Immunogenicity and Toxicity of Yellow Fever Vaccines: A Systematic Review

by

Nondumiso Siphosakhe Makhunga-Ramfolo

23343975

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DECLARATION

I hereby declare that this dissertation presented to the University of Pretoria for the Masters of Science in Clinical Epidemiology degree is my own work and has not been presented previously to any other tertiary institution for any degree

Authorship

First Author: Dr NS Makhunga-Ramfolo

Second Author: Prof P Rheeder

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ABBREVIATIONS

ACIP	Advisory Committee On Immunization Practices
AEFI	Adverse Event Following Immunisation
CDC	Centers For Disease Prevention And Control
CF	Complement Fixation
DIC	Disseminated Intravascular Coagulation
DRC	Democratic Republic Of Congo
ELISA	Enzyme Linked Immunosorbent Assay
FDA	Food And Drug Administration
GBS	Guillain Barre Syndrome
HI	Haemagglutination Inhibition
IFA	Indirect Fluorescent Antibody Test
ITT	Intention To Treat
IU	International Units
LNI	Log Neutralisation Index
NDoH	National Department Of Health
NTD	Neglected Tropical Diseases
OAE	Systemic Adverse Events
PFU	Plaque Forming Units
PP	Per Protocol
PRNT	Plaque Reduction Neutralization Test
RCT	Randomised Controlled Trials
RD	Risk Difference
RR	Relative Risk
SD	Standard Deviation
SyAE	Systemic Adverse Events
TNF	Tumor Necrosing Factor
VAERS	Vaccine Adverse Event Reporting System
VHF	Viral Haemorrhaigc Fever
WHO	World Health Organisation
YF	Yellow Fever
YF-AVD	Acute Viscerotropic Disease
YF-NVD	Acute Neurotropic Disease

ABSTRACT

Immunogenicity and Toxicity of Yellow Fever Vaccines: A Systematic review

Student:	**Nondumiso Makhunga-Ramfolo**
Supervisor:	**Prof. P. Rheeder**
Department:	**Division of Clinical Epidemiology** **Faculty of Health Sciences** **University of Pretoria**
Degree:	**MSc (Clinical Epidemiology)**

BACKGROUND

Yellow fever (YF) is a non-contagious, mosquito borne haemorrhagic fever caused by a single-strand RNA flaviviruses. YF is endemic in the tropics primarily in South America and Africa although the vectors are present in Asia, Europe, Pacific and Middle East. Human beings serve as viraemic hosts for mosquito infection. YF carries a high burden of disease, particularly in developing countries with up to 200 000 cases reported annually and a case fatality rate of 20-50%.The pathogenesis is poorly understood and little research has been conducted .There is no known cure or specific treatment for YF and prevention remains the mainstay the public health approach in terms of effectiveness and cost. The World Health Organisation (WHO) conventions have made vaccination mandatory for travel to endemic countries to prevent outbreaks and transmission to susceptible individuals.

YF vaccine is one of the oldest vaccines known and in use and is derived from an attenuated virus strain 17D originally produced in the 1930s. The vaccine has historically been considered effective and safe. However, severe life-threatening side effects to the vaccine have been reported in the past 20 years. Acute vaccine-related viscerotropic (AVD) and neurotropic (AND) side effects have been reported globally particularly in the elderly. The adverse reactions typically present as YF- like illness resulting in multi-organ failure with death as a possible outcome.

OBJECTIVES

To estimate the immunogenicity and toxicity of 17D and 17DD YF vaccines by summarizing the available data from randomised controlled trials.

STUDY DESIGN

A summary of randomized controlled trials (RCT) of YF vaccine immunogenicity and safety and tolerability was obtained using standard meta-analysis methodologies.

METHODS

A comprehensive literature search was conducted in order to identify trial that met with predetermined inclusion and exclusion criteria. Features of each study were noted taking into account the type of vaccine used, the duration of follow up, assignment to intervention, blinding and randomization methods. Three studies were eventually pooled and effect size estimates reported in each study were noted and analysed using meta-analysis software, MIX. Reports on the side effects post vaccination were summarized and analysed.

RESULTS

The difference in outcomes between the standard 17DD YF vaccines intervention, traded as Arilvax ® and the 17D YF vaccines traded as YF-Vax ® and Stamaril ® was negligible in terms of effect size. Effect sizes that considered the means between the treatment and control groups demonstrated a difference that favoured the control group viz. Arilvax ®. The pooled results also showed significant publication bias most likely attributable to the small number of studies considered. The pooled and annotated forest plot supported the available literature in confirming the effectiveness of YF vaccines in conferring immunity. A summary of tolerability events

CONCLUSIONS

This study has confirmed the effectiveness of YF vaccines in terms of immunogenicity and also demonstrated that YF vaccines are well tolerated and safe The small number of study units considered in this study presented challenges for analysis and for interpretation but highlighted the need for more research to be

conducted in this area. The results are in keeping with the existing body of evidence supporting the robustness of the immunological response to YF vaccination. The safety and tolerability of the vaccine established in this study was also consistent with known literature. There are important implications for further research and implementation that became evident such as the need for further studies to be conducted in African populations where the burden of disease is highest.

1   CHAPTER 1

1.1   Statement of the Problem

1.1.1   Purpose of the study

This dissertation was designed to be a comprehensive account on YF vaccines and their efficacy. It will further explore the history of YF vaccine development and more significantly its effectiveness by using meta-analytic techniques. Chapter Two will review what is known about YF in terms of the burden of disease globally and the clinical course. This will be followed by a focus on YF vaccines including the history and development. The discussion will also delve into the immunogenicity and safety of YF vaccines before discussing new developments in YF vaccination. The final section will provide the background to the study viz meta-analysis and the rationale for its use in this dissertation. Chapter Three will provide methodology on how the study was conducted including an outline of the search strategy, study selection process and statistical analysis. Chapter Four presents the results of the statistical analysis while Chapter Five will synthesize and contextualize the findings. The chapter will also conclude with a discussion on the limitations of the study and implications for further research and practice.

1.1.2   Aims

To investigate the immunogenicity and tolerability of yellow fever vaccines in healthy adults and children.

1.1.3   Objectives

1.   To compare the immunogenicity as measured by successful seroconversion among commercially available 17D YF vaccines and 17DD yellow fever vaccines
2.   To estimate the pooled effect size for 17D YF and 17DD YF vaccines
3.   To determine the frequency of side effects and adverse events following YF vaccination

1.2 Justification for the study

In the public health context, endemic infectious diseases e.g. YF, newly emerging diseases e.g. severe acute respiratory syndrome (SARS) and multidrug resistant TB (MDR-TB); and reemerging diseases e.g. West Nile virus and dengue [1] continue to pose a challenge particularly for low resource countries such as South Africa. Recently, infectious diseases such as anthrax have even been disseminated as part of biological warfare strategies, posing a global threat to nations. [2] Although Viral haemorrhagic fevers (VHF) are collectively classified as Category A agents of bioterror due to the public health impact they have, the presence of what is considered to be an effective vaccine for YF reduces it to a category C bioweapon. [3] In the foreseeable future, South Africa will become increasingly exposed to these infectious diseases as a result of economic development, increased international travel and human behaviour. In addition, the African urban population is predicted to triple in the next 40 years increasing the risk of urban yellow fever.[4]

YF is an important infectious disease on the African continent, where children account for 70-90% of cases. [5]YF is not endemic to South Africa and an outbreak could therefore have significant public health implications in terms of preparedness. It is evident from the literature that YF carries a large burden of disease particularly in Africa and results in significant morbidity and mortality. According to Fauci [1], while the risk remains theoretical the spread of YF into non-endemic areas, such as South Africa ,is possible as a result of cases of imported yellow fever, Fauci has further described the potential for an epidemic as requiring the right vector, 'the right microbe and suitable hosts'. Due to increasing interregional travel, people incubating the virus could transport it to other regions. [6] South Africa could therefore be affected if precautions are not put in place to prevent this.

Previously South Africa has experienced public health emergencies related to VHFs. In 1996, a South African nurse died from a case of Ebola hemorrhagic fever after nursing an infected patient from the Democratic Republic of Congo (DRC) who was in South Africa for treatment. [7] This was followed by the National Department of Health (NDoH) [8] put on alert as a result of a suspected Marburg virus thought to

have been imported from Angola which was experiencing an outbreak at the time.

Several countermeasures have been developed and produced to assist the public health response to YF transmission. However, in order to address possible YF outbreaks, resources must be utilized to understand the pathogenesis, transmission patterns and host susceptibility. Vaccines and diagnostics are therefore considered a critical component of the public health   response.

Several travel restriction are implemented internationally to prevent transmission of yellow fever into South Africa. A valid YF certificate ,indicating that the traveler has received vaccination ,is required if a traveler older than one year starts their travel from or is in transit through the yellow fever belt of Africa or South America for entry into South Africa . Yellow fever certificates are therefore considered a visa requirement for affected travelers in keeping with International Health regulations. [9] In order to protect its South African citizens, persons arriving without a valid yellow fever vaccination certificate are either be vaccinated immediately or held in quarantine as a precautionary measure.[10] In countries where YF is endemic use of the vaccine is mainly for primary prevention.[6] However, when coupled with insensitive, passive surveillance systems which are ineffective, this leads to poor control of epidemics. According to the WHO, millions of people, largely in West Africa, will be affected by an impending shortage of yellow fever vaccine by 2010 due to a lack of funding to replenish stock.[11] Outbreaks could significantly impact the public health infrastructure and create excessive demand for the limited supply of YF vaccine.[12] Little is known to date about management and treatment of YF in infected patients and in patients with serious adverse vaccine related side effects. There are currently no specific drugs to treat yellow fever or manage vaccine related side effects.[12]

According to Jefferson et al [13], despite the large volume of work that has been published on clinical trials on various vaccines, little attention has been given to summarizing vaccine quality in terms of efficacy, safety, efficiency, effectiveness and acceptability. In a search of the Cochrane Vaccines Field to identify and quantify studies summarizing vaccine quality, it was determined that knowledge gaps

existed. Only six (6) reports on yellow fever vaccine trials were registered in the Cochrane Controlled Trials Register. No existing or anticipated systematic reviews on YF were registered in the Cochrane database of Systematic Reviews and Database of Abstracts of reviews of effectiveness at the time of Jefferson's report.[13]

The advantages of using a meta-analysis to examine the immunogenicity and safety of YF vaccine are numerous and will be discussed in subsequent chapters. Utilising existing research, various studies will be pooled to determine the effect of vaccination in terms of the immunogenic effectiveness and the side effect profile.

2 CHAPTER 2

2.1 BACKGROUND

2.1.1 Overview of literature review

The aim of the literature review is to provide the reader with a synopsis of the global and geographical distribution of yellow fever .This section will then ensue to describe the yellow fever (YF) disease and its causative agents, determinants, prevention and treatment modalities. Significant emphasis will be placed on describing the prevention approaches particularly in relation to vaccines. The history of YF vaccines will be discussed with the view to explaining the origins of current available vaccines after which immunogenicity measures related to YF vaccines will be explored. The side effects of YF vaccines following vaccination with YF vaccines will also be considered in this chapter. Finally the discussion will focus on the current challenges and research needs in relation to YF vaccines.

2.1.2 Impact of yellow fever

It estimated by the World Health Organisation that there are 200000 cases of YF occur in endemic areas annually. [14,15,16] However, only a small percentage of these cases are identified resulting in underreporting.[17] Globally an estimated 30000 deaths are attributable to YF, with a significant mortality among unvaccinated travellers to endemic areas .[16] Case fatality rates of between 20 and 50% in infected patients who entered the toxic stage of the disease have been reported. [16] In 2002, WHO reported that of the 30000 YF related deaths that occurred in countries where vaccination is part of the national immunization schedule, 50% of deaths occurred in children under the age of five. [12]

The annual global incidence of YF is reported in figure 1. [18] Despite an increase in the overall vaccine coverage epidemics have continued to occur particularly between 1985 and 1995.

Figure 1 Global annual incidence of YF
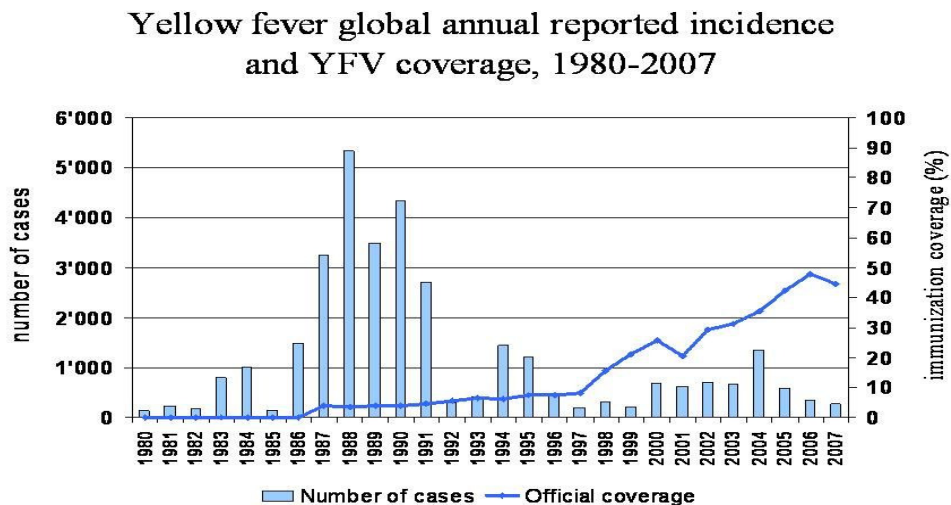
Yellow fever global annual reported incidence
and YFV coverage, 1980-2007

Of concern is the resurgence in YF that has been noted since the 1980s following a prior reduction in incidence. [14] Most cases of YF occur in sub-Saharan Africa with incidences as high as 20% during epidemics being reported. Whilst YF is considered endemic and epidemic in Africa and South America, particularly in the tropics, the potential for introduction in areas where the *Aedes aegypti* mosquito vector is present remains a concern.[16] This potential threat in non-endemic areas exists for regions such as the Caribbean, Europe [19], United States [20] and Asia[17].

2.1.3 Epidemiology of Yellow fever

2.1.3.1 Causative agent

YF is a non-contagious, infective viral haemorrhagic fever caused by an arthropod vector borne arbovirus from the flavivirus genus of the Flaviviridae family. [14, 15,21,22] The Flaviviridae family which contains over 70 related but distinct viruses[15,23,24] which are known to cause haemorrhagic fever and acute encephalitis.[25] YF is the prototype member of the genus. The virus is a positive sense, single stranded RNA genome consisting of a ribonucleoprotein core and a lipoprotein envelope. The envelope contains a single glycoprotein with type and group specific antigenic determinants.[22] The lipid bilayer that constitutes the viral envelope is derived from the infected cell with dimers from the envelope (E) protein on the surface. [22] The E

protein is the main target of the host's immune response and is responsible for the initial phases of infection of host cells.

YF is thought originate in Africa and was transported to the Americas as a result of the slave trade in the 1500s. [26,27] YF was the first flavivirus identified in Barbados as early as 1667 [28]following the first recorded epidemics in Mexico and Guadeloupe in 1648. [27]

In Africa three genotypes have been identified one represented by West African viruses and the others by Central and East African strains.[19, 20] There may be one or possibly two in South America which fall mainly into one major phylogenetic group. Unlike their African counterparts, the two South American genotypes do not segregate into discrete geographic distributions. It is suggested that since one genotype has not been recovered since 1974, it may have been lost.[14]

2.1.3.2 Yellow fever vectors

In Africa, the main vectors of yellow fever are mosquitoes of the genus *Aedes*, subgenera *Stegomyia* and *Diceromyia* with seven species which are thought to play an important role in nature: *Aedes (Stegomyia) aegypti, A. (Stegomyia) africanus, A. (Stegomyia) opok, A. (Stegomyia) luteoceph alus, A. (Stegomyia) simpsoni group, A. (Diceromyia) furcifer,* and *A. Diceromyia) taylori.*[14]

Vainio classifies *Aedes*-vectors into three categories according to their contact with humans. [14] The first category of vectors are domestic (i.e. around the household), mainly *A. aegypti.* The second category includes all other species of Aedes and is mainly wild. The final category is the semi-domestic which consists of wild vectors which can acquire domestic habits .The latter category consists mainly of *A. furcifer, A. africanus* and *A. luteocephalus.*[14]

Monkeys and galagoes (bush babies) to a smaller extent are the main vertebrate hosts. Over a maximum period of nine days the primate host develops a viraemia which results in a lifetime immunity following exposure. The link between humans and the wild cycle is through the monkeys that come to ground rather than remain in

the forest canopy. In savannah areas these monkeys are exposed to mosquito bites when they sleep in the canopy.[14]

2.1.3.3 Transmission of Yellow Fever

Vainio[14] describes two mechanisms of transmission that have been identified viz. vertical and horizontal.

It has been demonstrated by several authors that vertical transmission of the virus may occur as a venereal infection by females of males although this remains largely untested. Vertical transmission may possibly explain YF virus existence until the rainy season when the virus can theoretically be transmitted at the first blood meal without completion of the viral extrinsic cycle. This results in increased survival, drought resistance and persistent infection of vertebrates as the vector keeps the virus for extended periods.

Two mechanisms of horizontal transmission have been identified; maintenance cycles and amplification cycles. The degree of contact with the susceptible host and the associated ecological factors determine which cycle prevails. The maintenance cycle occurs when the vector-vertebrate contact is loose and is the more stable of the two cycles. This results in an endemic or enzootic form of yellow fever. In contrast, the amplification cycle results in increased circulating virus as a result of closer vector –vertebrae contact and manifests in epizootic or epidemic forms of yellow fever.[14]

Both intrinsic and extrinsic ecological factors have been identified in affecting horizontal transmission. In the invertebrate host the ability of the virus to cross the gut barrier of the mosquito and invade various tissues are considered to be intrinsic factors .There also extrinsic factors which are deemed independent of the virus. Intuitively, the invertebrate host must remain alive for a period that will be long enough to allow full development of the virus inside its body. This is due to the life cycle of the virus that requires that the vector becomes infected after a blood meal on an infected vertebrate host. Following replication in the tissues of the invertebrate

host and after, the virus must be inoculated with saliva into another vertebrate host e.g. monkeys and humans.

2.1.3.3.1 Transmission patterns in Africa

The vegetation patterns in Africa largely determine the transmission pattern as it determines the availability of the invertebrate and vertebrate hosts.

Mutebi [29]et al reports that the current endemic region, that encompasses 34 African countries with a total population of 500 million people, can be found between 15^0 north to 15^0 south of the equator. Endemic forms of yellow fever occur in the equatorial rain forest zone extending from Guinea in the west, to Uganda in the east, and south to Equatorial Guinea and northern Angola. This form of YF occurs year round and transmission is primarily between monkeys and *A. africanus.* Due to low virus activity, typically sporadic cases or focal outbreaks, predominantly monkey-to-monkey, with sporadic human infection occur.[14]

When extending outwards from the rain forest zone, into the savannah-forest mosaic and moist savannah, the rainfall decreases. Due to the increased presence of both vector and host populations, these regions are prone to high rates of transmission and repeated emergence of yellow fever activity particularly during the rainy seasons when enzootic *Aedes* reaches high densities. This results in cyclic epizootics in monkey populations and epidemics with interhuman transmission. This zone is also known as the intermediate zone of transmission. Concurrent vertical transmission in these mosquitoes ensures viral survival and there is continuation of epizootic waves. Most YF epidemics occur in this vegetation zone.

In the dry savannah zones the enzootic vector populations are low and active for a short period of time. In addition, the rainfall is very low due to the shortened rainy season making an epizootic outbreak unsustainable. However, the virus may be introduced into a cycle of interhuman transmission by *Aedes aegypti* typically if infected individuals move to villages in the dry savannah. Urban type transmission may also occur with resultant outbreaks of *A. aegypti-*borne yellow fever if the virus

is introduced into urban regions. The outbreaks may spread from village to village following the lines of communication used by humans. The virus can also be transported to non-endemic regions either by infected persons or by infected mosquitoes resulting in imported cases of YF [14,17].

West Africa contains all the YF hotspots in terms of incidence, [29] demonstrating the unequal YF incidence within the continent. Mutebi[29] et al also noted that large epidemics corresponded to civil unrest.

2.1.3.3.2 Epidemiology of Yellow fever in South America

Two types of epidemiological cycles have been identified in South America viz. jungle and urban. The latter is transmitted by *A. aegypti* while the jungle yellow fever is transmitted by the bite of *A. haemagogus* or other forest-breeding mosquito that was previously infected by feeding on an infected vertebrate host. Destruction of the urban breeding grounds for *A. aegypti* through a vast campaign resulted in eradication of the urban YF [14,17,22]. However; there has been subsequent re-infestation by *A. aegypti* in Central and South America occupying areas adjacent to endemic YF zones. Extensive vaccination campaigns and vector control have resulted in low virus circulation for a long period in the Americas when compared to Africa.

In South America YF affects mainly unvaccinated people who enter the forest for hunting, fishing, or wood cutting and become infected, making it an occupational disease. It estimated that about 80% of cases are reported in young adult male forest workers due to this.

2.1.3.3.3 Yellow fever in Asia

While it is hypothesized that YF can spread from East Africa to Asia, no cases have yet been documented despite numerous opportunities for introduction and spread .[14] Numerous reasons have been postulated for this but none provides a completely satisfactory explanation. It is feasible that yellow fever was never introduced to Asia, or humans vary in susceptibility, or there is cross-protection between flaviviruses, the maintenance cycle is absent, or there is variation in vector competence and

behaviour. Cross-protection from other flaviviruses may possibly account for the apparently lower susceptibility to YF of the Indian population.[14, 17]

It is postulated that Asian strains of A. *aegypti* may be less efficient vectors of yellow fever virus than African or American populations. Hindle's experiments in 1929 showed that one Indian strain of A. *aegypti* was a less effective vector than the African strains of mosquitos for the virus in question. However, studies conducted by Aitken and Tabachnick [30] demonstrated Asian populations of A. *aegypti* to be better vectors than West African populations. It was also shown by Miller et al [31] that in the presence of high population density an incompetent mosquito vector can initiate and maintain virus transmission resulting in an epidemic. Vector incompetence thus becomes less plausible as an explanation for the absence of yellow fever in Asia. In summary, it is not known why yellow fever never spread to Asia, but there is no evidence to show that this could not occur. All South- East Asia countries should therefore ensure that persons arriving from the Latin American and African countries at risk for yellow fever have a valid yellow fever vaccination certificates.

2.1.3.4 Risk factors for acquiring YF

Various factors have been identified as having significance in the susceptibility of individuals and populations to yellow fever. These include previous exposure to yellow fear and other flaviviruses, immunity, occupational exposure and racial and genetic factors.[1, 6, 14]
The immune status of a population will determine its susceptibility to YF. Previous exposure to YF either through a previous epidemic or mass vaccinations appears to confer protection. The case distribution will typically reflect this on second exposure or during the course of an epidemic. It is for this reason that YF is commonly included in the national Expanded Program on Immunisation (EPI) schedule in countries that are at risk.[2, 6,32]

In Africa, human behaviour such as monkey hunting and forestry practices is a significant risk factor that determines yellow fever transmission as these behaviours expose humans to infected monkeys. Additionally increased population growth

resulting in forest encroachment, migration, political unrest and wars and urbanisation all contribute to increased transmission. [14, 26]

In a study conducted by Hudson, he determined that the overall risk of contracting yellow fever in US travellers was 0.4 to 4.3 per million travellers with a ten fold increase in travel to West Africa than in South America. [20]

The role of genetic or racial factors in human responses to yellow fever infection is uncertain and no convincing evidence exists. Racial differences in the lethality of yellow fever have been investigated and demonstrated lower rates in blacks than whites during outbreaks in West Africa, tropical Africa and the US. It is unclear if this was due to acquired immunity or genetic factors. An association between HLA haplotype and disease severity has been found in patients with dengue haemorrhagic fever which is also caused by a flavivirus. TP Monath has motivated that racial differences in susceptibility to yellow fever will be resolved only by well-controlled epidemiological and serological studies in the setting of an outbreak affecting both races.[33] Hepburn et al [34]have also noted that racial differences in response to YF vaccine boosters with African-Americans having a lower response, although this may be attributable to self classification in racially mixed populations.

Anecdotal evidence suggests that reduced susceptibility to other flavivirus infections is conferred by distantly related viruses e.g. dengue haemorrhagic fever (DHF). [14] Cross immunity to several flaviviruses has been observed and makes this laboratory diagnosis difficult. However, it is hypothesised that cross-protection may be dependent on the specific virus causing primary infection, the interval between primary and secondary infection, and on quantitative and qualitative aspects of the immune response.[14]

2.1.3.5 Pathophysiology and clinical course of yellow fever

The case definition for YF is; an illness in a patient of any age with high fever, severe headache, neck and back pain, possibly accompanied by vomiting, abdominal pain, diarrhea, hematemesis, bloody diarrhea, jaundice, and epistaxis as described in a thesis by Onyango in 2004. [35] Due to the non-specific symptoms and

signs the differential diagnoses may be ;severe malaria (blackwater fever), leptospirosis, Borrelia recurrentis, typhoid fever, rickettsial infections, other influenza, viral hepatitis, Lassa fever, Marburg and Ebola virus diseases, Crimean-Congo hemorrhagic fever, Rift Valley fever, dengue and Congo-Crimean hemorrhagic fever.

Laboratory diagnosis can be performed by detecting viral antigen by a monoclonal antigen-detection ELISA or by serological diagnosis by measuring IgM antibodies through ELISA.

Monath has noted that only a descriptive account of the disease is available in literature. He has described the clinical course of yellow fever as outlined further.[36] The clinical presentation of YF disease varies from mild, non-specific to severe, fulminating disease.
Following inoculation, the virus replicates in the adjacent tissues and localised lymph nodes .Fixed macrophages in the liver are infected 24 hours after inoculation, followed by infection of the kidney, bone marrow, spleen and lymph nodes and myocardium.[37]

Hepatic disease is characterized by a unique feature of yellow fever; its mid-zonal distribution, with sparing of cells around the central vein and portal tracts. This distribution of hepatic lesions indicates that these cells are most susceptible to virus replication. The infected hepatocytes undergo degeneration typical of apoptotic cell death and distinct from the ballooning and rarefaction necrosis seen in viral hepatitis and tend to be a late event. Apoptosis may explain the virtual absence of inflammatory cells in yellow fever, preservation of the reticulin framework, and healing without fibrosis.

The renal pathology is characterised by eosinophilic degeneration and fatty change of tubular epithelium without inflammation. Direct viral injury is thought to have a role. Patients present with oliguria caused by pre-renal failure associated with hypotension. Acute tubular necrosis occurs as a terminal event. Abnormal glomerular function may be responsible for the albuminuria that is seen in these cases.

The late stage of illness is characterized by hypotension and shock. The shock syndrome may be as a result of a combination of cytokine dysregulation and bacterial sepsis. Tumour necrosis factor (TNF) and other cytokines and cytotoxic T cells involved in viral clearance may cause oxygen free radical formation, endothelial damage, microthrombosis, disseminated intravascular coagulation (DIC), oliguria, and shock. Myocardial fibers may be directly damaged by the virus contributing to shock.[36]

The quiescent incubation period lasts 3 to 6 days after the bite of an infected mosquito. This is followed by a period of fever, myalgia, headache and vomiting. In a study conducted in Nigeria, the average duration of acute illness was 17.8 days.[38]

In very mild yellow fever the only symptoms are fever and headache lasting from a few hours to a day or two. Monath describes the average fever as 39 ^{0}C and lasting 3.3 days.[36] Additional symptoms such as nausea, epistaxis, Faget's sign which is a relatively slow pulse in relation to constant or rising temperature, slight albuminuria, and subicterus. Moderately severe yellow fever is clinically diagnosable as more classic symptoms are present. These may include black vomit, possibly as a result of swallowed blood due to epistaxis, or uterine hemorrhages, jaundice and marked albuminuria.

Moderately severe and malignant attacks of yellow fever are characterized by three distinct clinical periods: the period of infection, the period of remission, and period of intoxication.
During the period of infection lasting approximately three days, large amounts of virus are present in the circulation due to increased multiplication of the virus. The patient may experience severe headache, nausea and vomiting, generalized aches and myalgia and is unable to sleep and irritable. The pyrexia may be higher than 39^{0}C to 40 ^{0}C. The nausea and vomiting are sometimes severe.

 During the period of remission, lasting a few hours to a couple of days, there is a marked decrease in the temperature to or toward normal and the patient may report

feeling much better.

Approximately 15-25% of people will progress to the third stage. Approximately 50% of these patients will die within 7-10 days following onset of symptoms. The remainder will have full recovery following convalescence characterized by severe weakness and fatigue. During the third stage of intoxication, lasting 3-4 days, the free virus usually is not detectable in the blood although the toxemia it produced persists. The classic symptoms of yellow fever, which are manifestations of this toxemia, become fully developed. The tongue has a characteristically bright red margin and tip and a furred center with gums become congested and bleeding under slight pressure. Three typical signs are elicited on the 3rd day or early 4th day; anuria, copious hemorrhage from the gastrointestinal tract, or delirium .When multiple organs have become affected the body's defenses is overwhelmed and the patient will die. Progressive tachycardia, shock, and intractable hiccups are considered ominous and terminal signs .The period of intoxication is the most variable of the three periods and at its maximum, it is much the longest. In mild infections it is not recognizable at all.

2.1.3.6 Treatment of yellow fever infection

No specific antiviral treatment exists for the management of infected patients. Passive antibodies e.g. interferons have been found only to be useful before or within hours of infection and therefore for post exposure prophylaxis e.g. in laboratory workers. Treatment is therefore primarily supportive. Monath [39]states the gold standard protocol comprises of maintenance of nutrition and prevention of hypoglycemia; nasogastric suction to prevent gastric distension and aspiration; intravenous cimetidine to prevent gastric bleeding; treatment of hypotension by fluid replacement and vasoactive drugs (dopamine); administration of oxygen; correction of metabolic acidosis; treatment of bleeding with fresh-frozen plasma; dialysis if indicated by renal failure; and treatment of secondary infections with antibiotics. Adherence to these recommendations in resource limited countries where YF typically endemic poses a challenge resulting in poor outcomes.

2.1.3.7 Prevention of Yellow fever

There are two main methods of preventing yellow fever namely vector control and vaccination.[9] WHO provides protocols and guidelines on assessing disease burden using a variety of methods such as disease surveillance, rapid assessments, or population-based studies.

2.1.3.7.1 Vector control

Yellow fever may be prevented by reduction of domestic breeding of vectors at adequately low levels although this may be a difficult undertaking. Vector control methods include community based environmental interventions e.g. spraying of breeding sites. 'Autocidal' ovitraps, mass-rearing and release of predatory Toxorynchites mosquitoes and placement of predatory fish in potable water (jars and cisterns) are among the more novel and innovative techniques of vector control. [40]

In preventing Yellow fever general precautions to avoid mosquito bites should be followed. These include the use of insect repellent, protective clothing, and mosquito netting. [41]

2.1.3.7.2 Yellow fever vaccine

In the past yellow fever was considered the third human disease to be effectively controlled by vaccine following small pox and rabies largely as a result of work conducted by South African born physician Max Theiler. [42] It is estimated that 100 million doses of YF vaccine are manufactured by six WHO approved institutes globally. [40,43,44]

Pugachev [45]et al has written that while the incidence and geographic distribution of flavivirus has increased there are few vaccines developed against Flaviviridae. Vaccines are only available for Japanese Encephalitis, tick-borne encephalitis, Kyasanur forest disease and yellow fever. Reemergence of yellow fever is due to incomplete vaccination coverage and mosquito reinfestation.

YF vaccines have been considered immunogenic, safe and well tolerated .[8,11,13,14] Yellow fever is also considered a good vaccine as a vector. The vaccine not only elicits a robust immune response but also provides long lasting, possibly life long protection against future infection following immunization more than 90 % of vaccines achieve protection within 10 days and 99% in 30 days. [3, 44] It is also believed that the neutralizing antibodies induced by YF vaccination can be correlated to future infection resistance as it effective against all 7 genotypes of wild-type YF. [44,46]

Figure 2 Countries with YF as part of the EPI schedules
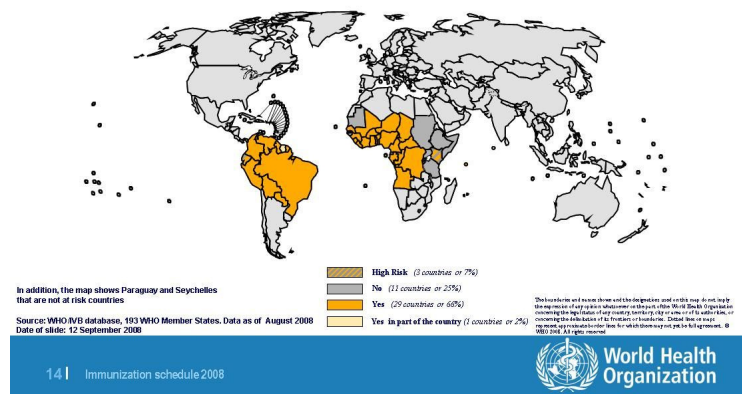
Currently a Yellow fever vaccination certificate is an entry requirement in 127 countries globally and is offered during mass vaccination and catch up campaigns for routine use as part of the Extended Programme for Immunisation (EPI) for infants in endemic countries. According to the Advisory Committee on Immunization Practices (ACIP), for persons 9 months and older travelling to or resident in endemic regions, revaccination every ten years is recommended .[14]

2.1.3.7.2.1 History and Development of Yellow Fever vaccines

One of the first strains of YF virus was isolated at the Institut Pasteur at Dakar, Senegal in 1927. The following year, the virulent organs from an infected monkey

were transported to European and American laboratories under the name of "French strain". [47] Subsequent trials on humans by simultaneous injection of a suspension of the French strain and a certain quantity of human immune serum were successfully conducted in 1931. This resulted in a successful subcutaneous inoculation of the modified French strain alone in a campaign. By 1941, YF inoculation by scarification became part of programme of compulsory immunization in French West Africa resulting in marked reduction in YF incidence and outbreaks in the region. [14, 17, 19, 47] Due to the high incidence of encephalitic reactions, particularly in children, this strain was discontinued in 1980.

Today, 17D strain, known as the Asibi strain [44], is the only type of YF vaccine produced. The origins of this strain can be traced back to 1937. Max Theierl, who received a Nobel Prize for his efforts, attenuated the virus in monkeys, mouse embryonic tissues and chicken embryonic cultures in more than 200 serial passages. [42] Querec et al [48] reported that in its 65 year history more than 400 million people have been immunized with 17D vaccine. The original 17D were unstable due to contamination by avian leukosis virus and has since been made avian leukosis free.[42]

The yellow fever 17D vaccine is currently manufactured in chick embryos according to WHO standards. [44] Production of 17D-204 vaccine in chick embryos has remained constant and largely unchanged for more that sixty years. There are three main substrains of YF17D available in vaccine today, traded as 17D-204,17D-213 and 17DD. The 17D vaccine is traded under the name Stamaril and YF-VAX .17DD is available and traded as Arilvax. One dose of vaccine contains between 104 and 106 pfu of virus. [44] As recommended by the WHO, safety testing is conducted in non-human primates as they closely reflect human infection. YF vaccine is convenient as it can also be administered as a single dose to recipients with minimal or no previous immunity to yellow fever .Moreover it readily accepts the introduction of foreign sequences into its genome without rejecting them and losing infectivity.[49] According to WHO regulations, new master and working seed lots shall be tested for viscerotropism, immunogenicity and neurotropism in a group of 10 test monkeys prior to production. Control test can then be conducted on the final lot in humans

through clinical trials. Clinical trials are expected to be conducted on award of a new manufacturing license. This could be considered useful in and beneficial especially in developing countries where it could be combined with a vaccine for another endemic pathogen, making it practical and more cost effective. Due to the fact that YF vaccine is a live attenuated virus it become effective as it has the replication capacity of live viruses.

2.1.3.7.2.2 Immunogenicity and the antibody response to Yellow fever vaccination

The innate and adaptive immune responses to YF vaccine remain poorly understood although recent research has provided additional evidence. [44]While the live attenuated 17DD-YF vaccine is considered to be an effective vaccine there is no comprehensive evidence to describe the immunological innate mechanisms by which 17DD acts. Recent evidence also suggests that the strength and quality of the adaptive immune response is largely determined by the innate immune system and that they represent a significant loop in the immune response against YF antigens. [50]What is evident is that YF vaccine induces a viraemia as the critical pathogenetic phase allowing antibodies to act on the organism[51]. Efficacy of the YF vaccine therefore correlates with measures of the subsequent immune response although this can be occasionally weak or in some cases uncertain. Plotkin [49] asserts that most vaccines' efficacy depends mainly on functional serum antibodies and to a lesser extent mucosal and cellular responses.

In considering vaccines, herd immunity has to be investigated. It is significant as it protects the unvaccinated where there are fewer infected individuals in a highly vaccinated population, unvaccinated persons are less exposed and eliminating the risk to unvaccinated where the infection is eradicated by a vaccine. While in natural exposures the challenge dose is not known, in artificial challenges, such as vaccination, it is easier to discern the effect of the dose. Plotkin [49] argues that protection is therefore a statistical concept in that when a particular titre of antibodies is considered protective, 'we mean under the usual circumstances of exposure, with an average challenge dose and in the absence of negative host factors'.

Adaptive immune functions may be classified as those mediated by B cells, CD4 T-cells and CD8 T-cells .B cells can be subdivided in to IgG and IgA antibodies. CD4 T cells are required to assist B cells and CD8 cells. The latter's main function is to kill HLA-matched infected cells. As a live attenuated virus vaccine YF induces the full range of functions.

The role of vaccine induced T cell responses, particularly CD8+ T cells, in the protective efficacy of the YF virus have been demonstrated in studies of cellular immune response following 17D vaccination. [50,52] In an article published by Barrett et al [44], it is stated that 17D YF vaccines elicit a potent CD4+ and CD8+ cytotoxic T-cell response directed against the YF structural (NS1,NS2B,NS3) and non-structural proteins.The CD8 Tcell response peaks within 2 of vaccination and is detectable up to 19 months.

Martins et al [21] investigated peripheral blood neutrophils, eosinophils, monocytes and natural killer cells with the intention of characterizing the kinetics of the innate immunity following 17-DD first time vaccination. The results showed an activation status of neutrophils and eosinophils with an associated increase in the frequency of neutrophils expressing the CD23 and CD28 marker and eosinophils expressing the CD28 and HLA-DR. There has previously been little information about the role of these cells in viral infections. It was further established that at day 30 post-vaccination, there was a later increment of CD28 and HLA-DR eosinophils were detected. The investigators concluded that the these cells not only have a pivotal role in controlling the infection but also induce an adaptive immune response underlying the protective immunity triggered by the 17DD YF vaccination in vaccines who did not experience any adverse effects.

Querec et al [48] reported that multiple toll-like receptors on dendritic cells are activated by the 17D vaccine and may be responsible for the broad spectrum of innate and adaptive immune response. Activated dendritic cells possibly migrate to regional lymph nodes stimulating a cell mediated and humoral adaptive immune response.

The main mediator of immunity elicited by the 17D vaccines is neutralising antibody

and has been unequivocally correlated with protection from disease in non-human primates. Neutralising antibodies develop in 98-100% of yellow fever 17D vaccines within 7 days of vaccination, providing protection for at least ten years although it may continue for 45 years. [34, 42, 44]

2.3.1 Measuring the antibody response to yellow fever

There are numerous serological methods used to study antibody response to YF vaccine and the detection of YF vaccine antibodies can be performed using several modalities. [51] WHO lists neutralisation, haemagglutination inhibition (HI), complement fixation (CF), Enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFA) as being the some of them.

Currently, detection and analysis of the immune response post-vaccination uses the Plaque reduction neutralisation tests (PRNT) as the gold standard as it is considered to be the most specific test. Neutralising antibodies are detectable using plaque reduction assays and mouse protection tests and are probably detectable for life. Plaque reduction assays are considered to be the standard currently and are more sensitive in the detection of neutralising antibody than the mouse protection tests which was never standardised due to the variability of the results. Potency of YF vaccine lots is typically assessed by plaque assays using the plaque reduction neutralization test (PRNT) with a minimum potency of 10^3 mouse LD_{50} per dose or its equivalent in plaque forming units (PFU).[53] This definition is being assessed by a collaborative study in order to improve the definition of potency.

ELISA, IFA and HI tests are additional tests that can be used. The latter determines IgG and IgM antibody levels for the presence of antibodies in sera for persons vaccinated against YF.[49] All except for CF appear within a week of yellow fever.

Utilising ELISA to detect IgM antibodies is the most useful test in detecting recent infection and diagnosis in cases demonstrating cross reaction. While the duration of IgM antibodies is variable it can be present as long as 18 months after immunisation. ELISA results correlate well with those found by neutralisation and it is increasingly preferred because it is quicker to perform than PRNT. [53, 54]

Niedrig et al [51] reported that in their study to evaluate IFA against PNRT in terms of sensitivity and specificity it was found that IFA could be a useful tool for the diagnosis during outbreaks .IFA also has the added benefit of being able to detect non-specific reactions making it useful in endemic areas. They also asserted that a cross reactive immune response could be differentiated by making a fourfold increase in titre in two consecutive sera mandatory before concluding a result. Therefore indirect IFA demonstrated similar sensitivity to PNRT with the benefit of being faster to perform. IFA performed using cells infected with YF virus can detect both IgM and IgG. IgG and IgM antibody level determination using immunoflourescence assay (IFA) can be used as additional markers to detect the presence of serum antibodies following vaccination.[55]

The haemagglutination inhibition (HI) and complement fixation (CF) tests are widely used for the diagnosis of natural infection and is therefore not suitable in assessing responses to yellow fever vaccines. While CF is more specific HI it is more useful in indicating a recent infection.

In the development of vaccines it is critical to define the immunological correlates of the protection conferred by the vaccine. Protection against YF have been found to have a correlation with antibody titres of 0.7 IU corresponding to a titre of 1/5.[51] Although this is the accepted cutoff for seroconversion , its origin is among non-human primates. In a report examining the definition of immunity, Amanna et al argues for more appropriate correlates of immunity to be determined.[56] However, the effectiveness of the protective immune response is analysed using PNRT which is considered the gold standard.

2.1.3.7.2.3 Tolerability and safety of yellow fever vaccines

Studies have indicated that YF vaccine is usually well tolerated by adults with serious adverse events rarely reported. [44,57,58] Systemic reaction is reported as being less than 0.2% [59,60] ,although it may be more common than thought. [60,61]

Adverse events following immunization (AEFI) is defined as 'signs or symptoms that follow application of a vaccine and that are believed to be caused by vaccine.[62] AEFI is monitored by a passive surveillance system known as Vaccine Adverse Event Reporting System operated by CDC and the Food and Drug Administration (FDA).[63] Temporal association between vaccination and the onset of adverse effects may inhibit accurate estimate of relative risk.[64] Fernandes et al argue that this definition therefore has a low specificity. [62] AEFI are typically mild and nonspecific.[65] The first cases of jaundice and encephalitis as side-effects of 17D vaccinations were recorded in Brazil.[14] In August of 1940, the practice of adding 10% normal human serum (necessary for the filtration of the virus) to the vaccine was given up. However, serum was used in preparing vaccine in the US, resulting in a major outbreak of hepatitis in the military in 1942. [15] The practice had resulted in the transmission of the virus of infectious hepatitis, which for many years contaminated yellow fever vaccine.

Between 1951 and 1952, the occurrence of postvaccinal encephalitis in 15 infants from UK, US and France formed the basis for a recommendation that excluded use of 17D vaccines in infants under six months of age. In 1958, the 17D vaccine was shown to induce very long-lasting immunity, providing the basis for new recommendations regarding reimmunization of travellers at 10-year intervals. [14, 17, 46]

Recent reports [11,16,17,31,66, 67] have indicated that YF vaccine can cause disease that resembles wild type YF virus infection described as viscerotropic disease (YF-AVD) and neurotropic disease (YF-AND) after YF vaccination. YF-AVD and YF-AND are more recent terms to describe post-vaccination multiple organ failure and post-vaccinal encephalitis respectively. It is reported that in a study by Vellozi et al it was determined that fatal adverse events were associated individual host factors controlling susceptibility to yellow fever. [67] This was also reported by Vasconcelos in a report of two cases in Brazil. In addition, some vaccinees had large variations in the acute phase response to the vaccination resulting in being classified as hypo- and hyperresponders. [68] This response is though to be genetically determined. Barrett also indicated that in addition to age and thymus disease, male gender may be a potential risk factor for development of SAE. [49] This was further confirmed by Lindsey et al whose study demonstrated a higher incidence of local inflammatory

events in female than in males.[65] Hepburn et al did not observe a gender effect in relation to YF vaccine booster response.[32] Niedrig also established that a stronger immune response after YF vaccination in men than in women is a well known fact although factors contributing to this remain unclear.[51]

The vaccine may not be administered in conditions including severe chronic illness, immunodeficiency or immunosuppressive therapy, and pregnancy although no teratogenocity during pregnancy have been reported The vaccine was also found to be harmless even for children and for women at any stage of pregnancy. Persons with egg allergy are not immunized as the vaccine is manufactured in chick embryos. [8, 18]

Early trials demonstrated that following vaccination mild reactions occurred five to eight days after vaccination in 10-15% of the persons vaccinated, with more intense reactions in only 1-2% .[21, 22] Laboratory studies indicated that about 95% of the vaccinated had acquired immunity as measured by specific antibodies.[14]

Infants and children are at greatest risk of death. [22,69] The YF vaccine is contraindicated in children below the age of nine months except in active epidemics where it may be used in children as young as four months. [5]

2.1.3.7.2.4 Precautions and contraindications for YF vaccine

Barrett identified five main considerations in determining suitability of a patient for YF vaccination.

- Age. Infants below 6 months should not be vaccinated while persons above the age of 60 may be at a higher risk of side effects. [70,71,72,73] Khromava et al reported that advanced age as a risk factor for AEFI can be concluded.[63] Weinberger et al attributed this decrease in post vaccination protection to immunosenescence.[74]
- Thymus disease. Thymectomy and thymus disease is a contraindication for vaccination as it increases the risk of AVD. [75]
- Pregnancy. YF vaccine is contraindicated in pregnancy as safety has not been well established. In a study conducted by Robert et al there was no

evidence of transplacental passage of YF vaccine virus although neutralizing antibodies were found to have crossed the placenta and were also found in the colostrum.[76] Unintentional administration of YF vaccine during pregnancy is not an indication for termination.[76] Teratogenicity was not noted in a report by Suzano et al that in cases of first trimester exposure to YF vaccine although were unable a link to early gestational losses could not be established.[77] This was in contrast to a study conducted by Nishioka that demonstrated an increased risk in spontaneous abortion.[78] However, YF vaccine may be given during unavoidable travel or during an epidemic.

- Immunosuppression. Immunosuppression due to disease e.g. leukaemia, malignancy or drugs eg corticosteroids have a theoretical risk and are not recommended. [79, 80] HIV infected people who do not have AIDS or a CD4>200mm^3 may be vaccinated although the neutralizing antibody response is muted.[81] In vaccinating HIV infected patients Rouken et al have issued a note of caution when YF vaccine is co- administered with the antiretroviral drug, maraviroc as it can increase severity of infection resulting in a risk for YEL-AVD.[82]

- Allergy. YF vaccine is contraindicated in persons who are hypersensitive to eggs as it is produced in embryonated chicken eggs. In a study of 102 HIV infected patients it was determined that HIV infected patients fewer patients generated neutralizing antibodies and the antibody titre was lower.[81]

Yellow fever vaccine safety was until recently considered undisputed with serious adverse events rarely reported .In cases where adverse events were reported, they were primarily allergy related mostly in individuals allergic to eggs. Serious adverse effects of YF vaccination can be classified as YEL-AND, previously known as postvaccine encephalitis and YEL-AVD, also known as postvaccine multiorgan system failure. [44] An international laboratory network of YF vaccine associated adverse events has been established to document and determine the pathogenesis of severe adverse events following YF vaccination through laboratory evaluation.[83]

2.1.3.7.2.4.1 Yellow fever neurotropic disease (YEL-AND)

YEL-AND typically occurs in first time vaccines approximately 2-30 days post vaccination and carries a case fatality rate of below 5%.Young children also had increased incidence of YEL-AND albeit at a low frequency of less than one per 8 million. Between 1990 and 2004, 11 cases of YEL-AND were identified among US citizens. [17,18] Most of these cases have been benign and self limited. Four of these cases had post-vaccinal encephalitis, four had Guillain Barre Syndrome (GBS) [65] and the remaining three had acute demyelinating syndrome. CDC published that four cases of acute encephalitis had been identified in adults between June 2001 and August 2002 in adults following administration of 250000 doses suggesting that the frequency of YEL-AND to be as high as 16 per million. In Europe it is estimated to be 1.3-2.5 per million based on the number of Arilvax [®] doses sold between 1991 and 2003.[18]

2.1.3.7.2.4.2 Yellow fever viscerotropic disease (YEL-AVD)

YEL-AVD is a severe acute illness with an incubation period of 2-5 days. [32,33] It is characterized by hepatitis, multi-organ failure and high mortality, mimicking wild-type YF in most respects, with viral antigen present in many tissues.[65] In endemic countries the presence of vaccine virus has to be confirmed by viral isolation in order to distinguish from wild-type virus.
As of May 2009 51 cases of YEL-AVD have been identified since it was first reported in 2001. Nine cases of AVD were reported in between 1996 and 2001, four in the USA, four in Brazil and one in Australia, eight of which were fatal. [18]

Post vaccination surveillance has subsequently been intensified in USA and Brazil as a result of the reported deaths. Syndromic investigations on data generated from passive surveillance poses the limitation of underreporting.[18] Simultaneous administration of vaccines has also been investigated by various authors and the evidence suggest serologic response to YF vaccine is not reduced.[37] Belsher et al has argued that pre-travel immunoglobulin co-administered with hepatitis A vaccine may have previously reduced the recognition of YEL-AVD.[84] In an article by Fletcher et al it is argued that the combination of measles and YF vaccine immunization as

part of the EPI programme should be revived. In a study by Ambrosch et al YF was combined with typhoid fever, subjects showed higher antibody titres against YF than when vaccinated with YF vaccine alone.[85]

The risk of YEL-AVD is 2.5 per one million doses for 17D-204 vaccine and estimated to be as high as 1 in 40000 doses among the elderly (>60 years old) although it does not seem to be limited to this age group. [32] In a trial Monath et al it is reported that the relative risk in the elderly ranges from 5.9 to 16.2 when compared to younger. It is now believed that the risk was underestimated in Brazil as the revaccinations and vaccinations of naturally immune individuals was affecting 50% of the population was not considered. The estimate is considered to be closer to 2.13 per million. The case fatality rate for YEL-AVD is approximately 60%.

YEL-AVD development is thought be due to viral and host facors.[32] Host genetic factors are therefore considered to be significant in increasing susceptibility although this requires further elucidation.[32] Acquired host factors are considered to be significant as an association between thymoma and thymectomy and YEL-AVD has been established. [33] In a review of vaccines for travel Lee estimated the risk of YEL-AVD to increase by three to four times in higher in patients who are not immunocompetent e.g. thymic dysfunction.[86] Eidex has reported that thymic changes may contribute to increase incidence of YEL-AVD particularly in elderly individuals and some genetic disorders such as DiGeorge's syndrome.[87]

Kitchiner [32] asserted that concurrent tetanus toxoid administration as a risk factor for YF-AVD may be a confounding factor ,with no trend toward association among YF-AND cases.

Mutations of the YF vaccine virus have not been found on analysis of genomic sequences of virus isolated from fatal human cases. In monkey and hamster models the biological properties of isolates have also remained unchanged.

As with wild type YF infection treatment of YEL-AVD is mainly supportive .Based on the 2003 Surviving Sepsis Campaign Management Guidelines Committee the use of SDS for the treatment of septic shock was recommended. [88] YF-AVD is therefore managed as septic shock with the use of stress dose steroid (SDS) treatment

administered at 200-300 mg/day.

2.1.3.8   New developments

Van Epps [46] reports that in research conducted by Rice and others, structural 17D genes were replaced with those from other Flaviviridae, including Japanese Encephalitis virus, and then used to generate neutralising antibody responses against the flaviviruses. This was known as the chimeric approach and would be used successfully against Japanese Encephalitis, Dengue and West Nile Virus. [22] Subsequently vaccines created by the insertion of gene fragments of non-flaviviruses have been explored .[23] Proof of concept was tested by Ricardo Galler et al using malaria protein and it was demonstrated that robust immune responses were elicited in mice that were inoculated with the experimental vaccines .The same approach is being tested for the development of cancer vaccines and yellow fever based HIV vaccines. Pugachev has also argued for a need to explore molecular approaches in making the vaccine safer.[45]

2.1.4   Background to meta-analysis

2.1.4.1 Historical context of meta-analysis

Although Karl Pearson is thought to have performed the first meta-analysis in 1904 it was thought to have first been described and defined by Gene Glass et al in 1976 as '…the statistical analysis of a large collection of analysis results from individual studies for the purposes of integrating the findings'.[89] Meta-analyses are being increasingly used in research as they are considered to not only review a large body of evidence systematically but are also able to produce an effect size measurement that can be generalised. [90,91] Meta-analysis may be conducted either from collecting aggregate patient data or from individual patient data. The former, which is completed from studies that have been published in literature by other investigators and remains the most common method of conducting meta-analysis [90] and forms the basis of this study. In contrast utilizing individual patient data is more costly and requires a greater deal of effort as it requires cooperation with the original investigators. Further work conducted by Glass resulted in the more rigorous

statistical techniques that are now being currently used .[89,91] Meta-analysis has been increasingly used in the medical field and is beginning to have major impact on clinical research policy and patient care.[91] In addressing important clinical issues meta-analysis is considered to be the highest level of evidence.

Meta-analysis has notable strengths and advantages as a study design as it allows for the researcher to review work that is not only important and significant but it also focuses on obtaining quantitative summary conclusions using standardized terms.[91] Modern researchers, policy makers and clinicians are overwhelmed with the volume of reports that are available on a specific research topic and a meta-analysis is a useful tool to summarise and simplify the body of evidence that exists in a particular field of interest .[90] The study design also allows the researcher to reduce the complexity of conducting research making the meta-analysis a simple and affordable means of studying a particular issue. This is particularly useful when one wants to conduct research for rare medical conditions or as done in this study for neglected diseases like YF. By summarizing data and conducting a quantitative analysis of research questions across studies, the added benefit of being more generalisable than individual outcome studies is realized .The rigorous and systematic methodologies that are followed in conducting a meta-analysis also yields more robust implications and have additional benefits of reaching conclusions that are more reliable and accurate as a result of the methodologies used. In instances where heterogeneity is identified new hypotheses about subgroups can be also be generated.

Despite the numerous strengths of the meta-analysis, the researcher should be aware of and identify possible limitations to the general applicability of the possible findings. [91] The results acquired from a meta-analysis depend very largely on the breadth of the substantive literature review that must be conducted by the researcher .This typically requires access to large bibliographic indexes, registries of studies and to some extent language skills. The quality of the clinical trials from which the meta-analysis will be compounded also becomes critical if the conclusions are to be generalized .[91] Restrictions in terms of age, sex and nationality in the clinical trails being analysed are among some of the critical constraints to

generalisability of findings of a meta-analysis .[91] Finney et al also states that in conducting a meta-analysis the researcher should be cognisant of the dependence of the results on the original data and therefore criteria for inclusion needs to be carefully defined. Of significance is that the researcher must also assume that the presented data in included studies is valid and has been uncorrupted or primary scientific data has not been falsified. [91]

2.1.4.2 Vaccinology and meta-analysis

The use of meta-analysis in vaccinology has grown substantially in recent years.[89] Jacobson et al reported that a significant body of work on vaccines dealt with 'efficacy, effectiveness, immunogenicity, safety, reactivity, acceptability, delivery, cost effectiveness or cost benefit' of active immunizations. The authors also determined that despite the increasing popularity of meta-analyses, databases are incomplete requiring the researcher to search more than a single database including file-drawer reviews and trail registries. Of significance, is the challenge of heterogeneity that is inherent in meta-analyses. This is found at the level of the individual studies in relation to study population, the study interventions, means of detecting and measuring the outcomes sought and the study components.[89] One of the key methods of addressing heterogeneity is by grouping studies according to methods used ie RCTs versus prospective observational studies. For this study the former was done ie only RCTs were considered.

Jacobson et al also expressed the challenge of assuring the quality of studies when conducting a meta-analysis which may be due to various combinations such as poor study design and publication bias. [89] This can be addressed by evaluating studies individually and selecting RCTs where bias control is less complex than with non-randomised trials. This is usually achieved with a quality indicator score. In this study the Jadad score was selected and the reasons are outline in a later section.

When conducting a meta-analysis a study protocol that outlining the major activities to be followed in conducting the meta-analysis must be generated.[90] The meta-analysis process has five major components: problem formulation, data collection,

data evaluation, analysis and interpretation and reporting. Prior to commencing a meta-analysis main questions need to be explicitly asked that relate to the identified topic. This dissertation has to this point elaborated on problem formulation including this significance of the problem .This section that follows will focus on methodologies for data collection and data evaluation.

2.1.4.3 Summary

Yellow fever poses a major public health risk to South Africa. As a neglected disease it may be used for against a civilian population causing unprecedented mortality and morbidity. Yellow fever carries a high burden of disease particularly in Africa where only a small proportion of cases are reported. There has been a resurgence of YF since the 1980's bringing into question the readiness of the public health system to cope with an outbreak. In recent years South Africa has seen cases of imported viral haemorrhagic fevers and should therefore be adequately prepared in the event of an outbreak.

While vector control is a critical component of prevention, vaccination remains the mainstay. The yellow fever vaccine has till now been considered a safe and effective vaccine but recent studies have reported that neurotropic and viscerotropic side effects can occur particularly in the elderly, children and immunocompromised individuals. Numerous studies have been conducted investigating the antibody response to YF and much remains unclear as to the exact mechanisms.

Many studies have been conducted on various groups which are at evaluating the immunogenicity and tolerability of yellow fever vaccines. However, quantitative proof of efficacy comparing 17D and 17DD YF vaccines remains a gap that this study will attempt to address through a meta-analysis. The following chapter will describe the methods used to determine this important public health issue,

3   CHAPTER 3

3.1   METHODOLOGY

This section will provide the strategy and steps utilized in conducting the meta-analysis on YF vaccine immunogenicity and safety. It will outline the search strategy, data extraction process, the assessment of data quality as well as data management. It will also elucidate on the statistical analysis methods employed.

3.1.1.1   Database search

A comprehensive literature search of multiple databases subscribed by the University of Pretoria was conducted. .PubMed, Oxford Journals, EBSCO –host, Cochrane Controlled Trials register, BMJ, Cochrane Reviews, MEDLINE, Elsevier Science Direct, Highwire, BMJ, Google Scholar, e-theses and e-dissertations, Wiley Interscience databases were searched. International health agencies e.g. WHO and CDC websites and publications were included in the search. Only real life randomized controlled trials studies of humans in clinical and non clinical settings were considered to be units of analysis. Applying the terms 'yellow fever vaccine', 'randomized control trials' 'tolerability' 'efficacy' and 'immunogenicity' 'side effects' and 'vaccination' ,vaccine' 'intervention research' with publication time limits from 01 January 1900 until 30 August 2008 , searches were conducted. Results of studies identified were recorded. Locating 'grey literature' such as dissertations was also conducted along with crosschecking of references. Randomised controlled trials reporting means, standard deviations or standard errors that were published between 1900 and 2008, in English were included. Studies published in French, Spanish or Portuguese were not considered due to lack of translation capacity.

3.1.2   Study Selection

The inclusion of studies was based on assessing the intervention, population definition, study design and outcome measures and this is described in the sections to follow.

3.1.2.1 Intervention

Only real life randomized control trial studies where 17D and/or 17DD yellow fever vaccination was the primary intervention met the inclusion criteria. Studies that examined chimeric vaccines or YF vaccines administered in combination with other vaccines were not considered.

3.1.2.2 Study design

No anecdotal case studies, epidemiological, cross sectional or cohort studies were considered. Cross sectional studies are unable to establish temporal sequence which is a key factor in assessing immunogenicity over time. Only randomised blinded vaccine trial were included i.e. subjects, investigators and laboratory personnel will be blinded to the vaccine type lot assignments.

3.1.2.3 Outcome measures

Inclusion required that immunogenicity and /or tolerability were outcome measures and defined as the endpoints. Studies that were published as separate papers for the same trial i.e. splicing, were examined as a single study. Studies that were included required that:

    a. Follow up of participants be done following inoculation

    b. Ethical approval to conduct the trial must have been sought or given at the time of publication and

    c. Vaccination of subjects be performed by intradermal, subcutaneous or scarification techniques

3.1.2.4 Population definition

Population parameters of the studies used in studies that considered subgroups e.g. immunocompromised subjects e.g. HIV, post- splenectomy and post-thymomectomy patients, pregnant women and animals were excluded. Studies were excluded where:

    a. Previous YF or other flavivirus vaccination in the preceding 30 days or Treatment with immunoglobulin or blood products was not established

b. Administration of other experimental drugs or vaccines, including yellow fever were part of the protocol

c. No postvaccination follow up was conducted

d. Pregnant women were included

3.1.3 Data Abstraction

A coding manual (see Appendix 1) was developed to provide a framework for recording study findings. The data abstraction was conducted independently by the author. Each study was allocated a unique identifier. Both descriptive and outcomes data was extracted for each of the studies identified. Information on the study design, publication type, outcome criteria, demographic descriptors of the subjects, method of assignment to the intervention , nature of the intervention , presence of a control or comparison group ,dosages of vaccines given , method of inoculation, and duration of follow up were noted. The effect size data and method of calculation, sample size, outcomes data (means, standard error or deviation, tests of significance) and subject attrition was recorded for identified studies. Population characteristics e.g. gender, age, setting and race were noted. Analysis methods used in each study e.g. per protocol (PP) or intention to treat (ITT) were recorded. When conducting a meta-analysis, the quality of the RCTs that have been included in the study has to be assessed to ensure that reported results are a valid estimate of truth, are accurate and can provide more realistic estimates of treatment efficacy. The results of a meta-analysis can be significantly affected by the quality of the original trials. Moher et al assesses various scales, defined as a 'continuum with quantitative units that reflect varying level of a trait or characteristic', and checklists, which have no quantitative score, which has been developed to assess RCT quality.[92] Quality was defined as 'providing information about the design, conduct, and analysis of the trial'. The quality of a trial is therefore dependent on the reporting of all the relevant elements assessed according to the definition. Moher et al further cautioned against the use of scales in assessing quality as many have not been developed with standard techniques. While many checklists are also weak in their development they are the most useful in quality assessment as they provide guidance to authors on how to report, in terms of masking, patient follow up ,statistical analysis and patient assignment. The Jadad score is commonly used in

assessing quality because it is easy to understand and incorporates all the important methodological quality e.g. randomization. However, the Jadad score has also been found to have some disadvantages as it places emphasis on what is reported rather than the actual methodologies.[93] In the study conducted by Bhogal at al the authors concluded that in cases where the levels of blinding, concealment allocation, intention to treat and attrition are not always feasible to assess due to the nature of the intervention the Jadad score was found to be less comprehensive than measure of methodological quality .This was apparent when assessing stroke rehabilitation literature where the PEDro scale was more valuable. The findings were in keeping with Clark et al who also expressed concerns about the poor level of interrater agreement when using the Jadad score. [94]

In the selected trials for this study, the nature of the intervention i.e. YF vaccination lends itself well to assessment with the Jadad score due to the process that have to be conducted in the trial.

3.1.4 Statistical analysis

An Excel spreadsheet was used to collect and summarise data which was imported to MIX. The meta-analysis was conducted using MIX comprehensive free software for meta-analysis of causal research data) Version 1.7 which was developed by Bax L, Yu LM, Ikeda N, Tsuruta H, Moons KGM.

4 CHAPTER 4

4.1 RESULTS

In this section graphs and tables will report and summarise the statistical analysis results in terms of 1) the study process (2) procedures and tests conducted to establish reliability and publication bias (3) findings in terms of effect size and (4) subgroup analysis. The framework for the reporting is based on the QUORUM statement for reporting meta-analyses of RCTs.[95]

4.1.1 Process and Trial flow

The aim of this research was to identify RCTs that evaluated the immunogenicity and tolerability of YF vaccines. A two phase process was conducted with the aim of indentifying studies that met the criteria (see Appendix 3). The process is summarized in Figure 3.

4.1.1.1 Phase 1

Using the pre-determined search terms 6807 articles were returned as results cumulatively. Abstract of studies were read and assessed against the inclusion criteria. Studies that did not explicitly meet the criteria but warranted further probing in full text articles were brought forward into Phase 2. By the end of Phase 1 eleven independent studies were further identified and reviewed and evaluated against the inclusion and exclusion criteria in Phase 2. The process flow is illustrated in Figure 3.

4.1.1.2 Phase 2

Studies that appeared to meet the criteria in Phase 1 were further interrogated and read using full text articles. It was established that 3 studies were reports from the same trial that examined the same patients but focused on different outcomes of the same trial. One study was a sub group analysis of a study that was already included. These articles were then coded using the coding manual (Appendix 1) and after final review only eight studies were deemed to provide sufficient information for further analysis. All extracted data was captured in a Microsoft Excel spreadsheet for.

Appendix 3 provides a list of studies included in both phases. Only one trial fulfilled all the criteria but did not report on the immunogenicity. It was included in the assessment of reactogenicity. Five trials were excluded on account of lack of sufficient data for analysis. A further three trials were excluded as they were not commercially available or popular substrains. Only three trials were considered in the final analysis.

Figure 3 Summary of review process -Trial Process Flow
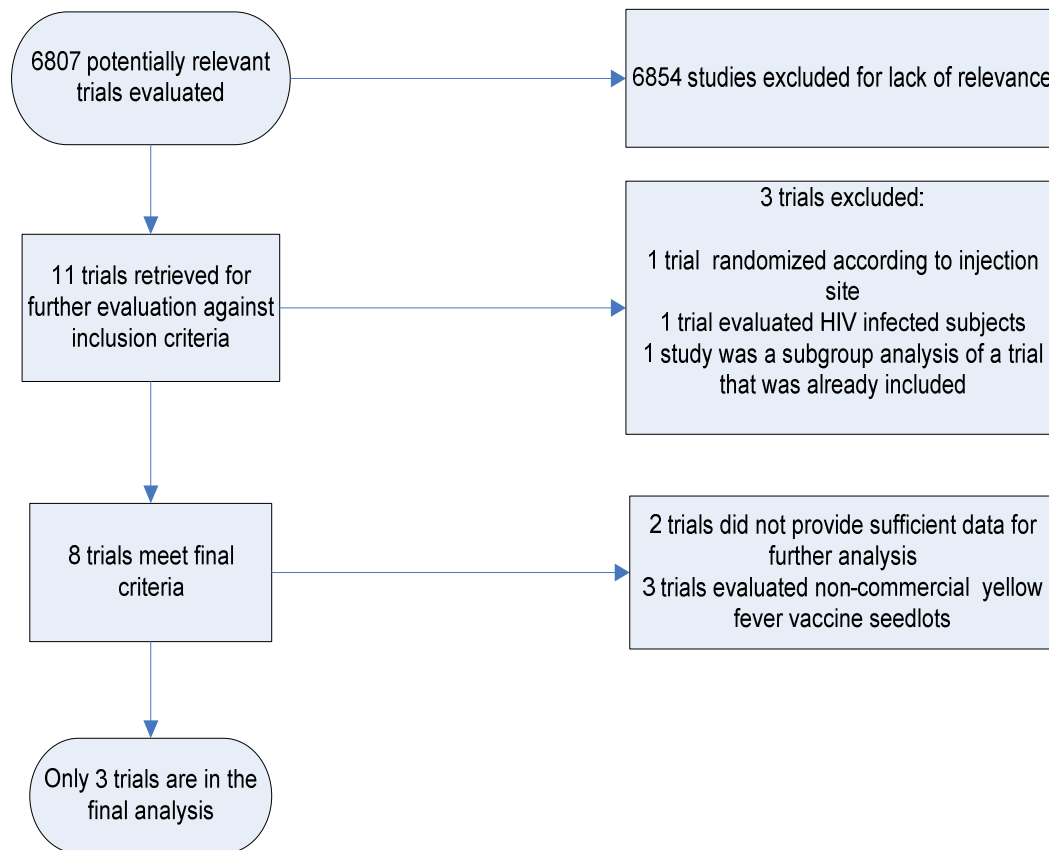
4.1.1.3 Quality assessment of studies

Using the JADAD method of assessing the quality of selected trials (Appendix 3), further grading of the 11 trials was conducted. A numerical score between 0–5 is assigned as rough measures of study design/reporting quality (0 being weakest and

5 being strongest). This number is based on the validated scale developed by Jadad et al. 96 By the end of this process on 3 trials were retained for further meta-analysis. The quality score of included studies is summarized in Table 1. The quality scores of the remaining studies were deemed to be good and acceptable for the purposes of further analysis.

Table 1 Jadad Quality scores of Selected Studies

Author	Jadad Quality score
Monath TP et al	3
Belmusto-Worn VE et al	4
Lang J et al	4

4.1.2 Study characteristics

The total individual study sample sizes ranged from 185-981 with a total sample size of 1740.
All the studies used randomization in the assignment process and studies evaluated immunogenicity and tolerability as outcomes of interest. All three studies included a treatment or intervention with a control. In all trials 17 DD (Arilvax) was the control intervention with the intervention being 17D vaccine substrains (Table 2). In calculating the effect size only subjects who were efficacy evaluable i.e. had serology that could be assessed were included.

Table 2. Numbers of studies and assignment groups

ASSIGNMENT OF INTERVENTION	NO OF STUDIES
Studies with treatment and control group	3
Studies with treatment, one control and one comparison group	0
Studies with treatment, one control and two comparison group	0

The settings in which studies were conducted differed between the studies. All the studies were multicentre based with study focusing on travel medicine units.

Table 3 Settings and participant profile of study

AUTHOR	COUNTRY	SETTING	PARTICIPANT PROFILE
Monath TP et al	United States	Multicentre outpatients	Healthy adults
Belmusto-Worn VE et al	Peru	Multicentre outpatients	Healthy children
Lang J et al	United Kingdom	Multicentre travel clinics and research centre	Healthy adults

Table 3 describes some basic study descriptors in terms of author, year of publication, total sample size, assignment method and duration of study. It is noted that none of the studies were conducted in Africa where the burden of disease is highest.

Table 4 Basic study descriptors

Author	Year	Intervention	Control	Primary Outcome/s	Sample Size	Study Design
Monath TP et al	2002	YF-VAX (17D)	ARILVAX (17DD)	Immunogenicity/ safety	574	RCT
Belmusto-Worn VE et al	2005	YF-VAX (17D)	ARILVAX (17DD)	Immunogenicity/ safety	981	RCT
Lang J et al	1999	Stamaril (17D)	ARILVAX (17DD)	Immunogenicity/ safety	185	RCT

4.1.2.1 Participants

4.1.2.1.1 Demographic characteristics

The mean age of the participants and the ranges is described in table 4. Two of the studies were conducted in adults, while one investigated vaccination in children. Table 4 also provides information on the racial characteristics of the subjects as well as data on gender composition of the studies.

Table 5. Mean age, gender and race of participants

AUTHOR	AGE		GENDER		RACE
	Mean age	Range	Male (%)	Female (%)	
Monath TP et al	38 years	Not reported	38.1%	61.8%	Caucasian 80%
Belmusto-Worn VE et al	4 yrs 11 mo	2yrs 5 mo	48.3%	51.7%	Caucasian 1.1% Black 0.1% Mixed 98.8%
Lang J et al	31 yrs 5mo	18-69 years	36.2%	63.8%	Not reported

4.1.2.1.2 Outcome measures

Criteria for reporting outcomes measures included reporting seroconversion rates according to WHO recommendations. In the selected studies it was noted that there were variations in the expression of the outcome measures. However, the differences were in the expression of the outcomes rather than measurements i.e. YF virus neutralizing antibody titres >=1:5 corresponds to the log neutralizing index (LNI) > OR = 0.7 .Outcomes measures that were used in the selected studies are documented in table 5.

Table 6 Outcome measures of vaccines used in trials

AUTHOR	OUTCOME MEASURE
Monath TP	Log $_{10}$ neutralising index (LNI)> or = 0.7
Belmusto-Worn VE et al	Log $_{10}$ neutralising index (LNI)> or = 0.7
Lang J et al	YF virus neutralising antibody titres > or = 1:10

4.1.3 Vaccine safety and tolerability

In a cross referenced study by Monath et al [73] a classification system for adverse events was described as shown in Table 7 where ;SyAE reports systemic adverse

events , OAE describes other systemic events . The table provides examples of adverse events but is not limited .All studies were included in the summary which enumerates events and not subjects. This is due to the fact that some subject experience more than one events.

Table 7. Adverse event classification

ADVERSE EVENT CATEGORY	INCLUDED ADVERSE EVENTS	NUMBER OF EVENTS IN ALL TRIALS
Neurologic (SyAE)	GBS, new onset seizures, encephalitis ,myelitis, altered mental state, facial or cranial neurologic deficits, parasthesias, vertigo, headaches	121
Multisystemic (SyAE)	Myalgias, arthralgias, rhabdomyolysis, elevated transaminases, respiratory distress, nausea, vomiting, diarrhoea, nephropathy, DIC+/- fever. Onset <2 weeks after vaccination .Duration >=72 hours	1486
Uncomplicated Neurologic/Systemic(OAE)	Cases that met the neurologic or systemic criteria but had a full and rapid clinical recovery in <72 hours	0
Nonspecific Events (OAE)	Dizziness, headache, nausea, vomiting or diarrhoea alone	1653
Hypersensitivity Reactions(OAE)	Rash, urticaria +/- fever, anaphylaxis, angioedema. Onset within 48 hours of vaccination	395
Local reactions (OAE)	Localised pain, swelling, erythema or warmth at injection site. Onset within one week of vaccination	1114
Reactions unrelated to vaccines(OAE)	1. A clear ,alternative diagnosis confirmed by laboratory criteria that accounts for symptoms and signs; sometimes this is an underlying illness 2. Another cause implied or stated in the physicians report. This includes inadvertent administration during pregnancy with no associated adverse event	Not reported

4.1.4 Statistical analysis

Meta-analysis may be used to investigate the combination or interaction of a group of independent studies, results from similar studies conducted at different centres. MIX ™ software was utilized to analyse the data. Using MIX™, the Mantel-Haenszel type method of Greenland and Robins is used to estimate the pooled risk difference for all strata, assuming a fixed effects model. A confidence interval for the pooled risk difference is calculated using the Greenland-Robins variance formula.

4.1.5 Studies' findings

The quantitative findings were analysed on MIX™ and summarized below. Statistical significance was considered when p values were below 0.05.

4.1.5.1 Input summary

Table 8 presents a summary of the risk differences between interventions using a fixed effects model. The risk difference describes the absolute change in risk that is attributable to the experimental intervention. If an experimental intervention has an identical effect to the control, the risk difference will be 0. If it reduces risk, the risk difference will be less than 0; if it increases risk, the risk difference will be bigger than 0. The risk difference cannot be above 1 or below -1. Switching between good and bad outcomes for the risk difference causes a change of sign, from + to - or - to +.The risk differences between the vaccines are summarized in Table 5.

Table 8 Summary of risk differences using the fixed effects model

INPUT SUMMARY					Fixed Effects Model	
			RD(MH)			
Study ID	Study Date	RD	95% CI	p	Weight Bar	Weights (MH)
Lang et al	1999	0.0109	-0.0187 to 0.0404	0.4706	III	11.32%
Monath et al	2002	0.0073	-0.0094 to 0.024	0.3944	IIIIIIIIII	35.13%
Belmusto-Worn	2005	-0.0436	-0.0794 to -0.0078	0.0169	IIIIIIIIIIIIII	53.54%

When using a random effects model using the Der Simeon Laird weighting method the following results are elicited.

Table 9 Risk difference when applying the random effects model

INPUT SUMMARY						Random Effects Model
RD(DL)						
Study ID	Study Date	RD	95% CI	p	Weight Bar	Weights (MH)
Lang et al	1999	0.0109	-0.0187 to 0.0404	0.4706	IIIIIIIIII	31.67%
Monath et al	2002	0.0073	-0.0094 to 0.024	0.3944	IIIIIIIIIIIII	40.94%
Belmusto-Worn	2005	-0.0436	-0.0794 to -0.0078	0.0169	IIIIIII	27.40%

Analysis using both the fixed and random effects models demonstrates that while the RD in the intervention is less than zero, it closely approached zero suggesting minimal differences between 17D and 17DD vaccines.

The Relative risk (RR) was also assessed and the results are displayed in the Table 10 below. A relative risk of 1 indicates no difference between the two groups in terms of their response to the two treatments being compared . In this study the comparisons are between subjects who received Arilvax (17DD) versus those who received 17D YF vaccine. Tables 10 and 11 both indicate insignificant difference between the RR of both groups. Belmusto et al shows only a negligible decrease in RR that was significant.

Table 10 Relative Risk when applying the fixed effects model

INPUT SUMMARY						Fixed Effects Model
RR(MH)						
Study ID	Study Date	RD	95% CI	p	Weight Bar	Weights (MH)
Lang et al	1999	1.011	-0.9812 to 1.0417	0.4742	IIIIII	11.59%
Monath et al	2002	1.0074	0.9905 to 1.0245	0.395	IIIIIIIIIII	35.83%
Belmusto-Worn	2005	0.9541	0.9175 to 0.9921	0.0184	IIIIIIIIIIIIIIIII	52.58%

Table 11 Relative Risk when applying the random effects model

INPUT SUMMARY					Random Effects Model	
			RR(DL)			
Study ID	Study Date	RD	95% CI	p	Weight Bar	Weights (MH)
Lang et al	1999	1.011	-0.9812 to 1.0417	0.4742	IIIIIII	32.24%
Monath et al	2002	1.0074	0.9905 to 1.0245	0.395	IIIIIIIIII	41.63%
Belmusto-Worn	2005	0.9541	0.9175 to 0.9921	0.0184	IIIII	26.13%

4.1.5.2 Publication bias

Publication bias in conducting a meta-analysis publication bias must be considered as the number of trials published may not equal the number of trials conducted i.e. published literature does not represent the total population of studies that have been completed on the subject.[97] Publishers may show bias towards studies demonstrating larger effect size, with significant results or research that is easily available. [98] As this may weaken the validity of the meta-analysis an extensive search for studies and unpublished documents was conducted. A skewed funnel shape would indicate bias between published and unpublished studies while symmetry would suggest no or little bias.

Publication bias would indicate a tendency to report on studies with significant findings i.e. positive publication bias rather than those with negative or inconclusive results. Rothstein at al further describe other potential mechanisms of bias that may arise that results from language e.g. selecting studies published only in English, availability i.e. selecting studies that are easily accessible, cost bias e.g. due accessing available or free studies , familiarity bias and outcome bias. These biases result in reporting an unrepresentative population of completed studies which threatens the validity of the reported results.

Berlin and Ghersi [98] suggested that open access measures would reduce the possibility of publication by putting forward two main recommendations. The creation

of a central database where all clinical trials being conducted are registered would eliminate the time-consuming activities related to identifying grey literature. In addition Berlin and Ghesi also suggested increased use of the prospective meta-analysis i.e. where different groups of investigators combine their findings when the trials are complete. The latter allows for a meta-analysis to be designed prospectively allowing for standardisation of tools and outcomes measures.

The most common way of determining publication bias is through a funnel plot which is a graphical depiction of effect size against the study size of individual studies.[98]The funnel plot usually depicts the treatment effect on the horizontal axis and a wight on the vertical axis. The weight may be the inverse standard error or sample size. In determining the presence and magnitude of publication bias overall estimates are plotted against the inverse of the standard error using a fixed effect model with Mantel –Haenzel weighting.

In a funnel plot the most precise estimated are at the top of the funnel with the least precise at the base. The commonest interpretation is that a symmetrical funnel is usually formed in the absence of publication bias [100] and if a funnel appears to be missing points there is potential bias. [99] Funnel plots are attractive to use as they are simple to assess visually. However this also means that they can be interpreted subjectively by the reviewer. Publication bias, heterogeneity, chance, choice of outcome measure and choice of precision measure may all influence and result in asymmetry.[99] Additionally Tang et al also concluded that the absence of how the funnel plot should be constructed ,which is currently arbitrary , means that Asymmetric funnel plots can be trimmed and filled with 'missing' studies that would estimate the true centre of the funnel. A funnel plot was used to evaluate publication bias and its potential impact. A funnel plot showing effect size (risk difference) in the horizontal axis and inverse of the standard error on the vertical axis was demonstrated using the MIX software.

Figure 4. Funnel Plot using fixed effect model-M-H weighting
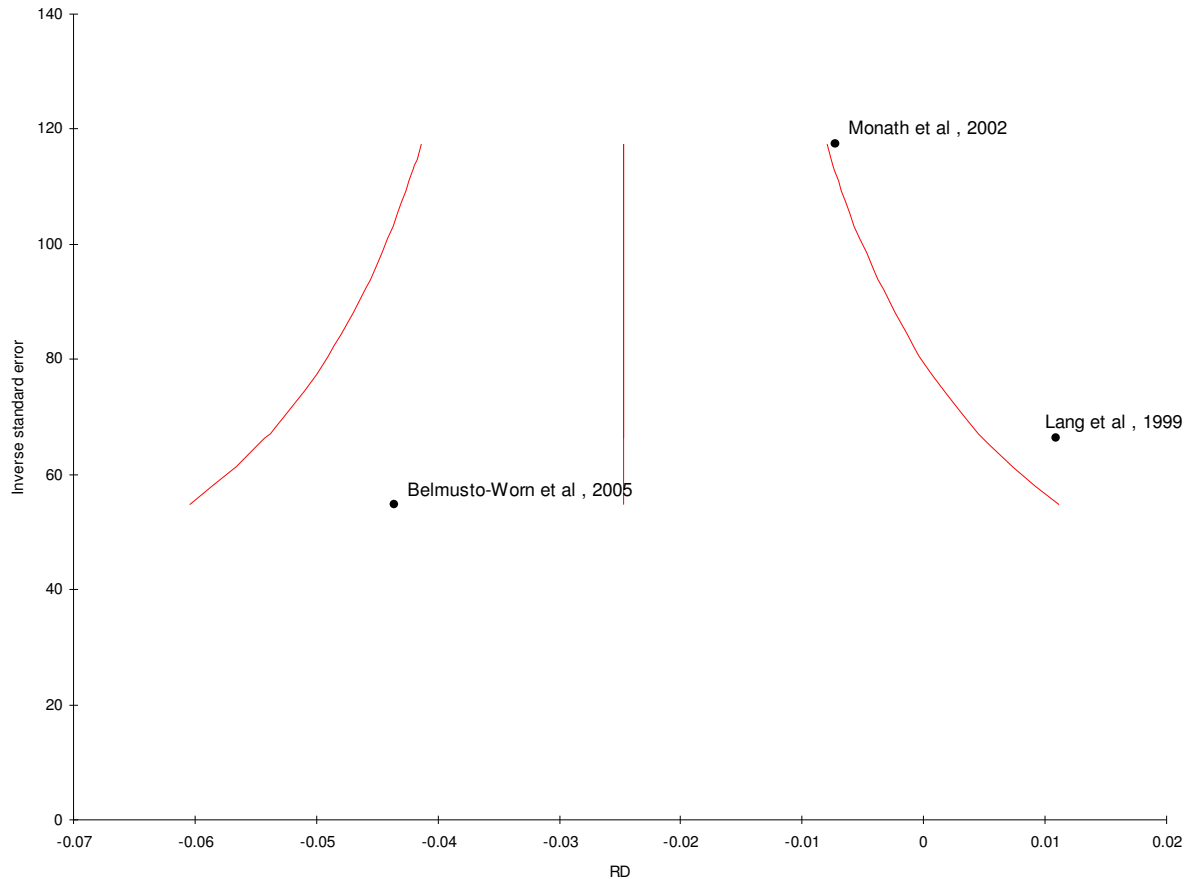
A funnel plot was used to assess publication bias.The funnel plots (Figure 4) indicates that in this study there was significant asymmetry which may be due to publication bias as most studies reported a positive significance. However, Terrin et al have also argued that asymmetry can be found in funnel plots where there are a small number of studies particularly where there are fewer than 10 studies being analysed as is the case in this study.[100] They further argue that visual inspection alone is inadequate for separating the effects of publication bias, heterogeneity and chance. Figures 5 and 6 clearly indicate asymmetry on visual inspection which may be due to publication bias, heterogeneity or chance.

Due to the small number of studies analysed it was not feasible to perform a trim and fill plot.

4.1.5.3 Effect size

The main aim of this study is to determine the overall effects of yellow fever vaccination in terms of immunogenicity of the vaccine as evidenced by changes in specific immune markers. The null hypothesis can be defined as the statistical hypothesis that states that there are no differences between observed and expected data. In this study the null hypothesis can be expressed as:

$H_{0:}$ δ=0 . This means that the effect size is zero i.e. there is no difference in effect size between the treatment and control groups in terms of seroconversion following vaccination. Effect size measures that will summarise the findings from the studies are reported in this section. In this study effect size was measured using the standardised mean difference and the correlation coefficient.

1. *Standardised mean difference*

The weighted mean average for seroconversion was calculated for the 17D and 17DD groups using the sample size as the weights where *w* is the sample size and *x* is the proportion of subject who seroconverted as expressed in Table 12.

$$\bar{x} = \frac{w_1 x_1 + w_2 x_2 + \cdots + w_n x_n}{w_1 + w_2 + \cdots + w_n}.$$

Table 12 Weighted and unweighted mean average of proportion of seroconverters

	Treatment Group (17D)	Control Group (17DD)
Unweighted Mean Average	0.964	0.977
Weighted mean average	0.942	0.968

Table 12 data indicates that 96.4% of subjects seroconverted when given 17D while 97.7 % seroconverted when inoculated with 17DD when pooled. However this represents the unweighted mean average that does not take the sample size of each study into account. When sample size is considered the weighted proportion of subjects who seroconverted when inoculated with 17D vaccines is 0.942 while the

proportion of seroconverted people in the 17DD group is 0.968.These proportions can then be used to calculate the correlation coefficient, Cohen's d.

2. Correlation coefficient

Measuring effect size is important when conducting a meta-analysis as it is a summary of the measure of the treatment effect.[99] Traditional tests of effect size e.g. *t* tests or *F* tests are inappropriate as they are to a certain degree a function of size and may therefore have large variations as sample sizes often differ. There are many acceptable methodologies for calculating effect sizes from research articles .Effect size estimates can be calculated using Cohen's d, Hedges g and Glass delta. The most common effect size estimate used in meta-analysis is the Cohen's d and will be selected for the purposes of reporting for this study.

When calculating effect size measures for two independent groups e.g. the experimental group (vaccinated) and control group (unvaccinated) Cohen's d can be used to assess where there was a positive or a negative effect size indicating improvement or deterioration respectively.

When examining the experimental and control group's effect size Cohen's D is a useful descriptive measure. The conventional values to describe Cohen's d effect size are small, where d= 0.20, medium where d= 0.50 and large where d=0.80.and can be calculated by dividing the difference of the two means divided by the standard deviation (S) using the following formula:

$$d = \frac{\bar{x}_1 - \bar{x}_2}{s}$$

where s is calculated using the following formula;

$$s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2}}$$

Table 13 . Summary of means and Standard deviations

	Treatment group	Control Group
Mean =	0.942	0.968
SD =	0.468	0.021
n =	705	1035

Cohen's d was computed using the pooled standard deviations using the data summarized in Table 13.

When computed using the 'effect size determination program' Cohen's d was calculated to be -0.087. The negative Cohen's d coefficient reflects group differences in a direction other than the expected direction. This means that the effect size between the treatment and control groups was small and also in the direction that favours of the control group i.e. Arilvax (17DD). However, in practice there was minimal difference between 17D and 17DD YF vaccines with the 17D control group showing better results.

4.1.5.4 Heterogeneity

A forest plot was produced using MIX software in order to assess heterogeneity and effect size .The chart is used to assess relative difference between the results of the studies included in the analysis. The vertical axis lists the studies in input order while the horizontal axis is a measure of the effect of each study including the confidence intervals.

  Forest plots depicting the pooled estimate are shown in figures 5 and 6. A vertical line representing no effect is also plotted. In analysing forest plots, if the confidence intervals for individual studies overlap with this line, it demonstrates that at the given level of confidence their effect sizes do not differ from no effect for the individual study. The pooled empirical value with its confidence interval is demonstrated by the diamond shape. If the points of the diamond which represents the pooled effect overlap the line of no effect the overall meta-analysed result cannot be said to differ from no effect at the given level of confidence.

The size of the square corresponds to the weight of the study in the meta-analysis. The confidence intervals for totals are represented by a diamond shape. The risk differences are displayed on a linear scale.

Figure 5 Annotated Forest Plot –Fixed effects –M-H
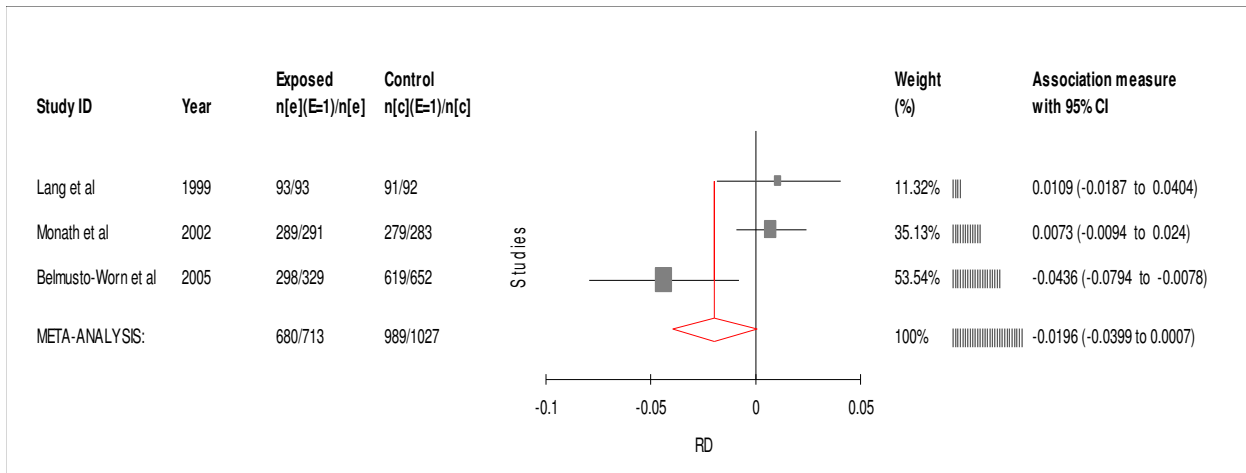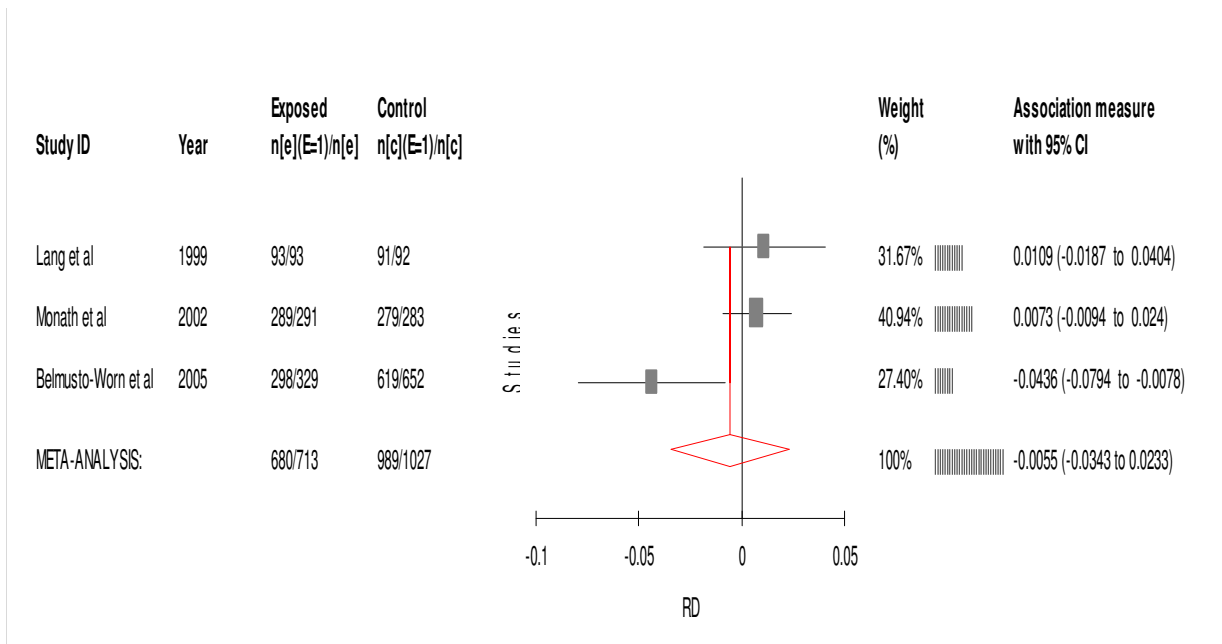

Figure 6 Annotated Forest Plot –Random effects

The forest plots indicate that the confidence intervals include zero suggesting no significant difference in effects of 17D and 17DD vaccines as interventions for Lang

et al and Monath et al. However the Belmusto –worn study does not include zero in the confidence interval when using the random effects model. Notably the pooled estimate includes zero for both the random and fixed effects models.

Heterogeneity was evaluated using the Cochrans' Q and the Higgin's H statistic. Cochrane's Q statistic is used to indicate the difference in two treatments applied to the same population. If the value of the statistic is high the null hypothesis of homogeneity is rejected. The low H statistic indicates high consistency among the study results. However, the high I^2 statistic shows a high percentage of the variation between the studies that is not explained by chance.

Table14 Summary of meta-analysis heterogeneity.

META-ANALYSIS	
General	
Number of studies	3
Number of participants	1740 (1740)
RD (MH) - Fixed effect model	
Meta-analysis outcome	-0.0196
95% CI low er limit	-0.0399
95% CI upper limit	0.0007
z	1.8902
p-value (tw o-tailed)	0.0587
Heterogeneity	
Q	15.721
p-value (tw o-tailed)	0.0004
H	2.8037
95% CI low er limit	1.6645
95% CI upper limit	4.7225
I^2	87.28%
95% CI low er limit	63.91%
95% CI upper limit	95.52%

5 CHAPTER 5

5.1 DISCUSSION

The purpose of the discourse outlined in this chapter is to summarise the findings that were elicited from the analysis of all data gathered in the context of known literature pertaining to YF vaccines. The findings are justifiable by prior research findings and the narrow field of interest resulting in a small meta-analysis with only three studies. By virtue of conducting a meta-analysis, only randomised control trials that were available on the university subscription could be and were included as per protocol. The implication for this was a study that had to accommodate the strict time limitations and available resources of a single student. This may result in instability in the study results because of few studies being considered.

The findings presented above could very well represent the first meta-analytic study on the immunogenicity and safety of YF vaccines to date and is therefore significant in this regard.

The results are based on a review of only three studies published in peer –reviewed journals examining immunogenicity and safety representing 1740 participants among the two outcomes viz immunogenicity and safety. Publication and selection biases in meta-analysis are more likely to affect small studies, which also tend to be of lower methodological quality. This may lead to "small-study effects," where the smaller studies in a meta-analysis show larger treatment effects. Small-study effects may also arise because of between-trial heterogeneity. Statistical tests for small-study effects have been proposed, but their validity has been questioned.[100] In this study Belmusto et al had the largest number of participants but also demonstrated the largest risk difference and relative risk when comparing treatment and control groups as opposed to the smallest study by Lang et al. This suggests that there are other factors that may be contributing to these differences beyond the sample size. These possible factors will be discussed later in this chapter.

The effect of race, gender and age is important as described in Chapter 2. The findings of this study show some marked differences in outcomes in terms of Belmusto which was a study conducted among children when compared to Lang and Monath et al. This suggests that the benefits are greater among children in terms of immunogenicity as evidenced by the relative risks and risk differences when compared to adults. Also of note is the racial composition of the Belmusto et al study which only comprised 1.1% of Caucasians when compared to Monath et al which comprised more than 80% Caucasians. While this is keeping with preliminary studies that suggest people of African or mixed Black descent have more muted responses in terms of achieving immunogenicity.

The findings of the meta-analysis were in keeping with the body of evidence that exists i.e. YF vaccine is highly effective and induces a robust immunological response.

5.1.1 Equivalence and Meta-analysis

The study design in equivalence studies typically compares two active interventions with aim of assessing whether the two interventions are equally effective. The null hypothesis states that there is no difference between the interventions. Typically it should be demonstrated that the treatment under investigation has for example less than 75% improvement of the effect of the control standard comparator for it to be considered non-inferior and equivalent.[101]There are some important considerations in conducting meta-analysis of equivalence trials. For equivalence studies the confidence intervals pertaining to the summary effect statistics in the meta-analysis assume greater significant than the statistical significance. It should also be considered that the interventions may be equivalent but equally ineffective.

5.1.2 Overall effect size

The results of this study are found to be in keeping other studies investigating the immunogenicity of yellow fever vaccines. All the studies reviewed showed that yellow fever vaccine was effective in conferring immunity to subjects. Rosenthal has suggested that assessing clinical meaning by comparing the results of meta-analysis

findings with other studies may deal with the issue of file-drawer studies.[98] The reporter was unable to find other meta-analyses investigating similar topics and therefore comparisons are made only with studies reporting individual outcomes.

5.1.3 Limitations of the study

According to the findings of this study both 17D and 17DD YF vaccines are effective in preventing YF infection. However, this should be interpreted with limitations in mind. The first limitation relates to factors that are due to the author and the nature of this dissertation.   The most significant of these relates to time and capacity. Due to the author having to conduct a meta-analysis alone within a limited timeframe for the purposes of completing a dissertation is limiting. Given time and additional personnel a more comprehensive and exhaustive process would have been followed that would have identified more studies for inclusion. The study should therefore be considered in this light.

A significant amount of research is conducted in Francophone, Lusophonic, Hispanophonic countries due to the geographic location of the YF belt. Due to limited resources available for translation only articles published in English were considered. This may have resulted in significant literature being excluded from the review.

The small size of the study units identified is also an important limitation. This may be due to the narrow area under investigation as YF as a disease is considered a neglected disease despite the burden of disease. An attempt was made to contact various authors known to experts in YF with the aim of identifying additional unpublished studies that could be added to this study to no avail. The communications are added as appendix 4.

The studies identified were largely funded by commercial interests with the aim of conducting the clinical validation process of new working seed lots. This may introduce bias and call into question the ethical robustness of the studies.

The study design i.e. meta-analysis has inherent limitations and weaknesses. TIN computing the effect size by using the pooled variance, there is an assumption that standardized effects are constant across the included studies.

Meta analysis also assumes that there is independence in the studies that are selected in terms of the methodologies used by researchers which are standardized and uniform. This may compromise statistical independence.

The nature of randomized control trials is such that there are selection and exclusion criteria which are determined by the researchers. This may have an impact on the result of the meta-analysis.

6 CHAPTER 6

CONCLUSIONS AND IMPLICATIONS

6.1 IMPLICATIONS FOR PRACTICE

This study adds to the limited information that is available on effectiveness of YF vaccine. The vaccine has been demonstrated to be safe and well tolerated. However, surveillance systems for monitoring YF activity in Africa remain poor and neglected. Strengthening of public health systems in order to mitigate and reduce the impact of YF outbreaks remains critical. Reporting systems for adverse side effects must be developed particularly in developing countries in order to improve the prescribing patterns. This may be addressed through education of health care workers particularly professionals e.g. doctors and nurses.

International health regulations are a pivotal control measure that needs to be reinforced. Due to the anticipated increase in travel between regions, it is critical that countries that are at risk have allocated the resources to manage incoming travelers from endemic areas.

6.2 IMPLICATIONS FOR RESEARCH

In his analysis of the data Monath noted that Non-Caucasian individuals had lower antibody titres as evidenced by lower mean LNI .[33] In the perusal of the literature, this factor appears not to have been explored fully by other authors. Most cases of Yellow fever epidemics have occurred in West Africa[29] yet research on the immunogenicity and safety of yellow fever specifically examining African populations are conspicuous by their absence. The role of race may be critical in assessing the efficacy of YF vaccine in African populations who are most at risk and would benefit from continued investigation.

All studies have emphasized the role of age in determination seroconversion rates and the role of increasing age particularly in the elderly seems to be undisputed. However, in comparing adults to children, the reasons for lower conversion rates among children remain unclear.[20] Given that YF is part of the EPI schedule additional research will be required to ascertain the relevance of current practice.

7 APPENDICES

7.1 Appendix 1 Coding manual

Bibliographic Information	
Identification of reviewer	
Date of extraction	
Title	
Authors	
Journal	
Publication Type	
Study Id Number	
Publication year	
Specific Notes and study descriptors	
Mean age	
Racial profile of subjects	
Gender profile of subject	
Care settings	
Other Population characteristics	
Methodology	
Study design	
ITT	
PP	
Blinding method	
Sample size	
Treatment group	
Control group	
Comparator group	
Placebo (Y/N)	
Reasons for attrition noted	

Outcomes	
Effect size name and type	
Tests of significance	
Outcomes measures	
Length of follow up	
Lost to follow up	
Missing data	
Total numbers	
P value	
Mean	
SD	
Effect Measures	
Notes	
Coding format and instructions for coders	

7.2 APPENDIX 2. Jadad Scale Criteria

This calculation does not account for all study elements that may be used to assess quality (other aspects of study design/reporting are addressed in tables and text). A Jadad score is calculated using the seven items in the table below. The first five items are indications of good quality, and each counts as one point towards an overall quality score. [Either give a score of 1 point for each "yes" or 0 points for each "no." There are no in-between marks.]

The final two items indicate poor quality, and a point is subtracted for each if its criteria are met. The range of possible scores is 0 to 5.

Jadad Score Calculation

Item	Score
Was the study described as randomized (this includes words such as randomly, random, and randomization)?	0/1
Was the method used to generate the sequence of randomization described and appropriate (table of random numbers, computer-generated, etc)?	0/1
Was the study described as double blind?	0/1
Was the method of double blinding described and appropriate (identical placebo, active placebo, dummy, etc)?	0/1
Was there a description of withdrawals and dropouts?	0/1
Deduct one point if the study was described as double blind but the method of blinding was inappropriate (e.g., comparison of tablet vs. injection with no double dummy).	0/-1
Deduct one point if the method used to generate the sequence of randomization was described and it was inappropriate (patients were allocated alternately, or according to date of birth, hospital number, etc).	0/-1

Randomization. A method to generate the sequence of randomization will be regarded as appropriate if it allowed each study participant to have the same chance of receiving each intervention and the investigators could not predict which treatment was next. Methods of allocation using date of birth, date of admission, hospital

numbers, or alternation should be not regarded as appropriate.

Double blinding. A study must be regarded as double blind if the word "double blind" is used. The method will be regarded as appropriate if it is stated that neither the person doing the assessments nor the study participant could identify the intervention being assessed, or if in the absence of such a statement the use of active placebos, identical placebos, or dummies is mentioned.

Withdrawals and dropouts. Participants who were included in the study but did not complete the observation period or who were not included in the analysis must be described. The number and the reasons for withdrawal in each group must be stated. If there were no withdrawals, it should be stated in the article. If there is no statement on withdrawals, this item must be given no points.

7.3 APPENDIX 3 Literature Review Process- Phase 1

NO	AUTHOR
1	Roukens AH, Vossen AC, Bredenbeek PJ, van Dissel JT, Visser LG . Intradermally administered yellow fever vaccine at reduced dose induces a protective immune response :a randomized controlled non- inferiority trial PLoS ONE 2008; 3 (4) ;1-7
2	Pfister M, Kursteiner O,Hilfiker H et al .Immunogenicity and Safety of Berna-YF Compared With Two Other 17D Yellow Fever Vaccines in a Phase 3 Clinical Trial .Am J of Trop Med.Hyg. 2005 ; 72(3) :339-346
3	Roche JC, Jouan A, Brisou B , Rodhain R, Fritzell B, Hannoun C. Comparative clinical study of a new 17D thermostable yellow fever vaccine. Vaccine 1986;4:163-165
4	Belmusto-Worn V E ,Sanchez JL,McCarthy K,Nichols R, Bautista AJ et al .Randomised double blind phase III pivotal field trial of the comparative immunogenicity, safety and tolerability of two yellow fever 17D vaccines .Arilvax and YF –VAX in healthy infants and children in Peru. Am. J. of Trop Med. and Hyg. 2007; 72(2) : 189-197
5	Monath TP ,Nichols R, Archambault WT, Moore L, Marchesani R,Tian J. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF –VAX) in a phase IIImulticenter double blind clinical trial .Am J Trop Med Hyg 2002;66(5) :533-541
6	Ripoll C, Ponce A, Wilson MM, Sharif N, Vides JB, Armoni J . Evaluation of two yellow fever vaccines for routine immunization programs in Argentina. Human Vaccine . 2008; 4(2): 121-126
7	Collaborative Group for studies with Yellow fever Vaccine .Randomised ,double-blind , multicentre study of the immunogenicity and reactogenicty of 17DD nad WHO 17D-213/77 yellow fever vaccines in children: Implications for the Brazilian National Immunizaion Program . Vaccine .2007 25 :3118-3123
9	Lang J, Zuckerman J, Clarke P, Barrett P, Kirkpatrick C et al . Comparison of the Immunogenicity and Safety of two 17D yellow fever vaccines. Am J Trop Med Hyg 1999 60(6) 1045-1050
10	Veit O, Niedrig M, Chapuis-Taillard C, Cavassini M , Mossdorf E, Schmid P et al. Immunogenicity and safety of yellow fever vaccination for 102 HIV infected patients. CID. 2009;48:659-666
11	Monath TP, Cetron MS, McCarthy K, Nichols R, Archambaul WT, Weld L et al . Yellow fever 17D vaccine safety and immunogenicity in the elderly. Human Vaccines 2007;1(5) 207-214

LITERATURE REVIEW PHASE 2

	EXCLUDED STUDIES	REASON FOR EXCLUSION
1	Roukens AH, Vossen AC, Bredenbeek PJ, van Dissel JT, Visser LG . Intradermally administered yellow fever vaccine at reduced dose induces a protective immune response :a randomized controlled non- inferiority trial PLoS ONE 2008; 3 (4) ;1-7	Only assessed reduced dose responses
2	Pfister M, Kursteiner O,Hilfiker H et al .Immunogenicity and Safety of Berna-YF Compared With Two Other 17D Yellow Fever Vaccines in a Phase 3 Clinical Trial .Am J of Trop Med.Hyg. 2005 ; 72(3) :339-346	
3	Roche JC, Jouan A, Brisou B , Rodhain R, Fritzell B, Hannoun C. Comparative clinical study of a new 17D thermostable yellow fever vaccine. Vaccine 1986;4:163-165	
6	Ripoll C, Ponce A, Wilson MM, Sharif N, Vides JB, Armoni J . Evaluation of two yellow fever vaccines for routine immunization programs in Argentina. Human Vaccine . 2008; 4(2): 121-126	Did not evaluate immunogenicity
7	Collaborative Group for studies with Yellow fever Vaccine .Randomised ,double-blind , multicentre study of the immunogenicity and reactogenicty of 17DD nad WHO 17D-213/77 yellow fever vaccines in children: Implications for the Brazilian National Immunizaion Program . Vaccine .2007 25 :3118-3123	Did not provide enough information for further evaluation
8	Camacho LAB, daSilva Freire M , da Luz Fernandes Leal M, Gomes de Aguiar S, Pereira do Nascimento J, Iguchi T et al .Immunogenicity of WHO-17D and Brazilian 17DD yellow fever vaccines : a randomised trial.Rev Saude Publica 2004;38(5):671-678	Camacho LAB, Gomes de Aguiar S, daSilva Pereira M , da Luz Fernandes Leal M, Pereira do Nascimento J, Iguchi T et al. Reactogenicity of yellow fever vaccines in a randomized , placebo controlled trial Rev Saude Publica 2005;39(3):413-420
10	Veit O, Niedrig M, Chapuis-Taillard C, Cavassini M , Mossdorf E, Schmid P et al. Immunogenicity and safety of yellow fever vaccination for 102 HIV infected patients. CID. 2009;48:659-666	Only evaluated safety and did not provide data on immunogenicity
11	Monath TP, Cetron MS, McCarthy K, Nichols R, Archambaul WT, Weld L et al . Yellow fever 17D vaccine safety and immunogenicity in the elderly. Human Vaccines 2007;1(5) 207-214	Sub group analysis of a previous study

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