settings. Countries decide which policies to implement in their settings. Some countries such as Brazil who generate data often make 'independent' policy decisions based on the wealth of evidence generated in-country. Countries derive this structural power from their independence and sovereignty. This independence may, however, be limited by the disease burden and what other stakeholders who have other leverage such as funders see as a priority. In instances where a disease has significant global health risk, global partners have 'power over' LMICs to push for the implementation of certain policies that include interventions against the disease. LMICs in Africa, however, may have difficulties in recommending an intervention without the support of the WHO at the global level compared to LMICs in South America with stronger regional support.

"…am talking of our scenario really in Kenya, I think it would be difficult for them to do that [introduce a policy locally without WHO's explicit backing] because of public confidence and I think they are quite resistant to doing things outside of the recommendations because of the potential for backlash in the headlines in the papers you know. The government gives us less vaccine and allegations of misconduct in that way, I think potentially that situation is different in uh for example countries like in South America…" YFP007.

Researchers and funders

Researchers as key stakeholders were described as individuals or research institutions with expertise in a certain field. Their key role is to generate high-quality evidence to be available to policymakers to inform policy decisions. Regional and national research institutes such as labs are involved in the identification of epidemics. In the case of YF, Uganda Virus Research Institute (UVRI) and Institute Pasteur de Dakar (IPD) are the regional reference labs for YF in Africa. Researchers were also described to have a significant role in vaccine policy advocacy alongside formal associations of practitioners such as paediatricians' associations, public health practitioners' associations.

"… my sort of perspective in this is that everybody has a role and especially if you have a platform where you can advocate for particular positions of course based on the right information and not misinformation; although that's another issue. I think advocacy groups are really important to be they associations, formal associations from medics for instance or other sorts of public health practitioners, vaccinologists and so forth…" YFP001.

Researchers work synergistically with global partners, including funders and donors such as the US – Centres for Disease Control (CDC), Médecins Sans Frontières (MSF), Foreign, Commonwealth & Development Office (FCDO), The Wellcome Trust, GAVI, the vaccine alliance (GAVI) and in some instances countries such as Brazil to determine the types of research that need to be done. Funders and donors have dispositional power due to the ultimate choice of whom to fund and what agendas they fund. In vaccine policy development, some funders predominantly fund vaccine research while others fund vaccine programs and initiatives. Global agencies allied to and supporting WHO's mandate often get priority in funding. For research on fractional doses of YF vaccines, the Wellcome Trust (WT), FCDO and European and Developing Countries Clinical Trials Partnership (EDCTP) have been involved in identifying evidence gaps and funding evidence generation including funding for epidemic response to the WHO's Global Outbreak Alert and Response Network (GOARN), United Nations Children's Fund (UNICEF) and some national research institutes.

Some funders were described by the interviewees as being agnostic to whatever the evidence shows while others are seen as being didactic based on their priorities and relationships with other actors.

"…I think for sure they are being challenged and that's why we are looking at what evidence gaps there are and to reach out and support on the decision either way… The Wellcome Trust is agnostic though, to you know, whatever the evidence shows…" YFP002.

Some funders such as the FCDO have additional roles, due to their political incline to support countries and consequently have a better reach worldwide and work collaboratively with other funders such as the Wellcome Trust to reach and support regional and national level actors.

GAVI and funders such as Bill and Melinda Gates Foundation (BMGF) fund vaccine access. Currently, vaccines are supplied to most LMICs through GAVI and UNICEF. BMGF, a key donor to GAVI, subsequently have the 'power to' influence the decisions made by GAVI in regard to vaccine access. Some interviewees indicated that funders and donors can be didactic and often have 'power over' some policymakers especially in LMICs where vaccine access may be less than equitable.

Some actors that work within humanitarian settings have key roles in supporting emergency response logistics during crises. Actors such as MSF and IRC are involved in proposing focus for research and policies. Although such stakeholders have an invaluable role to support countries for example to apply for emergency vaccines from the ICG during disease outbreaks, they are limited in their mandate.

6.3.2. Actor concerns and challenges

Interviewees identified potential barriers in the policy development and anticipated policy implementation processes of fractional doses of the YF vaccines. The concerns and challenges were related to the WHO's definition of fractional doses, the policy decision-making process, nature and completeness of evidence, vaccine production and characteristics and practical challenges anticipated in fractionating vaccines (see table 6.2).

Table 6. 2 of potential barriers to utilising fractional doses of the YF vaccines

Element of concern or perceived challenge	Barrier	Recommendation
Definition of fractional dose	WHO's definition is based on a standard dose that varies depending on manufacturer and lot and therefore inconsistent in terms	A descriptive definition of fractional doses to a more precise 'reduced' dose recommendation.

	Uncertainty regarding minimum dose requirement and longevity of immunity using fractional doses	Studies to model longevity of immunity using fractional doses.
The vaccine policymaking process	Vaccine decision-making processes are heavily subjective and political.	More transparency on how actors engage to make decisions related to vaccine policies.
Nature and completeness of evidence	The evidence to use fractional doses beyond the current recommendation in emergency settings is incomplete.	More research is required to address the knowledge gaps identified by stakeholders to be able to legitimise policies related to fractional doses.
	There is contradicting evidence regarding the role of booster vaccination on longevity.	
	There is a significant waning of immunity in children by their fifth year of life.	
Impact of fractional doses on vaccine manufacturing processes	Concerns that vaccine production and vaccination activities have slowed down due to prioritisation of COVID-19 pandemic-related activities.	There should be efforts to refocus priorities on YF outbreaks and strategies to eliminate epidemics.
	Fractional dosing may require that manufacturers change how they produce the vaccine. This change is lengthy and expensive while it provides no financial motivation as the vaccine is relatively cheap and the global market share relatively small.	The most ideal utilisation of fractional doses would be firmer recommendations for off-label use of the vaccines.
Perception of fractional doses by consumers	The consumer stakeholders (vaccinators and vaccinees) may view the trend to reduce the vaccines (vaccine frequency and actual vaccine dose) in the recent changes in YF vaccine policies to remove the booster and to use fractional doses as dose rationing.	Community engagement to educate the stakeholders on the dose-sparing strategies.
Practical concerns	While fractional dosing in the context of vaccination campaigns (pre-emptive and reactive) are more practical, fractionating vaccines in the context of routine immunisation may lead to vaccine wastage.	Plans and strategies such as batching vaccinations at vaccination points would minimise vaccine wastage as a barrier to implementing the use of fractional doses.

6.3.3. Actor position and attitude

Table 6.3 indicates whether the interviewee supports the prospective policy conditionally or unconditionally and their reasons or the specific conditions. All interviewees supported a prospective policy to allow wider use of fractional doses, although some people's support was conditional, usually based on completeness of evidence. Several interviewees had different personal positions to the organisation or institution they represented. For instance, YFP002 indicated the institution's approach was agnostic while they personally had reservations regarding the actual dose of the vaccine.

Table 6. 3 Actor position and attitude

Interviewee	Position	Comments
YFP001	Supports unconditionally	The stakeholder is involved in evidence generation and feels that there is sufficient evidence to support the wider use of fractional doses. *"…the advantage is extending the supply, more regions can be vaccinated and if you actually demonstrate, have the data to support uh routine use, it means you can achieve, you know hopefully achieve more widespread coverage because you have a lot more vaccine available…"*
YFP002	Supports with conditions	The organisation represented supports based on the available evidence but has specific conditions for individual support. *"…the biggest surprise to me when I started doing yellow fever funding was that people don't really know what's in the vaccine vial, so really how do you assure that from one supplier to the next and if it's a one-fifth dose so whatever it could be that you are actually giving people enough vaccine so is there something here in the actual manufacturing of the vaccines themselves in the first place…"*
YFP003	Supports with conditions	Supports the use of fractional doses during campaigns and argues that the wider use in routine immunisation requires specific programmatic considerations to reduce vaccine wastage. *"…well for now I see more as an outbreak use when there is no enough this is the way to go you know but because we still have gaps, how long the protection will cover, for how long the people will be protected and also the routine side I see the problem on the wastage of the vaccine…"*
YFP004	Supports unconditionally	Support is based on the review of current data; the interviewee argues that the current data is sufficient to make policy changes to allow wider use of fractional doses. *"…we miss little to be able actually to use this in the EYE strategy, so in the elimination strategy, during the mass campaigns, because I mean we know already its effective, we know it's going to save money, it will allow to cover more people, there is no change needed in terms of cold chain actually you will need less cold chain because you will add more doses with less vials…"*

YFP005	Supports with conditions	Supports the use of fractional doses as long it does not change the manufacturing process of the vaccines. *"…the places where fractional dosing as a strategy would tend to work, are ones I think where you don't put in question the production of it, and you say I am proposing this to you as a measure that can be put in place in the event of lack of stock or emergency… …… you are more likely to be successful in the campaign environment than would be in the routine environment…"*
YFP006	Supports unconditionally	Support based on the current evidence which although incomplete is sufficient to recommend wider s of fractional doses in the context of EYE. *"… if you can demonstrate comparable immunogenicity you know over 6 months or a year post vaccination I don't see that there is a barrier but you know there are plenty of people that were questioning that, anyway so that would be a right time for actually have a revised policy, it's based on just the normal schedule of updates…"*
YFP007	Supports with conditions	Support dose sparing strategy as long the evidence supports their use. There are other advantages, for instance, economic benefits for other vaccines such as PCV by reduction of the cost of the vaccines. *"…and manufacturers are very resistant to people marking around with the products, so also it might lobby them to finally reduce the price, … apparently this happened with Hib vaccine so they just reduced the price of the product and then actually it becomes less important to give a fractional dose uh and that's also a win in some ways makes it more sustainable…"*
YFP008	Supports unconditionally	Supports the use of fractional doses and emphasises appropriate science and policy communication among stakeholders. *"…I think the evidence is there, but I think the bigger concern is more outbreaks I think WHO would use fractional doses, as a routine way to vaccinate because I mean if you eventually find out that the fractional doses at whatever level whether at a 1000 international units or lower then you know there's nothing that's stops people from using fractional doses routinely even for children who get routine vaccination for yellow fever and even people who travel…"*
YFP009	Supports unconditionally	Supports the use of fractional doses with emphasis on the redefinition and standardisation of the fractional doses to ensure that vaccine efficacy and confidence is maintained. *"…Fractional doses are definitely a solution as long as it is above 1000 IU/dose…my concern is that you may go too low and not get a good response. If somebody takes a fifth of the dose and leaves the ampoule out such that it loses viral particles in hot climates and not get full protection, then the problems of confidence in the vaccine starts…"*

6.3.4. Vaccine policy content

The interviewees acknowledged that the policy content was described in the WHO's position paper and the IHR guidance for a single life-long standard vaccine dose for routine

immunisation and outbreak response. Confirming my findings from the systematised review chapter, most interviewees indicated that often vaccine policy form and focus includes an overarching policy that may be adapted to different situations. The content of the vaccine policy defines the formality of the policies. The content of policies is in turn defined by evidence and the vaccine characteristics.

The formality of the current vaccine policy.

Although all the participants were clear on the current guidance on the use of fractional doses, there was no consensus among the interviewees regarding the formality of the current recommendations for use of fractional doses. They considered them either formal, informal or exceptions to policy. On the other hand, those who defined the recommendation as a formal policy argued that the recommendation followed the WHO's formal policy process and was published as a vaccine position paper.

"…so, there is a vaccine position paper specific to fractional dose yellow fever and I can share with you if you haven't seen that. But in another sense, it is a formal WHO policy. [To produce this position paper] It went through all the same mechanisms as a formal WHO policy. We actually didn't have like a formal SAGE working group, we had more of informal working group but anyway yes, it is official policy…" YFP006.

The current recommendations are silent on booster doses following vaccination with fractional doses. Interviewees who considered the recommendations of informal policies or exceptions to formal policies cited incompleteness of the evidence and the circumstances for which these recommendations were made. Some interviewees felt that due to the COVID-19 pandemic, the WHO has delayed a revision on the vaccine position paper based on the growing evidence to firmly recommend the use of fractional doses beyond epidemic control.

Completeness and quality of evidence and its role in defining policy content

Evidence was widely acknowledged as the basis of policy decisions. Researchers who were interviewed noted however that there is a lack of clarity on the level of evidence that is adequate to influence policy. The mere availability of evidence (however complete and straightforward) does not guarantee its utilisation in vaccine policy development. The evidence needs to be communicated effectively to policymakers and other advocacy groups who have 'power to' directly influence decision-making processes. The most significant evidence gap highlighted by interviewees relating to current YF vaccine policies was related to the longevity of protection in adults and children following vaccination with fractional doses.

Evidence was described as valuable to actors such as researchers, funders, donors and policymakers to prioritise and promote innovative and sustainable interventions through vaccine policies. Although there is general guidance on the quality of evidence from the WHO's GRADE scale[287], interviewees indicated that often the threshold of evidence may differ based on the urgency for intervention. It was thought that the threshold is relatively high when changing vaccine policies for routine use as compared to vaccine interventions in the setting of emergencies. For example, interviewees observed that it took decades to generate evidence on the longevity of immunity post-vaccination with standard doses to recommend the removal of the ten-year booster dose. On the other hand, during the YF outbreak in Angola and DRC in 2015, due to the urgency to increase vaccine supply, a recommendation to use fractional doses was made fast and based on limited evidence. In the same way, during the 2016 Ebola outbreak, the Ebola vaccine was recommended for use before the evidence on vaccine efficacy was complete.

Several interviewees commented that it is not possible to have complete evidence since that requires that there are no information gaps and as such, the term 'complete evidence' may be fallacious. They added that regardless of how straightforward or complete the evidence is for vaccine policies, the policies themselves need to be formalised for them to be legitimate. One

interviewee revealed that the role of evidence, and how it is perceived, has shifted in the recent past due to the involvement of the private sector in vaccine production. As a result, the focus is now more on the minimum evidence required to allow for regulatory authority approval as compared to older vaccine models such as YF where the evidence was assessed more thoroughly.

"…the role of the private sector is relatively recent in vaccine development and one of the consequences is that both the structure and design of clinical trials is for licensure – not clinical trials to generate knowledge which are very, very different. There are different priorities put into those [licensure trials] and the first priority is of course efficiency. How quickly can I [the manufacturer] get to the minimum data set needed in order to submit this to a regulatory authority for approval so I [the manufacturer] can put it out in the market…" YFP005.

How vaccine manufacturing process, dosing and other characteristics define policy content

The need for a dose-sparing strategy was identified in response to persistent vaccine shortage, which is related to a vaccine manufacturing process that is not readily scalable to respond to outbreaks. Interviewees described the manufacturing system for the YF vaccine as fragile and complicated by the low number (four) of UN-prequalified vaccine manufacturers producing the vaccine. This low number was attributed to low-profit margins. Fractional doses allow for an extension of global supply without necessarily changing the manufacturing process or the vaccines, and so costs are not reduced for manufacturers. It was also argued that there is no financial incentive for vaccine manufacturers to change the manufacturing process or reformulate how they manufacture the vaccines. The complexity of the process of WHO's prequalification and regulatory approval was identified as further grounds for the reluctance of vaccine manufacturers to make these changes. Lack of or limited evidence on the efficacy of lower doses was felt by several interviewees to have little to do with the reluctance to change manufacturing processes.

"…yeah there will be revision by the regulatory and they require a lot of data. It's a long process and it costs. And then we have to go to WHO prequalification as well for a final… so

it's a long process …and I don't know… how interested the manufacturers are on their label change unless somebody push them to do it…" YFP003

"…from a manufacturing standpoint, it's not a question of generating data, right, like they know all of this Derick, it's not like they don't know this, it's just that they are really isn't a clear incentive to change…" YFP005

When the interviewees were asked about the need for a new YF vaccine given new technologies[288 289], such as mRNA vaccines, most interviewees did not see the need to change the current vaccine. Previous failures with an attempt to develop new vaccines in the 1980s were cited as a reason for hesitancy in changing the current 17D vaccine.

"…Why do you need a new yellow fever vaccine? Only if its thermal stable, … what will be the characteristics of a better vaccine than what exist today? Is it a vaccine that can be taken in your pocket [that]… is stable [and] can be used out of cold chain? …" YFP004

A few interviewees however thought that there is a need to update the vaccine formulation to take advantage of the current technology for a possibly more efficient vaccine compared to the current method of YF vaccine production which has not changed much since the 1930s. Acknowledging the high cost of developing new vaccines, some interviewees who supported new vaccines noted that there is no guarantee that new vaccines would be feasible given the small global market for YF vaccines that is limited to LMICs.

"…I think we need more products on the market to keep manufacturers in check in a way…and I think modern ways of manufacturing vaccines are often more efficient…although there are potential Zika vaccines that we never produced because of the lack of profit because it was only in low income countries " YFP007

Interviewees acknowledged current evidence shows that a fractional dose ($1/5^{th}$) is non-inferior to the standard dose as released by vaccine manufacturers[8]. Also, these fractional doses (one-fifth) have been defined by WHO as pragmatic and used for other vaccines such as Measles, Polio and Rabies vaccines. Nevertheless, some interviewees proposed a redefinition of the fractional doses of YF vaccines to be more descriptive or precise based on the characteristics of the vaccine.

Other interviewees were keen to note a difference between YF and other vaccines is that the other vaccines have a minimum-maximum recommendation which makes standardisation of the fractional doses easier.

"… the difference with fractional doses of YF versus for example Measles vaccine is that Measles vaccines have a min-max and therefore can be standardised with relative confidence…" YFP009.

6.3.5. Vaccine policy context

All the interviewees noted that vaccine policies are contextualised to regional and national settings post-formulation and reiterated the importance of understanding the context within which and for which vaccine policies are made as an important consideration when developing vaccine policies. For YF vaccine policies they derived their views from experience with previous YF and other VPD epidemics, current epidemic control strategies, routine vaccination programs and programmatic characteristics, as described in turn.

Experience from previous epidemics

Interviewees drew from their experiences of the YF and Ebola outbreaks as well as the COVID-19 pandemic, for instance, when considering vaccine policies to respond to emergency settings. Drawing from the 2016 YF epidemic in DRC, several interviewees mapped out elements they considered important in policy responses to emergencies. Epidemiological information and disease dynamics were cited as most important at global, regional and national levels to identify the most at-risk populations. However, actors identified relationships with implementing actors as influencing regional and national implementation of responses. One interviewee compared favourable actor relationships and support in responding to a YF outbreak in DRC with less than ideal support in Nigeria:

"…discussing with the team in Nigeria, a country endemic for yellow fever, … we could not intervene as we did with DRC. We know the vaccine are much less than what is needed, … the

coverage is always very 'very' low and at that point we needed political goodwill to intervene…they were not interested…" YFP005

Depending on the disease burden and risk during an outbreak, vaccine policies can be fast-tracked even with limited evidence to allow for authorisation for emergency use or off-label use. Interviewees cited experience with Cholera in Haiti, Ebola in Liberia and YF in DRC. For instance, in 2016, the SAGE constituted an informal working group to recommend the use of fractional doses.

"…we actually didn't have like a formal SAGE working group, we had more of informal working group but … it is official policy. The caveat so, so you know it does highlight that it should be done only on the context of limited supply in the kind of outbreak setting and it did specify that it does not meet the IHR requirements…" YFP006

When outbreaks occur, most interviewees observed that there is always an urgency to respond with vaccinations as soon as an outbreak is confirmed. This was described for YF and Ebola. Often guidance for this response is provided by the WHO and its agencies such as the ICG who provide the vaccines in an expedited process that requires significant efforts and coordination by actors.

"…for the outbreak response there is the need to request the vaccine through the ICG in a formal request and there is this epidemiological information that the country needs to give - all managed by the ministry of health; so, the national authorities. Then the ICG provides the answer and then the doses are sent according to the needs. And most of the time, the choice needs to be made because there are not enough doses and there are moments where (because the seasonality of the yellow fever etc), there are many outbreaks going on in several countries. And then it's up to the experts in the ICG then to decide, and sometimes they may decide to give all the doses requested, or decide according to the epidemiological information to concentrate to one at risk area or more at-risk populations…" YFP004

Based on the experiences during epidemics and emergencies, disease control strategies such as the EYE strategy have been developed to control disease and avoid future epidemics.

Epidemic control strategies

Interviewees noted with concern that YF vaccinations have previously not been as included in catch-up campaigns as often seen with Measles and Polio. Most interviewees described the EYE strategy, an attempt to refocus the control measures into a single strategy with a more targeted implementation of the current vaccination programs, as the most important strategy towards the control of YF epidemics. However, all these strategies require sufficient and reliable vaccine supply. All interviewees felt that the use of fractional doses is the most ideal approach to assure supply.

Pre-emptive and preventive vaccinations were identified as strategies that may be important to eschew YF outbreaks and epidemics. Pre-emptive campaigns require that there is the capacity to predict YF outbreaks which were described as difficult given dynamics of the disease and evolving disease factors such as vector capacity and global warming.

While acknowledging the importance of the EYE strategy, a few interviewees raised concerns about the unnecessarily long ten-year implementation period. This length, and limited provision of fractional doses in the strategy, may have resulted from the limited data on the use of fractional doses when the EYE strategy was developed. Since then there is more confidence in the wider use of fractional doses.

"…the data that we are generating … could potentially support an alternate policy decision and I remember looking at the EYE strategy … two or four years ago and seeing the work plan over 10 years to catch up the population to at least 80% covering and wondering why do we have to do over 10 years? and that was related to the supply constraints … and ideally we would actually be doing that much faster and if we have the vaccine doses…" YFP006

Altogether, the participants in the interviews noted that although the emergency response plans are important during the setting of an outbreak, policies and public health interventions towards strengthening routine immunisations would provide long-term solutions towards the control of YF.

Routine immunisation programs

Vaccination of children at 9-12 months of age and travellers from non-endemic regions to endemic regions are the routine immunisation process (i.e. the formal, procedural and long-term strategy of immunisation) often utilised in endemic regions. Most interviewees indicated that routine immunisation policies often consider complete evidence and would be expected to take more time to be legitimised and implemented compared to policies responding to emergency and epidemic settings.

"…Because so take for example like the minimum dose component with the vaccine interaction or something like that, ok, you are talking like ten to fifteen years from now before that would even become a question …" YFP006.

To improve the vaccination coverage within WHO's recommendations, interviewees indicated that there may be a need to consider preventive campaigns, including of adults, as part of routine immunisation for YF vaccines.

Programmatic characteristics

All the interviewees acknowledged that programmatic characteristics – i.e. elements of a system put in place to implement scheduled vaccination plans – are currently set up for the standard dose vaccination which may cause some potential bottlenecks for using fractional doses. The most important was the operationalisation of diluting the vaccines to desired fractions. Most interviewees argued that pragmatic fractions delivered in similar syringes as standard doses would be most favoured and would be convenient programmatically as they would require minimal systemic changes and financial implications. This contrasted with diluting vaccines to more specific syringes with more precise lower doses which would require significant training of the vaccinators. A vaccine policy that is easy to plug into the current programmatic settings would be easier to advocate for in terms of implementation. These considerations would also allow for a more holistic programmatic package that also includes

other interventions such as Vitamin A supplementation, deworming and nutritional status evaluation.

6.3.6. Vaccine policy processes

As I described in the introduction and methods sections, the focus of this policy analysis was on vaccine policy development macro processes; agenda-setting and policy formulation. Most of the interviewees highlighted that it is difficult to think about these initial heuristic processes in isolation. The key informants also identified micro-processes such as prioritisation, engagement through advocacy and coordination of policy actors, and communication of evidence and prospective policy changes as important processes that influence the direction of a policy change.

Reiterating the importance of the evidence the interviewees indicated that *agenda-setting* and *vaccine policy formulation,* is heavily influenced by the available evidence. The current evidence on the use of fractional doses ideally is presented to actors who then propel it onto the agenda at vaccine policy development forums. The SAGE working group through the *'*SAGE process'[243] of vaccine policy development (discussed in detail in the previous chapter) was identified as the most significant stage from which policies that consider wider use of fractional doses are formulated. The SAGE process was however criticized for having the potential of being subjective and dependent on individual actors' interests. Additionally, the process of *adoption* of the policies to different contexts was also described as subjective. Tracking the changes made on the primary policies may be neither a straightforward nor a transparent process.

"…in practice every country, every region is different and so there's issues around contextualizing some of those guidelines to a regional or national level, and where that information is in terms of the contextualizing that information to a national level, this needs to be more accessible because that's where that information is not as readily accessible unless you know which people to contact…" YFP001

Advocacy and lobbying

Interviewees noted that mere availability of evidence, however complete, does not guarantee a policy change and that advocacy plays a significant role in bringing fractional dosing policies to the attention of powerful actors who may be resourceful when lobbying for them. The interviewees identified several actors as ideal advocates of these vaccine policies. Some felt that researchers and professional bodies are most suited to advocate for specific policies especially if the policies utilises the evidence generated by them. Although other interviewees argued that in the context of emergency settings such as the COVID-19 pandemic, the status of disease and evidence evolves rapidly, and researchers and other advocates may be hesitant to declare support to specific interventions until more comprehensive evidence is available.

"…researchers are kind of leaning onto advocacy and its risky because if things change then you've already mapped yourself out as having a prior position…for example all the modelling evidence with COVID… but in the scenario where you got clinical trial data, its less risky because you're [researcher] is just advocating for the use of your data …" YFP007

Some interviewees described the ability to advocate and lobby for vaccine policies as a key source of power and legitimacy. Further, some interviewees pointed that political lobbying may have the potential to push vaccine manufacturers to allow off-label use of their vaccines in place of changing production processes or changing vaccine labels both of which require regulatory engagements and approvals from various regulatory bodies at the global and regional levels for re-prequalification of the YF vaccines.

Prioritisation

Prioritisation was identified as a key process in agenda setting especially in LMICs where there are often competing priorities on global and public health issues. Continuous assessment of disease status and continuous engagement and coordination of policy actors at every level was identified as a potential way to allow for the right priority areas to be mapped out correctly.

When adopting vaccine policies to different contexts, the vaccine supply status shifts priorities in the context of a YF epidemic to use fractional doses where there are shortages. The process of prioritisation may also benefit from advocacy and lobbying. Countries such as Canada were noted to have adopted the use of fractional doses for routine immunisation of travellers. Most interviewees also pointed that elite policy decision-makers will often prioritise focus on other vaccines than on changing already successful YF vaccines.

"… they [an elite policy maker] have bigger problems looking for new vaccines to other diseases such as COVID, influenza, dengue… if you take YF vaccine you want to change this vaccine that has been successful over years… they have [an elite policy maker] have bigger problems to prioritise other vaccines and that is how the issue of YF will fall out of priority. Not because it is not important but because there are competing priorities…" YFP009.

Research funders also confirmed that the research area priorities keep shifting based on the current evidence or lack thereof.

"…this has shifted priorities in our epidemics work and we [funder] formed these groups called priority areas on vaccines, snake bites and a few others and this was meant to connect more with the policies or the lack of policies out there …" YFP003

The majority of the interviewees indicated that since fractional doses of YF vaccines address the most important bottleneck in containing epidemics (the vaccine supply) they should be prioritised in the context of the EYE strategy.

Actor coordination, engagement and communication

The vaccine policy processes and the direction of vaccine policies to recommend wider use of fractional doses were described by interviewees to be dependent on how actors are managed through coordination efforts, continuous engagement and communication. The EYE secretariat, for instance, often hold regular meetings with stakeholders and other partners to update them on the status of the strategy. Such engagements were described by most interviewees as an important element towards keeping stakeholders informed through continuous assessment of interventions proposed in the EYE. These forms of engagement were

considered the most significant platforms to coordinate actors and communicate new evidence to policymakers. Some interviewees argued that this form of evidence communication may involve elements of advocacy as actors are likely to support policies, they are involved in developing. Communication of new or prospective policy changes was also identified as an important element towards reducing hesitancy in implementers and the communities that consume the vaccine interventions. This was described as a continuous process and allows implementing partners and consumers to anticipate these prospective policy changes and may play a role in the legitimisation of the policies.

6.4. Discussion

To the best of my knowledge, this study is the first to conduct a prospective vaccine policy analysis on a potential policy change to recommend the wider use of fractional doses of YF vaccines. The interviewees involved in this study represented global and regional partners from different aspects of the vaccine policy environment. Most of the actors interviewed were noted to be involved in generating evidence to support the dose-sparing strategy.

The focus of this stakeholder analysis was vaccine policy development – the initial two of the heuristic stages of policy; agenda-setting and policy formulation as has been described at the global level[290 234]. During the analysis, we found that it was, however, difficult to think about these policies in isolation and more practical to consider the policy processes as naturally iterative. These initial processes of vaccine policy involve prioritisation and development of courses of action towards a policy change. The processes are heavily dependent on human elements to steer the direction of policies and decision-making process[291]. The role of stakeholder engagement at the onset of policy development for potential policy changes and research agenda development was found to be invaluable in determining and anticipating the direction of such vaccine policies[245].

Actor roles for YF vaccine policies were found to be similar to other vaccines as described in the previous chapter. WHO and its agencies provide YF vaccine policy guidance, ensure access and support adoption and implementation of the guidance through the regional and in-country offices[216 229]. For YF these roles include developing and leading the implementation of public health strategies such as the EYE[116]. WHO work collaboratively with independent stakeholders such as researchers, funders, governments, technical bodies and other agencies to implement such strategies. The interaction of these stakeholders is heavily political and dependent on the actors' power dynamics (summarised in table 6.4), their relationships, perceptions and interests – all of which are shaped over time by their experiences[292].

Table 6. 4 power of actors involved in YF vaccine policies

Actor/stakeholder	**Form of power***	**Source of power**
The WHO and its agencies	Structural	The institutionalised mandate for vaccine policy development.
	Dispositional power	
	'Power to'	Authority to make decisions on vaccine utilisation and strategies.
	'Power over'	Mandate to make decisions over other stakeholders such as the recommendation of off-label use of fractional doses of the YF vaccines.
	'Power with'	Collaborative decision-making processes in consultation with other stakeholders
Vaccine manufacturers	Relational power	The relationship between the vaccine manufacturers and other stakeholders
	'Power to'	Autonomy to decide whether to change how they produce and package the vaccines
	'Power with'	The collaboration with others towards the provision of adequate doses of the vaccines through
Nations /countries and their technical independent bodies	Structural power	The sovereignty of states gives them the independence to endorse and legitimise vaccine policies and strategies to implement in their countries.
	'Power to'	
Funders	Structural power	Financial authority to set priorities on vaccines and vaccination strategies to be funded which turn provides them with dominance over other actors due to financial resources.
	'Power over'	
	Dispositional power	

	'Power to'	
Advocacy groups	'Power to'	Autonomy to decide what vaccine policies and vaccination strategies to advocate and lobby for.
Researchers	'Power to'	Epistemic authority to advance and interpret evidence.
	'Power with'	Collaboration with funders and other policymakers to advance and utilise evidence

* VeneKlasen and Miller's [265] and Arts and Tatenhove's[284]

The findings from this study show overwhelming support for the use of fractional doses to respond to outbreaks. There is general support towards the wider use of fractional doses beyond the current recommendation – for routine immunisation and vaccination campaigns. These recommendations however require amendments to implement the dose-sparing strategy beyond the current recommendation[293 22]. There were some barriers identified for achieving such a change. The most important was related to the amount and completeness of evidence on the longevity of immune protection following vaccination with fractional doses. The majority of children vaccinated with standard doses in their first year of life currently fall below the threshold of protection by their 5th year of life. Although current evidence shows that the fractional doses induce similar immune response[130], there are concerns that the response is not robust enough in children and HIV-infected individuals [5 155 294].

Another important barrier is the inconsistency of WHO's definition of fractional doses – interviewees felt that since vaccine manufacturers release doses between 5000IU/dose to doses as high as 70,000IU/dose, the current WHO's recommendation to use a pragmatic fifth of the standard dose varies widely depending on the vaccine manufacturer and vaccine lot[136 178 269]. There are recommendations to change how the vaccine is manufactured and packaged to allow for standardisation of fractional doses across manufacturers and vaccine batches. However, there is a reluctance to change how the vaccine is manufactured due to the complex technicalities of changing the vaccine manufacturing processes and the quality assurance of the new product for prequalification[295]. Further, there is no apparent financial motivation for

vaccine manufacturers to make these changes in the manufacturing of the vaccines. Not only are the YF vaccines relatively cheap the global market share for YF vaccines is relatively small. The majority of actors suggest that the WHO should reconsider the minimum dose required for the YF vaccine. Although the WHO's current minimum recommendation is 1000 IU/dose, the study reported in chapter 4 of this DPhil found that 500IU/dose is sufficient to induce protective immunity. According to the actors involved in this study, the WHO should consider firmer recommendations for off-label use of the current vaccines as they are. It is unlikely that vaccine manufacturers will change how they produce and package the vaccines.

Further confirming findings from the previous chapter, there are procedural requirements to change the current YF vaccine policies to include the wider use of fractional doses [243]. These steps or processes are iterative and may be influenced by several factors such as evidence of global health importance of the disease, specifically the importance of disease epidemiology [296] [297] and subjective human elements that reflect individual priorities and experiences of key actors [277] [291]. The formality of the processes defines the formality of the recommendations; they can be considered formal, informal or exceptions to policies. Vaccine policies to use fractional doses were considered informal recommendations since they did not follow formal processes to change policy and were reactive to emergency response and were made based on limited evidence.

While the role of evidence is significant in changing policy, there are varying thresholds for policy depending on the urgency and context for which the policies are made[287]. Researchers continually seek to use their research to change policy to improve population outcomes. However, although evidence is required to make new policies or change existing policies, the availability of evidence does not always indicate the need for a policy change, regardless of how valuable a prospective policy may seem to those researchers. Additionally, there are concerns related to the subjectivity of policy decision-making processes in practice for YF

vaccine policies and practical considerations of implementing policies that allow wider use of fractional doses, specifically for routine vaccination programmes. Thus, the role of scientific evidence is at best supportive of vaccine policies. In a review of how science can shape policy, Julia Rosen argues that scientific evidence can be the basis for good policy but rarely defines it [298]. More evidence will increase confidence in the use of fractional doses and will be the basis of firmer recommendations.

The vaccine policy-evidence interface is highly subjective and requires effective engagement between actors who generate the evidence and those that make policy decisions. Engagement platforms provide for coordination and communication across these two sets of actors and provide grounds for the stakeholders to discuss the disease problems and explore through advocacy and lobbying potentially important vaccine policy (changes). Vaccine policy advocacy and lobbying refer to the processes of providing information and the intention to influence policy actors to support a new policy or a change in existing policy [299]. While lobbying more specifically refers to the intentional influence on actors, advocacy refers to the general education on a certain issue. Advocacy most commonly uses alternative sources of power to explore potentially shifting the dynamics of power. Some policy analysts have argued that all lobbying is advocacy but not all advocacy is lobbying [300]. As described by Matti et al., these interactions are made easier through proper coordination of the actors and continuous advocacy and lobbying for policies that support public health strategies [301]. Advocacy and lobbying for the off-label use of fractional doses of YF vaccines will allow for the use of the dose-sparing strategy without the need to change how the vaccines are manufactured.

6.5. Strengths and limitations of the study

The primary strength of this stakeholder analysis involved key informants who represented vaccine policy actors at all processes of policy. Although the study interviewed a small number

of stakeholders (n=9), it offers an in-depth view of their roles, perspectives, challenges and positions towards a prospective policy change to recommend wider use of fractional doses. The study has a specific focus on fractional doses of YF while making references to similar diseases for the generalisability of the findings. Although there is an attempt to outline the different actors' power, a comprehensive power analysis as has been described by Topp et al., [302] is indicated.

6.6. Conclusion

The YF outbreak in Angola, DRC, Brazil, Nigeria and Ethiopia in recent years brought YF back to the focus of global actors as a disease of global health significance and contributed to the development of the EYE strategy. However, YF vaccines supply is a potentially significant bottleneck to achieving the goals of the EYE strategy. The dose-sparing strategy is a relatively straightforward solution to the persistent YF vaccine shortage and has been used previously for other diseases such as Rabies, Polio and Measles.

There is unanimous support for use of fractional doses in emergency contexts. However, there is a need for more complete evidence to support wider use for routine immunisation strategies. It is important to standardise the definition of fractional doses to ensure that it includes the minimum dose required to induce adequate immunity. This requires that vaccine manufacturers and policy-making stakeholders explore interventions that allow for this redefinition of fractional doses. Although the process of vaccine policy development is relatively systematised in theory, in practice the direction of the prospective policy change is dependent on the priorities and experiences of the policymakers and other vaccine policy stakeholders and how their interaction during these policy-making processes. Important policy changes can be supported by intentional efforts to continuously engage relevant actors with different levels and forms of power through coordination mechanisms and open and regular communication.

7. Concluding remarks

In this chapter, I will make remarks on my experience working on an interdisciplinary DPhil project, and its outputs; safety, immunogenicity and policies of fractional doses of YF vaccines as a dose-sparing strategy.

7.1. Interdisciplinary DPhil thesis experience

Mine was an interdisciplinary DPhil project that included quantitative and qualitative research that brought together clinical trials, lab work and policy analysis work. In theory, policies are made based on bodies of evidence including quantitative evidence such as epidemiology of diseases, efficacy and effectiveness of interventions[303]. The evidence is then synthesised and utilised for evidence-based policies. Nevertheless, the policymaking processes are actor-led and therefore rely heavily on the understanding of actor elements. For these reasons, the field of health policy and systems research leverages multiple disciplines, including clinical and behavioural research, population health research and social science[298 304]. Although interdisciplinary research approaches have been praised for countering the limitations of individual disciplines and providing a more holistic approach to global health issues, challenges have been identified[305]. The challenges for a DPhil include the ability to cover each disciplinary element in enough depth, and working across different epistemological assumptions (spanning more positivist and interpretivist paradigms)[306]. My overall approach was pragmatic, bringing together disciplines and methods to help answer my overall research questions.

In my training during this DPhil, considering my largely quantitative background, I had to put in significant work to understand qualitative research approaches, methods and understand the data that I generated. I had two quantitative researchers and a qualitative researcher as my DPhil supervisors. I also benefited from learning through peers who were primarily qualitative researchers. While most of my PhD fellow peers fit in a single department, I was involved with

three departments; the Clinical Research department for my clinical trials work, the Biosciences department for my lab analysis work and the Health Systems Research and Ethics department for my policy analysis.

The challenges were not without benefits as I learnt new skills and competencies. I learnt to be receptive and appreciative of multiple facets of evidence and knowledge and gained an ability to reflect on different points of view. One of the most valuable skills I have learned during this training was to think in a non-linear manner for a more holistic understanding of evidence, interventions and policies. Another important lesson was on the importance of theory in doing good quality qualitative research. Although interdisciplinary research is complex and considered difficult, flexibility and adaptability of researchers and systems can go a long way in integrating evidence and knowledge [307].

7.2. Overview of main findings

The main findings are summarised based on the objectives of the DPhil described in chapter 1. The findings are based on a literature review of available literature (chapter 2), as components of the clinical trials in different populations (chapter 3 and 4), the policy analysis work through a systematised review (chapter 5) and a stakeholder analysis (chapter 6).

7.2.1. Clinical trials

In a clinical trial preceding the studies detailed in chapters 3 and 4, we recruited 960 adults and tested the standard doses and fractional doses ($1/5^{th}$) of all the four pre-qualified vaccine manufacturers[135]. We demonstrated that fractional doses are non-inferior to the standard doses across all the vaccine manufacturers[8]. The vaccines from the Poliomyelitis and Viral

Encephalitidis and the Institut Pasteur de Dakar were tested in the trials reported in chapters 3 and 4 respectively.

7.2.1.1. Safety of fractional doses of 17D YF vaccines

Standard doses of the 17D YF vaccine have been shown to have an excellent safety profile[94 308]. Serious adverse events following vaccination with the 17D YF vaccines are extremely rare (one case in every 100,000 persons vaccinated)[8 96]. It has been suggested that the association between serious adverse reactions and primary vaccination may be due to the viremia that primary vaccinees experience following vaccination [309]. The transient 17D viremia is short-lived and is undetectable with the development of neutralising antibodies post-vaccination. Some studies in non-human primates have suggested an inverse relationship between YF vaccine dose and the magnitude and duration of vaccine viremia and therefore anticipated a higher risk of safety events [310]. While more studies may be warranted to assess the impact of low vaccine doses on safety events [128], the clinical trial studies described in this thesis showed that the fractional doses of the 17D YF vaccines were safe at varying dose concentrations between 250 IU/dose and ~67,608 IU/dose.

In the 3rd chapter of this DPhil, the fractional dose and the standard dose were ~67,608 IU/dose and ~13,521IU/dose respectively. Consistent with previous results on the YF vaccine safety there were no safety concerns as the local and systemic events reported were mild or moderate in severity and resolved spontaneously without sequalae[101 104 130 151]. None of the SAEs reported was related to vaccination. An increased risk of YEL-AND and YEL-AVD in HIV-infected populations with very CD4 levels has been reported [168 169]. The study recruited participants with CD4+ T cells >200 cells/ml and hence could not assess the safety of the vaccine in

individuals with CD4+T-cell counts (<100cells/ml). Therefore, the study cannot confidently confirm the safety of the fractional doses in this population.

In the 4th chapter, I assessed the safety of different doses of the vaccine; ~13000IU/dose, ~1000 IU/dose, ~500 IU/dose and ~250 IU/dose. All vaccine dose groups had similar trends in safety events with the events reported being mild or moderate and self-limiting. Similarly, SAEs reported were not related to vaccinations. The majority of the pregnant women who were inadvertently vaccinated or became pregnant within the first month of the trial voluntarily terminated their pregnancies and those who carried their pregnancies to term had normal pregnancies, babies and puerperium. Although the sample size was rather small, there was an indication that the vaccines are safe in pregnant women contrary to some reports of possible safety concerns described in literature[197-201]. Further studies in pregnant women are warranted. These results build onto the existing confidence in the safety of the vaccine in different populations at different doses.

7.2.1.2. Immunogenicity of fractional doses

The live-attenuated vaccines, for example, the Smallpox and YF vaccines, are considered to be some of the most successful vaccines[311]. Although the 17D YF vaccines have been used for decades, the mechanisms of protection are not completely understood [312]. There are no human studies to determine the correlates of protection. This is not unique to YF – Pulendran and Rafi noted that some of the successful vaccines have been empirically developed with little immunological insight. The immunological mechanisms are often studied and described retrospectively [139]. Although neutralising antibodies have been described as the surrogates of protection for most of these vaccines, Barrett argues that they are, in fact, correlates of protection [139 312].

Historically, the current measure of protection has been modelled from dose-response studies in NHPs[85]. There is no consensus on the actual threshold that is considered as protective in humans vaccinated with antiflaviviral vaccines. Some studies have described a $\log_{10}$ neutralisation index (LNI) of 0.7 as protective while other studies have described neutralising antibody levels as low as 1:10 as protective. Higher levels of antibodies (≥ 1:40) have been shown to protect against disease expression[203]. Often a fourfold increase in the antibody titre from pre-vaccination baseline to about a month post-vaccination is considered sufficiently immunogenic[85 313]. Although $PRNT_{90}$ titres are valuable, the WHO has proposed that all studies report $PRNT_{50}$ titres for comparability [185].

Staples et al. have described several observations that are supportive of the proposed mechanisms of protection. In their review, they noted that during epidemics unvaccinated populations are usually affected significantly more than the vaccinated populations in the lab and the field. They also noted that during outbreaks there is a rapid disappearance of cases when vaccination campaigns are initiated[80]. This has also been shown to be true when using fractional doses of the YF vaccine to respond to the YF outbreak in Kinshasa, the Congo [127 165]. For the clinical trials reported in this thesis, I considered titres of 1:10 as measured by $PRNT_{50}$ as a protective consistent with WHO's guidance [163 164].

In chapter 3, I described the immunogenicity of the standard and fractional doses (~67,608 IU/dose and ~13,521IU/dose respectively) in an HIV-infected population. Although both doses were significantly higher than the WHO minimum recommendation, the difference in vaccine concentration between the dose groups was significant (~54,087IU/dose). A robust immune response was demonstrated in both treatment arms with the standard dose inducing higher antibody titres at 10-days, 28-days and one-year post-vaccination. The fractional dose demonstrated non-inferiority at 28-days post-vaccination that was maintained at one-year post vaccination but failed to demonstrate non-inferiority at 10-days post-vaccination. These

findings were consistent with a preceding study conducted among HIV-uninfected individuals [8].

In chapter 4, I described the immunogenicity of three lower doses (~1000 IU/dose, ~500 IU/dose and ~250 IU/dose) against a standard dose (~13000IU/dose). High antibody titres were described in all the vaccine dose groups – with those elicited in the 500IU/dose and 1000 IU/dose groups marginally higher than those of the standard dose. The two lower doses (~500IU/dose and ~1000IU/dose) demonstrated non-inferiority to the standard dose while the ~250 IU/dose group failed to demonstrate non-inferiority.

These results support the use of the YF vaccine with doses as low as 500 IU/dose. Some gaps remain regarding the longevity of the immune protection in different populations including children using the different vaccine doses[5 84]. In addition, these studies have focused on humoral immunity; the quantity and quality of neutralising antibodies as a mechanism of protection. However, there are other proposed innate and adaptive mechanisms of protection and the role of cellular immunity that were not comprehensively described in these studies [139 314 315]. There is proposed work these mechanisms using systems serology and cellular immunology [175].

7.2.2. Policy analysis

The clinical and immunological evidence presented in the previous sections addresses some of the key research gaps that were identified by the WHO for use of fractional doses of the YF vaccines[7]. The availability of this evidence is a start to considerations for firmer and wider recommendations for the use of fractional doses. These recommendations and potential prospective policy changes involve complex health policymaking processes. Understanding the role of evidence in policy-making processes is limited especially at the heavily political

regional and global levels [291]. It is also important to note that evidence need not be scientific and can be based on experiences and observations that are heavily subjective[316]. At the interface between evidence and action, there is an interplay of other elements and actor interests which can ultimately influence other stakeholders, their networks and how policy processes unfold in practice [120].

In chapter 5, using a systematised review of available literature, I mapped out the processes, actors and other elements that influence the development of policies based on Walt and Gilson's policy triangle[133]. I described a systematised policy process outlined for vaccine policies at the global level by the WHO on how issues get on the agenda and policies formulated for interventions [243]. Although in theory, the processes are transparent and systematic, in practice, the pathways for policy development are complex and actor-driven, making them inherently subjective. In the review, I described differences in disease priorities especially at the global level depending on the populations affected. I noted that each vaccine policy and the process is unique, depending on the vaccine characteristics, disease burden (the urgency to develop or change policy), the affected population and stakeholders' priorities, interests and ability to lobby for policies. For vaccine policies for VPDs of epidemic and pandemic potential whose decisions are largely made at the global level, there is a risk of underestimating the burden of disease in LMICs as most of the stakeholders at the global level are from HICs. A need for targeted analysis involving actor factors of the vaccine policy and other elements that facilitate vaccine policy introduction or change was recommended to anticipate vaccine policy processes.

In chapter 6, I report a stakeholder analysis focusing on the interests, perceptions, position, attitude and role of these actors in the prospective policy for wider use of fractional doses. There is overwhelming support for the wider use of reduced doses of the 17D-YF vaccines. The support is based on the currently available evidence, albeit incomplete. However, it is clear

the process from evidence to the policy will be slow and other factors beyond the evidence play a significant role. Stakeholders felt that a priority is a need for the WHO to redefine fractional doses. The current recommendation of the fractional doses is by volume; a fifth of the standard dose while the WHO recommendations for standard dose are based on vaccine potency. I learned that it is unlikely that the vaccine manufacturers will change how they manufacture the vaccine for reasons detailed in the stakeholder analysis. A key issue is related to the regulatory pathways to licensure of new regimens and formulations of the vaccine. Ultimately the vaccine policy processes are heavily political with advocacy, lobbying, actor coordination and communication playing important roles.

7.3. Other control measures for YF

The vaccination and surveillance strategies for YF have been outlined comprehensively in the EYE strategy[116]. The EYE strategy fits within the WHO's Immunisation Agenda 2030 (IA2030), an agenda that aims to strengthen immunisation strategies and systems globally [317] [318]. The recurrent 17D YF vaccine shortage remains the biggest threat to achieving the goals of the EYE objectives to eliminate epidemics. Most emerging and re-emerging infectious diseases are zoonotic in origin. A one health approach, a cross-cutting approach of research, programmes, policies and legislation of interfaces between human, animal, plant and environmental health is a key consideration in the control of the diseases [319] [320]. Although vaccinations have been proven as an efficient and essential public health tool for control of VPDs[321] [322], there are other control measures discussed in chapter 2 of the literature review such as measures to combat global warming and vector control strategies that complement vaccination programs [22] [323]. Other complementary measures include primate reservoir vaccination.

NHPs (most importantly of genus *Callitrix spp. and Alouatta spp)* are the reservoirs of the YF. These reservoirs have been neglected in the fight against YF. Urban monkeys have been implicated for triggering epizootic urban outbreaks of YF in South America [324]. During the outbreaks in Brazil in 2017, there were many deaths of monkeys and most of these were linked to YF infections and led to revisiting the proposal for vaccinations of monkeys[325]. Although there are studies to understand the immunisation of NHPs, to improve the health of the NHP populations, its role in interrupting virus circulation and reducing the risk of spillover to humans is not fully understood[326]. With more efficacy and feasibility studies, there is a role of complementary vaccinations of NHPs to control recurrent outbreaks [326].

While the WHO has only approved the use of YF 17D vaccines [327], an increase in demand for the YF vaccines due to an upsurge of outbreaks has provoked new interest in developing new vaccines that can be scaled up to meet an increased global demand [328]. Leveraging on new technologies to develop more efficient vaccines, there are vaccines based on cell-culture and plant-produced subunit vaccines in the early stages of development [329 330]. An ideal alternative YF vaccine would at the least need to be non-inferior to the current 17D vaccines, production scalable to increase supply and safe across all populations. In the stakeholder analysis, YF vaccine policy actors were conflicted on the need for a new vaccine. Some actors felt that it is important to leverage the new technologies for a new vaccine while others felt that the current YF vaccine has an excellent immunogenicity and safety profile – close only to the Smallpox vaccine which was used to eradicate Smallpox globally [331]. Further, YF is endemic in low resource settings and the global market share for the vaccine is relatively small and therefore there may not be enough financial incentive to invest in a new vaccine[332].

7.4. The implication of the research and the findings

The studies reported in this project respond directly to the call by the WHO to generate more evidence on the use of fractional doses. I have advanced the evidence on immunogenicity, safety and policy processes for the use of fractional doses of the 17D YF vaccines. The success reported in different populations increases the confidence of using fractional doses in epidemic settings where there're vaccine shortages as guided by the WHO. The evidence further lays ground to consider the wider use of fractional doses beyond the WHO recommendation and specifically within the implementation of the EYE strategy for routine immunisation and mass campaigns. There are systematised processes for introducing new or changing existing vaccine policies. There is overwhelming support for the dose-sparing strategy based on the current evidence by actors and the significant barriers to implementing the strategy warrant further engagement, advocacy and lobbying to explore feasible solutions.

The successes in fractional doses of YF vaccines also provide confidence in the potential of fractional doses of epidemic and pandemic potential disease vaccines such as the COVID[333] and Ebola vaccine[264] or other infectious diseases such as the Malaria vaccine [334]. The buy-in to the dose-sparing strategies by policymaking and consumer stakeholders is dependent on the availability and completeness of evidence.

7.5. Evidence gaps and directions for future research

Important evidence gaps on fractional doses of YF vaccines remain. Table 7.1 details the general research gaps identified by the WHO in 2017 and research questions that have arisen since then[185].

7.5.1. Longevity of protection

One of the most important gaps relates to the IHR requirements that involve international travel is the confirmation of longevity of protection with fractional dosing, including the potential need for revaccination in both children and adults[185]. There have been concerns about waning immunity in children and that children vaccinated before their 2nd year of life fall below the WHO's threshold of protection by their fifth year of life[83 335 336]. This has been thought to contribute to growing immunity gaps in areas with a history of preventive campaigns. In addition to other serological studies elsewhere in endemic regions[335], studies in children at different ages who have been vaccinated in Kenya and Uganda with standard doses as part of EPI would be helpful to understand the protection status of these populations. In adults, however, fractional doses as low as 500IU/dose have been shown to protect vaccinees for at least eight years post vaccination[171]. Further studies to establish the actual duration of protection would be helpful when considering revisions to the current vaccination recommendations. Neutralising antibodies decrease more rapidly in persons infected with HIV [100 101 154]. Long-term follow up of participants from the trial in chapter 3 would provide evidence relating to the antibody profile over time comparing fractional doses and standard doses.

Table 7. 1 of a summary of research gaps on the use of YF vaccines

Observation	Research questions/gaps	Approach and expected outcome/output
There are four prequalified vaccines with varying potencies.	Can all prequalified vaccines be used as fractional doses subcutaneously?	Clinical trials in adults using all 4 prequalified vaccines found that fractional doses are non-inferior to standard doses across all the manufacturers[8].
About 80% of the HIV burden is in LMICs where YF is endemic. HIV-infected individuals may not respond as robustly as HIV-uninfected persons.	Are fractional doses immunogenic HIV-infected individuals?	*Chapter 3 of this DPhil found the fractional doses of the 17D-213 YF vaccine to be non-inferior to standard doses.* *Long-term follow up of this cohort would provide evidence on the longevity of antibodies.*
Fractional doses are proposed for use in emergency settings to respond to outbreaks.	How rapidly do fractional doses induce protective immunity?	*Although fractional doses have been recommended for outbreak control and have been used successfully to control outbreaks, we have demonstrated that less than 80% of the vaccinees achieve immuno-protection at 10-days post-vaccination.*
YF vaccine has a good safety profile but there are risks of neurotropic and viscerotropic safety concerns.	Are fractional doses of the YF vaccines as safe as standard doses?	*The results from Chapters 3 and 4 of this DPhil have demonstrated that fractional doses have a comparable safety profile to the standard doses.*
The current recommendation for use of fractional doses includes vaccination of children >2 years.	Are fractional doses sufficiently immunogenic in infants?	Several non-inferiority and dose-response trials in children 9-60 months are ongoing in Africa and South America.
A single standard dose provides lifelong immunity.	Is the long-term immunity affected by the fractional dose?	*Complementing evidence from previous studies, in chapter 3 I demonstrated that the immune-protection at one-year post-vaccination with fractional doses is comparable with standard doses. Work beyond this DPhil is planned to evaluate the longevity of antibodies at two years postvaccination.*
The response to YF vaccination can be affected by host factors such as genetics and environmental exposure such as other flavivirus infections.	What is the impact of various environmental exposures and genetic backgrounds on the response towards vaccination with YF? Are there differences between fractional doses and standard doses?	There are planned studies to assess the effect of cross-reactive Flaviviral antibodies on immunogenicity and comparison across populations in East Africa[135].
The immunogenicity of the YF vaccine is more robust in younger populations than in older populations.	Are fractional doses sufficiently immunogenic and safe in older populations?	Studies are needed in the older population (>60 years) to generalise the current findings in the younger population (<50 years).

The minimum potency for the YF vaccine has not been fully established.	What is the minimum vaccine potency requirement to induce protective immunity?	*Chapter 4 found 500IU/dose was non-inferior to a standard dose of ~13000IU/dose.*
Viremia kinetics are a marker of safety events for live-attenuated vaccines.	What are the viremia kinetics in a dose-response study in an African population?	There are ongoing studies to evaluate the viremia kinetics in a dose-response study in East Africa[135].
The YF serology assays require standardisation across different reference labs.	Standardisation and validation of serological tests	A YF serology assay standardisation network has been established under the EYE to standardise and validate the assay protocols across reference labs[337].
YF vaccines are often co-administered with Measles-containing vaccines during routine and immunisation campaigns.	Is there any interference when fractional doses of YF vaccines are co-administered with Measles-containing vaccines?	The study following the trial reported in chapter 4 will assess the interference of fractional doses of YF and Measles-containing vaccines in infants (9-12 months)[135].
There are un-prequalified vaccine manufacturers that have the potential for prequalification.	How do the safety and immunogenicity of non-prequalified vaccines compare with the prequalified vaccines?	Non-inferiority studies using WHO prequalified vaccines and the non-prequalified vaccines would be helpful to provide evidence on the safety and immunogenicity of the latter.
While most studies study and report on the humoral immune responses a better understanding of cellular immunity is crucial in understanding the immune mechanisms.	What constitutes cellular immunity protection with YF vaccination?	There is work on systems serology and cellular immunology using the study reported in chapter 4 [175].
There is a shift to an Afrocentric vaccine ecosystem.	What are the regulatory pathways for vaccines in Africa?	There is a need for policy analysis studies to map out the evolving regulatory pathways for vaccines in Africa in the wake of an Afrocentric vaccine ecosystem.
Fractional doses uptake during campaigns has been good but there are risks of misinformation that may result in vaccine hesitancy.	What is the general communities' understanding of fractional doses as a dose-sparing strategy?	Studies are needed to explore the understanding of fractional doses of YF as a dose sparing strategy and barriers for uptake within consumer communities.

I have indicated some of the gaps that have been addressed by ongoing research and this DPhil project in *italics and blue*.

7.5.2. Rapidity of immunogenicity

The WHO recommends that travellers are vaccinated at least 10 days before their travel to be protected. This recommendation is based on evidence that the immune protection of the YF vaccine is sufficient at ten days post-vaccination. Although the current evidence presented in a preceding study, chapter 3 and 4 indicate that the protection at 10-days was below the WHO's threshold of protection, the studies were not powered to assess this outcome[8]. Fractional doses of the YF vaccine have been utilised successfully to respond to outbreaks in DRC, Brazil and Nigeria indicating that they are, in fact, rapidly protective. Studies powered to assess non-inferiority of fractional doses at days would be able to describe how rapidly fractional doses induce protective antibodies across different populations.

Although children are often less affected in outbreaks and severity of YF, current immunisation programs target children under two years of age where the YF vaccine is often co-administered with a Measles containing vaccine either during catch up campaigns or routine EPI[338]. Children may not respond to the YF vaccine as robustly or as rapidly as seen in adults and further studies in children, especially those concomitantly vaccinated with Measles containing vaccines.

7.5.3. Mechanisms of immunological protection

As with many vaccines, there is a significant gap in the understanding of the correlates and surrogates of protection for the YF vaccines[339]. Generally, the mechanisms of protection of anti-Flaviviral vaccines are poorly understood [6]. With the recent rise in challenge studies as an opportunity to study the correlates of protection[340], such studies for YF may be ethically unviable. However, some lessons can be drawn from other vaccines such as the Human Papilloma Virus (HPV) vaccine. The HPV vaccine just like the YF vaccine does not have a true correlation to efficacy [341]. The understanding of the HPV vaccine has evolved over the last

decades. Initially, the vaccine was recommended for use in pre-teenage and teenage girls as a measure to reduce HPV-induced cervical cancer and later recommended for use in reducing HPV in young boys and therefore marked reduction in HPV burden through herd immunity[342]. Similar to the changes recommended for the YF vaccine, there was a shift towards reducing the doses from a previous recommendation of a three-dose regime to a single dose of HPV vaccination [248 343]. A recent study showed that a single dose of HPV vaccine is the same as multiple doses at 18 months post-vaccination [344]. This provides for an argument that alternative measures of efficacy can be used where correlates and surrogates of protection have not been defined[345].

While the immunological protection discussed in this project is based on humoral responses, the role of cellular immunity although invaluable is not completely understood[346]. Studies describing the phenotypic and functional characteristics of cellular immunity across different populations would be useful to understand cellular immunity and its mechanisms of protection.

7.5.4. Standardisation of vaccine potencies and serological assays

While there is a minimum recommendation by the WHO that guides the vaccine release potencies, there is no maximum recommendation. The vaccine manufacturers release their vaccines in significantly high and wildly varying potencies that make it difficult to standard vaccine doses by potency across different manufacturers. Discussions with stakeholders, reported in chapter 6, indicated that there is a very slim likelihood of the WHO revising their guidance to establish a maximum potency recommendation as this would change how the vaccines are made. However, for use of fractional doses, it is important to standardise lots across manufacturers for more standardised use of fractional doses. Lab studies that standardise

dilution of vaccines similar to the procedures discussed in chapter 4 would be informative to explore the use of more standardised delivery of fractional doses.

There are several WHO reference labs globally with two new labs in Africa (the Uganda Virus Research Institute (UVRI), Entebbe, Uganda and Centre Pasteur Cameroon, Yaoundé, Cameroon) in addition to the Institut Pasteur de Dakar. Standardised, accurate and timely molecular and serological diagnostic capacity is critical to the EYE strategy and in standardised immunological read-outs for immunological studies across regions. A Global YF laboratory network (GYFLN) has been set up to standardise the serology protocols across WHO-approved labs in Africa and globally [337].

7.5.5. Policy considerations for use of fractional doses

With the evolving vaccine landscape in the African context in the wake of the COVID-19 pandemic and its impact on the vaccine ecosystem, it is also important to map out and track the shift in regulatory pathways in Africa with the recent change towards an independent Afrocentric vaccine ecosystem. This information would be important to feed the evidence-to-policy processes within the ecosystem at the regional, national and sub-national levels for changes in YF vaccine policies and introduction of new vaccines and policies in the continent [286]. Last but importantly, studies in communities aimed at communication and evaluation of understanding of fractional doses of YF could have significant potential to promote uptake, anticipate and curb vaccine hesitancy related to the use of the dose sparing strategy [347 348].

7.6. Conclusion

YF remains a global health threat. There are concerns about vaccine supply during outbreaks and for the successful implementation of the EYE strategy among other regional and national

vaccination plans. This DPhil project provides robust evidence to support the use of fractional doses as a dose-sparing strategy when there are vaccine shortages during emergency settings such as outbreaks and even in routine use among HIV-infected and uninfected persons in endemic regions. The findings here demonstrate that fractional doses of YF vaccines are safe and immunogenic in these populations and the immune protection is potentially long-lived. I described processes, actors and elements involved when introducing new vaccines and changes to existing vaccines in LMICs. In a stakeholder analysis, additionally, I indicated that there is overwhelming support by stakeholders for the use of fractional doses during emergency settings and wider use but also a range of potential concerns and barriers to utilising the life-saving dose-sparing strategy.

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